RELATION BETWEEN TOLERANCES AND DISTRIBUTION OF TWO SPECIES OF EPHEMEROPTERA

by

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SUMMARY

In this study, tolerance limits for a number of environmental factors, taken both singly and in combination, have been determined in the laboratory for aquatic nymphs of two mayflies, Baetis harrisoni Barnard 1932 and Choroterpes (Euthraulus) bugandensis (Kimmins 1956). These limits have been examined in the light of available information on the ecology of the nymphs in order to see to what extent the distribution of each species might be limited by intolerable environmental conditions.

Baetis nymphs were found to be dislodged from rocks in the stream flow by current speeds in excess of 0.5-0.6 m/sec actually impinging on the nymphs and estimated 0.1 cm from the substratum surface. Choroterpes nymphs were dislodged by current speeds in excess of 0.3-0.4 m/sec estimated 0.1 cm from the substratum surface. Possible effects of flooding on nymphal populations of each species have been discussed in relation to the behaviour and physical habitat of the nymphs. Baetis nymphs live on rocks exposed to the current and numbers of them are washed away even in moderate floods. Choroterpes nymphs live on the undersurfaces of rocks and numbers of them are only washed away by floods sufficiently strong to roll these rocks.

Although widespread in southern African streams, Baetis nymphs were relatively intolerant of high temperatures (lethal limits for nymphs attempting ecdysis 25.4-25.8^{o}C in winter, 27.0-29.3^{o}C in summer). Higher temperatures than these were recorded in a Transvaal stream in which Baetis was not found. Choroterpes nymphs tolerated much higher temperatures (35.4-35.8^{o}C in winter, 35.9-36.2^{o}C in summer). High temperature tolerance of Baetis

nymphs was reduced in slow flowing water and at low dissolved oxygen concentrations, while that of Choroterpes nymphs was not. Conditions intolerable for Baetis nymphs might be expected from time to time in sluggish low lying streams. Conditions intolerable for Choroterpes nymphs will be rare in these streams.

Baetis nymphs entered chill coma at 5.7°C in summer and at 3.4°C in winter. Choroterpes nymphs entered chill coma at 6.5°C in summer and 5.5°C in winter. Even lower winter water temperatures than these are thought to occur in some of the river systems in which these species live.

The dissolved oxygen requirements of Baetis were found to vary tremendously with water current speed, temperature and the physiological state of the nymphs. The lethal limits for nymphs in ecdysis varied from above saturation in stagnant water to 3.2-5.2 mg/l (depending on temperature and on the nature of the flow) in 7-8 cm/sec water flow and to 0.6-1.1 mg/l (depending on temperature and flow) in 15-40 cm/sec flow. Choroterpes nymphs were not less tolerant during ecdysis than at other times. Their lethal dissolved oxygen concentrations varied with temperature but were only slightly higher in stagnant water (0.53 mg/l at 15°C, 0.60 mg/l at 20°C and 0.72 mg/l at 25°C) than they were in flowing water (0.44-0.48 mg/l at 15°C, 0.53-0.55 mg/l at 20°C and 0.69-0.71 mg/l at 25°C).

Their position on rocks in swift flowing water enabled Baetis nymphs to tolerate low dissolved oxygen concentrations in flowing water. Although they are tolerant of much lower dissolved oxygen concentrations, Choroterpes nymphs might conceivably be eliminated from polluted streams by oxygen lack

in the water between and beneath stones on the stream bed. Orthokinetic reactions to water of low oxygen content could assist both species in avoiding deoxygenated water.

Both species avoided deposited silt and settled in preference on stones.

Lethal low pH values for Baetis nymphs varied with temperature (pH 3.9 at 10°C, pH 4.0-4.3 at 15-20°C and pH 4.7-4.9 at 25°C). They were also increased slightly at low dissolved oxygen concentrations (pH 4.3-4.5 in 4mg/l oxygen at 20°C) and slow flow (pH 4.4-4.7 in 2.7 cm/sec flow at 20°C). Lethal low pH values for Choroterpes varied slightly with temperature (from pH 5.0 at 10°C to pH 5.3 at 25°C). For both species it was the low pH and not the free carbon dioxide concentration which was lethal. The distribution of these species in acid streams could be explained in terms of their lethal limits.

Baetis nymphs were more tolerant of alkaline water than were Choroterpes nymphs (lethal values pH 10.7-10.8 and pH 10.2-10.3 respectively).

Baetis nymphs were also more tolerant of high dissolved solids concentrations than were Choroterpes nymphs (lethal concentrations 12.2 g/l and 10.6 g/l respectively.

Free or "unionized" ammonia concentrations lethal for both species have been recorded in polluted streams. Baetis nymphs were found to be more tolerant of ammonia (lethal concentrations 7.9 mg/l N at 10°C, 7.5 mg/l N at 15°C, 7.3 mg/l N at 20°C and 7.0 mg/l N at 25°C) than were Choroterpes nymphs (lethal concentrations 3.5 mg/l N at 10-15°C, 3.7 mg/l N at 20-25°C). The absence of Choroterpes from a polluted stream could

be explained in terms of its ammonia tolerance. Both the past absence of Baetis from parts of this stream and its return after decline in the level of pollution could be explained in terms of its ammonia tolerance.

Both species were less tolerant of unionized ammonia at low dissolved oxygen concentrations than they were in well aerated solutions (lethal limits for Baetis 7.0 mg/l N not in ecdysis and 5.3 mg/l N in ecdysis at 4.5 mg/l oxygen as opposed at 7.2 mg/l N in aerated water, lethal limits for Choroterpes 2.7 mg/l N at 1.0 mg/l oxygen, 2.9 mg/l N at 3.0 mg/l oxygen and 3.7 mg/l N in 7.7 mg/l oxygen). Both species were less tolerant of ammonia in faster flowing water (lethal limits for Baetis 7.3 mg/l N at 7 cm/sec flow and 7.0 mg/l N at 22 cm/sec flow, for Choroterpes 3.6 mg/l N at 7 cm/sec flow and 3.3 cm/sec flow).

Copper salts were found to be toxic to nymphs of both species at concentrations considered possible in polluted streams, especially in water of pH less than 6.5. Lethal limits for Baetis nymphs were 2.6 mg/l Cu at 15^{o}C, 2.4 mg/l Cu at 20^{o}C and 2.2 mg/l Cu at 25^{o}C. Lethal limits for Choroterpes nymphs were 1.0 mg/l Cu at 15^{o}C, 0.9 mg/l Cu at 20^{o}C and 0.6 mg/l Cu at 25^{o}C. Lead and zinc salts were found to be far less toxic to these nymphs. The combined toxic effects of lead and zinc salts appeared to be additive.

GENERAL INTRODUCTION

This study was undertaken in order to gain an understanding of some of the ways in which the natural range of typical riverine invertebrates might be limited by different environmental conditions. Laboratory experiments have been carried out using nymphs of two common South African mayflies, Baetis harrisoni and Choroterpes (Euthraulus) bugandensis. In these experiments, the degree to which each species was able to tolerate extremes in a number of environmental factors, for instance temperature, dissolved oxygen and various poisons found in polluted rivers, has been investigated. Results are discussed in relation to what is known of the ecology of these species.

The need for this information arose with the development by workers in South Africa of procedures and criteria whereby the level of pollution in streams might be assessed on the basis of faunal data (for instance Harrison 1958 b, Allanson 1961, Chutter 1967 and in press, Pretorius 1969). The criteria drawn up by these authors have been based on their observations of the presence, absence and relative abundances of different macro-invertebrates under different conditions. It was hoped that information on the lethal limits for two representative species might lead to a better understanding of the ways in which natural environmental factors as well as both components and side-effects of pollution can influence the distribution at least of these species.

THE SPECIES STUDIED

Systematics and distribution

Baetis harrisoni Barnard 1932 is an easily recognized mayfly found

in most parts of South Africa. Its aquatic nymphs live in flowing water and may be found in places in considerable numbers. Table 1 is a summary of the information that appears to be available on its distribution in river systems in southern Africa and also indicates the sorts of biotopes in which the nymphs have been collected.

TABLE 1

A SUMMARY OF KNOWN DISTRIBUTION OF BAETIS HARRISONI

River system	Biotopes in which nymphs collected	References
South-western Cape Province		
Great Berg, Eerste and Breë Rivers, Cape Peninsula streams	streams, biotopes not described	Barnard (1932), Harrison (1950)
Great Berg, Krom and Kuils Rivers	abundant in the mountain torrent zone but present throughout the river system, mostly on stones in current, also on stones in backwaters ("probably strayed from the runs during the floods"), and among marginal vegetation ("during ..... flood periods")	Harrison and Elsworth (1958)
Southern Cape Province		
Palmiet, Storms, and Van Stadens Rivers and several other similar rivers in between	streams, on stones in current	Harrison and Agnew (1962) and A.D. Harrison (unpublished data)
Eastern Cape Province		
Swartkops River	upper river, on stones in current	Harrison and Agnew (1962), A.D. Harrison (unpublished data)

Sundays River	river and tributary, on stones in current	Forbes (1968)
Fish River	Amatola tributaries, biotopes not described	Crass (1947)
Buffalo and Nahoon Rivers	streams, on stones in current	Harrison and Agnew (1962), Thornton, Chutter and Hellwig (1967), P. B. Botha (unpublished data)
Rivers in the Transkei and East Griqualand (not specified)	biotopes not described	Crass (1947)
Natal		
Umzimkulu River	streams, on stones in current	S. J. Pretorius (unpublished data)
Umgeni River	almost throughout the system, mostly on stones in current, occasionally on marginal vegetation	Schoonbee (1964), Brand, Kemp, Pretorius and Schoonbee (1967)
Natal coastal rivers (some)	streams, on marginal vegetation (no stones in current present)	Brand, Kemp, Pretorius and Schoonbee (1967)
Tugela River	everywhere except the estuarine and upper source-waterfall zones, mostly on stones in current, occasionally on marginal vegetation	Oliff (1960a,b,) Oliff and King (1964), Oliff, Kemp and King (1965), Brand, Kemp, Oliff and Pretorius (1967)
Pongolo River	river, on stones in current	M. C. Roode (unpublished data)
Swaziland		
Usuthu River	river, on stones in current	P. B. Botha (unpublished data)

Orange-Vaal system		
Caledon River	streams, on stones in current	F.C. Viljoen (1970)
Vaal River	throughout catchment (particularly in the "stable eroding zone") on stones in current and occasionally also on stones in backwaters and on marginal vegetation	Chutter (1963, 1967)
Transvaal		
Jukskei-Crocodile River	streams, on stones in current and occasionally also on marginal vegetation	Allanson (1961)
Mutale River and other Soutpansberg streams	streams, on stones in current	J.D. Agnew (unpublished data)
Olifants River	Highveld streams, on stones in current	G.E. Venter, J.D. Agnew and H.P. Hofmeyr (unpublished data)
Crocodile-Incomati River system	streams, mostly on stones in current, occasionally also on stones in backwaters, exceptionally in a pool	Hughes (1966c), J.D. Agnew and J. Matthews (unpublished data)
Rhodesia		
Mazoe River	Highveld streams, on stones in current	Harrison (1966a,b)

Table 1 shows that Baetis harrisoni nymphs have been found in all of the major river systems in South Africa so far investigated in any detail. In all of the rivers in which they occur they have been found predominantly on stones in current. In most of these rivers they represent a numerically

significant component of the fauna of stony runs. Their presence at times in other biotopes has been ascribed to flooding and to other disturbances. (Harrison and Elsworth 1958). As Hughes (1966b) points out, these nymphs are found predominantly on the upper surfaces of rocks.

River systems in which Baetis harrisoni nymphs have been found to occur but not to be plentiful include the Sundays River, which is very slow flowing for long periods and whose water has a high salt content (Forbes 1968), and certain Natal coastal rivers, which are sluggish and sandy with almost no stones in current (Brand, Kemp, Pretorius and Schoonbee 1967). The rivers that do not appear in table 1 are mostly those that have not yet been investigated. The first impression to be gained from table 1 is therefore that Baetis harrisoni occurs in most, although not all, South African rivers in which stony runs are to be found.

Choroterpes (Euthraulus) bugandensis (Kimmins 1956) is also a widespread species. However, its nymphs are difficult to distinguish from those of Choroterpes (Euthraulus) elegans (Barnard 1932). As a result, the distribution records of these two species in South Africa have become very confused. The information that appears to be available on the distribution of each, and of Choroterpes (Euthraulus) that might be either bugandensis or elegans or both together, is summarized in table 2.

TABLE 2

SUMMARY OF KNOWN DISTRIBUTION OF

(a) Choroterpes (Euthraulus) bugandensis

(b) Choroterpes (Euthraulus) elegans

(c) Choroterpes (Euthraulus) sp. indet. either bugandensis or elegans

(a) Choroterpes (Euthraulus) bugandensis

River system	Biotopes in which nymphs collected	References
Uganda		
Lake Victoria, Nile River	"on stones in the Victoria Nile and also in more exposed situations in Lake Victoria"	Kimmins (1956), Corbet (1958, 1960), Tjønneland (1960)
Tanzania		
Pangani River	nymphs not collected	Gillies (1957)
Transvaal		
Pienaars River (near Pretoria)	to be described in this report	det. D.E. Kimmins for the present study

(b) Choroterpes (Euthraulus) elegans

River system	Biotopes in which nymphs collected	References
South-western Cape Province		
Olifants, Great Berg, Eerste and Breë Rivers	both flowing and still waters, mostly beneath stones	Barnard (1932), Harrison (1949)
Great Berg River	the main river, on stones both in current and in backwaters, nowhere common	Harrison and Elsworth (1958)
Southern Cape Province		
Grobbelaars River (Gouritz system)	stream, on stones in current and in marginal vegetation	Harrison and Agnew (1962)
Eastern Cape Province		
Buffalo River	streams, on stones in current	Harrison and Agnew (1962)

East Griqualand		
Sterkspruit and rivers around Kokstad	nymphs not collected	Crass (1947)
Natal		
Tugela River	in all zones except the estuarine and source-water-fall zones, the dominant species on stones in slow flowing water, also on stones in current, occasionally on marginal vegetation	Crass (1947), Oliff (1960a,b), Oliff and King (1964), Brand, Kemp, Oliff and Pretorius (1967)
Malawi		
Lake Malawi	nymphs not collected	Kimmins (1955)

(c) Choroterpes (Euthraulus) sp. indet. either bugandensis or elegans

River system	Biotopes in which nymphs collected	References
Natal		
Umzimkulu River	streams, on stones in current and on marginal vegetation	S.J. Pretorius (unpublished data)
Umgeni River	streams, mostly on stones in current, occasionally on marginal vegetation, once on stones in a pool	Schoonbee (1964), Brand, Kemp Pretorius and Schoonbee (1967)
Natal coastal streams (some)	streams, on marginal vegetation (no stones in current present)	Brand, Kemp, Pretorius and Schoonbee (1967)
Pongolo River	river, on stones in current	M.C. Roode (unpublished data)
Swaziland		
Usuthu River	river, on stones in current	P.B. Botha (unpublished data)

Orange-Vaal system		
Caledon River	streams, on stones in current and on marginal vegetation	Viljoen (1970)
Vaal River	throughout catchment, particularly on stones in backwaters most often "on stones with a cavity under the side away from the direction of flow"), also on stones in current	Chutter (1963, 1967, 1968)
Orange River	river, on stones in current	J.D. Agnew (unpublished data)
Transvaal		
Jukskei-Crocodile River	streams, on stones both in current and in backwaters	Allanson (1961)
Palala, Magol and Pafuri Rivers	streams, on stones in backwaters, and on stones in current	J.D. Agnew (unpublished data)
Olifants River	Highveld streams, on stones both in current and in backwaters	G.E. Venter and H.P. Hofmeyr (unpublished data)
Sabie-Crocodile-Incomati River system	streams, on stones both in current and in backwaters, in pools	Hughes (1966c), J.D. Agnew and J. Matthews (unpublished data)
Rhodesia		
Mazoe River	Highveld streams, on stones in current	Harrison (1966a, b), Harrison and Mason (1967)
Shangani River	stream, on stones in current	J.D. Agnew (unpublished data)
Mozambique		
Mazoe River	river, on stones in slow current	J.D. Agnew (unpublished data)
Malawi		
Shire River	tributary, on vegetation in current	J.D. Agnew (unpublished data)

Only Choroterpes elegans is known from the Cape Province. This species occurs in the slightly acid streams of the south-western Cape Province and in the alkaline streams of the southern and eastern Cape. It appears to be absent from the acid, peat-stained streams of the southern Cape (Harrison and Agnew 1962) and from the Sundays River (Forbes 1968). Elsewhere in South Africa most of the major river systems investigated have been found to contain relatively large numbers of one or both Choroterpes (Euthraulus) species.

Table 2 might at first glance give the impression that nymphs of the two Choroterpes (Euthraulus) species are to be found in a variety of biotopes. This is misleading. Chutter (1967) described the nymphs he found in the Vaal River as "cavity dwellers" that are found mostly in hollows on the undersurfaces of rocks. Conditions of this sort are to be found in backwaters, in stony rapids, in pools and even among marginal vegetation. It is not known that the physical habitats of Choroterpes bugandensis and Choroterpes elegans nymphs differ in any way. The greatest numbers of both species have been found in stony backwaters.

Range of conditions in which nymphs have been found

The range of biotopes in which Baetis harrisoni and Choroterpes (Euthraulus) nymphs have been collected has been described. Chutter (1969) has shown that the stones on which the nymphs live in streams of the Vaal River catchment can become covered by deposited silt and sand and the habitat of these species obliterated. However, he found the density of nymphs on stones in streams of the "stable eroding zone," in which no appreciable deposition occurred and on the stones not covered in streams of the "unstable

depositing zone," in which sand and mudbanks were constantly being built up and shifted, not to be greatly different. He also found Baetis harrisoni to be less tolerant of deposited silt than were Choroterpes (Euthraulus) nymphs. Harrison and Elsworth (1958) have suggested that numbers of Baetis harrisoni might be displaced both downstream and to other biotopes after the sudden deposition of sand and silt.

Both Baetis harrisoni and Choroterpes (Euthraulus) nymphs have been collected both in very clear and in very turbid water. Oliff (1960a) recorded suspended solids concentrations of 0.2 and 27.8 g/l on successive days in the Tugela River at Bergville, where both species were to be found.

Both Harrison (1958a) and Hughes (1966d) counted comparable numbers of Baetis harrisoni nymphs in sunny and in shady streams. However, in a series of laboratory experiments, Hughes (1966a, b) showed that the behavioural reactions to light of Baetis harrisoni nymphs were important in influencing the distribution of the nymphs in streams. His observations will be discussed in one of the sections of this report.

In the rivers they investigated, Harrison and Elsworth (1958), Allanson (1961) and Schoonbee (1964) found numbers of Baetis harrisoni nymphs as well as other animals in stony runs to be drastically reduced by severe floods. Details of the water current speeds during the floods are not available, but both Harrison and Allanson measured speeds of the order of 2 m/sec during floods. Oliff (1960a,b) describes floods in which he measured current speeds sometimes exceeding 3 m/sec. These lifted and rolled even quite large boulders and resulted in considerable reductions

in the densities of the fauna of stones in current, including both Baetis harrisoni and Choroterpes elegans. On the other hand, Chutter (1967) did not find numbers of Choroterpes (Euthraulus) sp. nymphs in the lower Vaal River to be significantly reduced by floods. The highest current speeds he measured were of the order of 1.2 m/sec.

Both species have also been collected in conditions of slow flow, but actual current speeds at the collection sites were not always measured. The lowest current speed I could find apparently relating to a Baetis harrisoni collection site was 8 cm/sec (Oliff 1960a). Choroterpes bugandensis are known from lacustrine conditions (Corbet 1960) where water current speeds must have been very slow indeed.

Seasonal changes in the abundance of Baetis harrisoni nymphs have often been ascribed to changes in water flow conditions. Reductions in numbers took place in winter at places in the Great Berg River (Harrison and Elsworth 1958) but in summer in the rivers in other parts of the country, because this was when the floods occurred. Elsewhere in the Great Berg and in the Umgeni River (Schoonbee 1964) during these periods the numbers of this species were not reduced, presumably because severe flooding did not occur. Similar effects have been noted at times of very low flow. In two temporary streams, Baetis harrisoni nymphs disappeared entirely during the dry season (Harrison 1958a, 1966a). The Kuils River dried out during summer, while the Rhodesian temporary stream studied dried out during winter.

Both Baetis harrisoni and Choroterpes (Euthraulus) nymphs have been collected in a wide range of water temperature conditions. By far

the highest temperature, 32.5°C, was recorded at a station in the Great Berg River where both Baetis harrisoni and Choroterpes elegans were apparently present (Harrison and Elsworth 1958). Oliff (1960a,b) recorded a range of -1.1°C to 28.9°C during one year in the Bushmans River where both of these species were frequently found. In spite of this apparently wide tolerance range, Harrison and Elsworth have pointed out that lowest numbers during winter of Baetis harrisoni nymphs in the Great Berg River were found at places where water temperatures were lowest. Numbers often rose when temperatures increased in spring. Chutter (1967) found winter water temperatures in streams at higher altitude to be lower than those in streams at lower altitude. He ascribes the relatively low numbers of Baetis harrisoni nymphs collected in the high lying streams during winter to the effects of low temperature. During summer water temperatures in streams at higher altitude were as high as those in streams at low altitude and numbers of Baetis harrisoni, if anything, exceeded those in the lower lying streams.

Dissolved oxygen concentrations in streams in which nymphs of Baetis harrisoni and Choroterpes (Euthraulus) spp. were collected generally appear to have been high. Allanson (1961) points out, however, that the high values that might be recorded during the day are not necessarily maintained at all times. Dissolved oxygen concentrations in the river he studied were markedly influenced by the effluent apparently released once a day from a sewage works. Concentrations fell each night from values approaching saturation to a minimum occasionally as low as 3 mg/l for 2-4 hours. Large numbers of Baetis harrisoni nymphs were found in the river where this occurred. I could not find an instance where Choroterpes (Euthraulus) nymphs had been

collected in water of comparably low dissolved oxygen content.

Neither Baetis harrisoni nor Choroterpes (Euthraulus) nymphs have been collected in the estuarine zones of any South African river. Only a very few records of these species in water of high total dissolved solids content are known. Forbes (1968) collected Baetis harrisoni in the Sundays River in water of 909 mg/l TDS content. Higher TDS concentrations have been measured in this river and in the Swartkops River (A.D. Harrison unpublished data) where this species was not found, and in the temporary Kuils River after Baetis harrisoni had disappeared from the stream (Harrison 1958a).

Harrison and Agnew (1962) have included Baetis harrisoni in a list of South African riverine invertebrates found both in acid and in alkaline waters. Choroterpes elegans, on the other hand, has only been found in alkaline streams. Baetis harrisoni nymphs have been collected in water ranging from pH 4.3 (Harrison and Elsworth 1958) to pH 9.5 (Schoonbee 1964). Choroterpes (Euthraulus) nymphs have been found in water ranging from pH 6.0 (G.E. Venter and H.P. Hofmeyr unpublished data) to pH 8.8 (Chutter 1967). Acid waters are found in South Africa in the peaty streams draining mountains of the Cape Fold Belt composed of Table Mountain Sandstone (Harrison and Agnew 1962) and in streams in Natal and the Transvaal receiving acid mine drainage (for instance Harrison 1958c). Essentially the same invertebrates have been found in comparable biotopes in these acid waters, except that the Cape streams contain a number of endemic species not found in the Transvaal or Natal.

Neither of these species has been found in a river heavily polluted with domestic sewage or industrial wastes. Harrison (1958b), Oliff (1960b),

Allanson (1961) and Chutter (1967) have all found exceptionally high densities of Baetis harrisoni nymphs in the stony runs of streams polluted with organic wastes. These authors have all attributed the increase in numbers to increased food supply, since the nymphs appear to be detritus feeders. Chutter (in press) describes Baetis harrisoni as the most tolerant of pollution of all South African mayflies. In the scheme he has drawn up for the assessment of water pollution based on the fauna of stony runs the abundance of this species is an important criterion used. Both Oliff (1960b) and Chutter (1967) have collected Choroterpes (Euthraulus) nymphs in streams they describe as mildly polluted.

Allanson (1961) has collected Baetis harrisoni nymphs in water containing high concentrations of ammonia, the highest being 59 mg/l total ammonia-ammonium. At higher concentrations (up to 189 mg/l) no Baetis harrisoni were found. The highest total ammonia-ammonium concentration in which Choroterpes (Euthraulus) nymphs have been collected appears to be 0.84 mg/l (Chutter 1967). This species is absent from the polluted sections of the river studied by Allanson. Wuhrmann and Woker (1948) have shown the toxicity of ammonia-ammonium solutions to be determined by the concentration of ammonia in solution and not by the ionic ammonium. This may be calculated from the total ammonia-ammonium concentration if the pH and temperature of the water are known, as will be shown in the relevant section of this report.

No information is available on the range of concentrations of such other toxic pollutants as, for instance, hydrogen sulphide and copper, in which Baetis harrisoni and Choroterpes (Euthraulus) nymphs may be found in South African rivers.

The general picture which emerges from this summary is that both Baetis harrisoni and the two Choroterpes (Euthraulus) spp. are very widespread in southern Africa. They occur in quite different but fairly clearly defined situations on and among stones in streams. Both have been found in a wide range of conditions of temperature, dissolved oxygen and water chemistry. Baetis harrisoni nymphs are found in most rivers where the nymphal habitat, in swift flowing water, is present. They appear to be excluded from parts of rivers that have a high salt content, that are very acid or that are heavily polluted. The range of the two Choroterpes (Euthraulus) spp. are probably also restricted by the availability of the nymphal habitat, by low pH values and by water pollution. A factor that appears to restrict the distribution of the species concerned in the southern and south-western Cape Province is the low pH of the water. Either species might perhaps also be excluded from parts of rivers that have a high salt content or that are moderately polluted.

Comparison with related mayflies

Baetis harrisoni is a typical representative of the family Baetidae. Its nymphs resemble in many ways those of the other species of the genus found all over the world. Their bodies are of streamlined, of torpedo-shaped form, their abdominal gills do not beat and the cerci are not as long as the body. Like most Baetis species they are found predominantly, if not exclusively, in flowing waters.

In contrast, the bodies of Choroterpes (Euthraulus) nymphs are dorsoventrally flattened, their gills beat rapidly and the cerci are slightly longer than the body. In these characters they resemble the nymphs both of

other Choroterpes species, and of several other Leptophlebiidae (Peters and Edmunds 1964).

Ambühl (1959) has studied the behaviour, oxygen uptake and minimal dissolved oxygen requirements of certain Baetis species under different conditions. His work is something of a classic and is discussed in some detail in the sections of this report dealing with water flow and with dissolved oxygen. Important information on the dissolved oxygen requirements of Baetis spp. is also provided by Fox and Simmonds (1933), who attributed the relatively high dissolved oxygen requirements of Baetis rhodani to the fact that its gills did not beat.

Ide (1935), Macan (1957), Pleskot (1958) and other workers have produced evidence to suggest that water temperatures might restrict the distribution of Baetis spp. and other mayflies in streams. The rivers they investigated appeared to be insufficiently warm in the upper reaches for certain species to complete their development and in the lower reaches to become lethally warm for certain cold-water species confined to the top. Whitney (1939) has found lethal temperatures for three Baetis spp. to lie within the range 18°C to 22°C.

Much less is known of the other species of Choroterpes. Gillies (1957) has described the nymphs of some of these from sluggish streams at low altitude in East Africa. One of them, Choroterpes (Euthraulus) tropicalis, was found in streams in which temperatures probably exceeded 30°C during the hot season and which became reduced to a series of stagnant pools in the dry season.

Comparison with other aquatic animals

A good deal of information is available on effects of various environmental factors upon other aquatic animals. Much of this, in particular the information that is available on the interaction of different factors, is relevant to the present study. The literature concerned is discussed in each of the sections of this report.

COLLECTION AND HANDLING OF NYMPHS

Collection sites

Baetis harrisoni nymphs for this study were collected in a tributary of the Jukskei-Crocodile River, the Braamfontein Spruit (on some maps the smaller of two Klein Jukskei Rivers) above the Rivonia-Ferndale road bridge north of Johannesburg, somewhere near station 13 of Allanson (1961). This small stream was perennial and numbers of Baetis harrisoni nymphs were available at most times of the year on rocks and boulders in the swift flowing water.

Choroterpes bugandensis nymphs were collected in the Pienaars River above the Pretoria-Cullinan road bridge east of Pretoria. Flow in this stream stopped for a short period each dry season but numbers of nymphs were always present on the undersurfaces of flattish stones on the stream bed.

Collection and transport to the laboratory

Nymphs were collected using a hand net, particular care being taken not to damage them. They were transported to the laboratory in plastic buckets containing two to three cm water. Choroterpes nymphs were very

easy to transport in this way. Great care had to be taken to exclude algae and other organic matter from buckets containing Baetis nymphs and to keep the water cool and in motion while in transit to the laboratory.

Mortality of Choroterpes nymphs as a result of collection and transport was rare. Individuals that did die had evidently been damaged. Among most batches of Baetis nymphs brought in to the laboratory, on the other hand, a few individuals died during the first 24 hours in the laboratory. The number dying varied and was not obviously the result of anything that had happened during collection or transport, and on average amounted to two to three per cent of the nymphs collected. In a later section it will be suggested that most of the nymphs that died had been disturbed at a time when they were starting to moult and that their death had resulted from some complication during ecdysis.

Maintenance of nymphs in the laboratory

On arrival in the laboratory, the nymphs were divided into random experimental groups as dictated by the design of the experiment planned. Each group was held in one to two cm water in a flat plastic container which was dark in colour and illuminated from above to prevent the Baetis harrisoni nymphs, which show a dorsal light response (Hughes 1966a), from being disorientated. Numerous flat stones covered with algae were placed in the water to provide food and substratum. In some cases Chlorella or Scenedesmus cells were added as a further source of food. The water was kept in motion at all times and water temperature controlled within a range of 1°C by suspending the trays in a water bath.

Nymphs of both species were seen to eat the food provided and appeared to be in good condition at all times. They could be raised to maturity in these containers.

Nymphs were brought into the laboratory around midday. Most experiments started the next day, the nymphs being transferred to experimental tubes during the afternoon. Strips of gauze were placed in the water and nymphs either settled on them or were caught on them while swimming. All of the nymphs in each group were used in the experiment, being transferred at random to the experimental tubes.

MEASUREMENT OF TOXICITY

Various measures of toxicity of substances to organisms are available. Most of these are population statistics and are based on the observation that individuals in a population differ both in their abilities to tolerate a given set of conditions for a certain time and in the times they will resist a lethal situation (Bliss 1935, 1937).

In dosage-mortality experiments, groups of animals are exposed for a standard period of time to different levels of the factor being studied and the mortality at each level is recorded. The level that would be expected to cause 50 per cent of animals to die within the exposure time is estimated from the resulting cumulative curve, referred to as the tolerance distribution or dosage-mortality curve. This level is termed the median lethal level (which might be a temperature or a concentration of poison) or LC_{50}, or the median tolerance limit or TL_m for a specified exposure time. The term "dosage" is used to denote the transformation of the dose which normalizes the tolerance distribution, most often the logarithm of the dose.

In time-mortality experiments, a single group of animals is exposed to a chosen level of the factor concerned and the precise time to death of each individual recorded. The time till 50 per cent of animals would be expected to be dead is estimated from the cumulative curve obtained, termed the resistance distribution or time-mortality curve. This time is referred to either as the median resistance time or MRT, or the median time of survival or MTS.

Logarithms of survival times have generally been found to be normally distributed (Bliss 1937), individual times to death have to be transformed to logarithms for the calculation of the time-mortality curve.

If it is found that no further mortality occurs after a certain time, the median lethal level for this time (or the level at which the median resistance time becomes infinite) is termed the "incipient lethal level" or the "threshold level" (Fry, Brett and Clawson 1942, Herbert and Merkens 1952).

The most usual method used to obtain dosage-mortality or time-mortality statistics is that of probit analysis. In this method, the sigmoid normalized dosage-mortality or time-mortality curve is transformed to a straight line by converting the proportion of animals dead to standard deviation units (normal deviates termed probits). Procedures for estimating parameters of the dosage-mortality curve are described by Finney (1952), and those for time-mortality are described by Bliss (1937). These are the methods used in this study. A number of short-cut procedures are also available (for instance Litchfield 1949) but these do not make allowance for natural mortality or for possible heterogenity among the animals being tested.

The experimental techniques described here can be used for any all-

or-nothing response to an environmental factor. Dose-reaction experiments lead to estimation of the median effective concentration or EC_{50}. Time-reaction experiments lead to estimation of the median reaction time.

Time-mortality experiments are generally very much easier to carry out than are dosage-mortality experiments. Far fewer test animals and tanks are needed (Herbert and Merkens 1952) and more complicated experiments involving interaction between several factors become feasible. However, it was necessary in the present study of mayfly tolerances to enclose the animals in a tube in order to control the nature of water flow around them. It was found to be impossible to distinguish with any certainty between dead and living animals when they were so enclosed. For this reason almost all experiments carried out in this study were of dosage-mortality design. Moreover, it turned out during the course of the study that these mayfly nymphs had to be disturbed at intervals throughout each time-mortality experiment. This in itself caused mortality of those nymphs of one of the species used that were undergoing ecdysis.

APPARATUS

All of the dosage-mortality experiments were carried out in the same apparatus. Certain of the details of the apparatus are described where this is relevant in the text. The following is a short general description.

The apparatus consisted of four sets of three tanks each. Each tank had a capacity of 40 litres and dimensions roughly 60 x 30 x 30 cm. Each tank could be aerated with suitable mixtures of gases. Proportions of carbon dioxide, compressed air and either nitrogen or oxygen were regulated using a Fisher and Porter "Flowrator" gas flow meter for each gas used.

In order to control levels of dissolved oxygen, suitable gas mixtures were bubbled through the water at a rate of 2 to 3 l/min. The tanks were closed off from the air using polythene sheeting, so that the gas mixture filled the space above the water and an equilibrium was reached with the gases dissolved in the water.

Each tank was supplied with a Braun "Thermomix" thermostat-heater. Any desired flow of water into each tank could be maintained. The chemical content of the water was made up by addition of chemicals before the start of each experiment. Desired pH values and carbon dioxide and bicarbonate concentrations were achieved first by addition of acid, alkali or bicarbonate, second by allowing the water to reach equilibrium with the carbon dioxide present in the aeration gas mixture and, third, by regular adjustment of pH using dilute acid or alkali.

During experiments, animals were held in these tanks in a series of perspex tubes (figure 1) of various diameters. The animals were kept in the tubes by a stainless steel gauze screen positioned at both ends. The tubes were connected to one another, usually in series but often in parallel, by means of B34 ground glass joints and glass connecting pieces.

In order to drive a current of water through these tubes, each tank was supplied with a perspex impeller box (figure 2) fixed to the frame of the tank.

The three impellers in each set of tanks was driven at the same speed by a "Klaxon" 1/6 h.p. series wound motor. This had an output of 800 - 2500 r.p.m. and the speed of the driveshaft was regulated first with a 1:3 belt-driven reduction gear and second with a "Kopp" variator. The final

output of the driveshaft could therefore be set at any value from 80 to 2500 r.p.m. An overall view of a set of three tanks is given in figure 3.

BOREHOLE WATER SUPPLY

The water used in this study was derived from a borehole next to the National Institute for Water Research building. Water was drawn off at a depth of about 30 m and was delivered to the laboratory through p.v.c. piping by means of a stainless steel pump. A constant level of water was maintained in the laboratory in a 2.3×10^3 litre "Proderite-lined tank. The water in this supply tank was strongly aerated. Water was delivered to the experimental tanks through p.v.c. and plastic tubing using a small stainless steel and perspex pump.

On arrival in the laboratory the water was found to be acid (pH 5.0 to 5.4). On contact with the air, and particularly when aerated, the pH rose and reached an equilibrium at pH 6.9 to 7.2 after 18 to 24 hours. During this time a reddish-brown precipitate of ferric oxide or hydroxide formed on the walls of the tank.

In order to get rid of the iron, the water was passed through an exchange column before it reached the supply tank. Iron was exchanged in this column for sodium and sulphate for chloride. The water emerged from the column with a pH value of 5.3 to 5.7 which again rose in the supply tank over 18 to 24 hours to an equilibrium at pH 6.8 to 7.1. This rise in pH appeared to be due to the release of carbon dioxide.

Routine chemical analysis of the borehole water were carried out from time to time by various persons working under the direction of Mr M.R. Henzen. Occasional determinations were also made by Dr F.W.E. Strelow

Figure 1

Perspex impeller, experimental tube and Venturi tube

a - impeller shaft

b - impeller box

c - B34 ground glass joint

d - experimental tube

e - direction of water flow

f - stainless steel gauze screen

g - Venturi tube

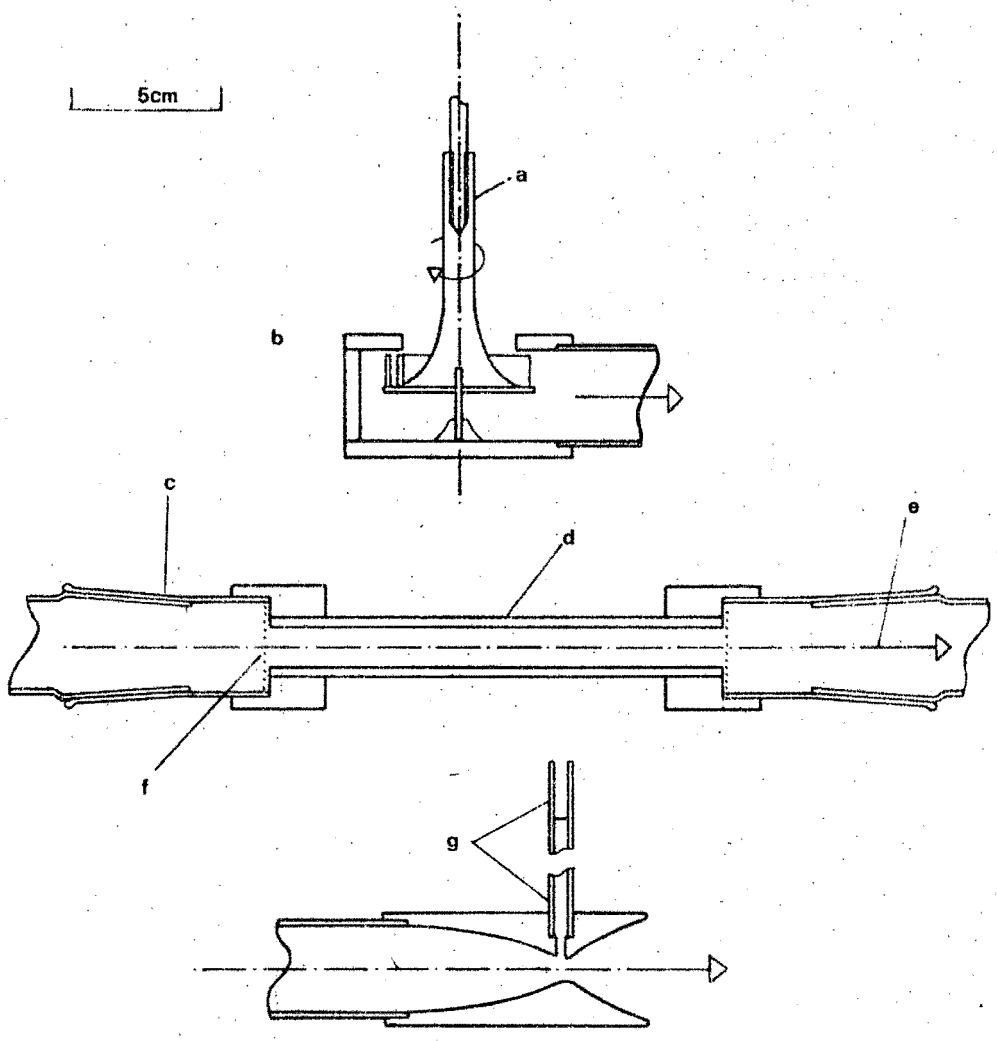

5cm
a
b
c
d
e
f
g

Figure 2

Experimental tank, showing impeller and tubes in position

a - impeller shaft

b - tank

c - water level

d - Venturi tube

e - experimental tubes

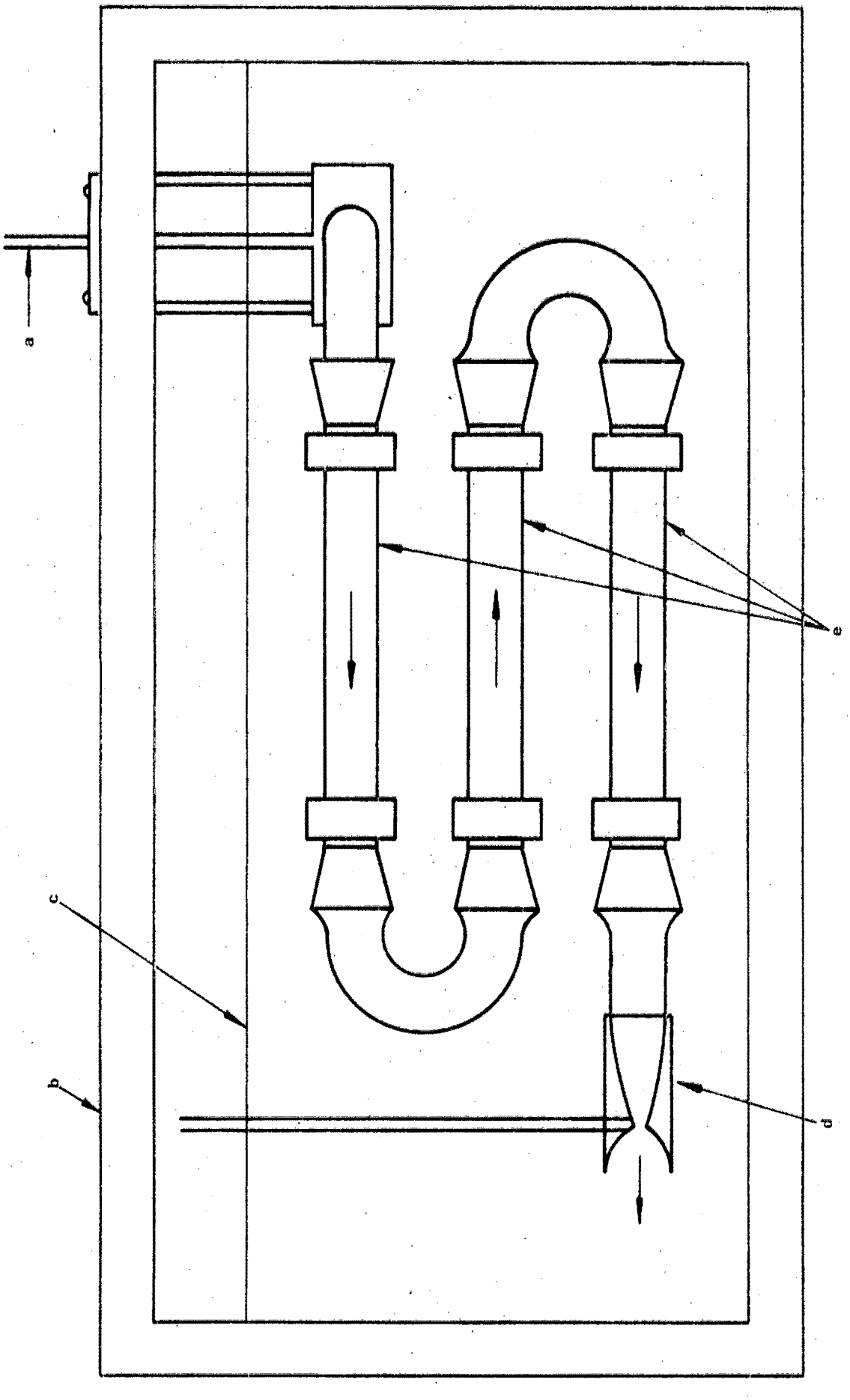

a
e
c
b
d

Figure 3

General view of a set of three experimental tanks

a - impeller box

b - gear

c - driveshaft

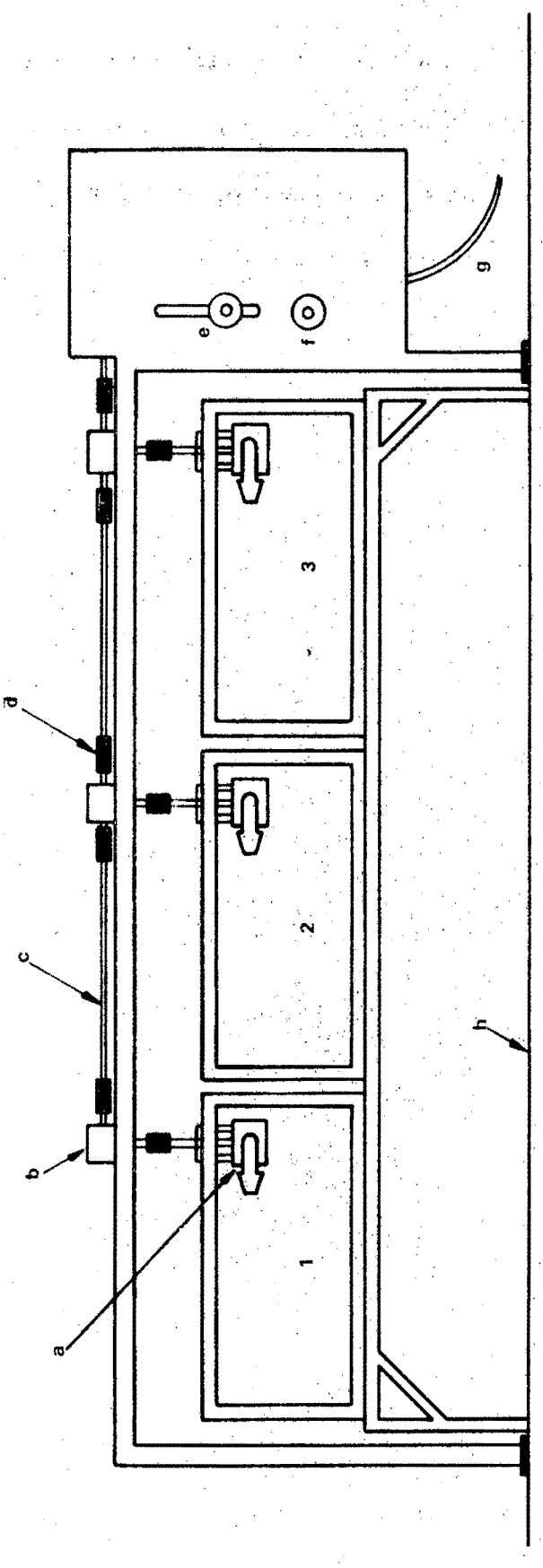
d - flexible coupling

e - variable drive

f - "Kopp" variator

g - flex

h - bench


a
b
c
d
e
f
g
h
1
2
3

of the National Chemical Research Laboratory and by Dr L.R.P. Butler of National Physical Research Laboratory of copper, lead and zinc dissolved in the water. Grateful thanks are due to these persons for these analyses. The chemical content of the water, as revealed from these analyses, is summarized in table 3.

TABLE 3

AVERAGE CHEMICAL COMPOSITION OF BOREHOLE WATER USED

pH	6.9 to 7.2
total dissolved solids (mg/l)	15 to 42
sodium (mg/l Na)	9.8 (6.2 to 14.1)
potassium (mg/l K)	< 0.1
calcium hardness (mg/l as $CaCO_3$)	1.9 (1.0 to 2.6)
magnesium hardness (mg/l as $CaCO_3$)	3.6 (2.2 to 5.1)
copper (µg/l Cu)	1 to 5
zinc (µg/l Zn)	20 to 30
lead (µg/l Pb)	10 to 30
chloride (mg/l Cl)	11.9 (7.2 to 17.9)
sulphate (mg/l SO_4)	not detectable
Kjeldahl-N	not detectable
phosphate	not detectable

PHYSICAL RESISTANCE TO FAST WATER FLOW BY NYMPHS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS

INTRODUCTION

Nymphs of Baetis harrisoni and Choroterpes bugandensis occur quite commonly in a number of Transvaal streams which are subject to periodic flooding in summer. From monthly records of relative numbers of aquatic macro-invertebrates in the Jukskei River near Johannesburg, Allanson (1961) has reasoned that large numbers of the first species are washed downstream during floods. Observations are reported here of the behaviour of nymphs of both species in fast flowing water under controlled laboratory conditions. From these observations estimates have been made of the water current speeds necessary to dislodge nymphs.

Several authors (for instance Macan 1957 for Ephemeroptera, Scott 1958 for Trichoptera, Grenier 1949 for Simuliidae) have found greatest numbers of different species to be concentrated in parts of streams where the water was flowing at particular speeds. Several of these species have also been found to congregate to some extent at certain current speeds in the laboratory (Phillipson 1956, Ambühl 1959). Quite marked differences in the composition of the invertebrate fauna have been found between places in stony streams differing but little in current speed (Knöpp 1952, Jaag and Ambühl 1964) and changes in the flow patterns of rivers have been found to result in striking faunal changes (Ravera 1951). Current speed has in fact been described by some authors as the most important single factor influencing the distribution of mayflies and other invertebrates within stream beds (Verrier 1948b, Ambühl 1959, Einsele 1960).

Water currents in torrential streams are capable of carrying away everything except the larger stones. These streams are inhabited only by animals able either to withstand such flows or to find sanctuary in cracks and crevices (Percival and Whitehead 1929). Torrential flows can occur from time to time in almost any stream and the effects of flooding on the fauna of normally moderately flowing streams can be so severe that recovery of the fauna can take several months (Pleskot 1962). Several species have been exposed to fast water flows in the laboratory (Dorier and Vaillant 1948, 1954, Harker 1953a, Philipson 1954, Stuart 1958, Bournaud 1963, 1965, Moore 1964). In general, animals found in fast flowing water in the field were better able to withstand fast currents in the laboratory than were animals from slower water. Some species from slower flowing water were found not to be able to withstand current speeds known to occur in fast flowing streams.

Current speeds of the water near the bottom are known only to be about a third to a half those in the free water (Hubault 1927, Scott 1958, Matalas and Conover 1965). Animals on the bottom apparently living in the current stream are really enclosed in a boundary layer of water one to four millimetres thick in which the water speed is further reduced (Ambühl 1959). The animals between and beneath stones over which water is flowing are in fact in a zone of "dead" water in which the water movements are even further reduced. It is possible to estimate the speed of water currents acting on animals in experimental tubes in a laboratory (Bournaud 1963, 1965) but extremely difficult to measure exact current speeds acting on animals in the field (Grenier 1949, Gessner 1950).

Many of the invertebrates of flowing waters have strikingly streamlined or flattened body forms. The possibly adaptive characters of these forms have been considered in some detail by, for instance, Steinmann (1907), Clemens (1917), Dodds and Hisaw (1924a), Popovici - Baznosanu (1928), Hora (1930) and Verrier (1953). As Nielson (1950, 1951) and Johnson (1959) have pointed out, most of those invertebrates that live on rocks exposed to the force of the current have bodies of streamlined form while those that live in the dead water in crevices or below rocks have bodies of flattened form. A variety of widely differing water flow conditions appear to be available around rocks in fast flowing streams and in the deadwater zone. It has been suggested that colonization of different situations in streams by invertebrates might be influenced more by water flow conditions than by any other factor (Sprules 1947). As Ambühl (1959) has shown, behaviour with respect to water flow is one of the most important adaptations to life in flowing water exhibited by stream invertebrates.

Of course, features of distribution of animals at moderate flow rates cannot be explained simply in terms of their resistance to fast water flow. Aggregations of different animals at particular water current speeds have been found to involve, for instance, behaviour characteristics of animals in current (Dorier and Vaillant 1954), the distribution of food supply (Scott 1958) and a great number of other biotic factors (Macan 1962). These complexities are beyond the present study.

MATERIAL AND METHODS

Nymphs of Baetis harrisoni and Choroterpes (Euthraulus) bugandensis were collected in the Braamfontein Spruit and Pienaars River, respectively,

as described in the introductory section. On arrival in the laboratory the nymphs were divided into random experimental groups which were held for a day in shallow plastic containers at pre-set temperatures before being used in experiments.

The apparatus used has been described. It consisted of a motor-driven impeller suspended in a 40 litre glass aquarium. The impeller could be driven at any desired speed between 800 and 2,500 r.p.m. and could maintain a controlled current of water through one or several perspex experimental tubes in which the animals were contained. These tubes were 15 cm long and were roughened on their inner surfaces to provide a foothold for the animals. They were joined to the impeller and to one another by means of B34 ground glass joints and were shut off at either end by a disc of stainless-steel gauze with openings about 0.1 cm across. Tubes of 0.75 cm and 1.6 cm internal diameter were used in the experiments described here.

In each experiment a single individual was placed in a tube and one or more short bursts of slow water driven down the tube to induce the individual to turn to face the direction of water flow from a position about half way down the length of the tube. The current speed was then swiftly raised to the desired fast speed and the length of time the nymph could maintain its position noted. Each individual in each experimental group was tested in this way. From the observed individual resistance times of the animals of each group, median times to washaway were calculated by probit analysis (Bliss 1937).

BEHAVIOUR OF NYMPHS

Both Baetis harrisoni and Choroterpes bugandensis nymphs were found

always to turn to face the source of the water current (positive rheotaxis). At slower current speeds, nymphs of both species were seen to move in almost any direction, apparently searching in a haphazard manner either for food or for a better position. However, Baetis nymphs tended to move forwards rather more than they moved backwards, possibly because they happened to be facing that way. Most Baetis nymphs tended to reach the upstream end of the tube after a while. They either stayed there or, more commonly, later made a darting swimming movement into the current and were carried downstream again.

Choroterpes bugandensis nymphs, on the other hand, tended to move sideways and backwards more than they did forwards. They almost all reached the downstream end of the tube in this way. Even though Baetis harrisoni and Choroterpes bugandensis differed in their behavioural reactions to current, it was found to be relatively easy to expose them to very fast water flows and to observe their resistance times.

CURRENT SPEEDS IN TUBES

Median times to washaway of Baetis harrisoni and Choroterpes bugandensis nymphs at different current speeds are shown in figures 4 and 5. These show that Baetis harrisoni nymphs were better able to withstand fast flows than were Choroterpes bugandensis nymphs. They also show that both species were more resistant to fast flow in the larger diameter tubes.

For each current speed tested, the relevant Reynolds number was calculated as follows:

Figure 4

Median times to washaway of Baetis harrisoni nymphs at different water current speeds (as average through tube) in two tubes of internal diameter 0.75 and 1.6 cm. 95% confidence limits are shown about each median value.

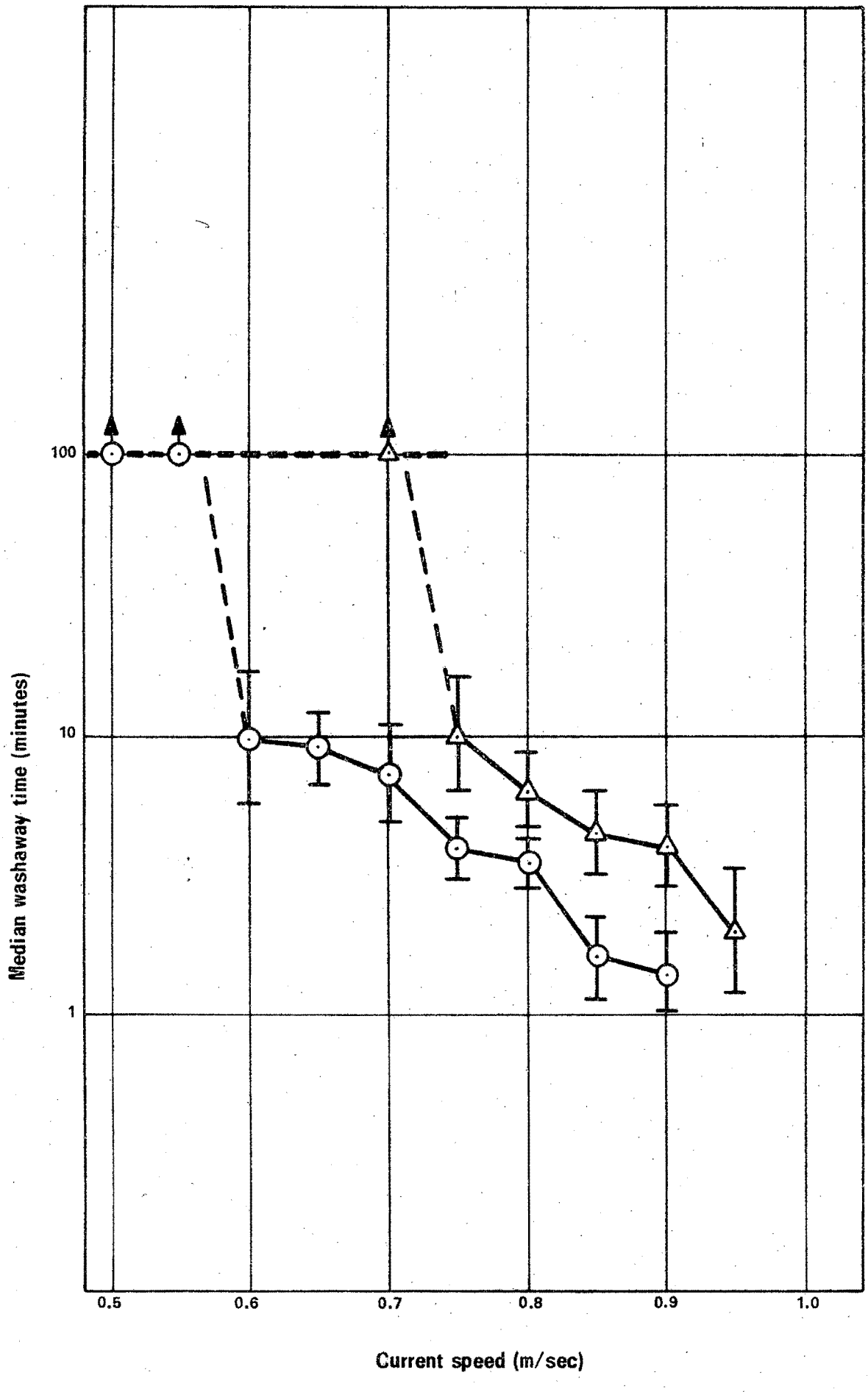
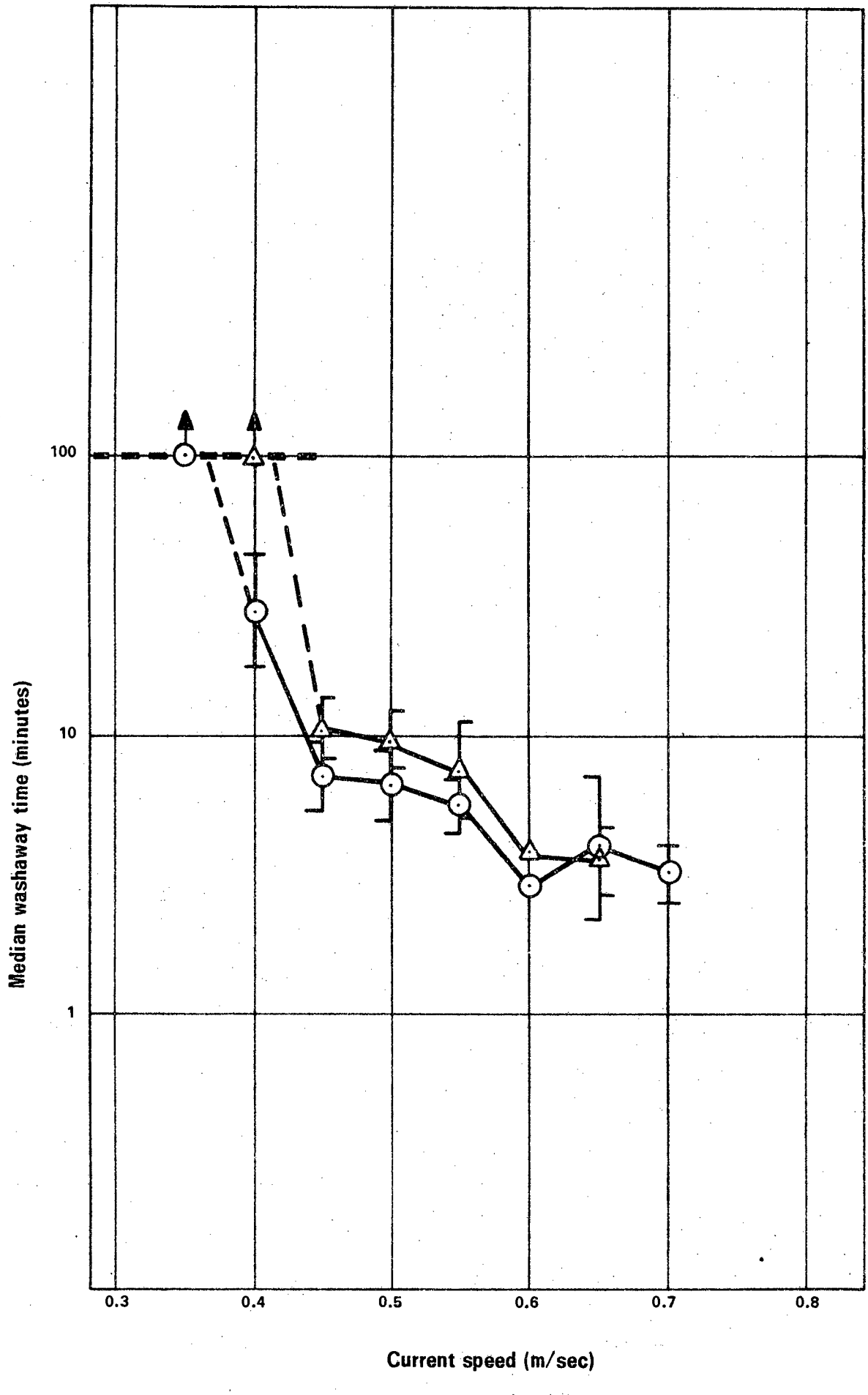

Median washaway time (minutes)
100
10
1
0.5
0.6
0.7
0.8
0.9
1.0
Current speed (m/sec)

Figure 5

Median times to washaway of Choroterpes bugandensis nymphs at different water current speeds (as average through tube) in two tubes of internal diameter 0.75 and 1.6 cm. 95% confidence limits are shown about each median value.


100
10
1
Median washaway time (minutes)
0.3
0.4
0.5
0.6
0.7
0.8
Current speed (m/sec)

$$Re = \frac{\bar{u}d}{\gamma}$$

where Re is the Reynolds number

$\bar{u}$ is the average current speed through the tube (cm/sec)

d is the internal diameter of the tube (cm)

γ is the kinematic viscosity of water (0.01 cm^2/sec at 20^oC).

It is assumed that flow was laminar at Reynolds numbers less than 2000 and turbulent at Reynolds numbers greater than 3000 (Krovetz 1965). The lowest Reynolds number recorded in any of these experiments was about 9000, so that flow in all of these experiments was turbulent. Flow in swift-flowing streams is also apparently always turbulent (Ambühl 1959). The turbulent nature of the flow was confirmed both in the experimental tubes and in a swift-flowing stream by releasing a very small droplet of dye into the flowing water. The dye swiftly diffused laterally through the column of flowing water. In laminar flows the droplet flowed as a thin line and did not mix with the surrounding water. The turbulent nature of the flow could also be seen by watching the bodies and particularly the anal cerci of animals in the tubes. It could be seen that these were being buffeted continuously by the water. The transition from laminar to turbulent flow could be observed by trailing a thread of fine cotton in the water. If the current speed was increased slowly from a slow speed at which the flow was laminar to a speed at which the flow became turbulent, the thread could be seen to start to be buffeted at the transitional speed when the flow became turbulent.

In a similar study of the resistance of a caddis larva to water current speed, Bournaud (1963) has calculated the force in dynes acting on animals at each current speed and Reynolds number. The formula he used

was of the following form:

$$F = ka\bar{u}^2$$

where F is the force acting on the animal (dynes)

k is a constant which may be calculated

a is the frontal area of the animal (cm^2)

This formula assumes that the animal, in this instance enclosed in a cylindrical case, approximates in shape to a regular cylinder facing end on to the current in the open flowing water, the speed of which is not influenced by boundary layer effects along the walls of the tube.

Since the mayfly nymphs used in the present study were not cylindrical in shape and were entirely enclosed within the boundary layer, Bournaud's formula could not be used. Instead, for each current speed and tube size, the current speed 0.1 cm from the tube wall, roughly the height of the nymphs used, was estimated as:

$$u = u_{max} \left(\frac{2h}{d}\right)^{1/7}$$

(Streeter 1958), where

u is the speed of the water (cm/sec)

h is the distance from the wall (0.1 cm)

u_{max} is the speed of the water along the central axis of the tube, calculated as:

$$u_{max} = \bar{u} + 4.07\sqrt{\frac{\tau}{\rho}}$$

(Goldstein 1938), where

τ is the shearing stress (g sec^2/cm), constant throughout the tube

ρ is the density of water (1.0 g/cm^3)

and where $\sqrt{\tau/\rho}$ was derived in turn from:

$$\bar{u} = 0.29\sqrt{\tau/\rho} + 5.66\sqrt{\tau/\rho}\log\frac{d\sqrt{\tau/\rho}}{\gamma}$$

(Goldstein 1938).

It is reasoned that the current speed 0.1 cm away from the wall approximated to the greatest speed to which any part of the animals was exposed. From this point outwards to the wall the current speed decreased to zero. In figures 6 and 7 the median resistance times of Baetis harrisoni and Choroterpes bugandensis nymphs at different current speeds and in the two tube sizes used are shown as a function of estimated current speeds 0.1 cm from the wall. As may be seen from these figures, the apparent difference between resistance times of nymphs in the two tube sizes disappears when actual current speeds acting on the animals are calculated in this way.

NYMPHS OF DIFFERENT SIZES

In tables 4 and 5 median washaway times of Baetis harrisoni and Choroterpes bugandensis nymphs of different sizes are compared. Each animal was killed after its resistance to flow had been tested. The length of each, excluding antennae and cerci, was then measured under a microscope. The largest class of nymphs used in this study was similar in size to that used in the previous experiments. From these results it appears that smaller nymphs of both species were slightly better able to resist fast water flows than were larger nymphs. Although median washaway times of nymphs of different sizes did not in all instances differ significantly from one another (Student's t test), a rank correlation test (Kendall 1955) carried out for each species indicated a statistically significant negative correlation between resistance time and body length ($p < 0.001$ for each).

ROUGH AND SMOOTH SURFACES

Stuart (1958) has suggested that mayfly nymphs might be able to withstand fast water flows better when clinging to a relatively rough surface providing a firmer foothold than could be obtained on a smooth surface. In order to get some idea of the magnitude of this possible effect, nymphs of Baetis harrisoni and Choroterpes bugandensis were exposed to fast flows in tubes with relatively smooth and relatively rough inner surfaces.

TABLE 4

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES

Body length (mm)	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
2.0 to 3.4	0.60	25	8.5 (8.2 to 8.9)
	0.80	25	4.1 (3.3 to 5.0)
3.5 to 4.9	0.60	25	8.3 (7.9 to 8.8)
	0.80	25	4.4 (4.1 to 4.8)
5.0 to 6.5	0.60	25	7.6 (7.0 to 8.3)
	0.80	25	3.8 (3.1 to 4.5)

The inner surface of the "smooth" tube was very lightly scratched all over, far more lightly than the tubes used for previous experiments but with a similar number of scratches. The "rough" tube was roughened inside with a similar number of scratches, but here the individual scratches were rather deeper than those in the tubes used for ordinary experiments.

Figure 6

Median times to washaway of Baetis harrisoni nymphs at different water current speeds (estimated 0.1 cm from wall) in two tubes of internal diameter 0.75 and 1.6 cm.

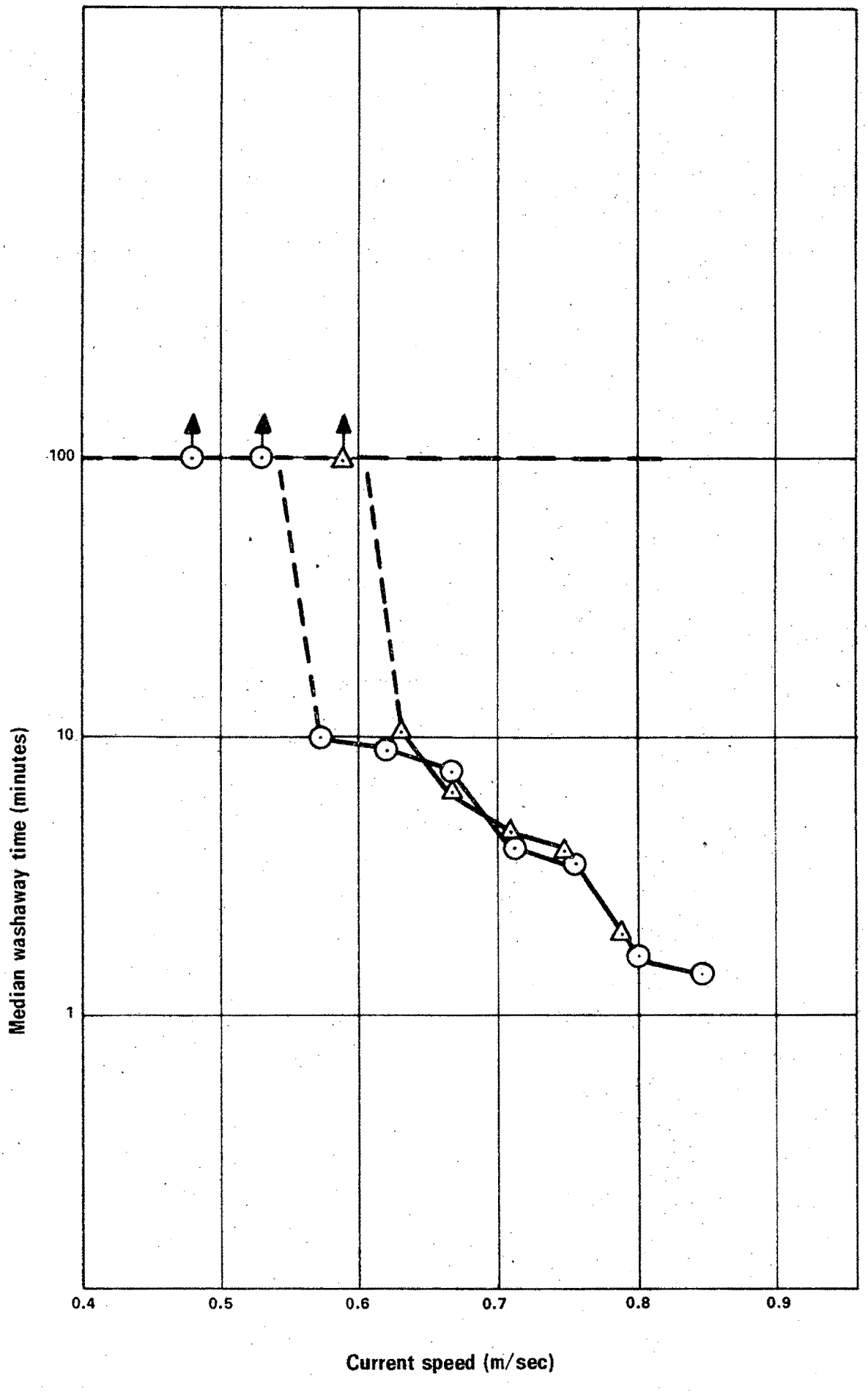

100
10
1
Median washaway time (minutes)
0.4
0.5
0.6
0.7
0.8
0.9
Current speed (m/sec)

Figure 7

Median times to washaway of Choroterpes bugandensis nymphs at different water current speeds (estimated 0.1 cm from wall) in two tubes of internal diameter 0.75 and 1.6 cm.

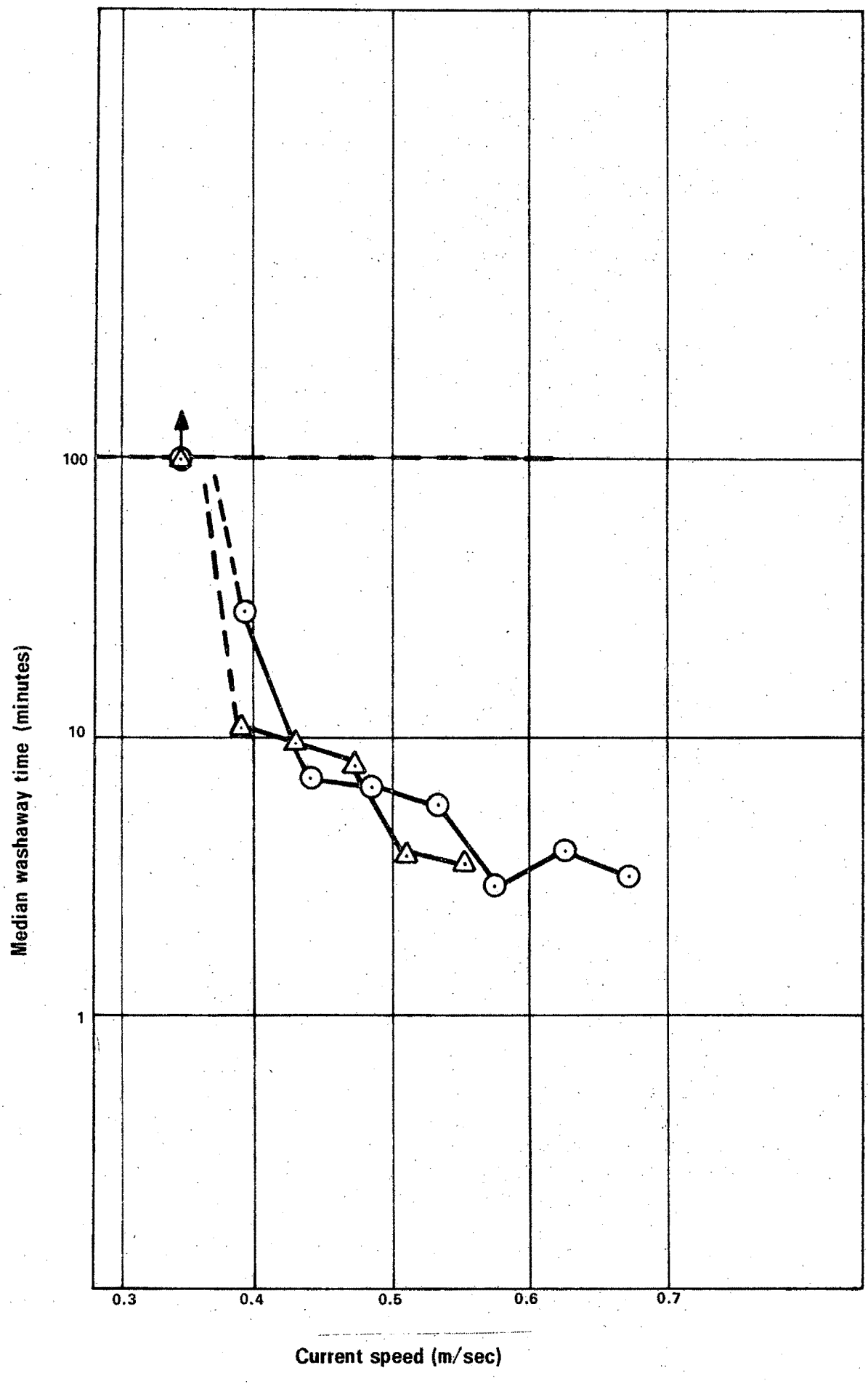

Median washaway time (minutes)
100
10
1
0.3
0.4
0.5
0.6
0.7
Current speed (m/sec)

TABLE 5

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF CHOROTERPES BUGANDENSIS NYMPHS OF DIFFERENT SIZES

Body length (mm)	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
1.6 to 2.9	0.50	25	9.2 (8.3 to 10.2)
	0.70	25	4.4 (3.6 to 5.2)
3.0 to 4.4	0.50	25	8.4 (7.9 to 8.8)
	0.70	25	4.1 (3.4 to 4.7)
4.5 to 6.0	0.50	25	7.1 (6.6 to 7.7)
	0.70	25	3.3 (2.7 to 3.8)

Median times to washaway of Baetis harrisoni and Choroterpes bugandensis nymphs to fast water flows in these rough and smooth tubes are shown in tables 6 and 7. As may be seen, the resistance times of nymphs of each species in the rough tube were rather similar to those in the smooth tube. These times did not differ significantly from one another. Both tubes apparently provided the nymphs with an adequate toehold. The claws of these animals were presumably covered by a "laminar sublayer" of the slowest moving water, even at the fastest and most turbulent flows. This layer should in these experiments have been about 0.02 to 0.04 cm thick (Streeter 1958). It seems likely that direct drag on the claws was negligible and that resistance depended on the ability of the animal to withstand the drag forces being exerted on the head and body.

TABLE 6

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF BAETIS HARRISONI NYMPHS ON ROUGH AND LESS ROUGH SURFACES

Surface	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
deeply scratched	0.60	25	7.5 (6.9 to 8.2)
	0.80	25	4.0 (3.5 to 4.5)
lightly scratched	0.60	25	7.2 (6.7 to 8.0)
	0.80	25	3.6 (3.0 to 4.2)

TABLE 7

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF CHOROTERPES BUGANDENSIS NYMPHS ON ROUGH AND LESS ROUGH SURFACES

Surface	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
deeply scratched	0.50	25	7.3 (6.8 to 7.8)
	0.70	25	3.3 (2.7 to 3.9)
lightly scratched	0.50	25	6.7 (6.1 to 7.3)
	0.70	25	3.2 (2.9 to 3.6)

In a further experiment, nymphs of each species were exposed to fast water flows in two further tubes lined with sand grains of fairly uniform

diameter. In each instance the sand grains covered about half the inner surface of the tube. The surface covered by sand grains had first been filed away to accommodate the sand grains without materially affecting the internal diameter of the tubes. Two tubes were used, one lined with sand grains 0.1 to 0.5 mm in diameter and the other lined with sand grains 0.8 to 1.0 mm in diameter, closely compacted together in each instance.

Median times of resistance to fast flow of Baetis harrisoni and Choroterpes bugandensis nymphs in each of the tubes lined with sand grains are shown in tables 8 and 9. As may be seen, nymphs of each species on sand grains appeared to be relatively less able to resist fast flows than were nymphs on the roughened perspex tested earlier. Nymphs on the sand grains of larger diameter were relatively less resistant than were those on the smaller sand grains. The observed differences between washaway times at the same current speeds in the two tubes were not statistically different (Student's t test) but a rank correlation test (Kendall 1955) revealed the frequency with which washaway times of each species on the smaller sand grains exceeded those on the larger sand grains at the same current speed to be significant ($p.< 0.01$). These results seem to suggest either that the relatively smooth sand grains provided less of a toehold than did the scratched perspex, or that unevenness of the wall changed flow conditions in the boundary layer in some way.

CURRENT SPEEDS IN RIVERS

Attempts were made using an impeller of 1 cm diameter described by Edington and Molyneux (1960) and recommended by Ambühl (1962) and also a Pitot tube similar to that described by Stuart (1958) to measure actual

current speeds over and among stones in the Braamfontein Spruit and the Pienaars River. Both instruments were calibrated against an Ott "Minor" current meter in open flowing water.

TABLE 8

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF BAETIS HARRISONI NYMPHS ON SAND GRAINS OF DIFFERENT SIZES

Sand grain diameter (mm)	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
0.1 to 0.5	0.60	25	4.8 (4.3 to 5.2)
	0.80	25	2.6 (2.4 to 2.8)
0.8 to 1.0	0.60	25	4.2 (3.8 to 4.7)
	0.80	25	2.4 (2.3 to 2.6)

TABLE 9

MEDIAN TIMES TO WASHAWAY IN FAST WATER FLOW OF CHOROTERPES BUGANDENSIS NYMPHS ON SAND GRAINS OF DIFFERENT SIZES

Sand grain diameter (mm)	Average current speed (m/sec)	Number of nymphs	Median washaway time (minutes), 95% confidence limits in brackets
0.1 to 0.5	0.50	25	6.0 (5.3 to 6.8)
	0.70	25	2.9 (2.4 to 3.3)
0.8 to 1.0	0.50	25	5.7 (5.2 to 6.3)
	0.70	25	2.6 (2.2 to 3.0)

The Edington and Molyneux impeller gave extremely sensitive and reproducible readings at all current speeds. It could be used to measure current speeds between rocks and in places inaccessible to the Ott. However, it was not really robust enough for field work and had frequently to be cleaned and recalibrated. The Pitot tube could be used to measure current speeds in places inaccessible to the Edington and Molyneux impeller, but was found to be highly insensitive and difficult to read. In fact, head fluctuation normally exceeded the head height to be measured. However, it did provide estimates of current speeds close to the surfaces of rocks.

A large number of measurements were made of current speeds in the water around rocks on which Baetis harrisoni and Choroterpes buganden-sis nymphs were found to be living in the Braamfontein Spruit and the Pienaars River at times of relatively fast flow. Counts were made of Baetis nymphs in randomly placed quadrats one foot square (30.5 cm x 30.5 cm) in the Braamfontein Spruit at a time of normal summer flow and flow measurements then made in the water above these quadrats. A brief summary of the results obtained is given in table 10. These results suggest that field populations of Baetis harrisoni nymphs were most concentrated in situations of relatively fast water flow, but that current speeds in the fastest sections of the river might have been faster than the nymphs could withstand.

From what is known of the distribution of current speeds in streams (Jeffreys 1925) one would expect speeds 0.1 cm above the surface of exposed rocks to be between about one third and a half of those 0.3 cm above the surface. The widest limits of expected speeds 0.1 cm above positions de-

scribed in table 8 where Baetis nymphs appeared not to be able to live would appear by this reasoning to be 0.3 to 1.2 m/sec. The actual speeds at these positions at the time that the observations were made probably lay between much narrower limits. Nowhere where nymphs were found would speeds in excess of 0.6 m/sec have occured 0.1 cm above the surface. In fact, they were probably rather less than this. Figure 3 shows that Baetis nymphs were unable to withstand speeds (again 0.1 cm above the surface) much in excess of 0.5 m/sec. It therefore seems possible to explain the absence of nymphs from exposed situations in terms simply of the intolerably fast water flows found there. Moreover, at times of lower flow nymphs were collected at all of these places.

TABLE 10

SUMMARY OF COUNTS OF BAETIS HARRISONI NYMPHS ON LARGE ROCKS IN DIFFERENT WATER CURRENT SPEEDS

Rock surface	Current speed (m/sec)			Number of samples	Number of nymphs (mean and range)
	5 cm above surface	1 cm above surface	0.3 cm above surface		
flat, parallel to flow	3 to 3.5	1.4 to 2.0	0.9 to 1.7	3	0/1/1
	2 to 3	0.8 to 1.8	0.6 to 1.1	12	36 (28 to 82)
	1 to 2	0.5 to 1.5	0.3 to 0.8	17	21 (6 to 74)
	0.3 to 1	0.2 to 0.5	0.0 to 0.3	8	20 (12 to 31)
flat, inclined toward flow	3.1, 3.2	2.3, 2.7	1.9, 2.3	2	0
	2.2	1.8	1.2	1	93
	1.2,1.4,1.4	0.8,1.1,1.3	0.5,0.7,0.7	3	23/13/17
	0.1 to 1	0.1 to 0.6	0.0 to 0.3	5	11 (2 to 25)
rounded facing flow	2 to 3	1.5 to 1.8	1.0 to 1.2	4	5 (0 to 16)
	1 to 2	0.8 to 1.2	0.6 to 0.8	7	6 (0 to 19)
	0.2 to 1	0.1 to 0.8	0.0 to 0.5	5	9 (4 to 11)

While Baetis harrisoni nymphs were found mostly on exposed rock surfaces and on the upper surfaces of stones, Choroterpes bugandensis nymphs, during the day at least, were to be found under flat stones. A brief summary of current speeds measured above and beneath these stones at a time of normal summer flows is given in table 11. Still other stones inhabited by Choroterpes nymphs were too inaccessible for the impeller. The currents flowing under them appeared to be even slower. Highest numbers of nymphs appeared to be found under stones over which relatively swifter water was flowing, although the rate of water flow under these stones was apparently not faster than under stones in slower flowing water.

Comparison of tables 10 and 11 shows that the Pienaars River was generally slower flowing than the Braamfontein Spruit. When the observations reported here were made, the current speeds close to the rocks would nowhere have been too fast for Choroterpes nymphs. In spite of this, the nymphs were all found under stones and away from the current.

TABLE 11

SUMMARY OF COUNTS OF CHOROTERPES BUGANDENSIS NYMPHS UNDER INDIVIDUAL STONES (COUNTS EXPRESSED PER SQUARE FOOT UNDERSURFACE OF STONE) IN DIFFERENT CONDITIONS OF WATER FLOW

Current speed (m/sec)		Number of samples	Number of nymphs (Mean and range)
5 cm above stone	under stone		
0.52 to 0.72	< 0.05 to 0.20	17	44 (5 to 117)
0.23 to 0.49	< 0.02 to 0.17	24	37 (12 to 94)
0.2 to 0.15	< 0.02 to 0.06	31	24 (3 to 43)

SUMMARY

1. Baetis nymphs were able to withstand faster rates of water flow (0.5 to 0.6 m/sec for 100 minutes) than were Choroterpes (0.3 to 0.4 m/sec for 100 minutes)

2. Nymphs in tubes of different internal diameters were similarly resistant to current speeds estimated to be flowing 0.1 cm away from the wall, although the average speeds flowing through these tubes differed.

3. Smaller nymphs of both species were a little more resistant to fast flows than were larger nymphs.

4. Roughness of the inner surface of the tube did not affect the resistance of nymphs, but compacted sand grains apparently provided a slightly less suitable surface for attachment than did scratched perspex.

5. Water current speeds which would have been too fast for either species were measured at places during flooding in one of the rivers in which observations were made.

6. The largest numbers of Baetis nymphs were found in the field on exposed rocks in current speeds approaching the fastest they could withstand. Nymphs of this species were absent from situations where the flow measured 5 cm above the stones exceeded about 3 m/sec, that measured 1 cm above the stones exceeded 2 m/sec and that measured 0.3 cm above the stones exceeded 1 m/sec. These flows are estimated to be equivalent to about 0.3 m/sec 0.1 cm above the stones.

7. Choroterpes nymphs in the field were found under rocks and therefore out of the current. Larger numbers were found under rocks over which swifter water was flowing. The water flow where nymphs were found nowhere approached speeds that might wash them away.

EFFECTS OF FLOODS ON POPULATIONS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

INTRODUCTION

Observations are reported here of the effects of snap floods on populations of nymphs of Baetis harrisoni and Choroterpes bugandensis in two Transvaal streams. The first species was studied in the Braamfontein Spruit north of Johannesburg, a stony stream subject to occasional fairly severe floods. The second species was studied in the Pienaars River, a more sluggish stream east of Pretoria. Measurements of actual water current speeds could only be made in the free-flowing water during floods, but an effort has been made to predict possible current speeds which might act on animals under such circumstances.

Results presented in the previous section have shown Baetis harrisoni nymphs to be able to maintain their positions in relatively swift flowing water. However, during fast flows in the Braamfontein Spruit the current speeds across at least some of the more exposed rocks were too strong for the nymphs. It seems not unlikely that even more rocks might be exposed to such fast flows during floods, and that numbers of these nymphs would be washed downstream as was suggested by Allanson (1961).

Choroterpes bugandensis nymphs were found in the previous section only to be able to resist rather slower water current speeds. These animals are normally found on the undersurfaces of stones in current and would presumably be affected only by floods sufficiently strong to disturb these stones.

FLOODS IN THE BRAAMFONTEIN SPRUIT

On several occasions when rain had been forecast in the catchment of the Braamfontein Spruit, counts were made of Baetis harrisoni nymphs in a stretch of river where nymphs were not normally collected for other purposes. On two such occasions rain did fall and flooding occured within the next few days. Water current speeds were measured during the flood on each occasion. After a fortnight or so, when the water had subsided, further counts were made for comparison.

Counts of nymphs were made in each instance using a Surber sampler (Surber 1936). This consisted of two square metal frames with sides one foot (30.5 cm) in length joined at right angles to one another along one margin of each frame. In use, one frame is laid flat on the stream bed. The other stands upright on the downstream side of the horizontal frame, at right angles to the direction of stream flow, and supports a long conical net which trails downstream. The animals in the area enclosed by the horizontal frame are washed into the net and counted. Samples were taken at a number of pre-selected points. The points were visited in consecutive order starting with the point furthest downstream. Care was taken that collection at one place did not disturb the animals at places still to be sampled. When they had been counted all the animals were released again.

The place at which these observations were carried out was selected because of the large number and variety of stones present. Several of these stones were marked using paint. After each flood it could be ascertained which had been moved by the flood.

Counts of Baetis harrisoni nymphs made in this way before and after two floods are shown in table 12. On each occasion, the numbers of nymphs were found to have been considerably reduced during the floods. Although the flood observed during November had been more violent, the proportional reduction in numbers appeared in each case to have been about the same.

TABLE 12.

NUMBERS OF BAETIS HARRISONI NYMPHS (PER SQUARE FOOT) COLLECTED BEFORE AND AFTER FLOODING IN THE BRAAMFONTEIN SPRUIT

Month	Flow before flood (m/sec)		Flow during flood (m/sec)		Numbers before flood	Numbers after flood	Reduction in numbers (per cent)	Diameter of largest stones moved (cm)
	5 cm above rocks	1 cm above rocks	5 cm above rocks	1 cm above rocks				
November	0.5 to 1.8	0.2 to 1.0	1.3 to 4.5	0.8 to 3.0	7 25 65 68 23 16 27 102 63 10	11 4 17 6 9 6 3 28 15 0	77	50
January	0.5 to 1.5	0.3 to 0.8	1.5 to 3.0	1.2 to 1.8	46 23 20 13 48 48 36 23 35 27	13 2 1 5 7 0 13 4 7 2	83	15

FLOODS IN THE PIENAARS RIVER

Counts of Choroterpes bugandensis nymphs were made in the Pienaars River in the same way that counts of Baetis harrisoni had been made in the Braamfontein Spruit. Counts were made on several occasions in expectation of flooding. On three occasions floods occurred within a few days. Current speed measurements were made at the height of the floods and counts of nymphs made again when the floods had subsided in order to determine the effect of flooding upon the Choroterpes population.

The counts made before and after these three floods are shown in table 13. As may be seen, one severe flood resulted in a drastic reduction in Choroterpes numbers. On the other hand, neither of the other two floods, both of which were moderate, had much effect on the numbers of nymphs present.

DRIFT

Riverine invertebrates tend to drift downstream apparently being carried passively by the current. Species appear to differ greatly in their tendencies to do this. This evidently depends on the degree to which they move about on rocks in the flowing water, on their behavioural reactions to current (Müller 1954, Besch 1967) and on their diurnal activity rhythms (Müller 1966, Waters 1968). The phenomenon of organic drift in streams is beyond the present study, but it is necessary here to distinguish drift which occurs at normal flows from washaway which occurs during flooding.

TABLE 13

NUMBERS OF CHOROTERPES BUGANDENSIS NYMPHS (PER SQUARE FOOT) COLLECTED BEFORE AND AFTER FLOODING IN THE PIENAARS RIVER

Month	Flow before flood (m/sec)		Flow during flood (m/sec)		Numbers before flood	Numbers after flood	Reduction in numbers (per cent)	Diameter of largest stones moved (cm)
	5 cm above rocks	1 cm above rocks	5 cm above rocks	1 cm above rocks				
October	0 to 0.5	0 to 0.1	0.4 to 2.6	0.1 to 1.2	16 45 44 29 45 31 9 3 1 78	10 45 33 16 21 61 37 5 10 27	12	10
November	0 to 1.0	0 to 0.4	0.5 to 3.5	0.1 to 2.1	23 54 20 36 45 27 5 18 49 21	10 0 0 8 6 1 0 3 2 0	85	20
April	0.1 to 0.5	0 to 0.2	0.3 to 3.0	0.1 to 1.4	23 16 49 7 36 3 74 12 27 23	85 12 32 18 5 19 13 50 5 12	8	10

Drift of Baetis harrisoni was observed while that of Choroterpes bugandensis was not. On three seperate occasions for each, two Surber samplers were wedged in the flowing water in the Braamfontein Spruit and Pienaars River and examined at dusk and in the early morning for nymphs washed downstream into them. Numbers of Baetis harrisoni nymphs were found on each occasion to have been caught in the nets both during the night and during the day. No Choroterpes bugandensis nymphs were caught.

CURRENT SPEEDS ACTING ON NYMPHS

Laboratory results reported in the previous section have shown Baetis harrisoni and Choroterpes bugandensis nymphs to be washed away by water flow rates in excess of 0.5 m/sec measured 0.1 cm from the surface to which they were attached. Field results showed that nymphs of this species were absent, or almost so, from those rocks in the Braamfontein Spruit exposed to water currents estimated tc exceed about 0.3 m/sec 0.1 cm above the rock surface. The current speeds measured in the free water above these rocks exceeded 3 m/sec. Large numbers of Baetis nymphs were found on rocks exposed to slightly slower flowing water.

RESISTANCE OF BAETIS IN THE FIELD

In order to observe resistances of nymphs to fast water flows in the field, a number of rocks of suitable sizes and shapes were selected which could be placed and manoeuvred in streams of fast flowing water. Most of these observations were carried out using a large piece of smooth slate, a flat rock of similar proportions but with a rough and pitted surface, and a smaller round stone, also with a rough surface.

A number of observations were made in the Braamfontein Spruit using these stones. Baetis harrisoni nymphs were captured and a number encouraged to attach themselves to the rock being used. On each occasion, the rock was manoeuvred into the faster flowing water and the reactions of the nymphs observed.

The two flat stones could be placed horizontally over a round stone on the stream bottom and the downstream end tilted upwards as required by means of a crowbar and using the rounded stone as a fulcrum. In this way, the upper surface was exposed to increasingly fast rates of water flow.

The round stone was firmly glued at opposite poles to two stout metal rods by means of epoxy resin glue. The rods pointed outward from the stones in opposite directions but were in fact fairly accurately in line with one another. This stone could be suspended in a flow of water with the metal rods at right angles to the direction of flow. It could then be rotated at will in the current.

The rates of water flow were measured in each case 0.3 cm above the rock face, using a Pitot tube similar to that described by Stuart (1958). It was reasoned in the previous section that current speeds 0.1 cm above the surface, and impinging on the animals themselves, would be perhaps a little more than a third of the speed measured 0.3 cm above the surface.

The speed at which nymphs were observed to be dislodged from the stones are listed in table 14. As may be seen, nymphs were found to be washed off the two rough-surfaced rocks at current speeds of 1.4 to 1.8 m/sec measured 0.3 cm off the rocks. These speeds must have been equivalent to speeds of 0.5 to 0.6 m/sec, perhaps a little more, 0.1 cm

above the rocks. These speeds are in very good agreement with laboratory observations reported in the previous section. The differences between washaway speeds on the flat rock parallel to the flow, the flat rock inclined to the flow and the round stone in the flow may presumably be ascribed to differences between the height of the boundary layer under these different conditions, as has in fact been described by Ambühl (1959).

TABLE 14.

CURRENT SPEEDS (MEASURED 0.3 cm ABOVE THE STONES) AT WHICH BAETIS HARRISONI NYMPHS WERE WASHED OFF STONES

Shape	Position in stream	Number of nymphs	Average washaway speed (m/sec)
Smooth slate	horizontal	24	1.0
	inclined ± 15° to flow	17	0.8
Flat rock	horizontal	39	1.8
	inclined ± 15° to flow	42	1.5
Round stone	in main stream	14	1.4

Table 14 also shows that animals on the flat piece of slate were much less resistant to fast flow than were animals on the rougher stones. They appear, in fact, to have been washed away by current speeds little more than half as fast as those found necessary to dislodge animals in the laboratory. This was surprising, since it seems unlikely that the slate

was so smooth as not to provide the nymphs with the toehold they required. Perhaps, in part, flow in the boundary layer over the smooth slate was faster than it was over the rougher rocks. Perhaps some factor not evident at the time affected the behaviour of nymphs on the slate.

It was noted during these observations that Baetis harrisoni nymphs did not appear to be affected in any way by knocks or bumps given to the rocks on which they were sitting. However, when the round stone suspended in the flow on two metal rods was rotated sharply through an angle of 90^{o} in the direction of water flow at least half of the nymphs on the stone were washed off on each occasion. When the stone was rotated through 360^{o} almost all of the nymphs were dislodged.

To sum up, many Baetis harrisoni nymphs appeared in the field to live on parts of stones exposed to the full force of the stream. The results presented here appear to confirm that a current of 0.5 m/sec or more actually impinging on the nymphs was necessary in order to dislodge them. Current speeds in excess of this appear to occur 0.1 cm above exposed rocks during floods and nymphs on these rocks might be expected to be washed away when this occurs. Furthermore, it seems that many of the nymphs on stones dislodged or rolled during flooding will almost certainly be washed away. Directions of water flow around stones which are rolled in the current evidently change too rapidly for the nymphs to be able to adjust their positions.

RESISTANCE OF CHOROTERPES NYMPHS IN THE FIELD

Similar observations were made using the same rocks transported to the Pienaars River and using Choroterpes bugandensis nymphs. The

current speeds observed to dislodge Choroterpes nymphs from these rocks are shown in table 15. As may be seen, these nymphs were also found to be able to withstand considerably faster current speeds when on natural rock surfaces that they could when on smooth slate.

TABLE 15

CURRENT SPEEDS (MEASURED 0.3 cm ABOVE THE STONES) AT WHICH CHOROTERPES BUGANDENSIS NYMPHS WERE WASHED OFF STONES

Shape	Position in stream	Number of nymphs	Average washaway speed (m/sec)
Smooth slate	Horizontal	47	0.8
	inclined ± 15° to flow	25	0.6
Flat rock	Horizontal	55	2.0
	inclined ± 15° to flow	40	1.5
Round stone	In main stream	16	1.5

Comparison of tables 14 and 15 shows, surprisingly, that Choroterpes nymphs in the field appeared to withstand faster current speeds than did Baetis nymphs. Laboratory observations reported in the previous section indicated that Choroterpes nymphs were not able to withstand current speeds in excess of 0.35 m/sec flowing 0.1 cm above the surface on which they were living. The water flowing 0.1 cm from the rough rocks when the observations shown in table 15 were made must have been at .

least 0.5 to 0.7 m/sec and were probably faster.

Only one possible explanation seems to suggest itself to account for this apparent discrepancy. Perhaps Choroterpes bugandensis nymphs were able to manoeuvre themselves on these rocks into pockets of slower flowing water in a way they could not in the laboratory. Uneven rock surfaces might create such small pockets. If Choroterpes nymphs move into these pockets while Baetis nymphs do not, this might be explained in terms of their different behaviour in flowing water.

As in the case of Baetis nymphs, Choroterpes nymphs did not appear to be affected in any way by bumps or knocks given to the rocks on which they were sitting. When the round stone with a number of nymphs on it was rotated through 90^{o} in the direction of water flow about one nymph in five was observed to be dislodged. Choroterpes nymphs seemed therefore to be far less markedly affected by changes in direction of water flow than were Baetis nymphs.

In table 13 it was seen that numbers of Choroterpes nymphs were reduced only during floods in which larger stones were dislodged. Presumably it was only when the stones were rolled that nymphs were exposed to the full force of the current.

SUMMARY

1. Numbers of Baetis nymphs were found to be severely reduced during two floods.
2. Numbers of Choroterpes nymphs were only seen to be severely reduced during one flood sufficiently strong to disturb stones on the

stream bed. The numbers of nymphs were not much affected by two other slightly less violent floods.

3. Numbers of Baetis nymphs were observed to drift downstream with the current during the day and night. No Choroterpes nymphs were observed in the drift.

4. Both Baetis and Choroterpes nymphs withstood far faster current speeds when they were on natural stone surfaces (1.4 m/sec or faster, measured 0.3 cm above the stones) than they did when they were on smooth slate (1.0 m/sec or less, also measured 0.3 cm above the stones).

5. Baetis nymphs were easily dislodged by changes in the direction of water flow. Choroterpes nymphs were less susceptible to such changes.

MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS AT HIGH TEMPERATURES

INTRODUCTION

Harrison (1965a), in discussing zoogeographical affinities of South African riverine invertebrates, concluded that a vast majority of species were of tropical origin. He further sub-divided the members of this Pan-Ethiopian element of the fauna principally on the basis of their presumed temperature tolerances. In this study the upper lethal temperatures of nymphs of two mayflies are examined. From their wide distribution both would qualify as eurythermal members of Harrison's Pan-Ethiopian element. Baetis harrisoni is a very common species in southern African streams and rivers, while Choroterpes bugandensis appears to be widespread in south-eastern Africa. The results presented here indicate that even widespread African mayflies like these can differ quite remarkably in their tolerances of high temperatures.

The distribution of a number of Ephemeroptera has been found to be influenced very markedly by water temperatures. Pleskot (1951) and Kamler (1965) have shown streams differing from one another principally in the ranges of their water temperatures to be inhabited by different mayfly species. Like Bretschko(1964), they also found growth rates and emergence of these species to be very markedly affected by temperatures. Macan (1960) made very careful observations of temperature ranges in several streams and found the distribution of a mayfly to be limited to those streams where the temperature did not exceed about 18^{o}C.

Whitney (1939) determined upper lethal temperatures for nymphs of two mayflies in the laboratory and found these lethal temperatures to be correlated with the temperature ranges to which these animals were exposed in the field. Marlier (1949), Muirhead-Thomson (1951), Nebeker and Lemke (1968) and others have investigated lethal temperatures of other aquatic insects. They have also found different species to differ in their temperature tolerances and observed upper lethal temperatures to be correlated with temperatures in the natural habitats of these species.

Whitney (1939) found smaller mayfly nymphs to be more tolerant of high temperatures than were larger nymphs. He suggested that this might have resulted from their different metabolic rates. The young of certain fish have also been found either to be more tolerant than were larger fish (Bishai 1960, 1965) or to acclimate to high temperatures more rapidly than larger fish (Davis 1955). Other species of fish appeared to be equally tolerant at different stages (Keiz 1953, Matutani 1960). In one species of fish the sexes have been found to differ in their heat tolerances (Freeman 1960).

Several workers have compared high temperature tolerances of different aquatic invertebrates and of different populations of the same species collected from different biotopes and localities. Mayfly nymphs from swift-flowing streams have been found to have higher metabolic rates and to be less tolerant of high temperatures than nymphs from ponds and sluggish streams (Fox and Simmonds 1933, Fox, Simmonds and Washbourn 1935, Fox, Wingfield and Simmonds 1937, Whitney 1939). The same has also been found to be true for other aquatic invertebrates (Walshe 1948) and for fish (for instance

Flörke, Keiz and Wangorsch 1954). It has in fact been widely held that eurythermal animals have generally lower metabolic rates than do stenothermal species (Schlieper and Bläsing 1953), although recent work has shown this not to be strictly true (Pattée 1965).

Observations have been made during the present study of mortality of Baetis harrisoni and Choroterpes bugandensis nymphs in the presence of different concentrations of dissolved calcium, since increased calcium in tissues appears to result in a reduction in metabolic rate (Halsband 1953). Whole animals might conceivably respond similarly. This phenomenon has been held, for instance, to explain the increased high temperature tolerance of Paramecium cultures observed in the presence of small amounts of dissolved calcium (Grigoryan 1964). The calcium content of earthworms has also been found to increase with acclimation to high temperatures (Saroja and Rao 1965).

Mortality of Baetis and Choroterpes nymphs has also been compared here in water containing different concentrations of sodium chloride. Tolerances by many different animals of high salinities and high temperatures have been found to be correlated. High temperature acclimation of earthworms has been found to involve changes in water balance (Rao and Ramachandra 1961) as has death of goldfish at high temperatures (Houston 1962, Heinecke and Houston 1965). Moreover, salmon smolt have been found to be more tolerant of high temperatures in brackish water (Alabaster 1967). Dehydration has in fact been held to increase high temperature tolerance (Christophersen and Precht 1953, Precht, Christophersen and Hensel 1955, Remane and Schlieper 1958).

MATERIAL AND METHODS

Nymphs of Baetis harrisoni and Choroterpes (Euthraulus) bugandensis were collected and brought to the laboratory as has been described elsewhere. The animals were held in well-aerated water at 20°C in the laboratory for 24 hours before being used in the experiments.

The apparatus used has also already been described. It consisted of 12 aquaria, each with an impeller capable of driving a controlled volume of water through one or several experimental tubes containing animals. The temperature in each tank was controlled using a Braun "Thermomix" thermostat-heater. The water in each tank was aerated strongly, and the dissolved oxygen content measured from time to time. In all of the experiments described here experimental tubes with an internal diameter of 2.6 cm were used. Current speeds in these tubes were controlled at 15 cm/sec, at which speed the flow in tubes of this size was turbulent (Re = 3900).

In each experiment, a number of nymphs were held for 1000 minutes at each of a number of different temperatures in the lethal range, and the resulting mortality recorded. From the observed numbers dead and alive at the end of the experiment, median lethal temperatures were calculated by probit analysis as described by Finney (1952). As part of each experiment, a number of nymphs were held at a high but non-lethal temperature under conditions otherwise similar to those being exposed to lethal temperatures and their mortality noted.

The experiments involving Choroterpes bugandensis were carried out during March and April. Those involving Baetis harrisoni were carried out during May and June.

MORTALITY AT SUB-LETHAL TEMPERATURES

No mortality was observed among any of several quite large groups of Choroterpes bugandensis nymphs held in experimental tubes for 1000 minutes at various sub-lethal temperatures. However, a few dead or dying individuals were invariably found among similar groups of Baetis harrisoni nymphs held at sub-lethal temperatures.

All of the Baetis harrisoni nymphs found dead or dying at sub-lethal temperatures were noted to have attempted ecdysis during the experiment. In those that had just started to moult, the outer skin was pulled free from the body in places. In these individuals the body could often be seen to have retracted from the outer skin in places. In other individuals the body had clearly separated from the outer skin. Where ecdysis had advanced still further the loose outer skin had split between the wing buds. In some, the body had begun to protrude from the split. In others, the thorax was flexed and strongly protruded dorsally. In normal ecdysis the head and legs were freed first and the abdomen pulled free of the old skin last. Animals which died during this last stage of ecdysis had often succeeded in pulling their abdomens free of the old skin but had either their mouthparts, or some of their legs, occasionally even the whole head, caught in the old skin.

By counting the number of empty nymphal skins in each experimental tube, the number of successful ecdyses which had taken place could invariably be established. No animals were found to have died after emerging successfully from the old skin. Ecdyses were more frequent at higher temperatures and among small nymphs. On average, about 14 per cent

of all ecdysis of Baetis nymphs at sub-lethal temperatures resulted in death.

LETHAL TEMPERATURES

After each experiment the length of each animal, excluding the antennae and cerci, was measured under a microscope. Median lethal high temperatures found for Choroterpes bugandensis nymphs of three different sizes are shown in table 16. These animals were all found to be relatively tolerant of high temperatures. The smaller nymphs were found to be slightly more tolerant than were larger nymphs ($p < 0.01$).

TABLE 16

1000-MINUTE MEDIAN HIGH LETHAL TEMPERATURES FOR CHOROTERPES BUGANDENSIS NYMPHS OF DIFFERENT SIZES

Body length (mm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
1.6 to 2.9	160	35.25 35.5 35.75 36.0 36.25 36.5 36.75 37.0	36.1 (35.9 to 36.2)
3.0 to 4.4	160	35.0 35.25 35.5 35.75 36.0 36.25 36.5 36.75	35.9 (35.8 to 36.1)
4.5 to 6.0	160	34.75 35.0 35.25 35.5 35.75 36.0 36.25 36.5	35.4 (35.2 to 35.7)

Conventional estimates of lethal temperatures for Baetis harrisoni could not be made. When relative numbers of nymphs dying were plotted on probability paper as a function of the logarithm of temperature the expected straight line approximation of the cumulative tolerance probability distribution curve was not obtained. Satisfactory straight lines were obtained in each instance from experiments using Choroterpes bugandensis and are necessary for estimation of the median lethal temperature using probit analysis. In each instance involving Baetis harrisoni nymphs, however, a sinuate curve was obtained. This sort of phenomenon is normally taken to reveal a heterogeneous distribution of tolerances in the population (Finney 1952).

Examination of Baetis harrisoni nymphs dying at high temperatures in these experiments revealed that a majority had died during ecdysis. They showed exactly the same symptoms as had those that had died in ecdysis at sub-lethal temperatures. Animals attempting ecdysis during the experiment were evidently far more sensitive to high temperatures than were animals that did not attempt ecdysis. Once again, by counting and matching up the nymphal skins found in the tubes it was possible to divide each group of Baetis nymphs into those that had attempted ecdysis and those that had not. Numbers dead and alive could be counted separately in each case for animals attempting and not attempting ecdysis. Plots on probability paper were made separately for each group and satisfactory approximations to straight lines obtained in each instance for each of the two groups.

A typical example is illustrated in figure 8. Here, total mortality

Figure 8

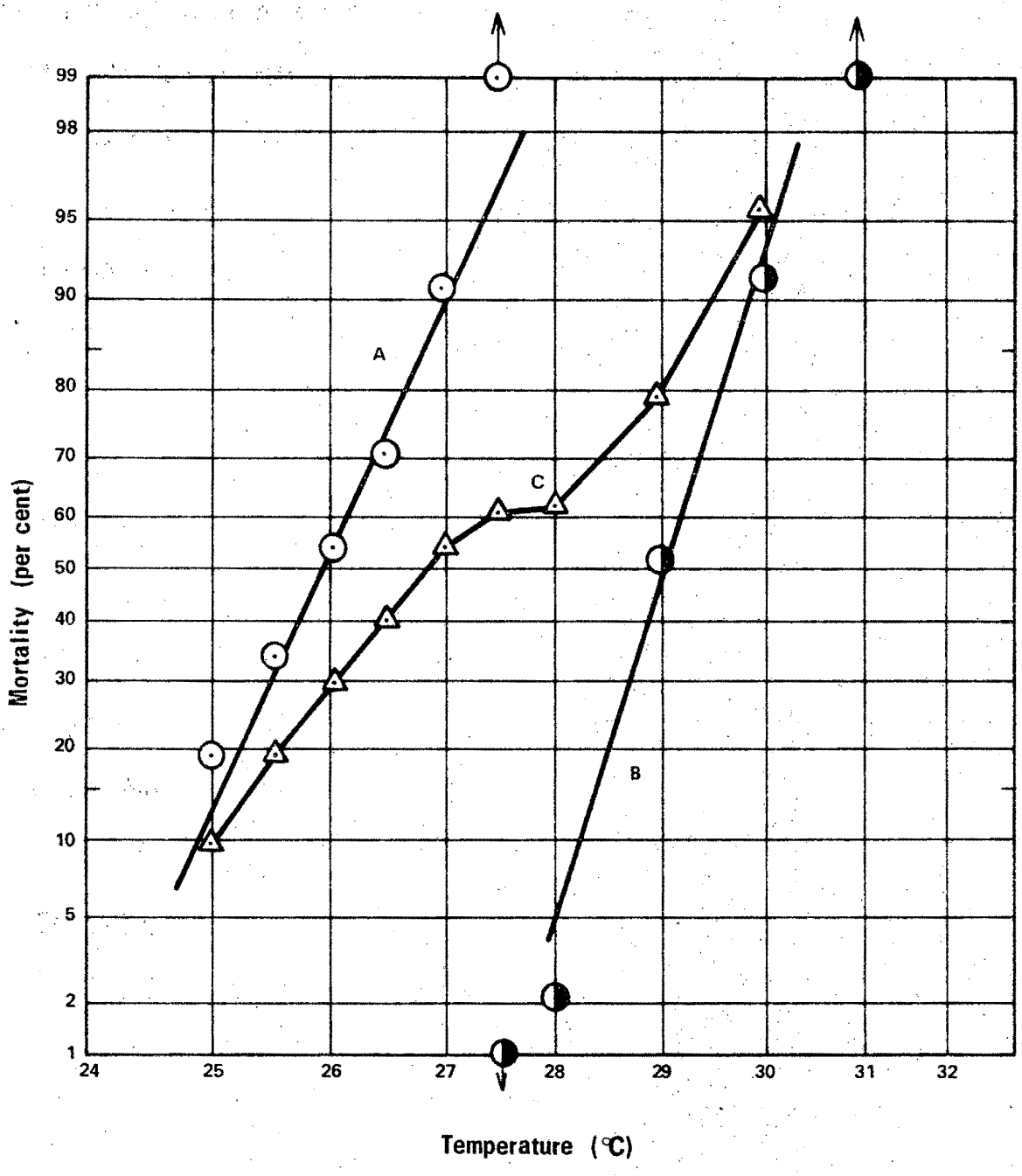
Mortality of Baetis harrisoni nymphs during 1000 minutes at high temperatures.

(A) - nymphs attempting ecdysis during exposure to high temperature.

(B) - nymphs not attempting ecdysis during exposure.

(C) - total.

Mortality shown per cent of number of nymphs exposed and plotted on probability paper.


99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
Mortality (per cent)
24
25
26
27
28
29
30
31
32
Temperature (°C)
A
B
C

per cent for each experimental group is shown at a number of test temperatures (open triangles and line C). The points do not fall on a straight line. Mortality of animals attempting ecdysis during the experiment has been plotted in the same way (open circles and line B) as has mortality of animals not attempting ecdysis (shaded circles and line B). The lines drawn through each series of circles were fitted by probit analysis and the line for animals in ecdysis was corrected and balanced for natural mortality. Correction for natural mortality was not necessary in the case of animals not in ecdysis, since no mortality of these animals at sub-lethal temperatures was observed.

Median lethal temperatures estimated from the probit lines for animals in and out of ecdysis and estimated in this way for Baetis harrisoni nymphs of different sizes are listed in tables 17 and 18 respectively for those attempting and not attempting ecdysis during the experiments. These results show that animals of all sizes tested were considerably more sensitive to high temperatures during ecdysis than were animals at other times ($p \ll 0.001$). Most significantly, Baetis harrisoni nymphs were significantly less tolerant of high temperatures than were Choroterpes bugandensis nymphs (cf. table 16). It is also to be noted that smaller Baetis nymphs were more tolerant of high temperatures than were larger nymphs ($p < 0.01$).

FOOD SUPPLY AND TEMPERATURE TOLERANCE

Two experiments were carried out in which mortality at high temperatures of the following three groups of Baetis harrisoni and Choroterpes bugandensis nymphs was compared:

TABLE 17

1000-MINUTE MEDIAN LETHAL HIGH TEMPERATURES FOR BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES ATTEMPTING ECDYSIS DURING THE EXPERIMENT

Body length (mm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
2.0 to 3.4	114	25.0 25.5 26.0 26.5	25.9 (25.8 to 26.1)
3.5 to 4.9	93	25.0 25.5 26.0 26.5	25.3 (25.2 to 25.5)
5.0 to 6.5	111	24.5 25.0 25.5 26.0	25.4 (25.2 to 25.5)

(a) nymphs supplied with algal food during the 24 hours before the experiment but not during the experiment itself

(b) nymphs supplied with food both before and during the experiment

(c) nymphs deprived of food both before and during the experiment.

The median lethal high temperatures for these three groups are compared in tables 19 and 20 for Baetis harrisoni nymphs attempting and not attempting ecdysis during the experiment and in table 21 for Choroterpes bugandensis. These results suggest that the temperature tolerances of these species were reduced if food was not supplied before the experiment ($p < 0.001$) However, their tolerances appear not to have been affected by the presence or absence of food during the experiment.

TABLE 18

1000-MINUTE MEDIAN LETHAL HIGH TEMPERATURES FOR BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT

Body length (mm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
2.0 to 3.4	138	28.0 28.5 29.0 29.5	28.6 (28.4 to 28.9)
3.5 to 4.9	139	28.0 28.5 29.0 29.5	28.8 (28.6 to 28.9)
5.0 to 6.5	150	27.5 28.0 28.5 29.0	28.5 (28.2 to 28.8)

TIME MORTALITY

In order to be able to observe times to death of nymphs at high lethal temperatures, nymphs had to be mechanically stimulated at intervals of time. If not stimulated, Baetis harrisoni nymphs became motionless after some time at temperatures in the lethal range. They had to be very lightly prodded to see whether or not they were still alive.

Choroterpes bugandensis nymphs at high temperatures were found to beat their gills very rapidly at first. The rate of gill movement then decreased with time. After some time at temperatures within the lethal range the movements stopped, at first for short periods and then for longer and longer periods. After a while, it became impossible to tell which animals were still alive without prodding them.

TABLE 19

EFFECT OF FOOD SUPPLY (BEFORE AND DURING EXPOSURE) ON 1000-MINUTE UPPER MEDIAN LETHAL TEMPERATURES FOR BAETIS HARRISONI NYMPHS ATTEMPTING ECDYSIS DURING THE EXPERIMENT

Food supply	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
before but not during experiment	79	25.0 25.5 26.0 26.5	25.4 (25.1 to 25.7)
both before and during experiment	82	25.0 25.5 26.0 26.5	25.5 (25.2 to 25.7)
neither before nor during experiment	73	23.0 23.5 24.0 24.5	23.9 (23.5 to 24.3)

TABLE 20

EFFECT OF FOOD SUPPLY (BEFORE AND DURING EXPOSURE) ON 1000-MINUTE UPPER MEDIAN LETHAL TEMPERATURES FOR BAETIS HARRISONI NYMPHS NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT

Food supply	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
before but not during experiment	60	28.0 28.5 29.0 29.5	28.6 (28.2 to 29.0)
both before and during experiment	73	28.0 28.5 29.0 29.5	28.7 (28.3 to 29.1)
neither before nor during experiment	64	27.0 27.5 28.0 28.5	27.9 (27.5 to 28.4)

TABLE 21

EFFECT OF FOOD SUPPLY (BEFORE AND DURING EXPOSURE) ON 1000-MINUTE UPPER MEDIAN LETHAL TEMPERATURES FOR CHOROTERPES BUGANDENSIS NYMPHS

Food supply	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
before but not during experiment	160	35.0 35.5 36.0 36.5	35.7 (35.5 to 35.9)
both before and during experiment	160	35.0 35.5 36.0 36.5	35.6 (35.4 to 35.8)
neither before nor during experiment	160	33.0 33.5 34.0 34.5	34.0 (33.7 to 34.3)

For these time-mortality experiments, nymphs were placed in shallow open trays made of nylon gauze, each tray being suspended in a tank in which the temperature was maintained at a selected lethal level. A flow of water of some 5 ml/sec was introduced into the tray from the circulation pump of the thermostat-heater in order to keep the tray inflated and the water in the tray in motion. The nymphs were supplied with Chlorella or Scenedesmus cells as food during this experiment and kept under observation for 3000 minutes. Quiescent nymphs were frequently lightly touched and numbers of nymphs dead and alive noted at frequent intervals. A control group of each species was maintained at a sub-lethal temperature.

Time-mortality curves could not be drawn up for Baetis nymphs attempting ecdysis since the times when different nymphs started ecdysis could ob-

viously not be determined. It was also apparent from observations carried out at sub-lethal temperatures that mortality of moulting nymphs was greatly increased by mechanical disturbance. A group of nymphs was held for 1000 minutes at 20°C in a gauze tray and prodded from time to time in the way nymphs in the time-mortality experiment were prodded. No nymphs not attempting ecdysis during the experiment were found to die. Of the nymphs attempting ecdysis 34 per cent died. This mortality is suggested to have been due to mechanical disturbance.

The fact that these nymphs were sensitive to physical disturbances seems to indicate that the "natural" mortality of Baetis nymphs invariably observed at sub-lethal temperatures in these experiments might have been due at least in part to handling of the nymphs in the preparation of the experiment.

Mortality of nymphs was observed at each of several temperatures. Median survival times calculated by probit analysis (Bliss 1937) for each temperature are illustrated in figures 9 and 10 respectively for Baetis harrisoni nymphs not attempting ecdysis during the experiment and for Choroterpes bugandensis. Mortality of nymphs of both species in lethal temperatures was found to continue right through the 3000 minutes of observation. Further mortality might have occurred if the experiment had been continued. This means that the "incipient upper lethal temperatures" or "threshold" temperatures for these species, at which no further mortality would be expected even during greatly extended exposure time, must be rather lower than the 1000-minute median lethal temperatures reported here.

Figure 9

Median times of survival at high temperatures of Baetis harrisoni nymphs not attempting ecdysis.

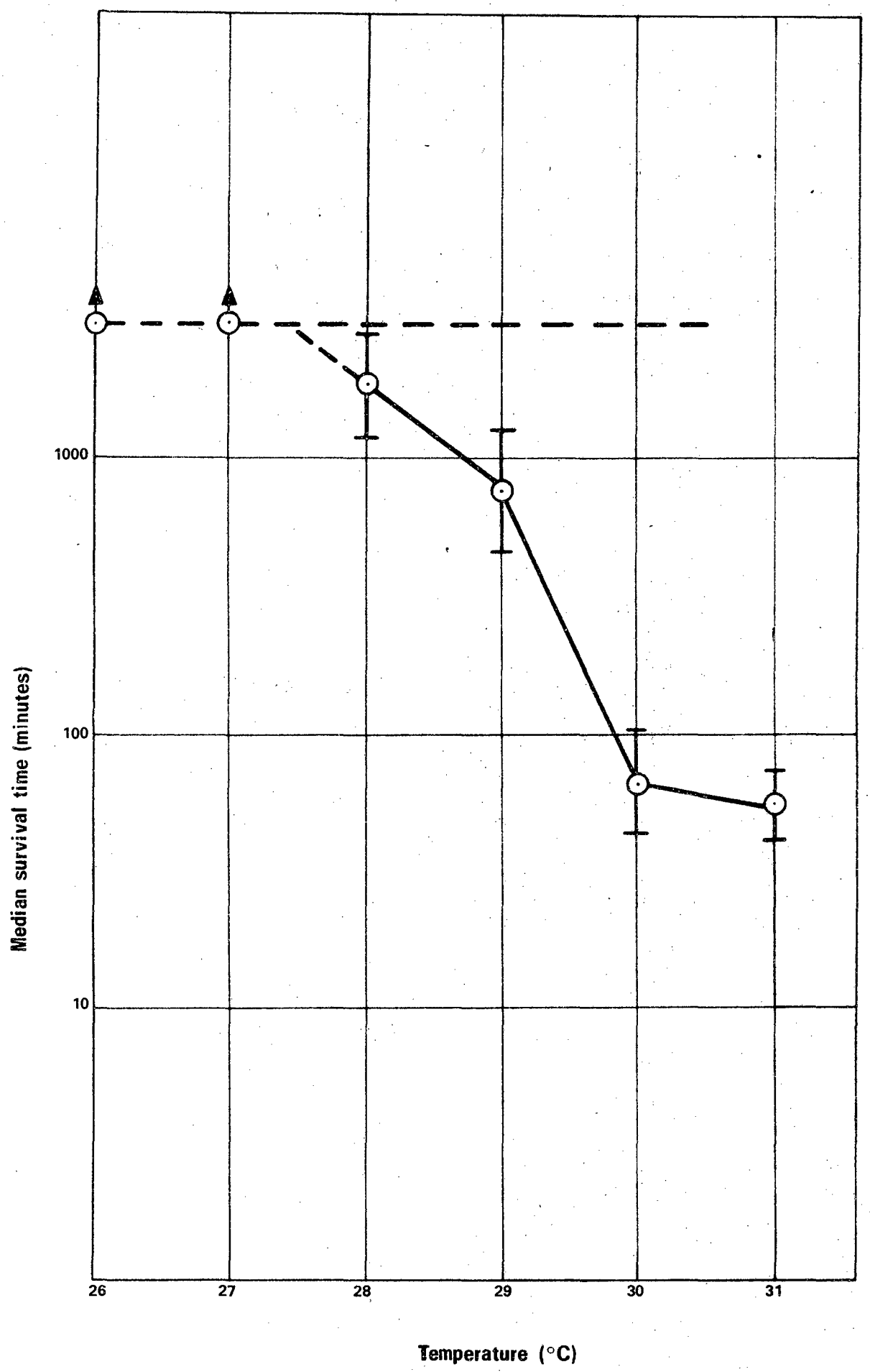
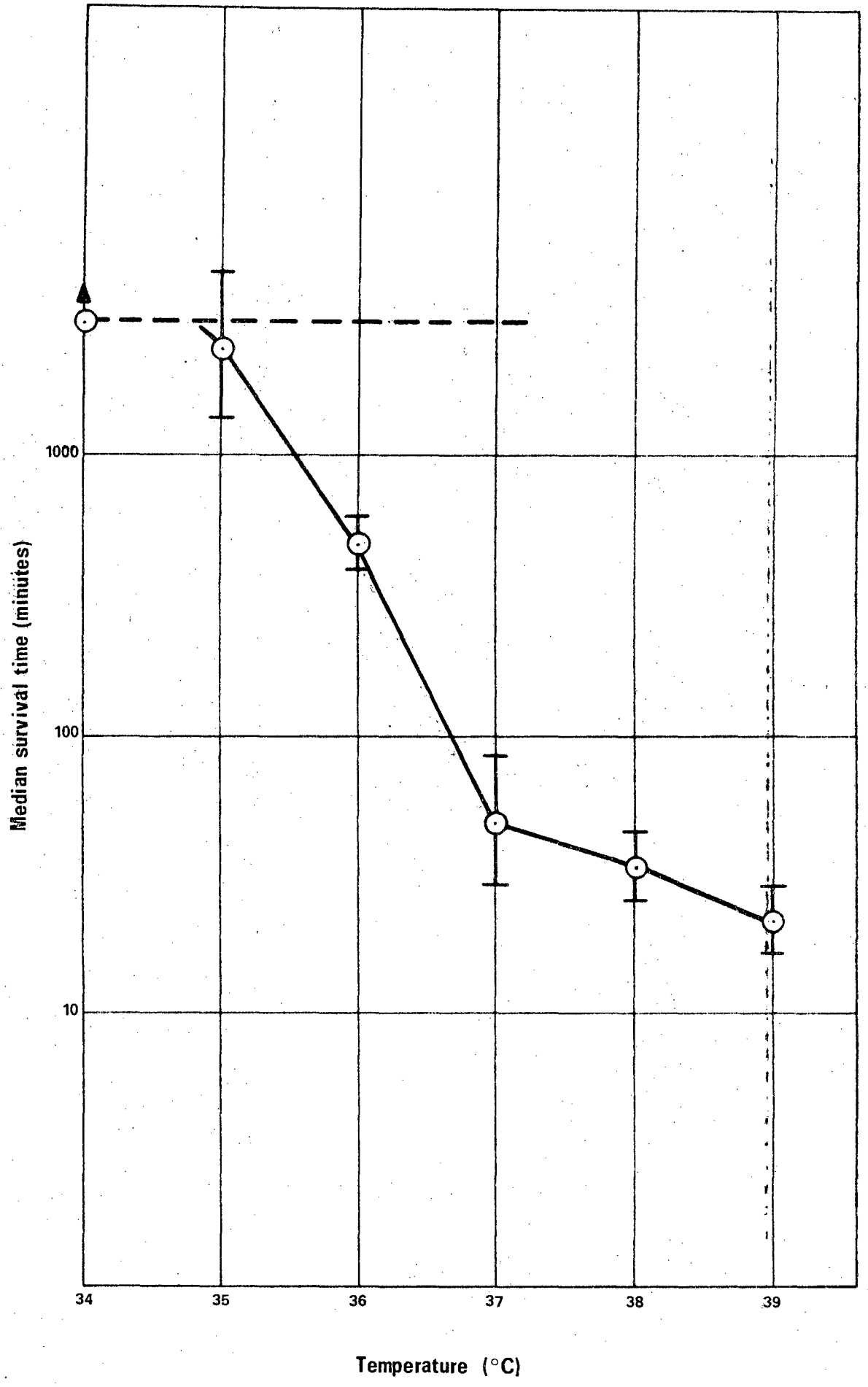

1000
100
10
Median survival time (minutes)
26
27
28
29
30
31
Temperature (°C)

Figure 10

Median times of survival of Choroterpes bugandensis nymphs at high temperatures.


Median survival time (minutes)
1000
100
10
34
35
36
37
38
39
Temperature (°C)

TOXICITY OF RUBBER AND PUTTY

In table 22 median lethal high temperatures for Baetis harrisoni and Choroterpes bugandensis nymphs are compared for two aquaria prepared in different ways. One of the tanks was completely lined with p.v.c. sheeting and the rubber tubing of the thermostat-heater replaced with p.v.c. tubing. In the other, the putty of the tank was not so covered and the rubber tubing was left in position. Nymphs in the two tanks appeared to be equally tolerant of high temperatures. No evidence of toxicity of the putty or rubber tubing could be detected.

CALCIUM

Two experiments were carried out in which the mortality of Baetis harrisoni and Choroterpes bugandensis nymphs in three different concentrations of dissolved calcium ions was compared. The first solution used was the same borehole water used in all previous experiments, in which the dissolved calcium content was found to be 1.1 mg/l. In the second solution the dissolved calcium content was raised to 40 mg/l and in the third solution the calcium content was raised to 400 mg/l. In each instance this was achieved by addition of calcium hydroxide. The water was then neutralized using dilute hydrochloric acid and bubbled for 24 hours using air which had been passed over potassium hydroxide, the pH being adjusted and allowed to reach equilibrium at pH 7. Thereafter, animals were exposed for 1000 minutes in each of these solutions to temperatures in the lethal range.

TABLE 22

EFFECT OF EXPOSURE TO RUBBER AND PUTTY ON 1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

Rubber and putty	Number of nymphs	Test temperatures (°C)	Lethal temperatures (°C), 95% confidence limits in brackets
exposed	70	24.5 25.0 25.5 26.0	25.5 (25.1 to 25.8) (Baetis attempting ecdysis)
	69	27.5 28.0 28.5 29.0	28.6 (28.5 to 28.7) (Baetis not attempting ecdysis)
	120	34.5 35.0 35.5 36.0	35.4 (35.1 to 35.6) (Choroterpes)
not exposed	76	24.5 25.0 25.5 26.0	25.4 (25.5 to 25.6) (Baetis attempting ecdysis)
	71	27.5 28.0 28.5 29.0	28.5 (28.3 to 28.7) (Baetis not attempting ecdysis)
	120	34.5 35.0 35.5 36.0	35.5 (35.2 to 35.7) (Choroterpes)

The median upper lethal temperatures estimated from these experiments for Baetis harrisoni and Choroterpes bugandensis nymphs in different calcium solutions are given in tables 23 and 24. These results show that, for each species, the upper lethal temperature was not significantly

affected by the presence or absence of calcium ions although, in both instances, animals in 40 mg/l calcium were found to be slightly more tolerant than were animals in lower and higher calcium concentrations.

TABLE 23

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR BAETIS HARRISONI NYMPHS IN DIFFERENT CALCIUM SOLUTIONS

Dissolved calcium (mg/l)	Number of nymphs	Test temperatures (°C)	Lethal temperatures (°C), 95% confidence limits in brackets
1.1	108	24.5 25.0 25.5 26.0	25.3 (25.1 to 25.5) (attempting ecdysis)
	108	27.5 28.0 28.5 29.0	28.4 (28.2 to 28.6) (not attempting ecdysis)
40	91	24.5 25.0 25.5 26.0	25.6 (25.3 to 25.8) (attempting ecdysis)
	106	27.5 28.0 28.5 29.0	28.6 (28.3 to 28.9) (not attempting ecdysis)
400	71	24.5 25.0 25.5 26.0	25.5 (25.3 to 25.6) (attempting ecdysis)
	119	27.5 28.0 28.5 29.0	28.4 (28.1 to 28.6) (not attempting ecdysis)

TABLE 24

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT CALCIUM SOLUTIONS

Dissolved calcium (mg/l)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
1.1	120	34.5 35.0 35.5 36.0	35.4 (35.2 to 35.6)
40	120	34.5 35.0 35.5 36.0	35.7 (35.5 to 35.9)
400	120	34.5 35.0 35.5 36.0	35.6 (35.5 to 35.7)

TOTAL DISSOLVED SALTS

Two further experiments were carried out in which mortalities of Baetis harrisoni and Choroterpes bugandensis nymphs in different concentrations of total dissolved solids were compared. The solution used were:

1. untreated borehole water having a total dissolved solids content of 0.03 g/l and a sodium content of 0.007 g/l.
2. borehole water to which sodium chloride had been added to give a total dissolved solids content of 5 g/l and a sodium content of 2.3 g/l
3. borehole water to which sodium chloride had been added to give a total dissolved solids content of 10 g/l, the sodium content of this

solution being 5.8 g/l

These solutions were made up as before and animals were exposed in them for 1000 minutes to temperatures in the lethal range.

The median upper lethal temperatures found in these experiments for Baetis harrisoni and Choroterpes bugandensis in different salt concentrations are given in tables 25 and 26. These results indicate that the salt content of the water had no apparent effect on the lethal temperatures of the two species.

TABLE 25

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR BAETIS HARRISONI NYMPHS IN DIFFERENT SALT CONCENTRATIONS

Total dissolved solids (g/l)	Number of nymphs	Test temperatures (°C)	Lethal temperatures (°C), 95% confidence limits in brackets
0.03	98	24.5 25.0 25.5 26.0	25.4 (25.2 to 25.6) (attempting ecdysis)
	109	27.5 28.0 28.5 29.0	28.5 (28.3 to 28.7) (not attempting ecdysis)
5	105	24.5 25.0 25.5 26.0	25.5 (25.2 to 25.7) (attempting exdysis)
	112	27.5 28.0 28.5 29.0	28.3 (28.1 to 28.5) (not attempting ecdysis)
10	83	24.5 25.0 25.5 26.0	25.4 (25.2 to 25.5) (attempting ecdysis)
	81	27.5 28.0 28.5 29.0	28.3 (28.1 to 28.5) (not attempting ecdysis)

TABLE 26

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT SALT CONCENTRATIONS

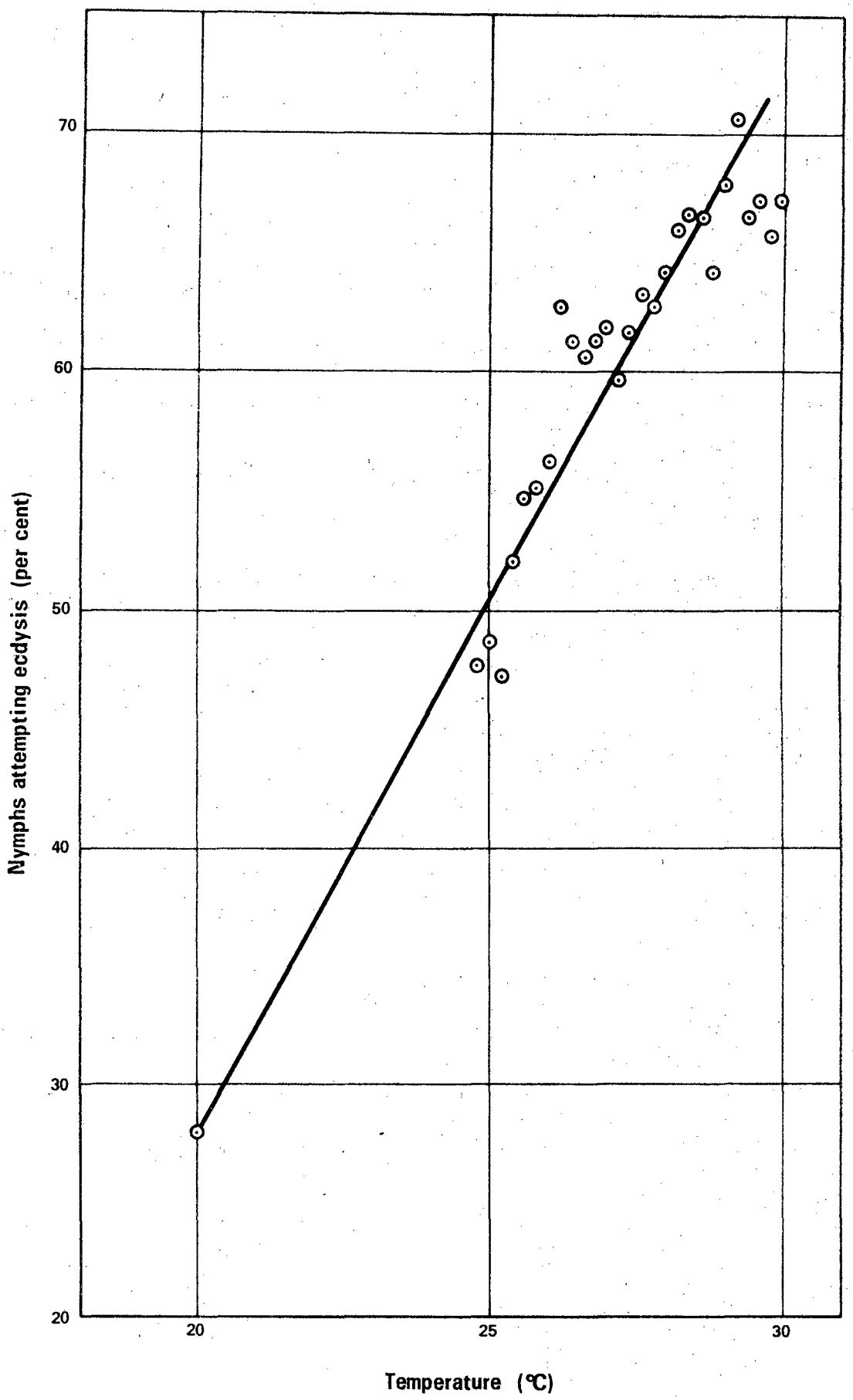
Total dissolved solids (g/l)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
0.03	120	34.5 35.0 35.5 36.0	35.6 (35.4 to 35.7)
5	120	34.5 35.0 35.5 36.0	35.5 (35.3 to 35.8)
10	120	34.5 35.0 35.5 36.0	35.6 (35.5 to 35.8)

FREQUENCY OF ECDYSIS OF BAETIS

In all dosage-mortality experiments involving Baetis harrisoni, numbers of nymphs attempting and not attempting ecdysis were counted separately in order that the lethal temperature could be calculated separately for each group. Relative numbers attempting ecdysis in experimental tubes at a number of different temperatures in the lethal range and at a sub-lethal temperature (20°C) are shown in figure 11. All of these animals had been held in the laboratory for 24 hours at 20°C before being exposed to these temperatures. As may be seen, the numbers of nymphs attempting ecdysis during 1000 minutes increased rapidly with increase in temperature. In certain insects, short exposure to high temperature has been

Figure 11

Ecdysis of Baetis harrisoni in experiments at different temperatures (mostly in the upper lethal range) during 1000 minutes


Nymphs attempting ecdysis (per cent)
70
60
50
40
30
20
20
25
30
Temperature (°C)

found to delay ecdysis (Okasha 1968). No evidence of any such delay was seen in these experiments.

SUMMARY

1. Choroterpes nymphs tolerated higher temperatures than did Baetis nymphs. 35.4°C was lethal for large Choroterpes nymphs and 25.4°C lethal for large Baetis nymphs in ecdysis.

2. Baetis nymphs were more susceptible to high temperatures during ecdysis (25.4°C being lethal for large nymphs) than at other times (28.5°C being lethal for large nymphs). Choroterpes nymphs appeared to be equally tolerant at all times.

3. The frequency of ecdysis of Baetis nymphs was found to increase markedly with temperature.

4. Larger nymphs of both species were less tolerant of high temperatures than were smaller nymphs.

5. Nymphs starved and fed during experiments were equally tolerant of high temperatures but nymphs starved for 24 hours before the experiments were less tolerant than those fed before the experiment.

6. Upper lethal temperatures of nymphs in different calcium concentrations and in water of different salinities did not differ significantly from one another.

ACCLIMATION TO HIGH TEMEPRATURES BY BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS, AND OBSERVED SURVIVAL OF NYMPHS IN THE FIELD

INTRODUCTION

A considerable volume of evidence is available to show that the temperature tolerances of most aquatic animals are influenced to a large extent by the temperatures at which they have been living. The literature has been extensively reviewed, for instance, by Fry (1947, 1958, 1964), Precht, Christopherson and Hensel (1955), Prosser (1955, 1958, 1964), Bullock (1955), Brett (1956) and Precht (1960, 1964). Observations are reported here of survival of Baetis harrisoni and Choroterpes bugandensis nymphs in the field at high temperatures. In order to judge to what extent lethal high temperatures might limit the natural ranges of these species, or might cause occasional mortalities of nymphs, the relative abilities of these two species to acclimate to different temperatures in the laboratory have also been investigated.

Lethal temperatures for fish have been found to change seasonally (Schlieper and Blăsing 1953, Kirberger 1953, Hoar 1955). Similar changes in temperature tolerance have been found to take place with acclimation in the laboratory, different animals being found to compensate in different ways and at greatly differing rates to changed temperatures (Bullock 1955, Kinne 1963). The temperature tolerances of some fish were found also to be affected by changes in photoperiod (Fry, Hart and Walker 1946, Hoar and Robertson 1959). Interest in the present study has been restricted to temperature acclimation.

The temperature tolerances of a fish, Lebistes, and of an insect,

Drosophila, have been found to be influenced by temperatures at which early development took place (Gibson 1954, Maynard - Smith 1957). Poljansky (1959) and Harnisch (1960) have also found effects of compensatory responses to temperature changes undergone by Protozoa and by Chironomus larvae to persist for some time. Changes in temperature tolerance can also take place over several generations when species are introduced into new areas (Shkorbatov 1964, Brun 1966).

A great deal of published information is available on physiological changes which can take place during the acclimation of poikilothermic animals to different temperatures. The changes that have been described are complex, involving physiological changes at many levels (Precht 1964). These aspects are beyond present study. However, the fact that a number of different phenomena are involved can cause acclimation to take different forms under different circumstances (Brett 1946). Several instances are available in the literature where animals have appeared not to acclimate to changed temperature (Fox, Wingfield and Simmonds 1937). Whitney (1939) has found the lethal temperatures of two mayflies to be unaffected after forty hours at raised temperature. The resistance of a protozoan has been found alternately to go up and down as different processes evidently took place during acclimation to higher temperature (Dregol'skaya 1963). Newell and Northcroft (1967) have shown that "activity" and "maintenance" metabolism of various invertebrates respond differently to changes in temperature.

Detailed studies of its temperature tolerances have shown a fish, Salvelinus fontinalis, to live in the field at temperatures right up to its lethal limits (Fry, Hart and Walker 1946). It is not easy from available infor-

mation to see to what extent other animals might be able to live at high sub-lethal temperatures. Information available on the temperature relations of Crenobia alpina shows just how difficult it can sometimes be to compare laboratory and field data on lethal limiting temperatures. Bläsing (1953) has found this species to survive 48 hours at 25°C but, curiously, its "incipient" upper lethal temperature (in the sense of Fry 1947) for prolonged exposure lies between 12° and 14°C (Beauchamp 1935, Pattée 1966).

Little is known of the effects of sub-lethal high temperatures themselves on aquatic animals. Heat stress is known to cause physical deformation in Aëdes (Anderson and Horsfall 1963) and Cocking (1957, 1959) has concluded that roach lose weight at temperatures a few degrees below their lethal temperatures. However, Fry (1964) has pointed out that Cocking's results need not necessarily be explained in terms of deletarious effects of sub-lethal temperatures. Effects of prolonged exposure to sub-lethal temperatures are really beyond the scope of this study. In the case of Baetis harrisoni, however, consideration has been given here to possible mortality of nymphs in ecdysis at temperatures below the lethal temperatures.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected in the Braamfontein Spruit and Pienaars River and divided at random into groups to be held in the laboratory in flat trays for different periods of time at different controlled temperatures, as has been described in the introductory section. Thereafter, groups were exposed to temperatures in the lethal range. From observed survival and mortality, median lethal temperatures

were calculated using probit analysis (Finney 1952). The apparatus used in these studies has also been described. Groups of animals were held in this apparatus in well-aerated water under controlled conditions of temperature and water flow. In the experiments described here, tubes of internal diameter 2.6 cm were used through out through which a water current speed of 15 cm/sec was maintained. At this speed through these tubes the water flow was always turbulent (Re = 3900).

SEASONAL CHANGES

As has already been reported, Baetis harrisoni nymphs in ecdysis were found to be considerably less tolerant of high temperatures than were nymphs not in ecdysis. In the experiments described in this section, as before, it was established which individuals had attempted ecdysis during the experiment and which had not. From relative mortalities of each, median lethal temperatures were estimated separately for animals in and out of ecdysis. These are shown, respectively, in tables 27 and 28. In each of these experiments, the nymphs were held in the laboratory for a day at the same temperature at which they were collected before the start of the experiment. The results show that Baetis harrisoni nymphs at different times of the year differed from one another in their temperature tolerances, summer nymphs being more tolerant of high temperatures than were winter nymphs ($p < 0.001$).

In table 29, median upper lethal temperatures for Choroterpes bugandensis nymphs collected at five different times of year are compared. The temperature tolerances of these nymphs also differed at different times of the year ($p < 0.01$), but in this case only by a small margin.

The nymphs of each species tested in October were collected at a time of exceptionally high water temperatures. The survival of nymphs observed at these temperatures in the field is to be discussed later. These nymphs were found to be more tolerant than any other nymphs had been. It seems likely that the median upper lethal temperature for each species determined on this occasion approximated to the "ultimate" upper lethal temperature (Fry, Brett and Clawson 1942), the highest which could be achieved by living at high temperatures.

TABLE 27

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES DETERMINED AT DIFFERENT TIMES OF YEAR FOR BAETIS HARRISONI NYMPHS ATTEMPTING ECDYSIS DURING THE EXPERIMENT

Month	River temperature at collection (°C)	River temperature range during previous month (°C)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C) 95% confidence limits in brackets
July	11.0	8 to 13	293	24.0 24.5 25.0 25.5 26.0 26.5	25.8 (25.7 to 26.0)
August	15.3	14 to 16	127	25.0 25.5 26.0 26.5	25.4 (25.2 to 25.6)
October	28.2	22 to 25	194	28.5 29.0 29.5 30.0	29.3 (29.1 to 29.5)
February	23.1	20 to 24	118	27.0 27.5 28.0 28.5	27.9 (27.6 to 28.2)
April	15.4	15 to 18	217	26.0 26.5 27.0 27.5	27.0 (26.8 to 27.2)

TABLE 28

1000- MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR *BAETIS HARRISONI* NYMPHS NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT, DETERMINED AT DIFFERENT TIMES OF YEAR

Month	River temperature at collection (°C)	River temperature range during previous month (°C)	Number of nymphs	Test temperatures (°C)	Lethal temperature(°C) 95% confidence limits in brackets
July	11.0	8 to 13	208	28.0 28.5 29.0 29.5 30.0	28.9 (28.8 to 29.0)
August	15.3	14 to 16	94	28.0 28.5 29.0 29.5	28.6 (28.3 to 28.9)
October	28.2	22 to 25	152	31.0 31.5 32.0 32.5	31.7 (31.4 to 32.1)
February	23.1	20 to 24	83	30.0 30.5 31.0 31.5	30.8 (30.5 to 31.1)
April	15.4	15 to 18	213	29.5 30.0 30.5 31.0	30.2 (30.0 to 30.4)

ACCLIMATION OF WINTER NYMPHS

Nymphs of each species were collected during late winter at a time when water temperatures varied between about 14° and 16°C. Groups of these nymphs were first held in the laboratory for 24 hours at 15°C and then held at different temperatures for a further one, two and three days before being exposed to high temperatures in the lethal range. Median lethal temperatures were estimated by probit analysis for each group. The

TABLE 29

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR CHOROTERPES BUGANDENSIS NYMPHS, DETERMINED AT DIFFERENT TIMES OF YEAR

Month	River temperature at collection (°C)	River temperature range during previous month (°C)	Number of nymphs	Test temperatures (°C)	Lethal temperature(°C) 95% confidence limits in brackets
August	17.2	11 to 18	356	35.0 35.25 35.5 35.75 36.0 36.25 36.5 36.75	35.8 (35.7 to 35.9)
October	31.6to32.5	23 to 29	404	35.5 35.75 36.0 36.25 36.5 36.75 37.0 37.25	36.2 (36.1 to 36.3)
January	19.1	18 to 23	180	35.05 35.75 36.0 36.25 36.5 36.75	35.9 (35.8 to 36.0)
February	22.6	20 to 24	120	35.5 36.0 36.5 37.0	36.0 (35.8 to 36.2)
August	16.0	14 to 16	120	35.0 35.5 36.0 36.5	35.6 (35.4 to 35.9)

lethal temperatures found after acclimation to different temperatures for different lengths of time are shown in figure 12 for Baetis harrisoni nymphs attempting ecdysis, in figure 13 for Baetis harrisoni nymphs not attempting ecdysis and in figure 14 for Choroterpes bugandensis nymphs.

Figure 12

1000-minute median upper lethal temperatures for winter Baetis harrisoni nymphs attempting ecdysis during exposure to high temperature, after acclimation in the laboratory at 15°C, 20°C and 24°C for different times. Lethal temperatures for summer nymphs shown by broken lines.

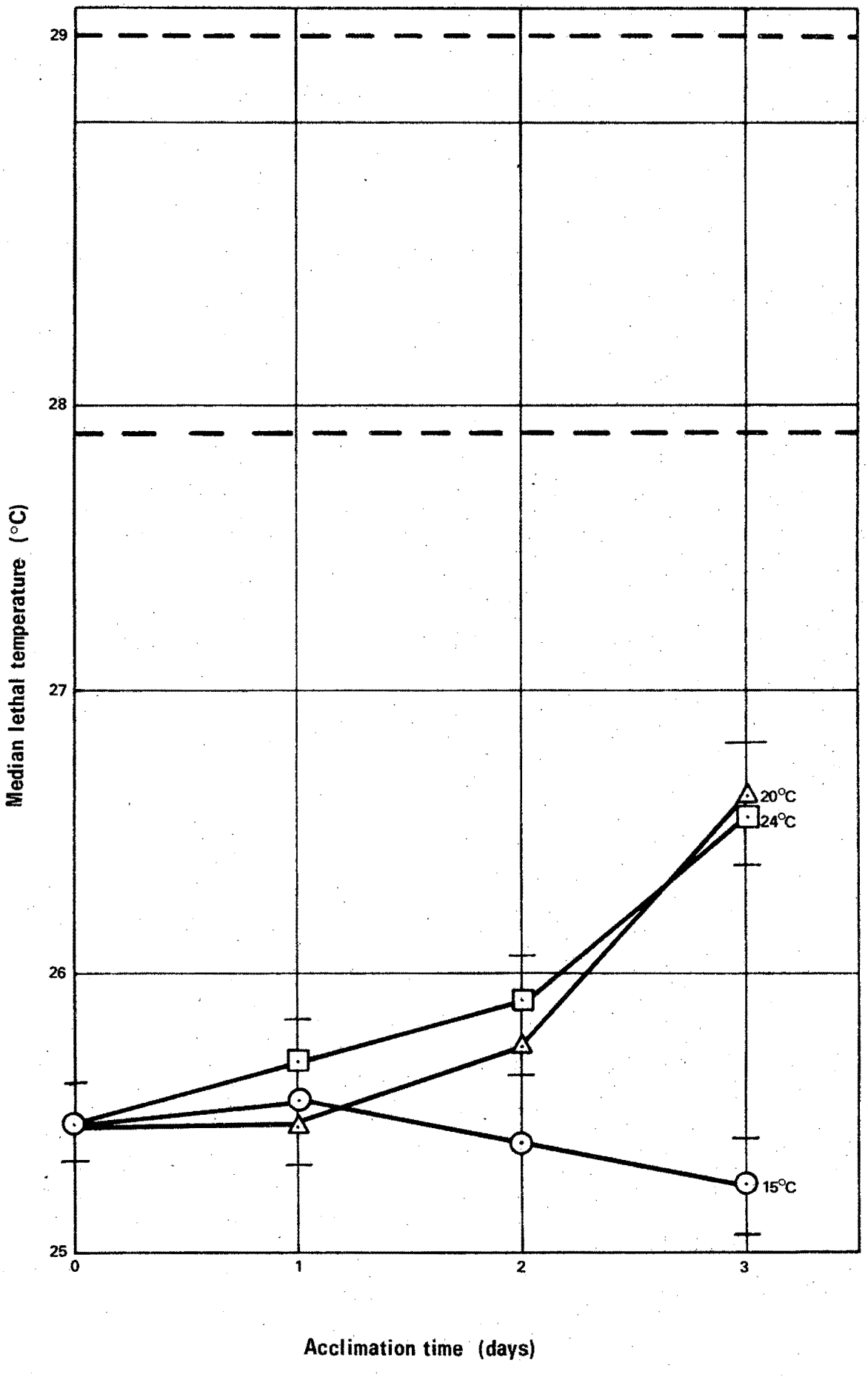

29
28
27
26
25
0
1
2
3
Median lethal temperature (°C)
Acclimation time (days)
20°C
24°C
15°C

Figure 13

1000-minute median upper lethal temperatures for winter Baetis harrisoni nymphs not attempting ecdysis during exposure to high temperature, after acclimation in the laboratory at 15°C, 20°C and 24°C for different times. Lethal temperatures for summer nymphs shown by broken lines.

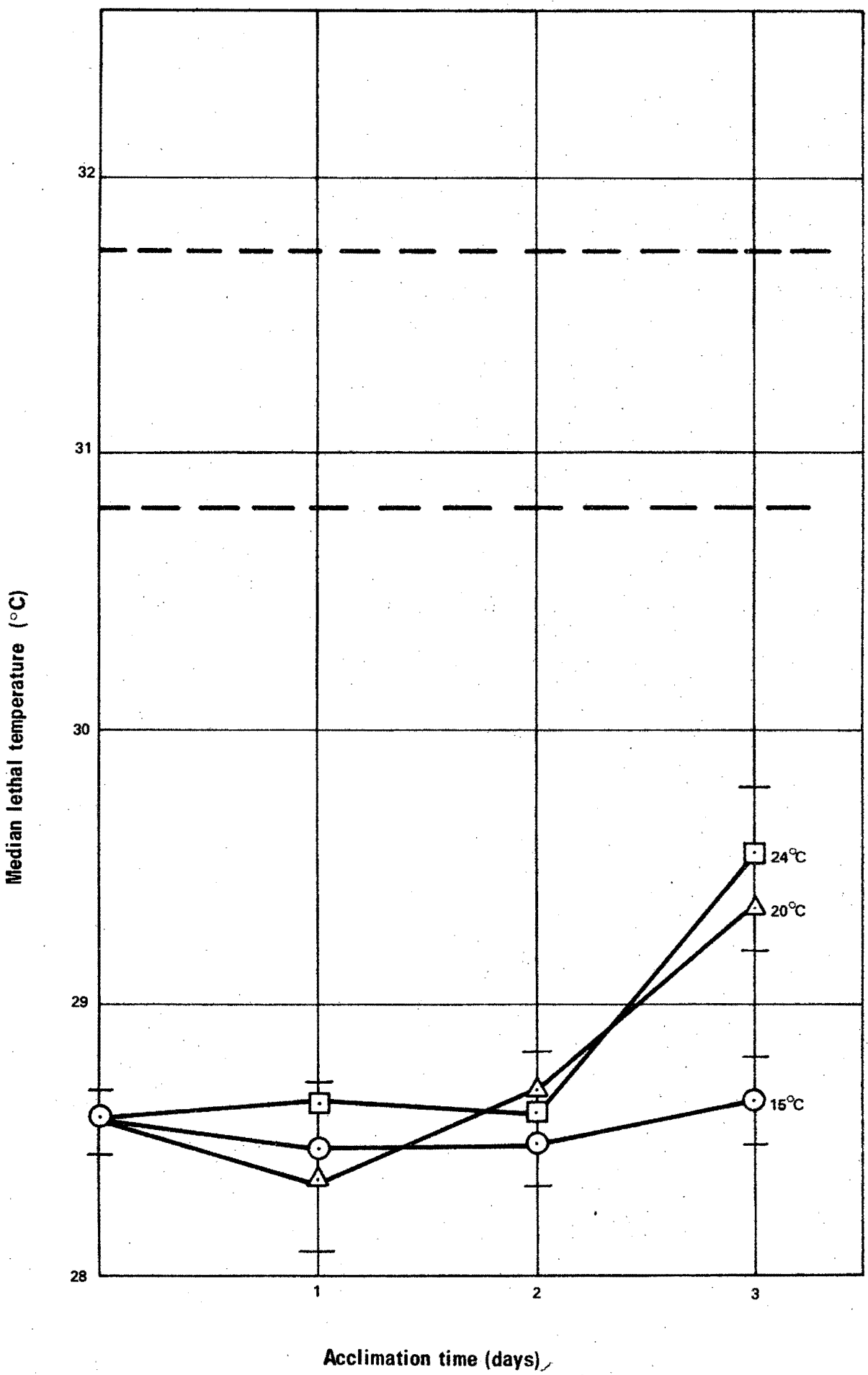
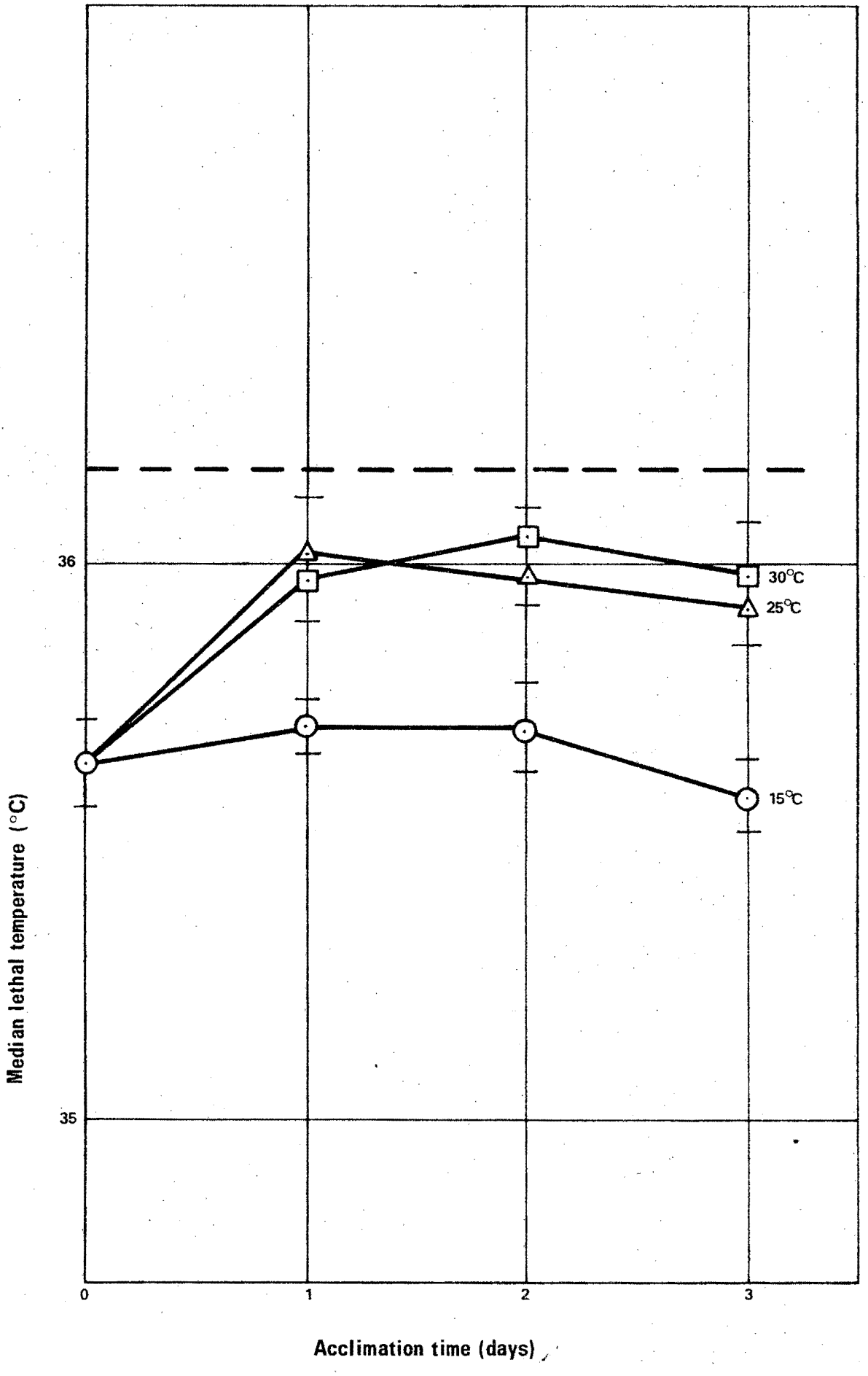

32
31
30
29
28
Median lethal temperature (°C)
24°C
20°C
15°C
1
2
3
Acclimation time (days)

Figure 14

1000-minute median upper lethal temperatures for winter Choroterpes bugandensis nymphs after acclimation in the laboratory at 15°C, 25°C and 30°C for different times. Lethal temperature for summer nymphs shown by broken line.


36
35
30°C
25°C
15°C
0
1
2
3
Median lethal temperature (°C)
Acclimation time (days)

Baetis nymphs held in the laboratory at 20°C and 24°C became increasingly tolerant of high temperatures on succeeding days, whether they attempted ecdysis during the exposure to high temperature or not ($p < 0.01$). However, the lethal temperatures on each day of nymphs held at 20°C and at 24°C did not differ significantly from one another. The high temperature tolerances of nymphs not in ecdysis did not change significantly after being held in the laboratory at 15°C. Nymphs attempting ecdysis appeared to become less tolerant after the second and third days in the laboratory at 15°C ($p < 0.025$).

Choroterpes nymphs held in the laboratory at 25°C and 30°C were found to have become more tolerant of high temperatures than nymphs not held at these temperatures ($p < 0.01$). However, nymphs held at 25°C and at 30°C and nymphs held at these temperatures for different periods of time did not differ significantly in their temperature tolerances. The upper lethal temperatures of nymphs held in the laboratory at 15°C also did not change significantly.

In each of these figures, median lethal temperatures for summer nymphs are indicated by broken lines for comparison. As may be seen, neither species achieved through laboratory acclimation the degree of high temperature tolerance shown by summer nymphs. Baetis harrisoni nymphs after three days in the laboratory were still considerably less tolerant than were summer nymphs ($p \ll 0.001$). However, the summer nymphs with which these are being compared here had been living at temperatures higher than any of the acclimation temperatures at which winter nymphs were held, so that a strict comparison is perhaps not possible.

Numbers of winter nymphs transferred immediately to temperatures of 25°C or higher were found to die, since this is very close to the lethal temperature for winter nymphs. However, the fact that the upper lethal temperatures of Baetis nymphs were found still to increase after two days acclimation might indicate that full acclimation would have taken longer than just a few days. Alternatively, temperature tolerances of these species are influenced by other factors in summer.

ACCLIMATION OF SUMMER NYMPHS

In two similar experiments, summer nymphs of each species were collected during February when water temperatures at times of collection ranged between 20°C and 24°C. Groups of these summer nymphs were first held for a day at 20°C and were then held for 1, 2 and 3 days at different temperatures in the laboratory before being exposed to temperatures in the lethal range.

Median lethal temperatures after acclimation for different times at different temperatures for Baetis harrisoni nymphs in ecdysis are shown in figure 15. Those for Baetis harrisoni nymphs not in ecdysis are shown in figure 16 and those for Choroterpes bugandensis nymphs are shown in figure 17. In all three figures, lethal temperatures for winter nymphs are shown by broken lines, for comparison.

The median upper lethal temperatures, both in and out of ecdysis, of summer Baetis harrisoni nymphs decreased on successive days after being held in the laboratory at 10°C and 15°C. Although the lethal temperatures estimated on successive days were not significantly different from

one another, a rank correlation test (Kendall 1955) revealed their tendency to decline with time to be significant ($p < 0.01$). The lethal temperatures of nymphs that had been held at $10^{o}C$ and $15^{o}C$ were not significantly different from one another but a rank correlation test also confirmed the statistical significance of the observation that the median lethal temperatures of nymphs that had been held at $15^{o}C$ were invariably higher than those of nymphs that had been held at $10^{o}C$ ($p < 0.01$). The changes in upper lethal temperature of nymphs held at $20^{o}C$ were not statistically significant.

It should be noted that the changes in median lethal temperature of Baetis nymphs observed were small, the greatest change being of the order of $0.7^{o}C$. The lethal temperatures of these summer nymphs did not ever drop anywhere near those of winter nymphs.

The median upper lethal temperatures of Choroterpes bugandensis nymphs held at $10^{o}C$, $15^{o}C$ and $20^{o}C$ were very close to one another. No two values were statistically different from one another. However, rank correlation tests suggested that both the observed slight decline in lethal temperatures during the course of the experiment and the observation that lethal temperatures of nymphs that had been held at $15^{o}C$ were always a little higher than those of nymphs that had been held at $10^{o}C$ might have been statistically significant ($p < 0.05$). The changes in upper lethal temperature of nymphs held at $20^{o}C$ were not statistically significant.

The changes in upper lethal temperatures of Choroterpes nymphs were exceedingly small, all within $1^{o}C$. These changes were smaller than the error of individual estimates. The lethal temperatures of these summer nymphs also did not on any occasion fall to within the range of lethal temperatures for winter nymphs.

Figure 15

1000-minute median upper lethal temperatures for summer Baetis harrisoni nymphs attempting ecdysis during exposure to high temperature, after acclimation in the laboratory at $10^{o}C$, $15^{o}C$ and $20^{o}C$ for different times. 95% confidence limits are shown about each median value. Lethal temperatures for winter nymphs shown by broken lines.

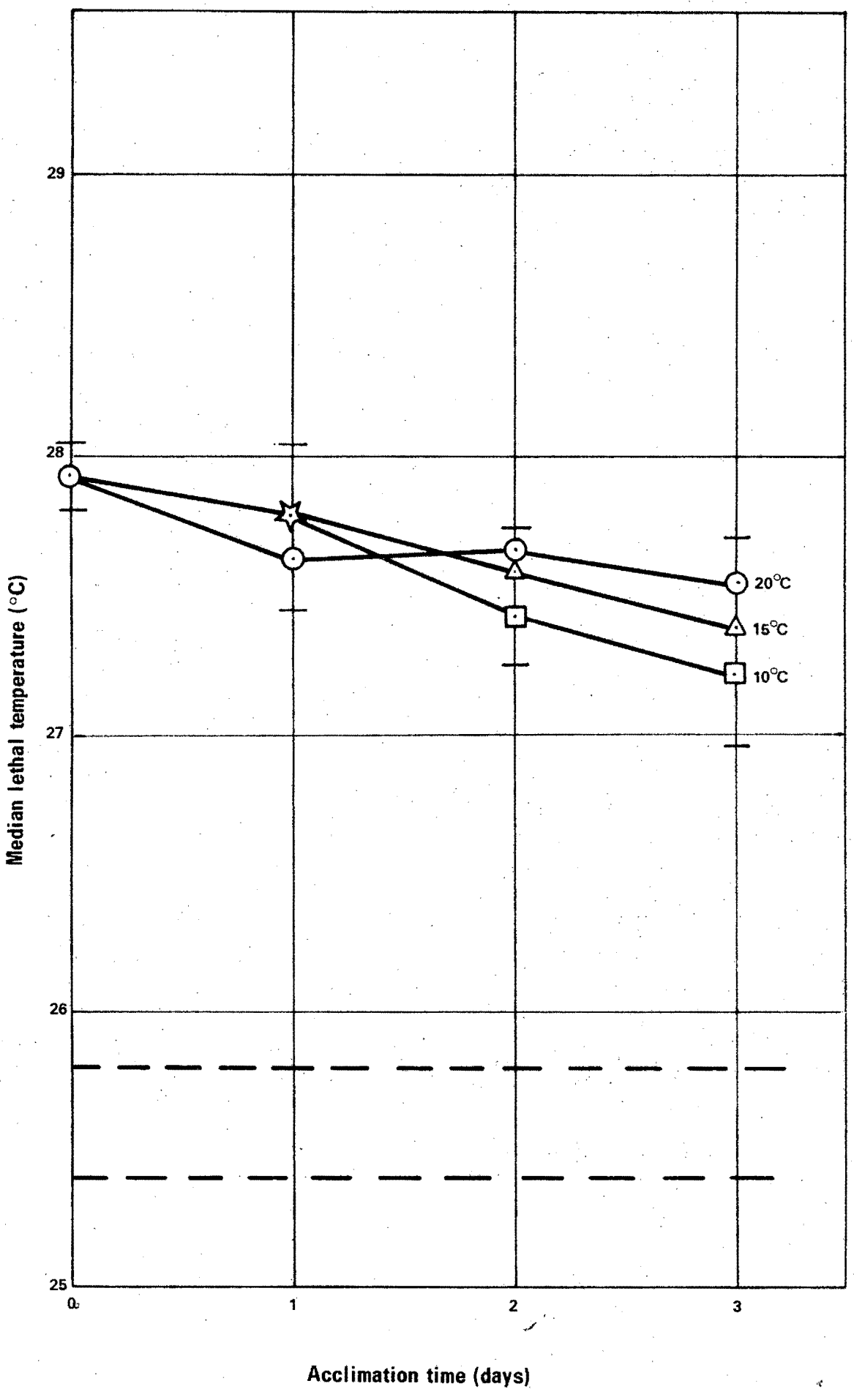

29
28
27
26
25
0
1
2
3
Median lethal temperature (°C)
Acclimation time (days)
20°C
15°C
10°C

Figure 16

1000-minute median upper lethal temperatures for summer Baetis harrisoni nymphs not attempting ecdysis during exposure to high temperature, after acclimation in the laboratory at $10^{o}C$, $15^{o}C$ and $20^{o}C$ for different times. 95% confidence limits are shown about each median value. Lethal temperatures for winter nymphs shown by broken lines.

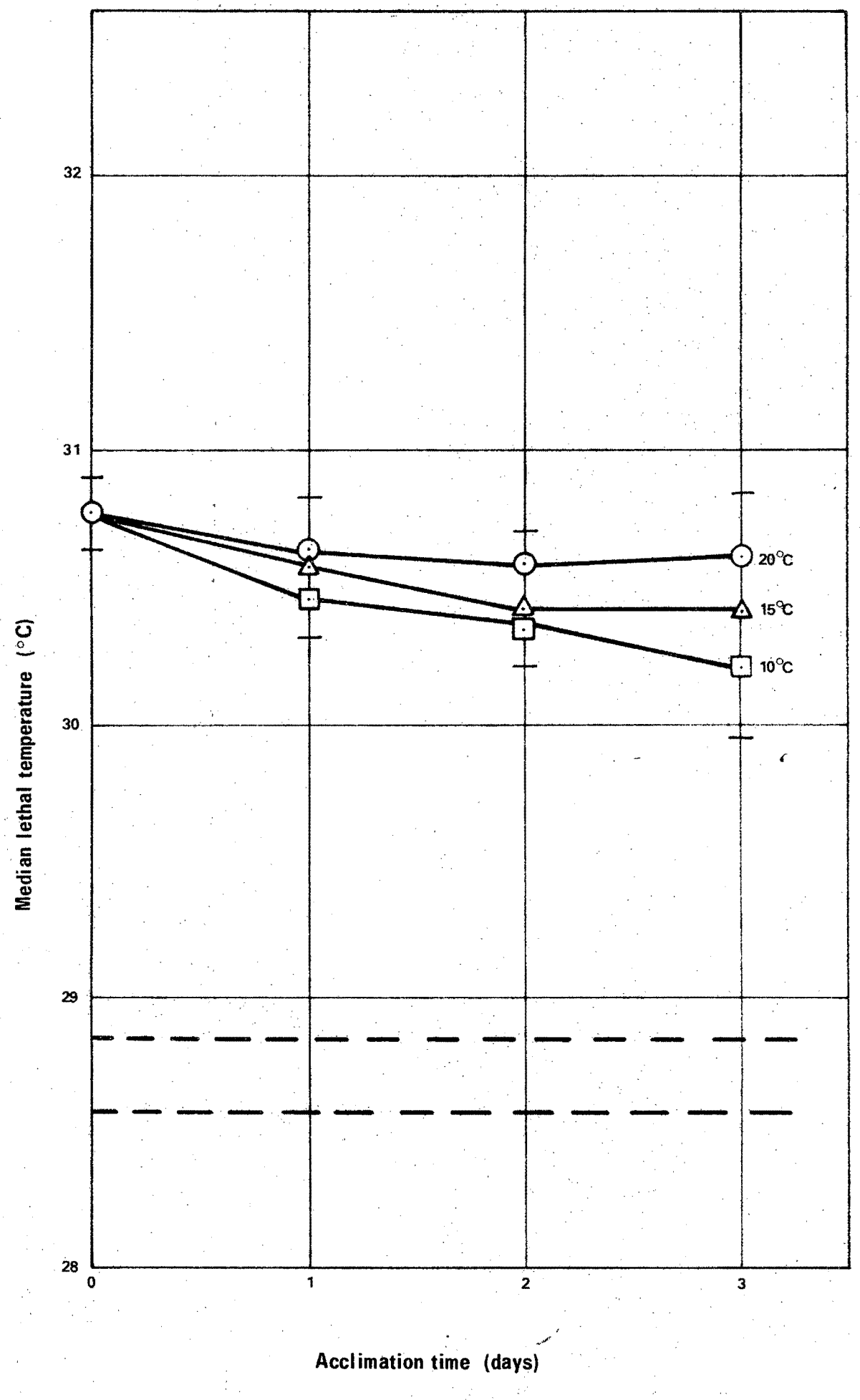
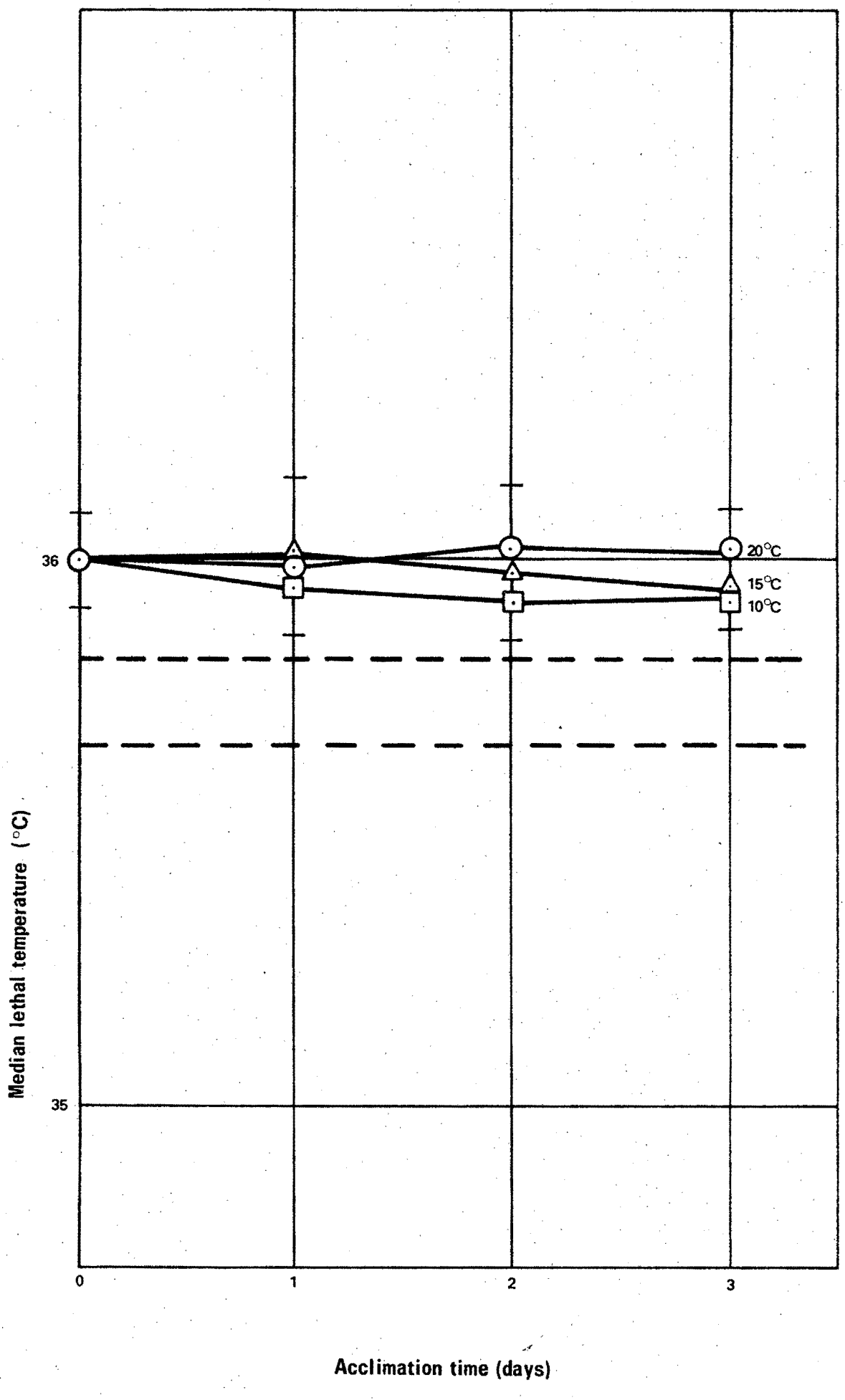

32
31
30
29
28
0
1
2
3
20°C
15°C
10°C
Median lethal temperature (°C)
Acclimation time (days)

Figure 17

1000-minute median upper lethal temperatures for summer Choroterpes bugandensis nymphs after acclimation in the laboratory at $10^{o}C$, $15^{o}C$ and $20^{o}C$ for different times. 95% confidence limits are shown about each median value. Lethal temperatures for winter nymphs shown by broken lines.


36
35
20°C
15°C
10°C
0
1
2
3
Median lethal temperature (°C)
Acclimation time (days)

FIELD OBSERVATIONS

Highest water temperatures in Transvaal rivers occur most frequently during early summer before the first rains (Chutter 1967). Stream flows at this time are generally lowest. On one occasion in October, during a particularly hot dry spell, temperatures in some of the smaller stagnant pools of the Pienaars River near Pretoria were found to rise during the day to levels between 31.6°C and 32.5°C. Numbers of Choroterpes bugandensis nymphs were found in these pools, apparently unaffected by the high temperatures. These nymphs were collected and brought back to the laboratory. After being held overnight at 32.5°C they were exposed to a number of temperatures in the lethal range as was described earlier. The median lethal temperature estimated on this occasion is in fact that shown in table 29.

On another day during the same month a temperature of 28.2°C was recorded in the Braamfontein Spruit near Johannesburg. Numbers of nymphs of Baetis harrisoni were present. This temperature was well above the median lethal temperature found earlier for winter nymphs. Quite large numbers of nymphs were to be found in the stream. Many were seen spontaneously to release their hold on the substratum and to drift short distances downstream, their bodies arched backwards slightly and their legs outstretched. Those that landed in a stagnant corner swam back into the flowing water with three or four jerking body movements. No nymphs were found dead, but a large number were obviously behaving abnormally.

In a stagnant pool cut off from the main stream, the temperature on this day was found to be 29.6°C. Nymphs transferred from the river to this pool all died within 20 minutes. A number of nymphs collected at this

station on this occasion were brought back to the laboratory and held there at 25°C for 24 hours. They were then exposed to lethal temperatures and their median upper lethal temperature estimated. These are the figures given earlier in tables 27 and 28. It seems clear from these observations that temperatures were reached in the Braamfontein Spruit on this occasion which were only very slightly below those which would have killed off the Baetis harrisoni nymphs. This was a rather rare occasion associated with warm weather and very low water flow. Efforts were made during the same period to find other streams within reach of Pretoria in which similar and perhaps even higher temperatures prevailed. The highest temperature measured during this period in any other stream in which Baetis harrisoni were found was 26.8°C. However, temperatures higher than 30°C were measured in several slow-flowing streams where Baetis harrisoni nymphs could not be found.

SUMMARY

1. Summer nymphs of both species were more tolerant of high temperatures than were winter nymphs. Median upper lethal temperatures for Baetis nymphs ranged from 27°C to 29.3°C in ecdysis and from 30.2°C to 31.2°C out of ecdysis in summer, from 25.4°C to 25.8°C in ecdysis and from 28.6°C to 28.9°C out of ecdysis in winter. Those for Choroterpes nymphs ranged from 35.9°C to 36.2°C in summer and from 35.6°C to 35.8°C in winter.
2. Median upper lethal temperatures determined using nymphs collected during a heat wave (29.3°C in ecdysis and 31.7°C out of ecdysis for Baetis, 36.2°C for Choroterpes) are assumed to approximate the

ultimate upper lethal limits for these species.

3. Upper lethal temperatures for winter Baetis nymphs rose about 1°C and those of Choroterpes rose about 0.3°C when they were held in the laboratory at higher temperatures than those at which they had been living in the field.

4. Upper lethal temperatures of summer Baetis nymphs fell about 0.4°C to 0.6°C and those of Choroterpes nymphs fell about 0.05°C (if at all) when they were held in the laboratory at temperatures lower than those at which they had been living in the field.

5. Very high water temperatures (up to 32.5°C) were observed in the Pienaars River during a period of hot dry weather. These high temperatures had no apparent affect on the Choroterpes nymphs. They were considerably higher than the lethal temperature for Baetis nymphs.

6. Baetis nymphs were found on one occasion in the Braamfontein Spruit at temperatures at which they were barely able to survive (up to 29.6°C). These temperatures would have killed winter nymphs.

MORTALITY AND SURVIVAL OF LAST INSTAR NYMPHS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS AT HIGH TEMPERATURES

INTRODUCTION

Results presented earlier have shown that nymphs of Baetis harrisoni were far more sensitive to high temperatures during ecdysis than they were at other times. Nymphs of Choroterpes bugandensis, on the other hand, appeared to be equally tolerant at all times. Last instar nymphs were not included in this earlier study, but for both species larger nymphs were invariably found to be less tolerant than were smaller nymphs. Since Ide (1935) found some Ephemeroptera could not be bred out at higher temperatures and since both Macan (1960) and Pleskot (1962) have described mayfly nymphs to be least tolerant of high temperatures during their last instar and final moult, observations were made here of mortality and survival of last instar Baetis harrisoni and Choroterpes bugandensis nymphs at high temperatures.

Both Lyman (1944) and Macan (1963) have pointed out that most Ephemeroptera undergo their final nymphal moult and emerge as flying subimagines at night. They point out that this might protect at least certain species from exposure of their last instar nymphs to maximal diurnal temperatures. Pleskot (1958) has also shown that Baetis rhodani emergence is stopped by heat. She does not suggest how this occurs, but it is known that heat can delay ecdysis in certain insects (Okasha 1968).

PATTERNS OF EMERGENCE

Tjønneland (1960) has shown that subimagines of Choroterpes bugandensis emerge during the early evening and that the subimaginal moult takes

place fairly shortly after sunrise the next morning. This pattern of emergence was also shown by Choroterpes bugandensis both in the Pienaars River and in the laboratory during the present study.

Less is known of patterns of emergence of Baetis harrisoni. Crass (1947) reported that emergence of this species took place during the day. During the present study both in the Braamfontein Spruit and in the laboratory, subimagines were observed to emerge during early and mid-morning. On one occasion, emergence was even observed during the afternoon. Most, however, emerged during the early morning.

MATERIAL AND METHODS

Last instar nymphs of Baetis harrisoni and Choroterpes (Euthraulus) bugandensis were collected and transported to the laboratory in the same way that other nymphs were brought in for experimantation. These nymphs were exposed to high temperatures in shallow trays made out of nylon gauze and suspended in tanks which were held at constant temperature. A slow flow of water was supplied into each tray from the outlet pipe of the thermostat-heater. Chlorella or Scenedesmus cells were provided as food. Satisfactory conditions for the maintenance of these nymphs appeared to be provided in these trays. If left there long enough they emerged, usually without difficulty.

Relatively fewer last instar nymphs of Baetis harrisoni were found to die at normal temperatures than were smaller nymphs. Possibly the last instar nymphs were easier to handle because of their size. As before, the median lethal temperature calculations were compensated and balanced for

"natural" mortality observed at normal temperatures (Finney 1952).

LAST INSTAR NYMPHS IN CLOSED TUBES

All last instar nymphs held in the experimental tubes in which smaller nymphs were exposed to high temperatures died if kept long enough in the tube. All the Baetis harrisoni that died did so after the onset of ecdysis. Nymphs starting ecdysis became quiescent and stayed motionless for very long periods of time. These nymphs all had black wing buds. The Baetis that died during the experiment showed the same signs of ecdysis described earlier for younger nymphs dying at high temperatures. They had loose outer skins and this outer skin had often split. The Choroterpes last instar nymphs also died in the tubes but did not show these symptoms. Most of those that died did so before the nymphal skin had split between the wing buds.

MORTALITY IN OPEN TRAYS

The experiments in which Baetis harrisoni and Choroterpes bugandensis final instar nymphs were exposed to lethal temperatures in open trays were carried out over a 1000 minute period of time, during which all the nymphs either emerged or died. Food was provided but the animals were not observed to feed. The lethal temperatures calculated from observations made in these experiments are summarized in table 30.

These experiments were carried out in August at a time when stream temperatures fluctuated fairly widely between 14°C and 18°C. The median upper lethal temperatures for final instar Baetis harrisoni and Choroterpes bugandensis nymphs shown in table 30 were lower than were those of

smaller nymphs tested during the same month (tables 21 and 23 of the previous section). This difference was about 2°C for Choroterpes and was significant. For Baetis the difference was only 0.2°C and was not statistically significant, especially since the tests were carried out on different days of this month.

TABLE 30

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES FOR FINAL INSTAR BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

Mayfly	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
Baetis	65	24.5 25.0 25.5 26.0	25.2 (24.8 to 25.6)
Choroterpes	52	33.0 33.5 34.0 35.0	33.6 (33.3 to 33.9)

RATE OF EMERGENCE

Simultaneously with the experiments in which last instar nymphs were exposed to lethal temperatures, parallel groups selected at random for each species were held at 20°C. Times to emergence both of these nymphs and of the nymphs at high temperatures were observed. Relative numbers of Baetis harrisoni nymphs found to have emerged in the laboratory after different times at 20°C and at lethal temperatures, as well as the relative numbers of Choroterpes bugandensis emerging on each of the three successive

nights at different temperatures, are shown in table 31. None of these observations appear to suggest that ecdysis of either species might be delayed or stopped at very high temperature.

TABLE 31

EMERGENCE OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS IN THE LABORATORY AT DIFFERENT TEMPERATURES

Mayfly	Temperature (°C)	Daily emergence (per cent of total)		
		first day	second day	third day
Baetis	20.0	71	29	0
	24.5	82	18	0
	25.0	91	9	0
	25.5	78	22	0
	26.0	80	20	0
Choroterpes	20.0	62	26	12
	33.0	59	30	11
	33.5	70	16	14
	34.0	62	35	3
	34.5	61	21	18

SUMMARY

1. Upper lethal temperatures of last instar Choroterpes nymphs were about 2°C lower than those of smaller nymphs at the same time of year. Upper lethal temperatures of last instar Baetis nymphs were not significantly lower than those of smaller nymphs.

2. Ecdysis and emergence did not appear to be delayed in either species at increased temperature.

EFFECTS OF LOW TEMPERATURES ON NYMPHS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS

INTRODUCTION

Low temperatures have been widely considered important in limiting the range of freshwater animals, Jubb (1962), Poynton (1962) and Harrison (1965a) believe the southerly distribution of certain fish, frogs and riverine invertebrates which also occur in tropical Africa to be limited in South Africa by low winter temperatures. Widespread mortalities of Tilapia mossambica, and sometimes of other fish as well, occur quite commonly during cold spells in parts of South Africa (Jubb 1962, Ernst 1965).

Observations are reported here of chill coma and mortality at low temperatures of nymphs of two common South African mayflies, Baetis harrisoni and Choroterpes bugandensis. This investigation was not prompted by any previous evidence that these species might from time to time be exposed to lethal temperatures. However, previous work had indicated that one species, Baetis harrisoni, might occasionally be exposed to lethal high temperatures. Observations of the effects of low temperatures on these species were then made for completeness.

Salt (1961a, b) and Kinne (1963) list a large number of fish and other aquatic animals of relatively warm climates which are known to become comatose at water temperatures greater than $0^{o}C$, especially if they have been living at fairly high temperatures (Mellanby 1958). A number of different poikilothermal animals are subject to chill coma at low temperatures. Symptoms in insects (for instance Payne 1926) and fish (for instance Doudoroff

1942, 1945) are similar and appear to indicate that it is the central nervous system which is primarly affected (Stroganov 1962). Animals in chill coma lose equilibrium completely, lie motionless and after a while no longer respond to any stimuli.

In fish, Doudoroff and others have distinguished two forms of chill coma. Primary chill coma occurs soon after transfer to low temperature. Some of the fish succumbing to primary coma recover after a while at the same temperature. Secondary coma occurs much later and fish succumbing to it do not recover unless transferred to warmer water. Pitkow (1960) found that recovery from primary chill coma was facilitated by raising the dissolved oxygen content of the water. He reasoned that this overcame the shortage of oxygen supply to the brain caused by cessation of opercular movements. Increased oxygen did not make these fish either less susceptible to secondary coma or less liable to die while in secondary coma.

The causes of cold death of poikilothermic animals are obscure (Payne 1927). Death of animals in secondary coma apparently depends on the time spent in chill coma (Stroganov 1962). Referring to insects, particularly terrestrial ones, Bursell (1964) has suggested that cold death might result from some metabolic upset. Doudoroff found that the marine fish he worked on were more resistant to secondary chill coma when they were in isotonic mixohaline water. Wikgren (1953) found fish to lose salts at low temperatures. Houston (1962) and Ernst (1965) have both found freshwater fish to gain water and become bloated at low temperatures. This process started before their fish entered secondary chill coma and continued even after death. Dilution of the blood seemed a likely cause of death

in these fish. It also seems likely, as Eliassen et al (1960) have pointed out, that osmoregulatory failure in fish at low temperatures might result from metabolic depression caused by the cold.

Nymphs of Baetis harrisoni and Choroterpes bugandensis were exposed to low temperatures in waters of different salt content in the present study in order to see to what extent osmoregulatory effects might affect their cold tolerances.

Cold tolerances of different animals have been found to be altered by acclimation to very different extents. Some insects held in the laboratory at relatively cooler temperatures have been found to become much less susceptible to chill coma (Mellanby 1958, Colhoun 1960) and to survive colder temperatures (Mellanby 1960). Other insects and invertebrates, on the other hand, have not been found to be affected by laboratory holding temperatures (Payne 1926, Scholander et al 1953). Brown (1929) found Cladocera collected at different times of the year to differ greatly in their temperature tolerances but found no evidence of acclimation of these animals in the laboratory. Rates of acclimation to decreased temperature have also been found to differ greatly. Doudoroff (1942) found that it took at least 20 days for a fish to become fully acclimated to a decrease in temperature, while Mellanby (1939) found an insect to require only 1 to 3 days for full acclimation to take place.

Acclimation to changed temperature conditions is known to involve compensatory changes in metabolic rates. Sayle (1928) found the oxygen uptake rates of dragonfly nymphs transferred to warmer and to cooler water, respectively, to increase and to decrease. After a while they then both

returned to the same intermediate value. Presumably for this reason, Dehnel and Segal (1956) found the metabolic rates at low temperatures of cold-acclimated insects to exceed those of warm-acclimated ones.

Rao (1962) has shown that several different processes take place during cold acclimation. Some of these changes involve the nervous system (Prosser 1966) and changes in metabolic rate appear to be associated with a number of different biochemical changes (Serfaty and Laffont 1965, Rao 1966a, b).

MATERIAL AND METHODS

Nymphs of Baetis harrisoni and Choroterpes bugandensis were collected and brought in to the laboratory as has been described elsewhere. Random groups of nymphs were held in the laboratory for 24 hours at selected temperatures and were then transferred to experimental tubes and exposed as described in the relevant sections below to selected low temperatures in the lethal range. The same apparatus was used in these experiments as was described for observing the effects of fast water flow on nymphs. In all of the tests described here a water current speed of 10 cm/sec was maintained through the experimental tubes. Experimental tubes of 1.6 cm internal diameter were used throughout. The water flow at this speed through these tubes was therefore laminar (Re = 1600).

BEHAVIOUR AT LOW TEMPERATURES

In one series of experiments carried out both in summer and in winter, numbers of nymphs were held in the laboratory for 24 hours at 20°C and at 10°C. These were temperatures which were of the same order as

those in which they had been living in the field on these two occasions. In each case, random groups of these nymphs were placed in experimental tubes, still either at 20°C or at 10°C. Each tube was then transferred directly to one of a series of experimental tanks at a pre-set low temperature in the lethal range and immediately coupled to the impeller which then drove a current of water through the tubes.

When exposed to low temperatures in this way, the nymphs either became quiescent or entered a state of chill coma similar to that described by Colhoun (1960). In this latter state they lost equilibrium, released their hold on the substratum and drifted with the current. During the succeeding three hours or so, many of these comatose nymphs were observed to recover, to re-establish their hold on the substratum and to remain in a quiescent state until the end of the experiment. None of the individuals which recovered were observed again to enter coma. Some comatose nymphs transferred to water at 15°C or 20°C instantly recovered. Others did not and died. The sequence of these events observed at different low temperatures is illustrated in figures 18 and 19 for summer and winter Baetis harrisoni nymphs and figures 20 and 21 for summer and winter Choroterpes bugandensis nymphs.

In another similar series of experiments also carried out in summer and in winter, nymphs in their experimental tubes were again transferred to the experimental tanks at the same temperature as that at which they had been held. The temperature in each experimental tank was then lowered over six hours to a pre-selected test temperature. These animals reacted to low temperature in much the same way as had those directly transferred to cold water. Their reactions are shown in figures 22 and 23 for Baetis

harrisoni and figures 24 and 25 for Choroterpes bugandensis.

In each of these experiments, more nymphs were seen to be comatose during the first four hours of exposure to cold water after direct transfer from warmer water than after gradual decrease in temperature. The shock of direct transfer evidently caused a greater number of nymphs to enter coma. From four hours after the beginning of exposure to low temperature, on the other hand, similar numbers of nymphs directly transferred and subjected to gradual temperature decrease were found to be comatose.

CHILL COMA TEMPERATURES

From the numbers of nymphs recorded in figures 18 to 25 to have been comatose after different exposure times, median effective temperatures were estimated by probit analysis (Finney 1952). These were the temperatures at which 50 per cent of nymphs would have been expected to be comatose after each exposure time considered. Median effective temperatures estimated in this way for Baetis harrisoni are shown in figure 26, while those for Choroterpes bugandensis are shown in figure 27.

Baetis harrisoni nymphs are revealed in thses results to have been more tolerant of low temperatures throughout than were Choroterpes bugandensis nymphs. Winter nymphs of both species were significantly more tolerant than were summer nymphs. Almost no shock effect was evident in the case of winter nymphs, presumably because the difference between holding and test temperatures was small. In all cases shock only appeared to affect results within the first four hours.

Figure 18

Numbers of summer Baetis harrisoni nymphs comatose (expressed per cent) at intervals of time after direct transfer to low temperature. Stars represent superimposed points.

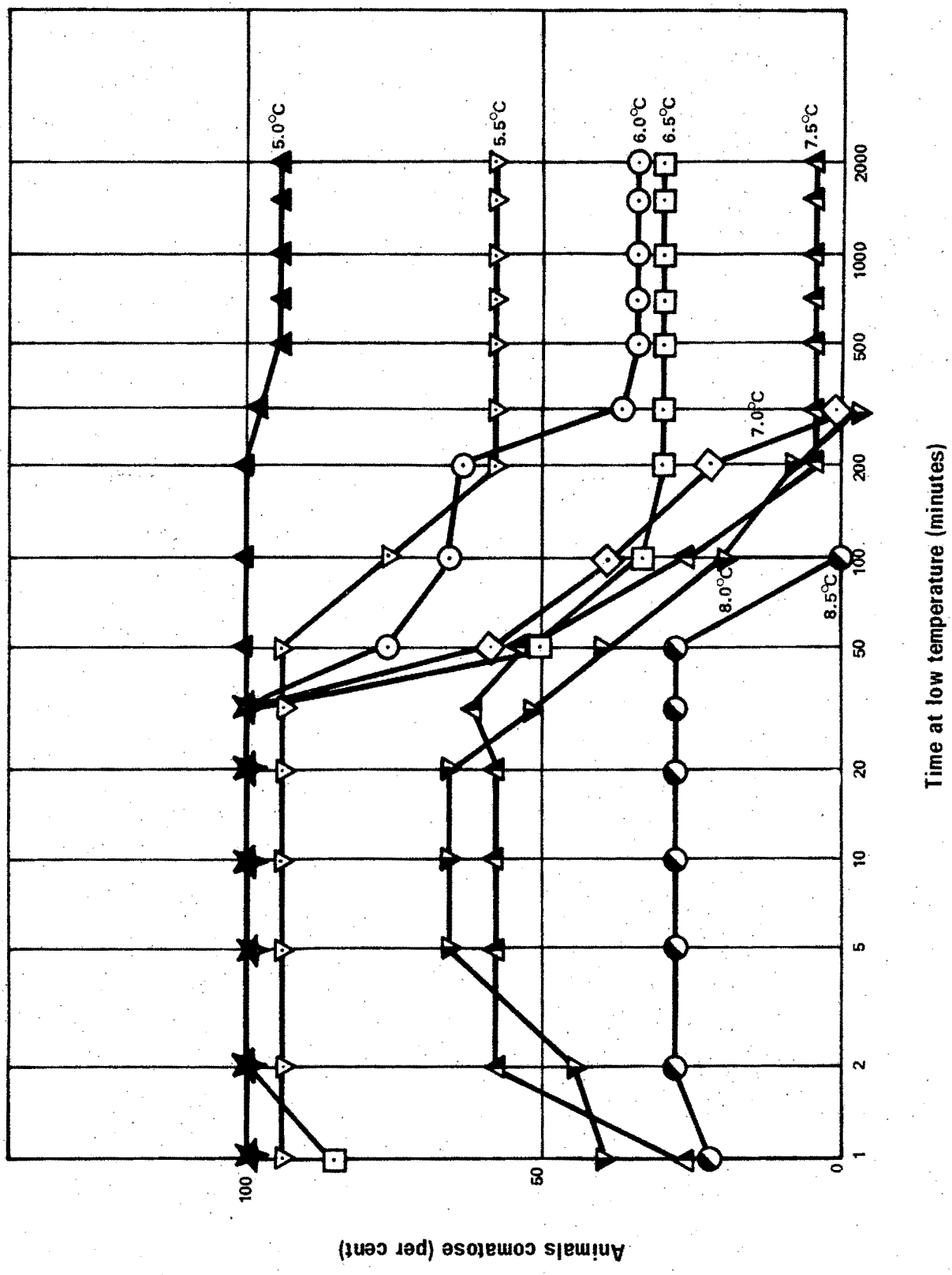

5.0°C
5.5°C
6.0°C
6.5°C
7.5°C
7.0°C
8.0°C
8.5°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 19

Numbers of winter Baetis harrisoni nymphs comatose (expressed per cent) at intervals of time after direct transfer to low temperature.

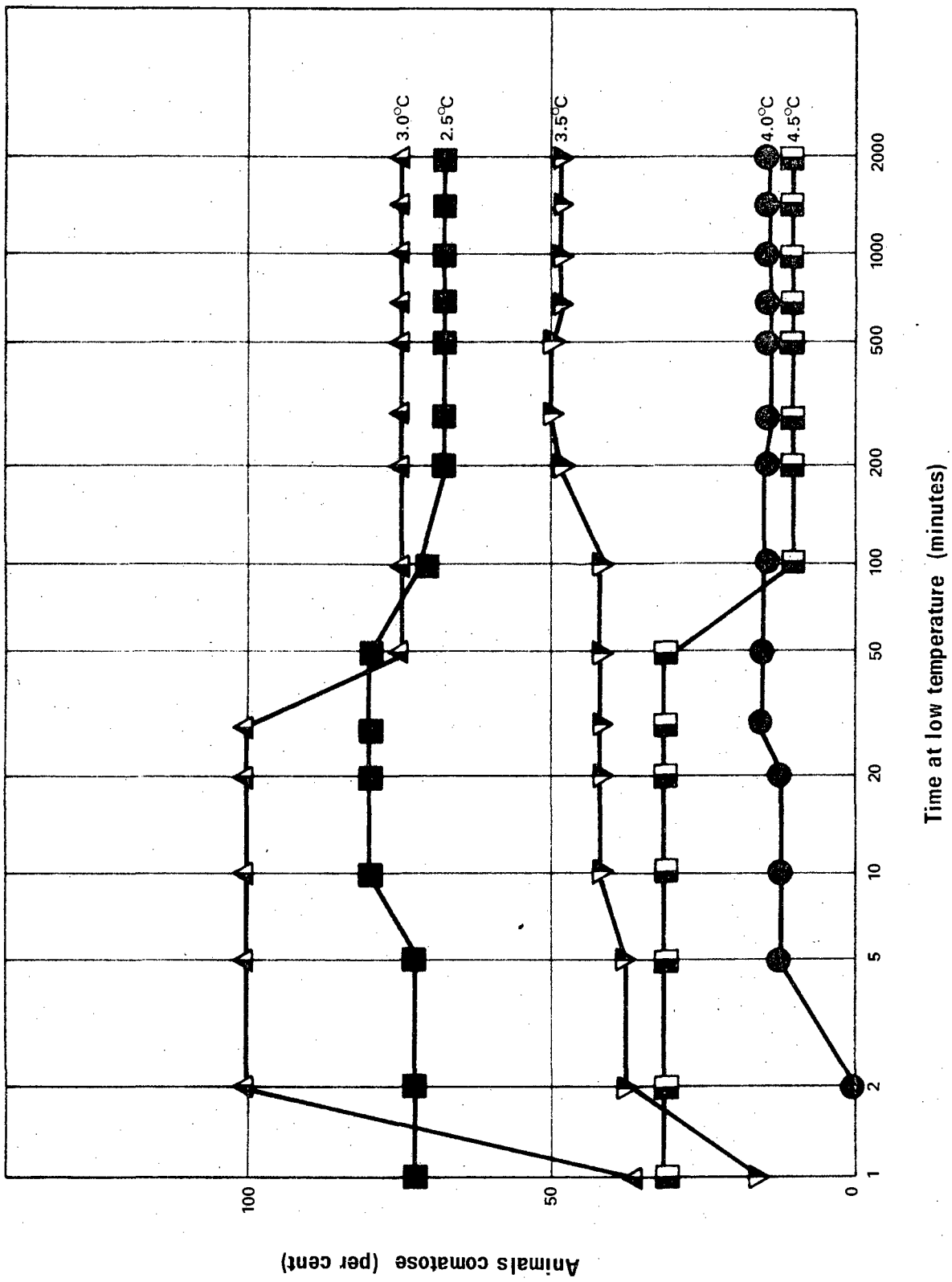

3.0°C
2.5°C
3.5°C
4.0°C
4.5°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 20

Numbers of summer Choroterpes bugandensis nymphs comatose (expressed per cent) at intervals of time after direct transfer to low temperature.

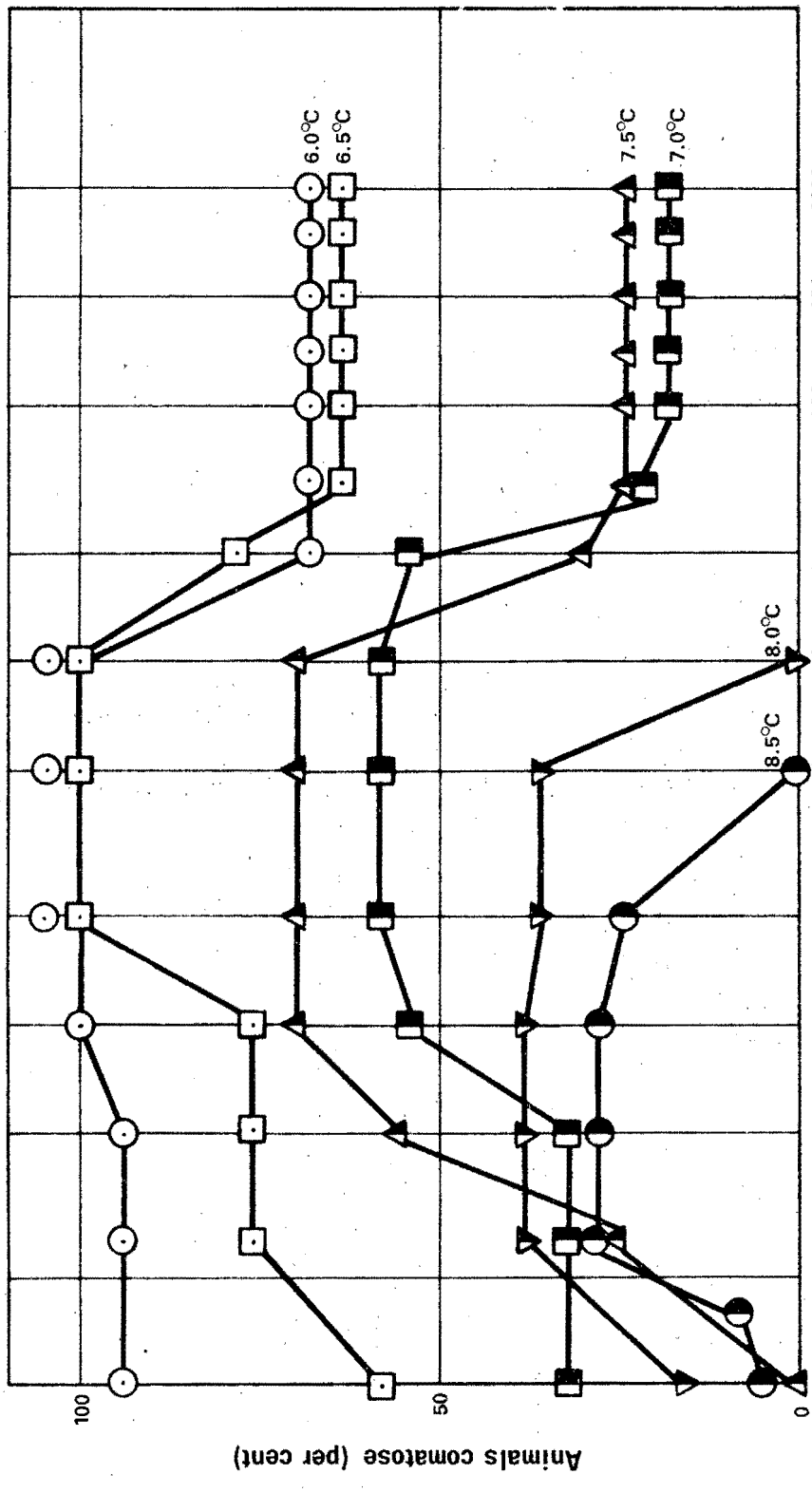

6.0°C
6.5°C
7.5°C
7.0°C
8.0°C
8.5°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 21

Numbers of winter Choroterpes bugandensis nymphs comatose (expressed per cent) at intervals of time after direct transfer to low temperature.

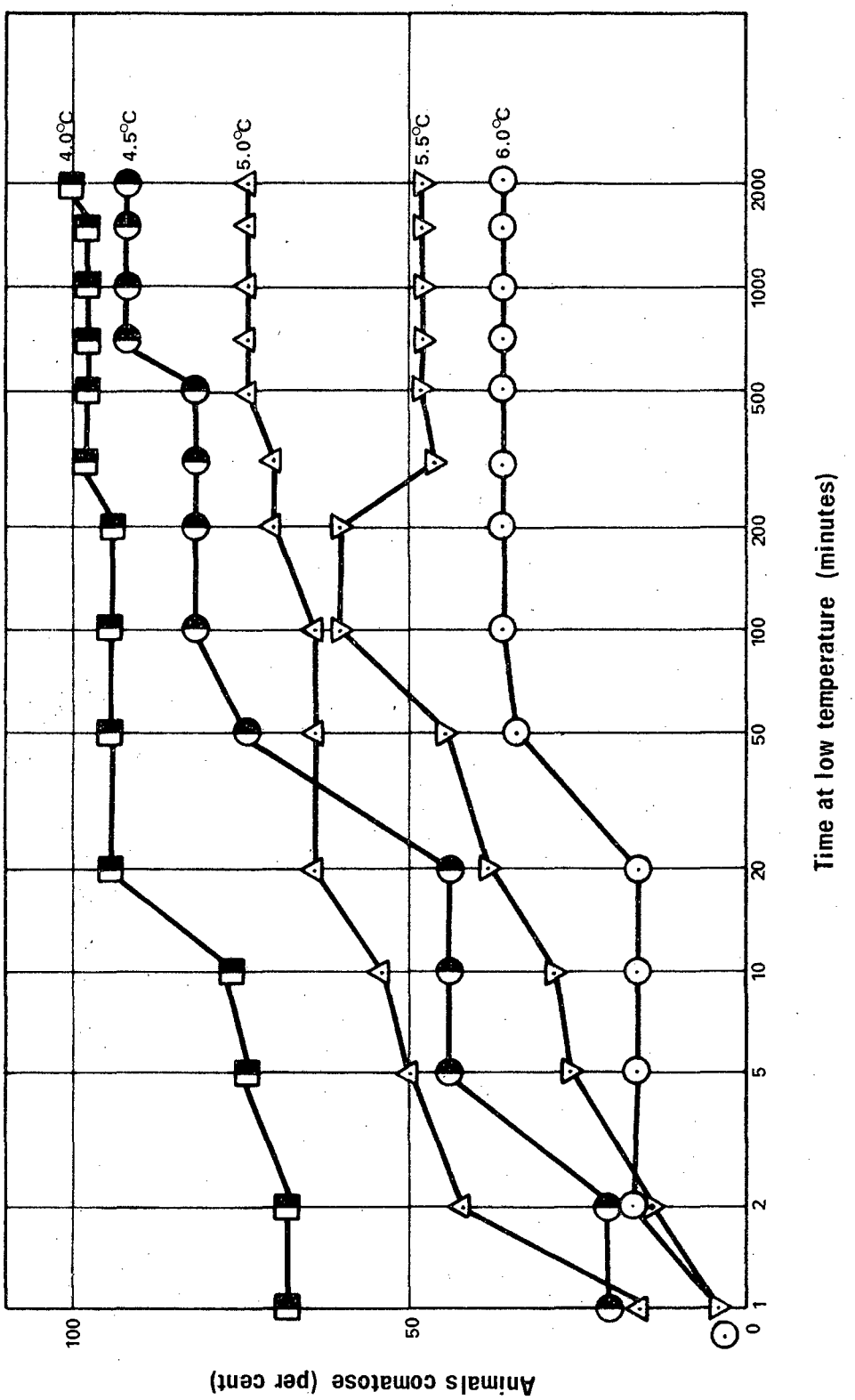

4.0°C
4.5°C
5.0°C
5.5°C
6.0°C
100
50
0
Animals comatose (per cent)
0
1
2
5
10
20
50
100
200
500
1000
2000
Time at low temperature (minutes)

Figure 22

Numbers of summer Baetis harrisoni nymphs comatose (expressed per cent) at intervals of time after drop in temperature over six hours. Stars represent superimposed points.

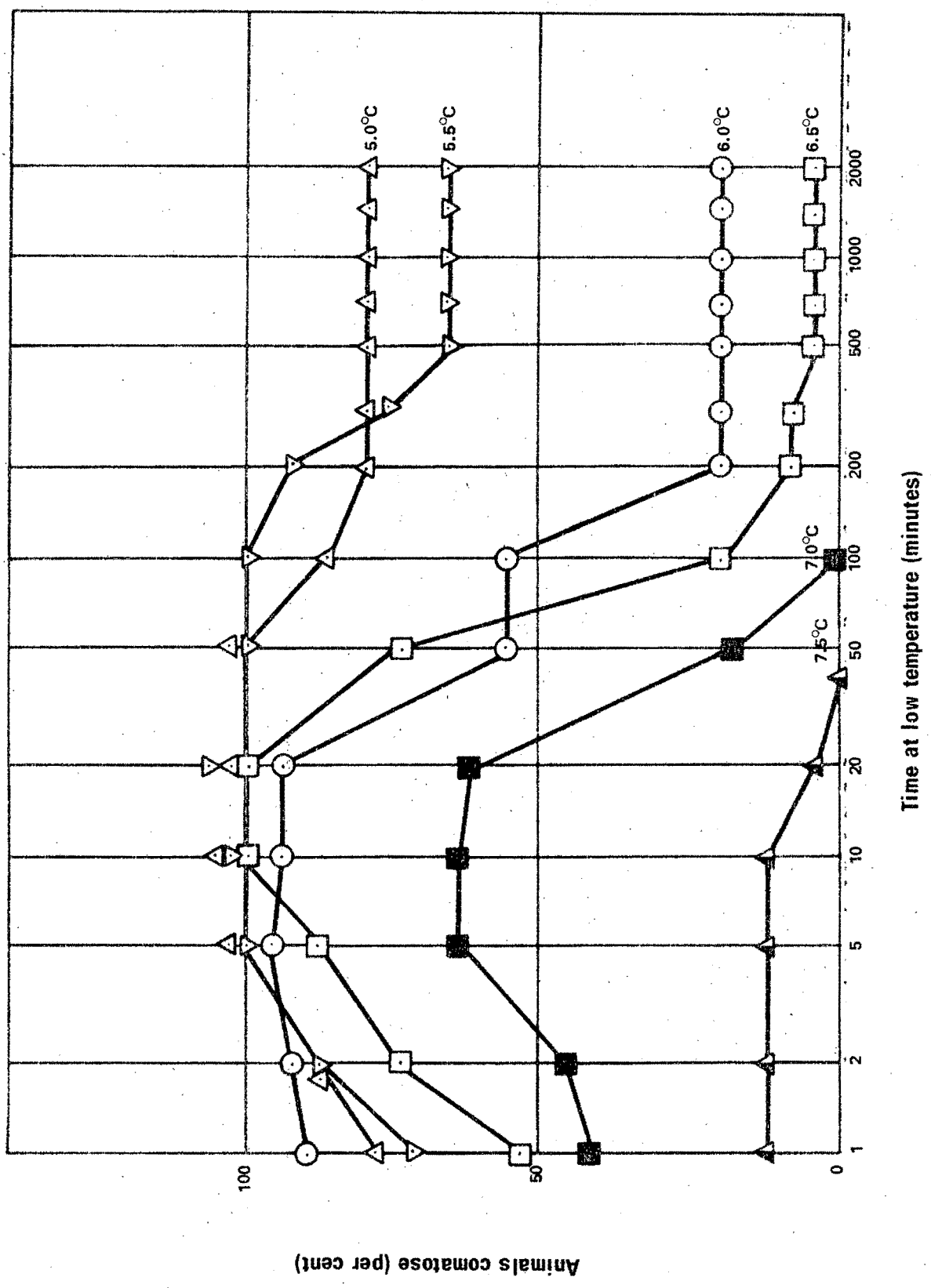

5.0°C
5.5°C
6.0°C
6.5°C
7.0°C
7.5°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 23

Numbers of winter Baetis harrisoni nymphs comatose (expressed per cent) at intervals of time after drop in temperature over six hours.

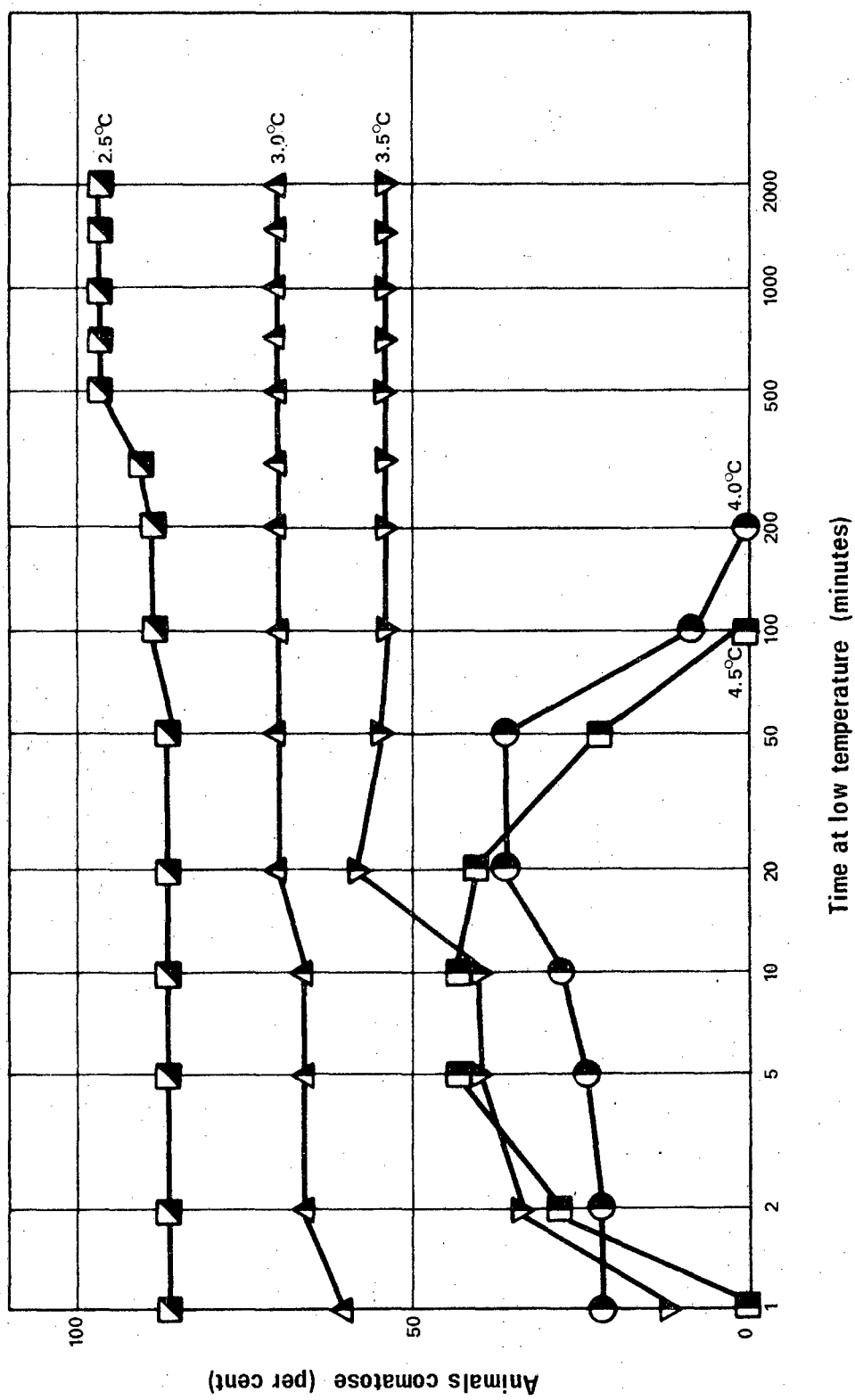
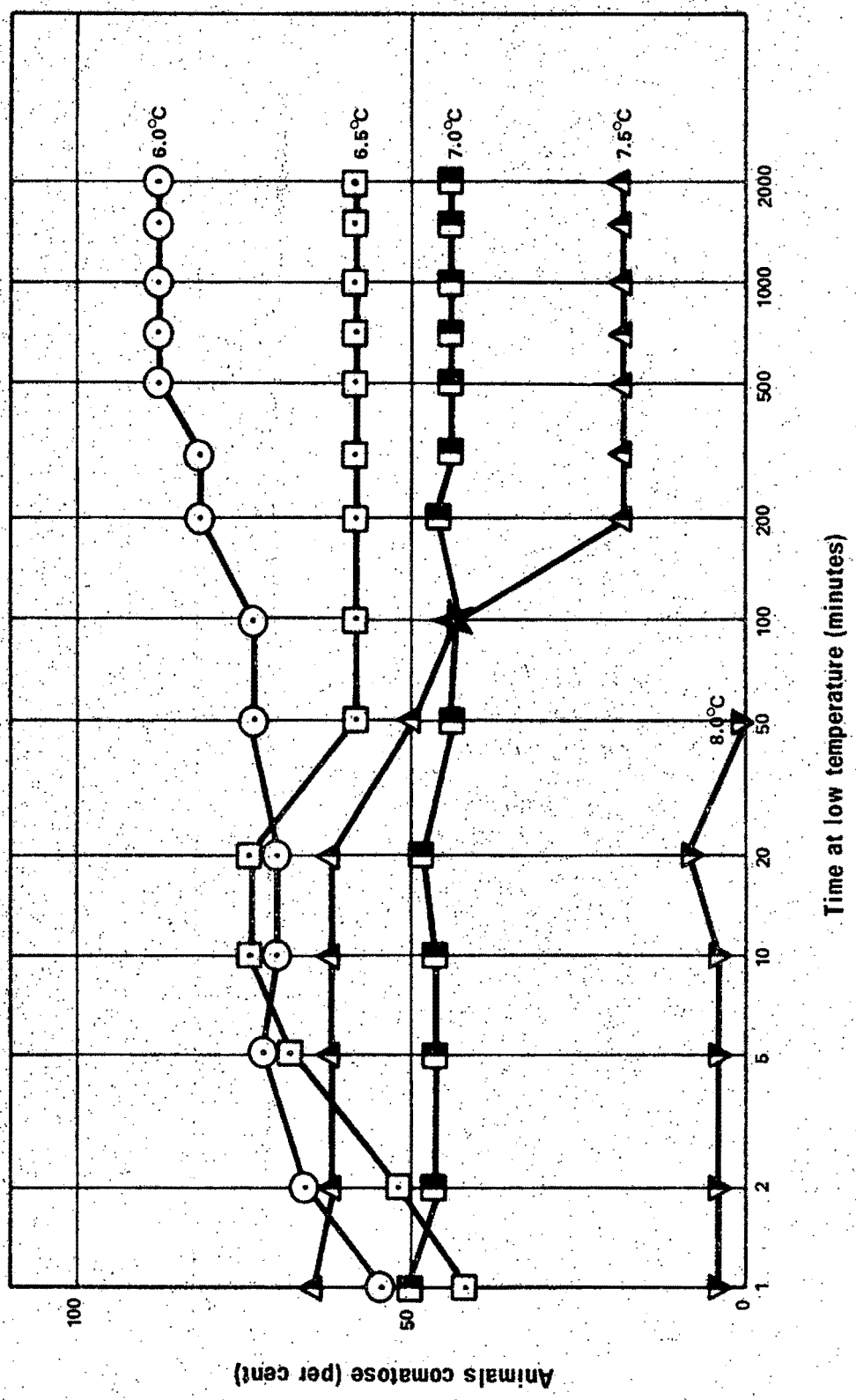

2.5°C
3.0°C
3.5°C
4.0°C
4.5°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 24

Numbers of summer Choroterpes bugandensis nymphs comatose (expressed per cent) at intervals of time after drop in temperature over six hours.


6.0°C
6.5°C
7.0°C
7.5°C
8.0°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

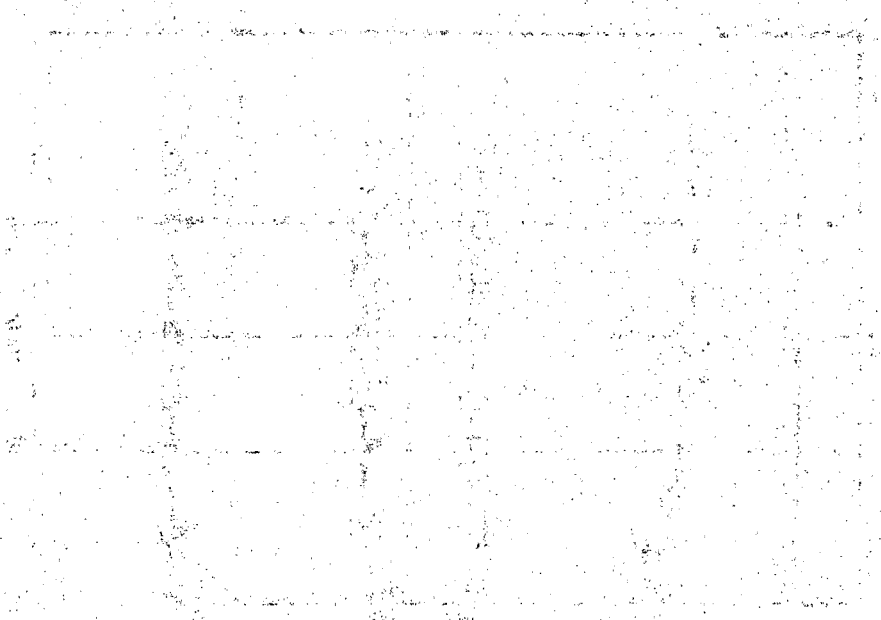


Figure 25

Numbers of winter Choroterpes bugandensis nymphs comatose (expressed per cent) at intervals of time after drop in temperature over six hours.

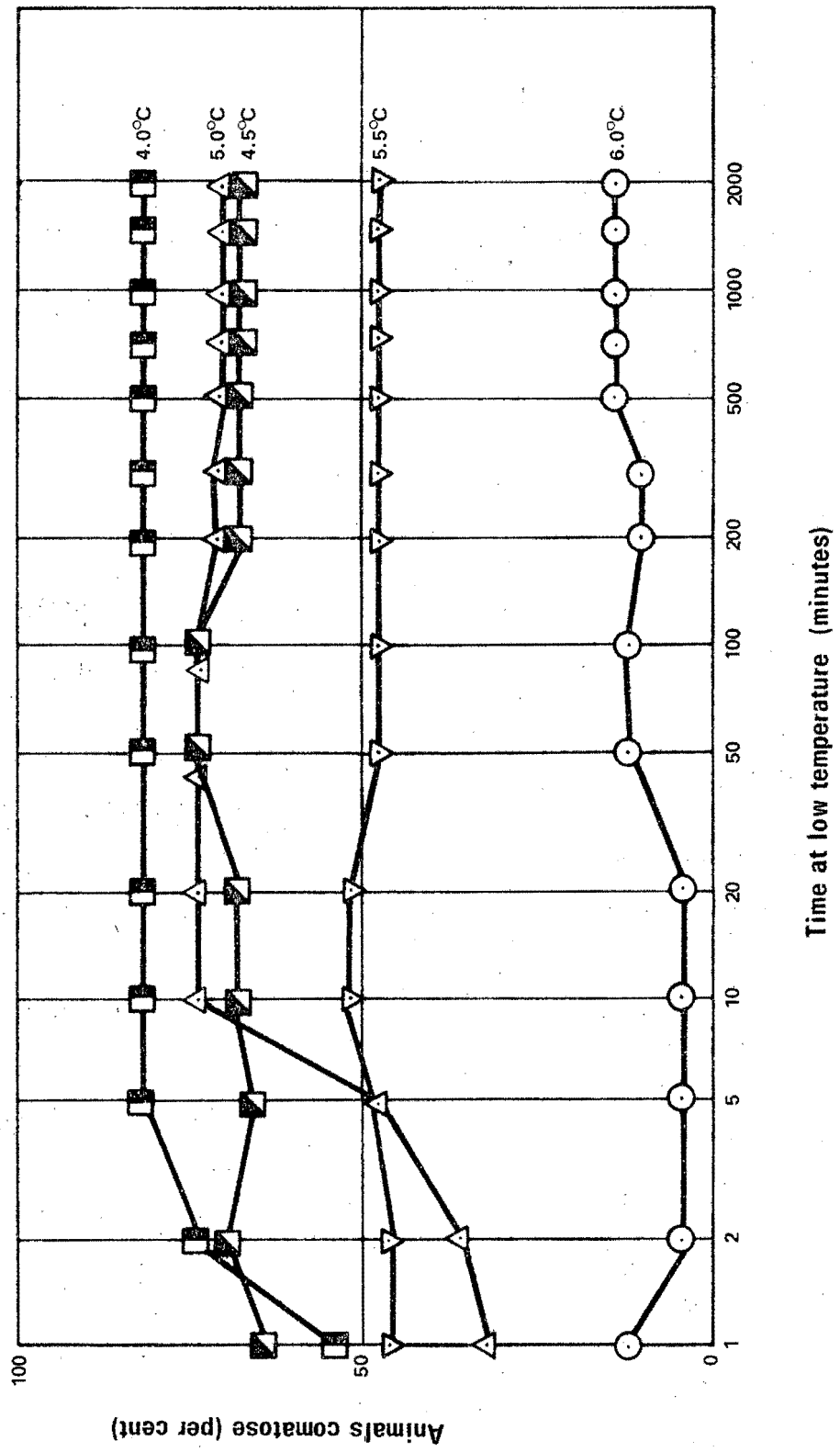

4.0°C
5.0°C
4.5°C
5.5°C
6.0°C
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Animals comatose (per cent)
Time at low temperature (minutes)

Figure 26

Median chill coma temperatures for Baetis harrisoni nymphs, calculated for different times of exposure to low temperature as follows:

open triangles - summer nymphs transferred directly to low temperature

open circles - summer nymphs, temperature decreased over six hours

closed triangles - winter nymphs transferred directly to low temperature

closed circles - winter nymphs, temperature decreased over six hours

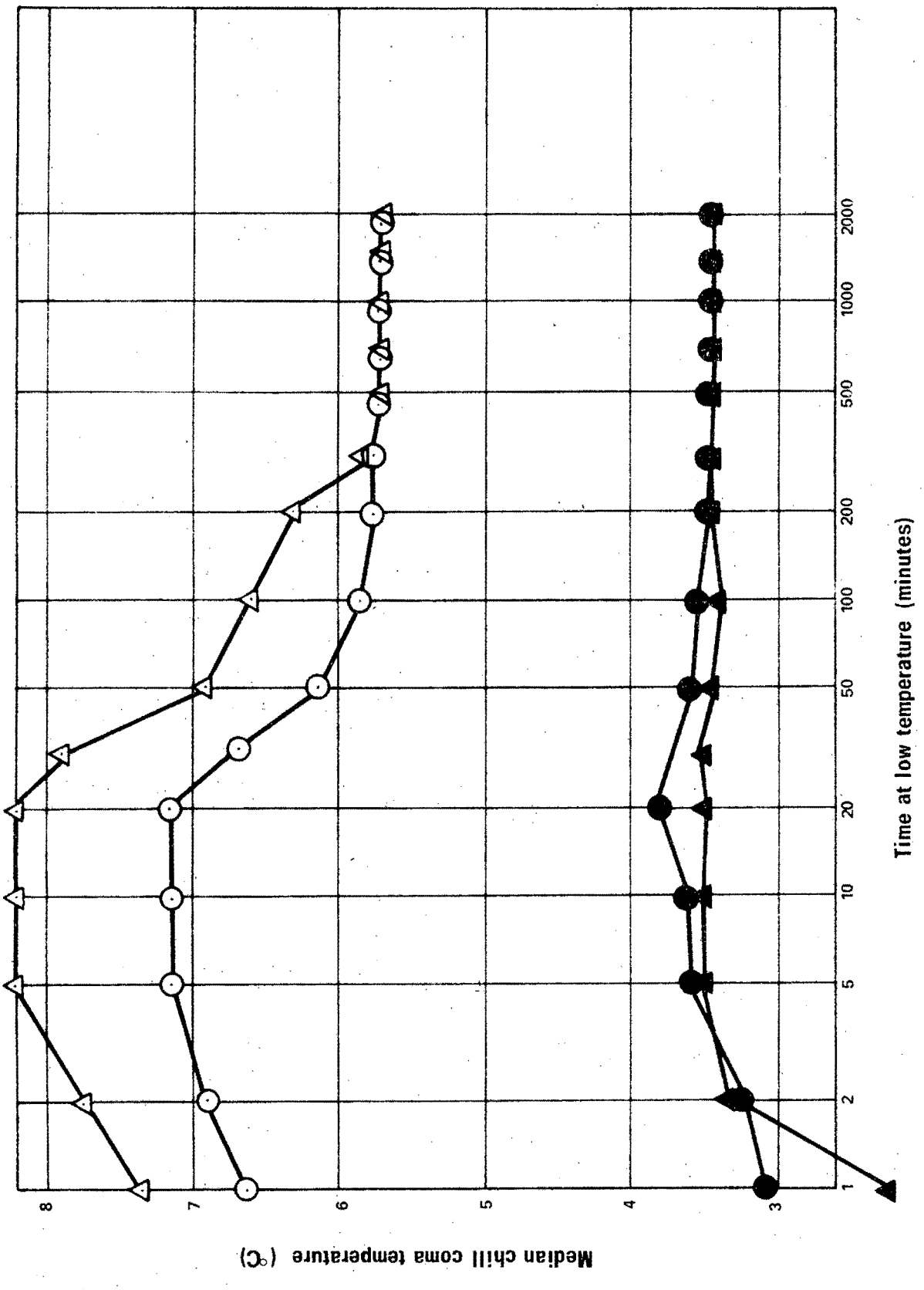

Median chill coma temperature (°C)
8
7
6
5
4
3
1
2
5
10
20
50
100
200
500
1000
2000
Time at low temperature (minutes)

Figure 27

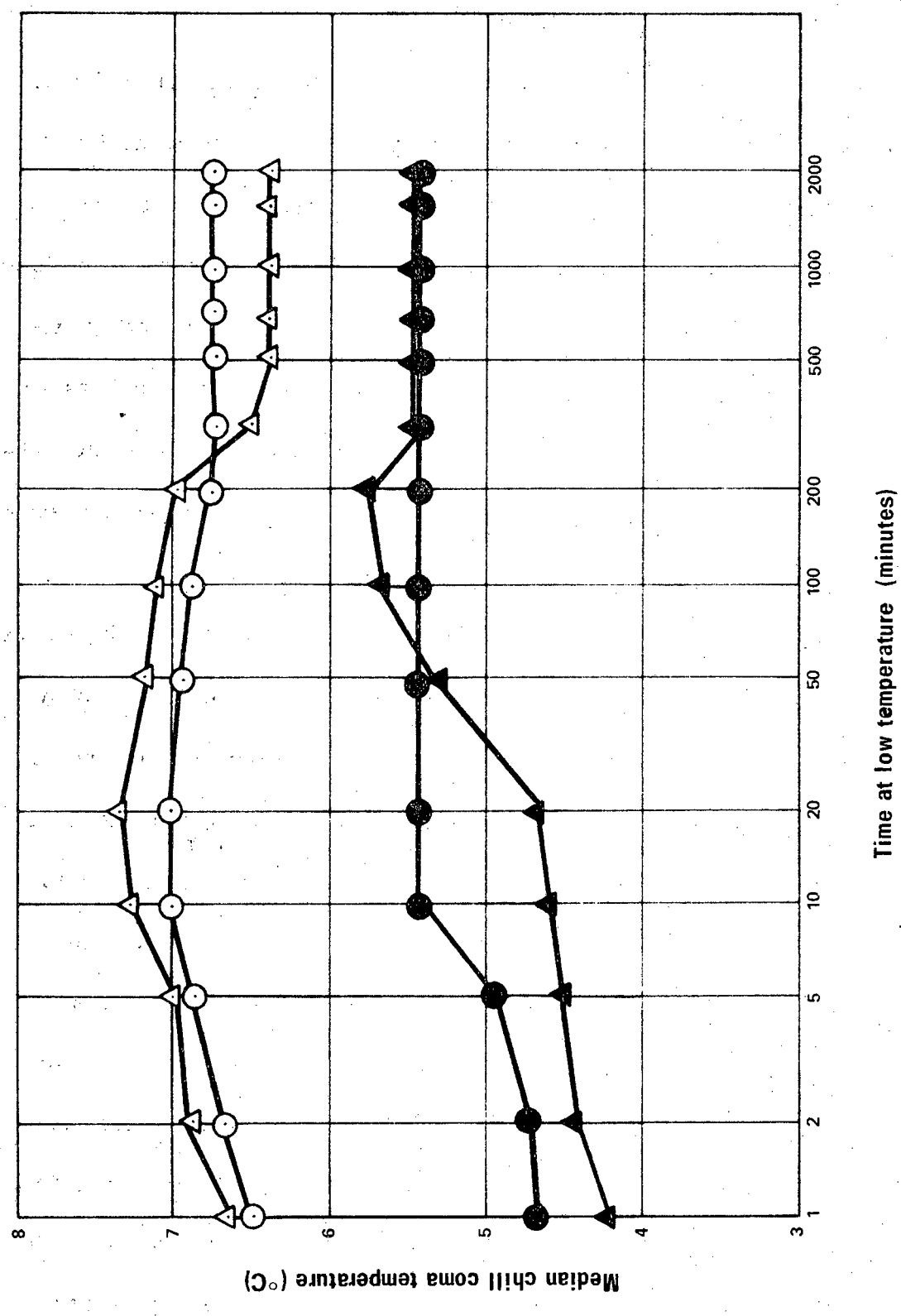
Median chill coma temperatures for Choroterpes bugandensis nymphs, calculated for different times of exposure to low temperature as follows:

open triangles - summer nymphs transferred directly to low temperature

open circles - summer nymphs, temperature decreased over six hours

closed triangles - winter nymphs transferred directly to low temperature

closed circles - winter nymphs, temperature decreased over six hours


Median chill coma temperature (°C)
8
7
6
5
4
3
1
2
5
10
20
50
100
200
500
1000
2000
Time at low temperature (minutes)

MORTALITY AT LOW TEMPERATURES

In an experiment carried out in summer only, Baetis harrisoni and Choroterpes bugandensis nymphs brought in from the field were held in the laboratory at 20°C for 24 hours. Groups of these nymphs were then transferred in experimental tubes directly into four test temperatures. At intervals of time thereafter groups were removed from the experimental tanks. Numbers of nymphs in these groups found to be comatose and numbers recovering when transferred to water at 20°C were noted. The mortality observed in these experiments of Baetis and of Choroterpes nymphs are shown, respectively, in figures 28 and 29.

It was found in these experiments that some nymphs of each species died quite early on in the experiment. Others survived for a relatively long period in a comatose state before dying. The proportion of animals found dead increased with time of exposure and all of the nymphs still in coma after four hours would apparently have died eventually if left at the test temperature. A distinction might therefore be drawn between the comatose state from which nymphs were observed to recover at the same temperature, perhaps resulting from shock at sudden exposure to low temperature, and the similar state from which nymphs apparently were not able to recover if they were not transferred to warmer water. The first began within five minutes or so of transfer to cold water and was restricted to the first four hours of exposure. The second could also apparently occur within a relatively few minutes, but continued until death.

In fish, Doudoroff (1945) and others have distinguished a primary chill coma state occurring relatively soon after exposure, from which individuals might

or might not recover at the same temperature, and a secondary coma only occurring much later. The two kinds of coma found here might superficially be compared to the primary and secondary chill coma of fish. However, in fish these two states differ clearly from one another not only in time, but also in the degree to which they are influenced by dissolved oxygen concentration and salinity (Pitkow 1960). In the mayfly nymphs studied here no such clear-cut distinction can be drawn.

NYMPHS OF DIFFERENT SIZES

In another summer experiment carried out for Baetis harrisoni and for Choroterpes bugandensis, nymphs of different sizes were held in the laboratory for 24 hours at 20°C and then transferred in experimental tubes directly into water at four different test temperatures. Numbers in coma were noted at intervals of time thereafter. At the end of the experiment the length of each individual, excluding antennae and cerci, was measured under a microscope. The results obtained are shown in figures 30 and 31 for Baetis and Choroterpes respectively. These results indicate that for both species the different size groups did not differ greatly in their susceptibilities to chill coma, but that the larger nymphs (body length 5.0 to 6.5 mm for Baetis and 4.5 to 6.0 mm for Choroterpes, as were used in all other experiments) were less susceptible to chill coma than were the smaller nymphs.

Figure 28

Numbers of Baetis harrisoni nymphs dead (expressed per cent of animals in chill coma) after different times at the following low temperatures:

circles - 4.5°C

triangles - 5.5°C

squares - 6.5°C.

Stars represent superimposed points.

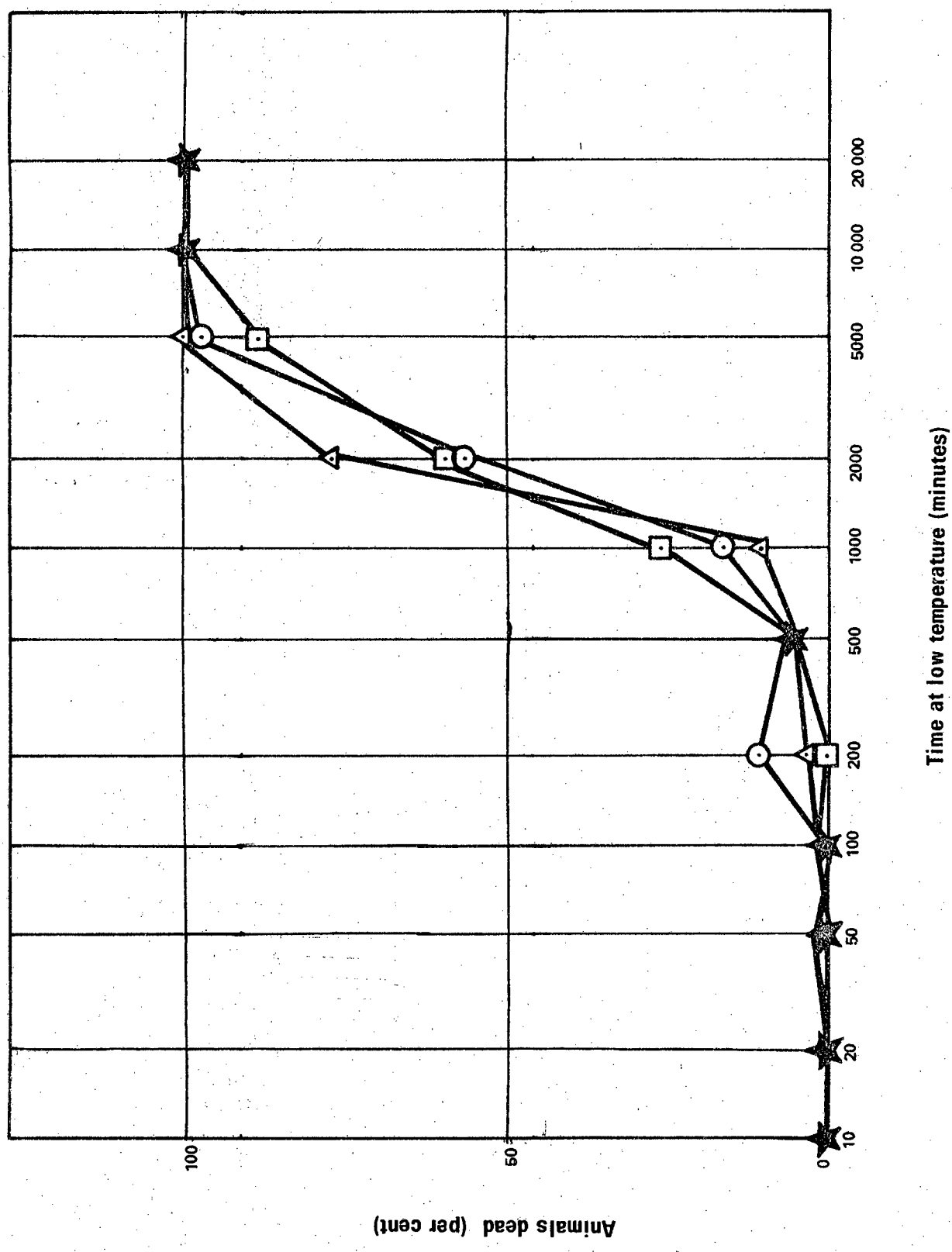

Animals dead (per cent)
100
50
0
10
20
50
100
200
500
1000
2000
5000
10 000
20 000
Time at low temperature (minutes)

Figure 29

Numbers of Choroterpes bugandensis nymphs dead (expressed per cent of animals in chill coma) after different times at the following temperatures:

circles - 5.0°C

triangles - 6.0°C

squares - 7.0°C.

Stars represent superimposed points.

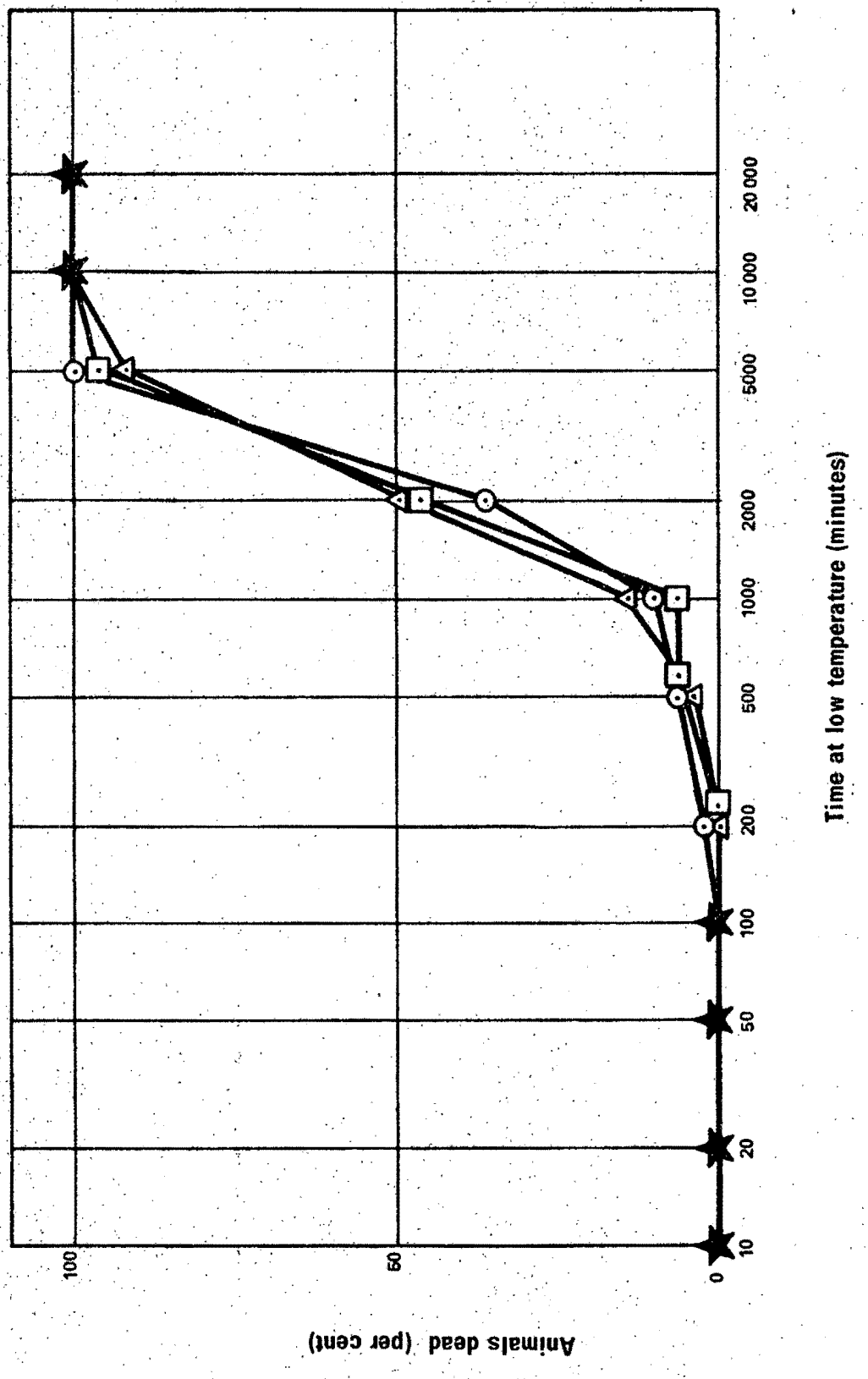

Animals dead (per cent)
0
50
100
10
20
50
100
200
500
1000
2000
5000
10 000
20 000
Time at low temperature (minutes)

Figure 30

Numbers of summer Baetis harrisoni nymphs of different sizes comatose (expressed per cent) after different times at low temperature. Sizes as follows:

closed triangles, circles and squares	-	body length 2.0 to 3.4 mm
shaded triangles, circles and squares	-	body length 3.5 to 4.9 mm
open triangles, circles and squares	-	body length 5.0 to 6.5 mm.

Temperature as follows:

triangles (point up)	-	5.5°C
triangles (point down)	-	6.0°C
circles	-	6.5°C
squares	-	7.0°C.

Stars represent superimposed points.

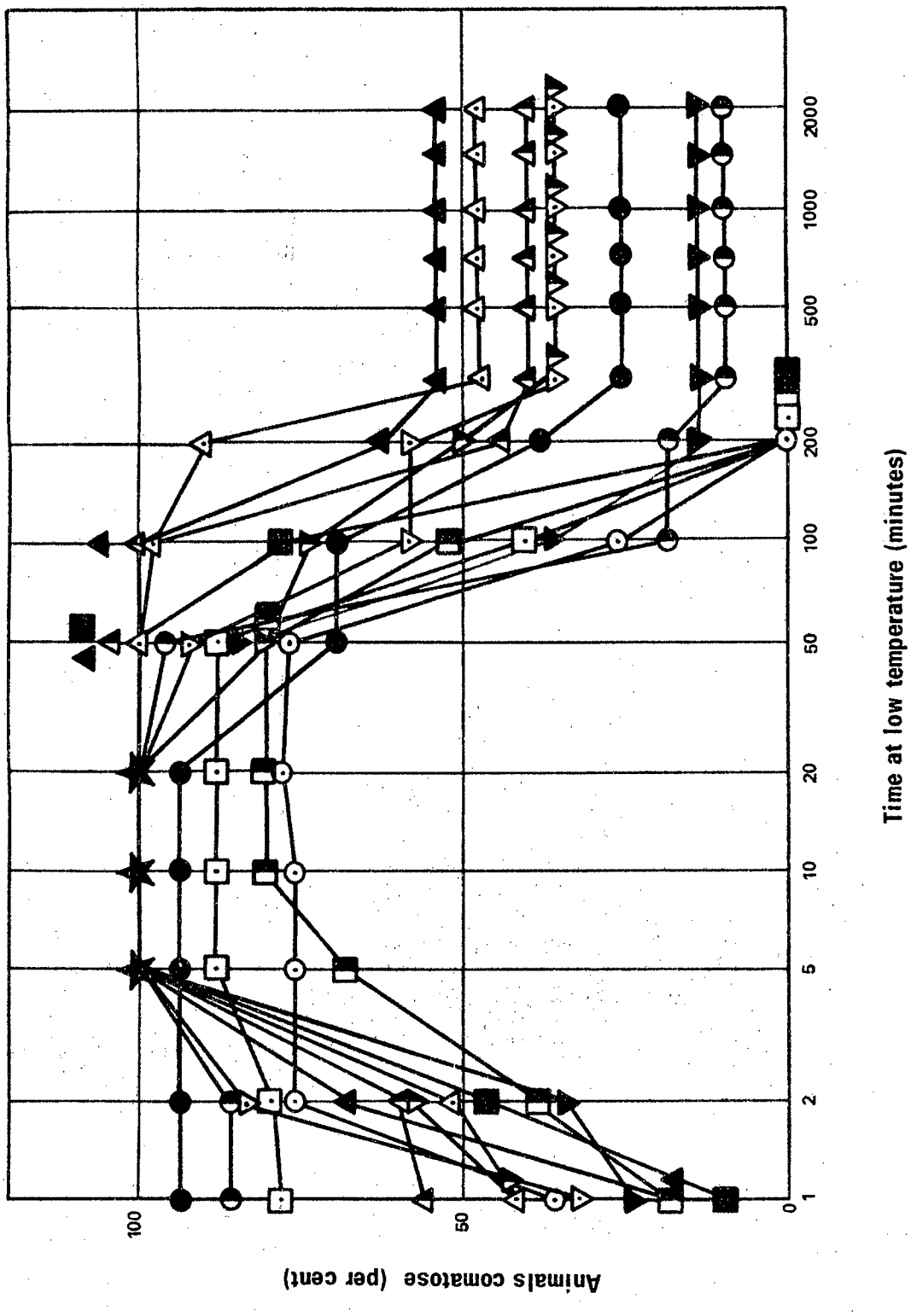

Animals comatose (per cent)
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Time at low temperature (minutes)

Figure 31

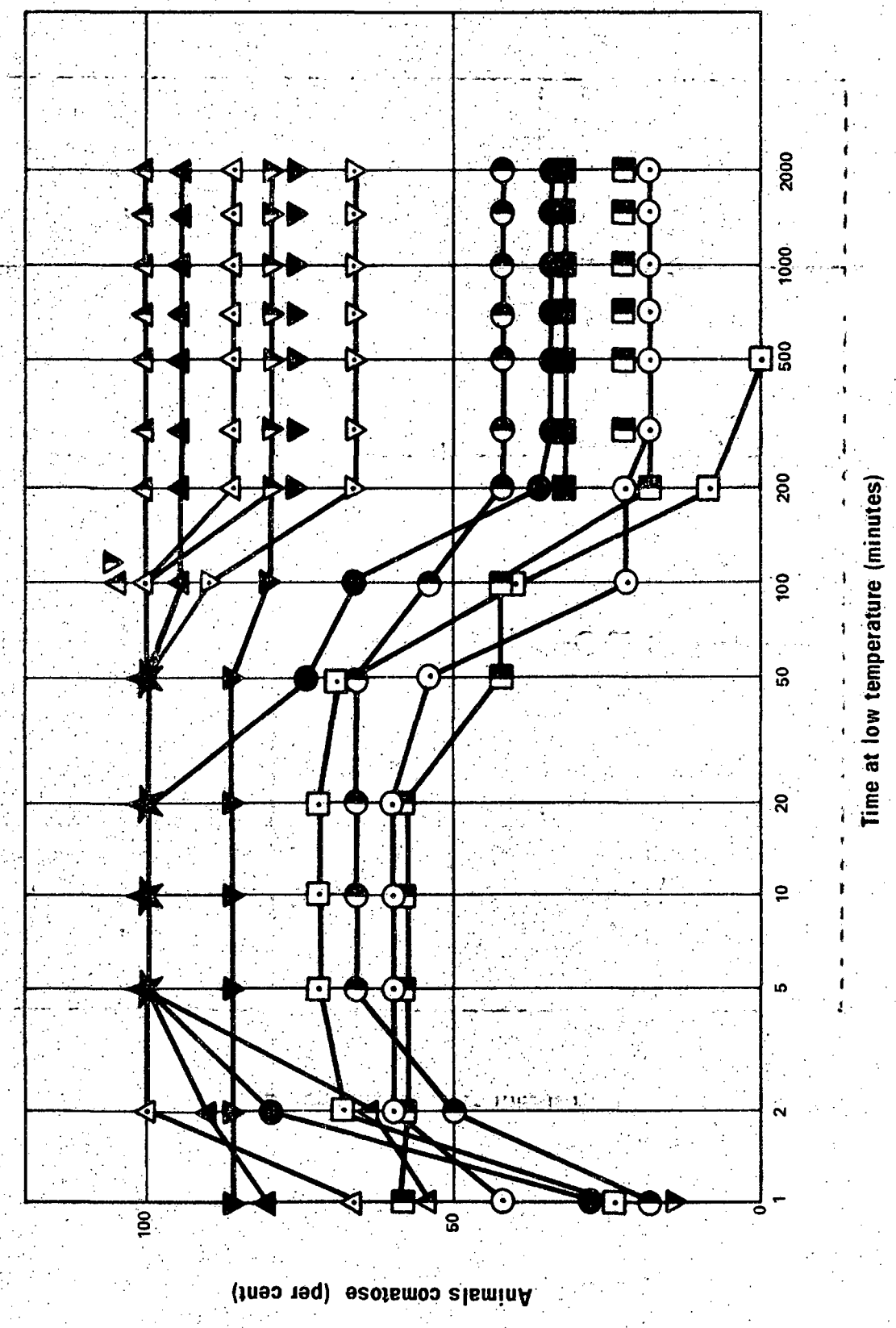
Numbers of summer Choroterpes bugandensis nymphs of different sizes comatose (expressed per cent) after different times at low temperatures. Sizes as follows:

closed triangles, circles and squares	-	body length 1.6 to 2.9 mm
shaded triangles, circles and squares	-	body length 3.0 to 4.4 mm
open triangles, circles and squares	-	body length 4.5 to 6.0 mm.

Temperatures as follows:

triangles (point up)	-	$6.0^{\circ}C$
triangles (point down)	-	$6.5^{\circ}C$
circles	-	$7.0^{\circ}C$
squares	-	$7.5^{\circ}C$.

Stars represent superimposed points.


Animals comatose (per cent)
100
50
0
1
2
5
10
20
50
100
200
500
1000
2000
Time at low temperature (minutes)

COLD ACCLIMATION

Comparison of figures 18 and 22 with figures 19 and 23, and of figures 20 and 24 with figures 21 and 25 shows that summer nymphs of both species tested were much more susceptible to chill coma than were winter nymphs. Experiments were therefore carried out to see to what extent nymphs gained or lost cold tolerance when held in the laboratory at different temperatures. Separate experiments were once again carried out using Baetis harrisoni and Choroterpes bugandensis nymphs.

For each species, summer nymphs were collected and held in the laboratory for 24 hours at 20°C. Thereafter, the population was divided at random into four groups, one of each of which was then transferred to trays of water in which temperatures were maintained at 10°C, 15°C, 20°C and 25°C. Random subgroups of each were removed from each of these after one, two, three and four days and transferred in experimental tubes to four test temperatures in the low lethal range. Numbers in coma and numbers not recovering in water at 20°C were then noted at the end of 1000 minutes' exposure for each subgroup.

Median effective chill coma temperatures for 1000 minutes' exposure were calculated by probit analysis from these results for each acclimation temperature and time. The effects of acclimation on chill coma temperature are shown in figure 32 for Baetis harrisoni and in figure 33 for Choroterpes bugandensis.

These results show that acclimation to low temperature by summer nymphs of both Baetis and Choroterpes took place when these animals were held in the laboratory at 10°C and 15°C. Nymphs held at these tempera-

tures progressively gained in cold tolerance. The median chill coma temperatures of Baetis nymphs held at 20°C and of Choroterpes nymphs held either at 20°C or at 25°C, on the other hand, did not alter significantly. The chill coma temperatures of nymphs held at 10°C and at 15°C did not differ significantly from one another. Baetis nymphs held for a day at 25°C were found to have lost cold tolerance (not shown in the figure), possibly because 25°C was close to the lethal temperature for this species and had a deleterious effect on these nymphs.

For comparison with these results, the 1000-minute median chill coma temperatures for winter Baetis and Choroterpes nymphs, estimated respectively from figures 19 and 23, have been shown in figures 32 and 33 as broken lines. Summer Baetis acclimated at 10°C for four days were still found to be significantly less tolerant of low temperature than the winter nymphs had been. It seems possible that winter Baetis nymphs might differ from summer nymphs by more than merely a short-term physiological adjustment to environmental temperature. The chill coma temperature of summer Choroterpes acclimated in the laboratory at 10°C for four days, on the other hand, did not differ very greatly from that of winter nymphs. Acclimation appears to be sufficient to account for the observed difference in cold tolerance of winter and summer nymphs of this species. The rate of gain in cold tolerance of Choroterpes with acclimation at 10°C and 15°C, however, was rather lower than that of Baetis.

A similar pair of experiments was carried out during winter to see to what extent winter nymphs might lose cold tolerance while being held in the laboratory at higher temperatures. One experiment again dealt with

Figure 32

1000-minute median chill coma temperatures for summer Baetis harrisoni nymphs held in the laboratory at the following temperatures:

circles - 20°C

squares - 15°C

triangles - 10°C

95% confidence limits shown about each median value. The median chill coma for winter nymphs shown by a broken line.

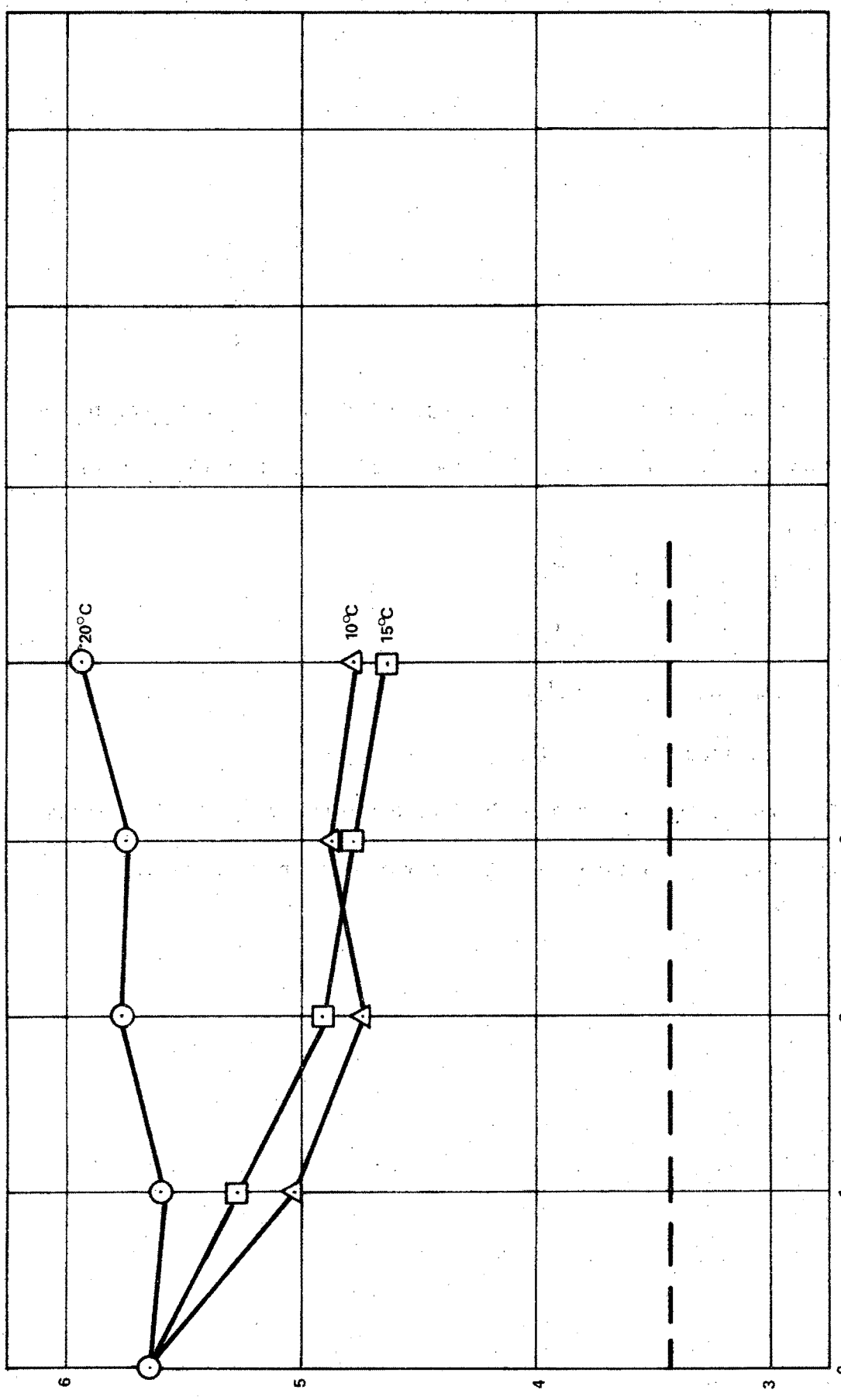

20°C
10°C
15°C
6
5
4
3
0
1
2
3
4
Median chill coma temperature (°C)
Acclimation time (days)

Figure 33

1000-minute median chill coma temperatures for summer Choroterpes bugandensis nymphs held in the laboratory at the following temperatures:

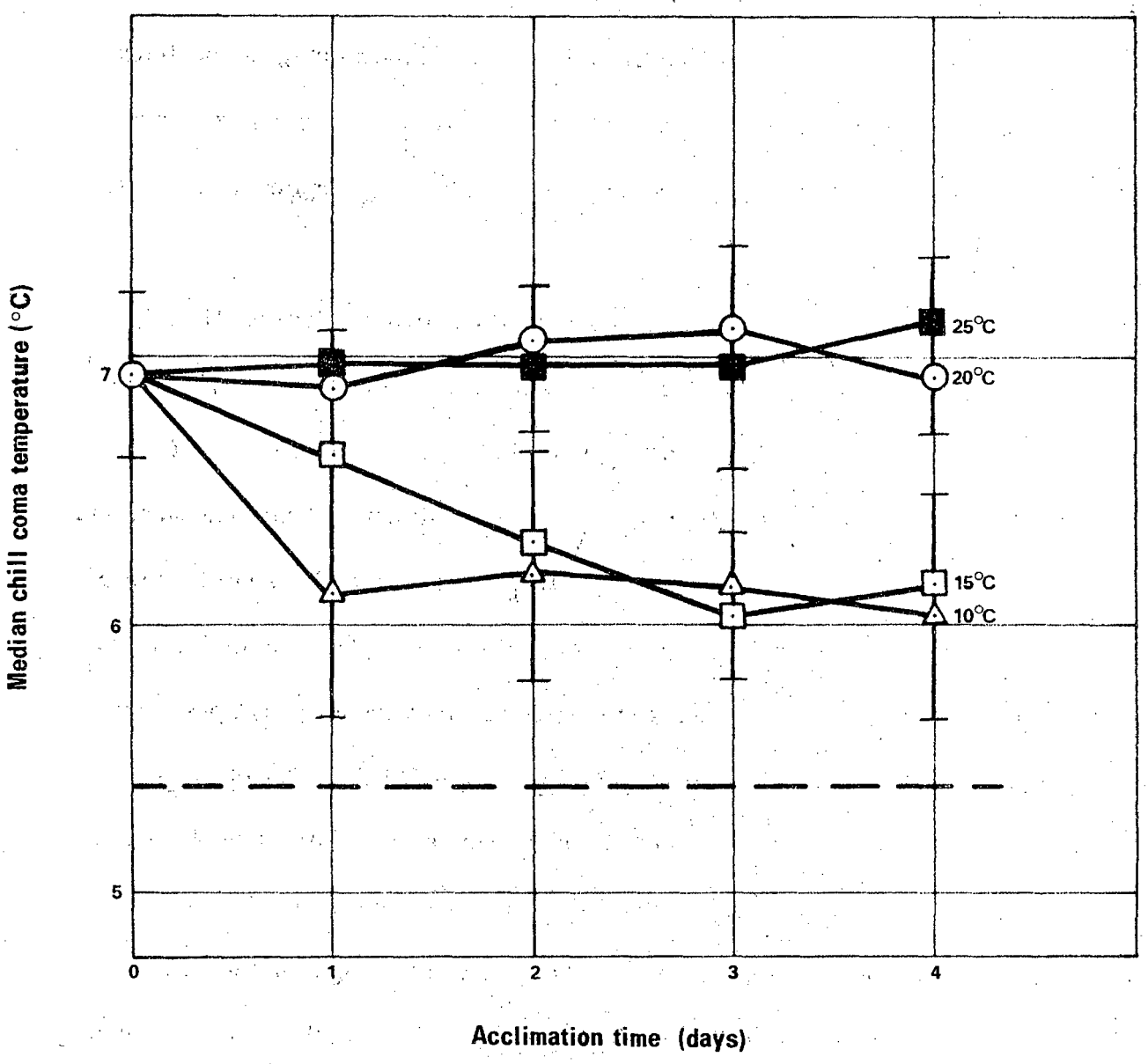
diamonds - 25°C

circles - 20°C

squares - 15°C

triangles - 10°C.

95% confidence limits shown about each median value. The median chill coma temperature for winter nymphs shown by a broken line.


Median chill coma temperature (°C)
7
6
5
0
1
2
3
4
Acclimation time (days)
25°C
20°C
15°C
10°C

Baetis harrisoni and the other with Choroterpes bugandensis. Nymphs were collected and held for 24 hours at 10°C, a temperature a little below actual stream temperatures at the time of collection. Thereafter, groups were transferred to water maintained at 10°C, 15°C, 20°C and, in the case of Choroterpes only, 25°C. They were held here for one, two, three and four days before being transferred directly to low temperatures in the lethal range. As before, numbers of nymphs comatose after 1000 minutes' exposure were counted and median chill coma temperatures estimated from these observations.

The results of these experiments are illustrated in figures 34 and 35. As may be seen, the median chill coma temperatures of nymphs held at different temperatures did not differ very greatly. Very few of the values obtained could be demonstrated to differ significantly from one another. However, a rank correlation test (Kendall 1955) confirmed the significance of the observation that the chill coma temperatures of nymphs held in the laboratory at 10°C and 15°C were consistently lower that those of nymphs held at higher temperatures ($p < 0.01$ for Baetis, $p < 0.001$ for Choroterpes). From this it seems that the chill coma temperatures of winter nymphs were increased as a result of the time they spent at 20°C and 25°C, but that the changes in chill coma temperature that occurred were small, of the order of 0.2°C for Baetis and 0.4°C for Choroterpes. None of the winter nymphs held here at summer temperatures was found to be as intolerant of low temperatures as had summer nymphs previously been found to be. To illustrate this point, the median chill coma temperatures for summer nymphs held at 20°C estimated from figures 17 and 21 have been indicated in figures 34 and 35 by broken lines.

SALINITY AND DISSOLVED OXYGEN

Pitkow (1960) has shown that death during primary chill coma of the freshwater fish he studied was caused or at least aggravated by oxygen lack. Doudoroff (1942, 1945) and Ernst (1965), on the other hand, found that secondary chill coma in the marine and euryhaline fish they studied was influenced by the salinity of the water. This sort of information can be of great importance in providing clues as to the mechanisms of lethal effects of low temperature on the animals concerned. Separate experiments were carried out for Baetis harrisoni and for Choroterpes bugandensis in order to compare the cold tolerances of these nymphs in different salinities and dissolved oxygen concentrations.

1000-minute chill coma temperatures for summer Baetis harrisoni nymphs held in the laboratory at 20°C for 24 hours and then exposed to different low temperatures in the lethal range in water with dissolved oxygen contents of 4 mg/l and about 10 mg/l, and in water containing 31 mg/l and 500 mg/l total dissolved solids are shown in table 31. Equivalent data for Choroterpes bugandensis are shown in table 32. The median chill coma temperatures estimated at the two levels of dissolved oxygen and at the two salinities were not significantly different from one another.

FIELD OBSERVATIONS

Low water temperatures may be expected in Transvaal streams either during winter cold spells or after hailstorms. The lowest temperature recorded during this study in the Braamfontein Spruit was 6.5°C on one occasion in winter. In the Pienaars River a temperature of 7.5°C was recorded on one

Figure 34

1000-minute median chill coma temperatures for winter Baetis harrisoni nymphs held in the laboratory at the following temperatures:

circles - 20°C

squares - 15°C

triangles - 10°C.

95% confidence limits are shown about each median value.

The median chill coma temperature for summer nymphs is shown by a broken line.

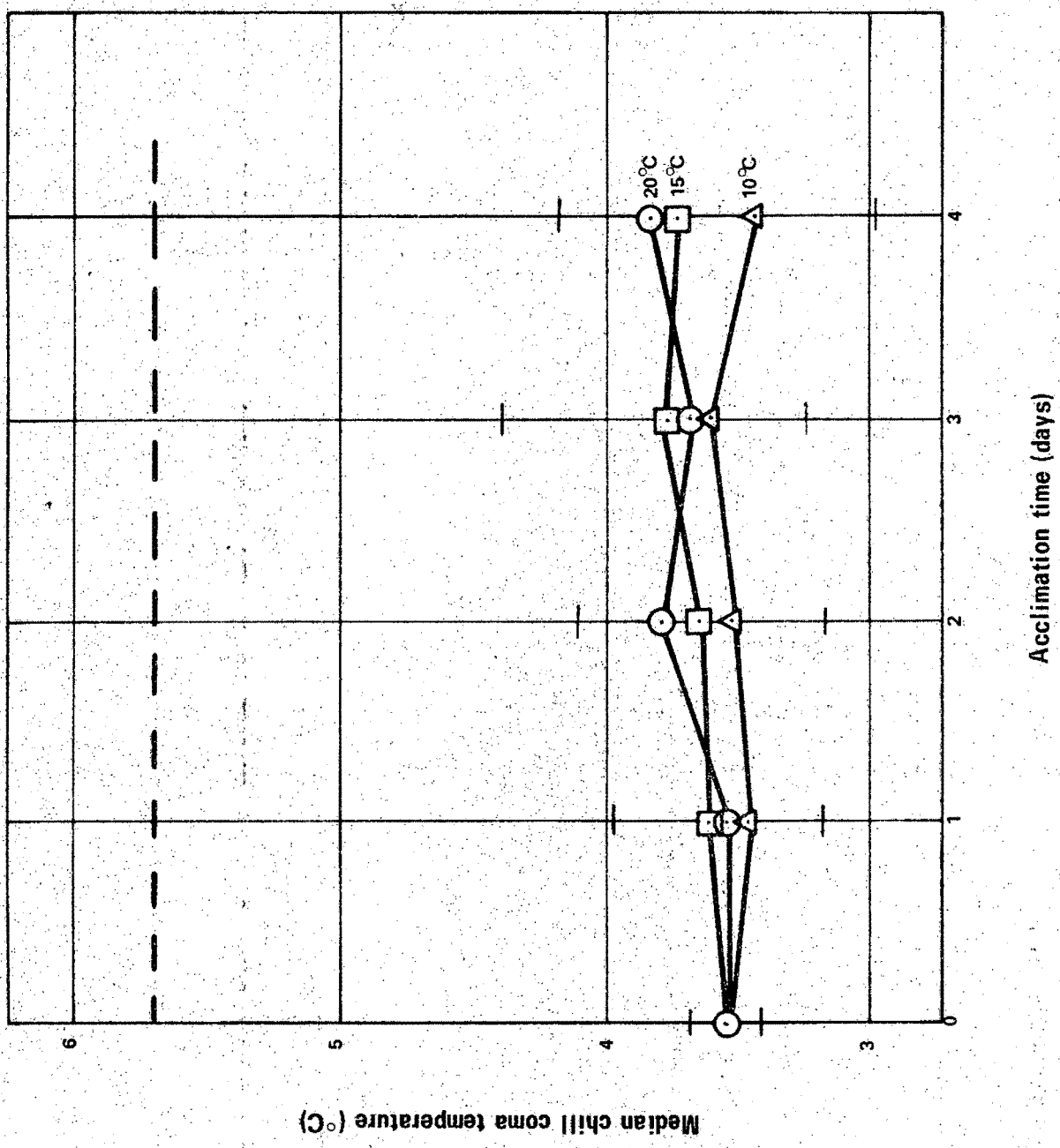

20°C
15°C
10°C
Median chill coma temperature (°C)
Acclimation time (days)
6
5
4
3
0
1
2
3
4

Figure 35

1000-minute median chill coma temperatures for winter Choroterpes bugandensis nymphs held in the laboratory at the following temperatures:

diamonds - 25°C

circles - 20°C

squares - 15°C

triangles - 10°C.

95% confidence limits are shown about each median value. The median chill coma temperature for summer nymphs is shown by a broken line.

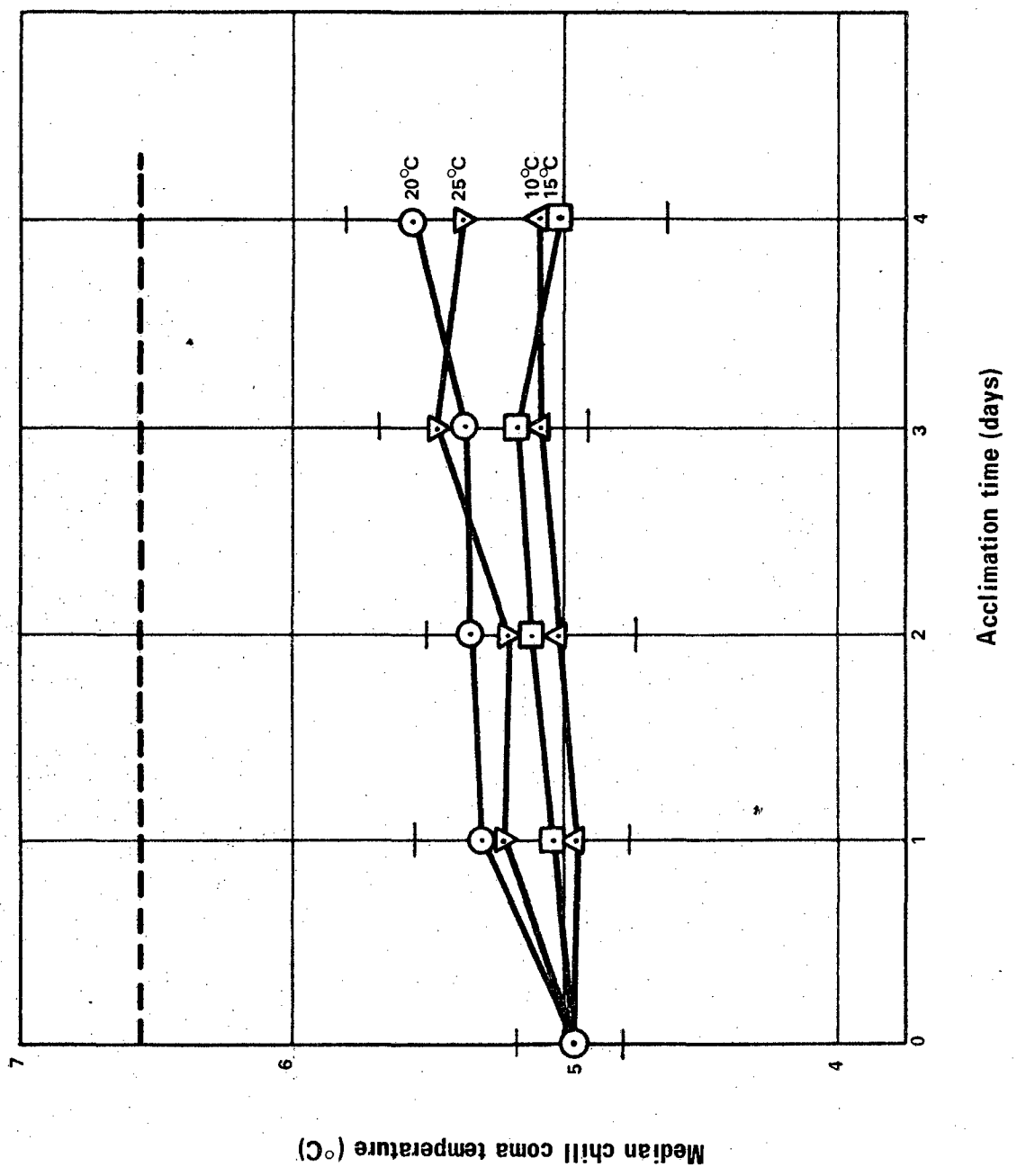

20°C
25°C
10°C
15°C
7
6
5
4
0
1
2
3
4
Median chill coma temperature (°C)
Acclimation time (days)

TABLE 31

1000-MINUTE MEDIAN CHILL COMA TEMPERATURES FOR SUMMER BAETIS HARRISONI NYMPHS, IN WATER CONTAINING 4 mg/l AS OPPOSED TO ABOUT 10 mg/l DISSOLVED OXYGEN, AND IN WATER CONTAINING 500 mg/l AS OPPOSED TO 30 mg/l DISSOLVED SOLIDS

Dissolved oxygen (mg/l)	Dissolved solids (mg/l)	Number of nymphs	Test temperatures (°C)	Median effective chill coma temperature (°C), 95% confidence limits in brackets
$\pm$ 10	30	160	5.0 5.5 6.0 6.5	5.6 (5.2 to 6.0)
4	30	160	5.0 5.5 6.0 6.5	5.9 (5.4 to 6.4)
$\pm$ 10	30	160	5.0 5.5 6.0 6.5	5.8 (5.4 to 6.2)
$\pm$ 10	500	160	5.0 5.5 6.0 6.5	5.6 (5.2 to 6.0)

occasion, also in winter. These particular temperatures would not have caused mortality of winter nymphs of either species, but could cause mortalities of summer nymphs if they persisted for some time. Much lower temperatures than these are thought to occur from time to time in these streams and in other rivers in which these species live. Allanson (1961)has seen ice on the Jukskei River in winter and Oliff (1960a) has recorded temperatures below freezing in the Tugela system. Both the Pienaars River and the Braamfontein Spruit were visited after fairly heavy hailstorms, but temperatures in

TABLE 32

1000-MINUTE MEDIAN CHILL COMA TEMPERATURES FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS, IN WATER CONTAINING 4 mg/l AS OPPOSED TO ABOUT 10 mg/l DISSOLVED OXYGEN, AND IN WATER CONTAINING 500 mg/l AS OPPOSED TO 30 mg/l DISSOLVED SOLIDS

Dissolved oxygen (mg/l)	Dissolved solids (mg/l)	Number of nymphs	Test temperatures (°C)	Median effective chill coma temperature (°C), 95% confidence limits in brackets
$\pm$ 10	30	160	6.0 6.5 7.0 7.5	6.7 (6.1 to 7.3)
4	30	160	6.0 6.5 7.0 7.5	6.6 (6.2 to 7.0)
$\pm$ 10	30	160	6.0 6.5 7.0 7.5	6.5 (6.2 to 6.8)
$\pm$ 10	500	160	6.0 6.5 7.0 7.5	6.5 (6.0 to 7.0)

the lethal range were not recorded. The lowest water temperatures measured in the Pienaars River after a hailstorm was 13.1°C. This was well above the chill coma temperature for Choroterpes bugandensis and nymphs in the river appeared to have suffered no ill effects. On this occasion the temperature subsequently rose again to 18°C within two hours. In all probability, low water temperatures after hailstorms do not persist for very much longer than a very few hours.

This information is a little inconclusive, but it does seem that temperatures in the lethal range might easily occur from time to time during exceptionally cold spells in winter. It is not inconceivable that numbers of nymphs might be killed during such spells, if they are of sufficiently long duration. The laboratory results seem to indicate that exposure to low temperatures of a few hours only, while they might cause nymphs to go into coma, would not be lethal.

SUMMARY

1. Baetis and Choroterpes in cold water either became quiescent or else within minutes entered a state of chill coma in which they lost equilibrium, drifted with the current and did not respond to physical stimulation.
2. Some of the nymphs that became comatose at low temperature recovered at the same temperature within about four hours and did not become comatose again. Other did not and did not recover if kept at the same temperature.
3. Some comatose nymphs immediately recovered when transferred to warmer water, even after prolonged periods in coma. The proportion of nymphs recovering in warmer water depended on the time they had been at low temperature rather than the low temperature itself. Median times to death of nymphs in coma were of the order of 1700 minutes for Baetis and 2500 minutes for Choroterpes at all temperatures tested.
4. Baetis nymphs were more tolerant of cold than were Choroterpes nymphs, 1000-minute median chill coma temperatures being 5.7°C in summer and 3.4°C in winter, 6.5°C in summer and 5.5°C in winter for Choroterpes.

5. Smaller nymphs of both species were slightly more susceptible to chill coma than were larger nymphs.

6. Neither dissolved oxygen concentration nor salinity materially affected the abilities of nymphs of either species to tolerate low temperatures.

7. Winter nymphs of both species were significantly more tolerant of cold than were summer nymphs (see 4 above).

8. Summer nymphs became more tolerant of low temperatures when held for one or more days at temperatures cooler than those in which they had been living in the field, the median chill coma temperature of Baetis nymphs being reduced by $1^{o}C$, that of Choroterpes nymphs being reduced by $0.8^{o}C$.

9. Winter nymphs became slightly less tolerant of low temperatures when held for one or more days at temperatures higher than those in which they had been living in the field. Changes in chill coma temperature that were observed were of the order of $0.2^{o}C$ for Baetis nymphs and $0.4^{o}C$ for Choroterpes.

MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS AT LOW CONCENTRATIONS OF DISSOLVED OXYGEN

INTRODUCTION

It has long been known that aquatic animals differ widely in their abilities to tolerate low concentrations of dissolved oxygen, and that low oxygen concentrations, usually associated with the decomposition of organic matter, make certain situations unavailable to less tolerant species (Hynes 1960). Well aerated streams are characteristically inhabited by animals that appear to be relatively sensitive to oxygen lack, notably the nymphs of certain Ephemeroptera (Hubault 1927, Verrier 1948a). Poorly oxygenated waters, on the other hand, are often inhabited by animals able to survive oxygen lack, of which the larvae of certain Chironomidae are perhaps the best known (Harnisch 1951, Thienemann 1954). Observations are reported here of mortality and survival at low concentrations of dissolved oxygen of nymphs of two common South African mayflies, Baetis harrisoni and Choroterpes bugandensis and the significance of these observations is discussed in relation to what is known of the ecology of the two species.

Studies of the dissolved oxygen requirements of different freshwater animals have been undertaken by a number of workers. It has also been recognized that the nymphs at least of certain Ephemeroptera require movement of the water around them in order to obtain sufficient oxygen (Avel and Avel 1932, Verrier 1948a). The same applies to the larvae of certain Trichoptera (Philipson 1954) and to the nymphs of some Odonata (Zahner 1959) and Plecoptera (Knight and Gaufin 1964, DeWitt 1964). In fact, Ruttner (1926)

speaks of the "respiratory value" of flowing water. Ambühl (1959) has shown that minimal dissolved oxygen concentrations required by certain aquatic larvae and nymphs, most notably the nymphs of a species of Baetis, decrease rapidly with increase in water current speed in the range 0 to 6 cm/sec. He also found that rates of oxygen uptake by these animals that were favoured by water flow increased with increase in current speed. He reasoned that the availability of oxygen to his animals was limited by the rate of diffusion of oxygen to them. Animals in stagnant water evidently became surrounded by oxygen-depleted water. In flowing water the oxygen-depleted water would have been swept away and replaced by oxygegenated water.

Of all aquatic animals, most is known of the oxygen uptake rates and requirements of freshwater fish (Fry 1957). It is of interest to note that Shepard (1955) found the lethal low oxygen concentration for one species of fish, Salvelinus fontinalis, to decrease somewhat for animals held for a few days at relatively low but non-lethal oxygen concentrations. Among similar individuals transferred back to well oxygenated water he found the lethal oxygen concentration to have increased again. This reversible adjustment to oxygen conditions appeared to be brought about by a change in the oxygen capacity of the blood and not by a change in the rate of oxygen uptake. This would be impossible for the nymphs of Baetis harrisoni and Choroterpes bugandensis since, like many other aquatic insects, these have a closed tracheal system. Any adjustment to oxygen conditions by these animals would therefore have had to have been brought about in a

very different way to that shown by Salvelinus.

Certain chironomid larvae able to live in bodies of water which become anaerobic have been found to possess several interesting adaptations which enable them to survive these conditions. Some of these possess haemoglobin which Walshe (1950) has shown to store up oxygen which can be used during times of temporary oxygen lack. Other have been found to various extents to be able to utilize anaerobic metabolic pathways during temporarily anoxic conditions (Harnisch 1936, Augenfeld 1967). Certain of these and other animals have in fact been found to be killed by high dissolved oxygen concentrations (Harnisch 1951) or to grow more rapidly in relatively low oxygen concentrations than they did in well aerated water (Fox and Taylor 1955). Both Baetis harrisoni and Choroterpes bugandensis appear only to live where oxygen is present. It seems unlikely that they should share these features with the Chironomidae and other animals of anaerobic waters. However, these aspects were not investigated in the present study.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected as before and populations were held at 20°C in oxygen-saturated water in the laboratory for a day before being used in experiments.

Dissolved oxygen concentrations were maintained at selected levels in the experimental tanks by bubbling through the water suitable mixtures either of nitrogen and air or of oxygen and air. The tanks were carefully covered, so that the space above the water became filled with the gas mixture supplied and the proportions of nitrogen and oxygen in the water and in the gas mixture

were allowed to reach an equilibrium. The required proportions of gases in the mixture were calculated in advance and adjusted both during a 48 hour equilibration period and during the experiment itself. Dissolved oxygen concentrations in each experimental tank were measured at frequent intervals during an experiment. Oxygen concentrations normally remained within 0.1 mg/l of desired values, although occasional deviations of 0.2 mg/l were observed. The very lowest oxygen concentrations were the easiest to maintain.

Experimental tubes of differing internal diameter were used in order to obtain different water current speeds and laminar or turbulent flow characteristics as these were required. A small number of tubes were connected in series in each tank. In some instances, two tubes were connected in parallel in order to get half the flow rate that would have been realized in a single tube.

Stagnant conditions could not be maintained in the tubes, since respiration of the nymphs over 1000 minutes was found to alter the dissolved oxygen concentration in the water trapped in the tubes. Tests requiring non-flowing water had therefore to be carried out in nylon gauze trays 10 cm x 10 cm suspended in the experimental tanks.

Water quality and temperature were carefully controlled in these experiments in the way described earlier. Experiments were carried out over 250 or 1000 minutes and numbers of nymphs dead and alive counted at the end of these exposure times. Median lethal dissolved oxygen concentrations were calculated from these observations by probit analysis (Finney 1952).

CHOROTERPES

Choroterpes bugandensis nymphs were exposed to a number of different low levels of dissolved oxygen at three current speeds for each of laminar and turbulent flow and for exposures of 1000 and 250 minutes. Median lethal low concentrations of dissolved oxygen estimated from this experiment for each current speed are shown in tables 33 and 34. As may be seen, nymphs of this species were found to be able to tolerate relatively low concentrations of dissolved oxygen. The lethal oxygen concentrations in stagnant water (i.e. in the gauze trays) were significantly higher than those in flowing water (i.e. in the tubes) for both 1000 and 250 minutes' exposure. The lethal concentration in 2.6 cm/sec laminar flow for 1000 minutes' exposure was also significantly higher than those at faster flows. For each exposure time, the rest of the lethal concentrations estimated did not differ significantly from one another.

The experiment that yielded these results could not be carried out on one day and was carried out in four parts on different days. The different combinations of flow and oxygen concentration to be tested were distributed at random among the four days on which the parts of the experiment were conducted.

Under normal conditions, the abdominal gills of Choroterpes bugandensis nymphs beat continuously and rhythmically at a fairly rapid rate. At concentrations of dissolved oxygen below 1.5 mg/l the gills beat more slowly and stopped beating for short periods from time to time, especially after prolonged exposure. At concentrations near the lethal limit the gills beat only in weak intermittent bursts. Animals in lethal levels of oxygen were seen

TABLE 33

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT 20°C AND IN DIFFERENT WATER CURRENT SPEEDS

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
laminar	2.6	2.6	156	0.5 0.6 0.7 0.8	0.62 (0.59 to 0.65)
laminar	6.8	1.6	160	0.4 0.5 0.6 0.7	0.56 (0.54 to 0.58)
laminar	12.0	1.2	160	0.4 0.5 0.6 0.7	0.55 (o.53 to 0.57)
turbulent	<0.2	open	160	0.6 0.7 0.8 0.9	0.75 (0.72 to 0.78)
turbulent	6.5	5.0	160	0.4 0.5 0.6 0.7	0.58 (0.53 to 0.63)
turbulent	12.0	2.6	160	0.4 0.5 0.6 0.7	0.54 (0.51 to 0.58)

to stay motionless for long periods, but to give very occasional and very short bursts of gill fluttering. Many were found able to survive for several hours in this condition. When transferred to aerated water they immediately recovered and started beating their gills normally again.

TABLE 34

250-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT 20°C AND IN DIFFERENT WATER CURRENT SPEEDS

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
laminar	2.6	2.6	160	0.3 0.4 0.5 0.6	0.47 (0.43 to 0.51)
laminar	6.8	1.6	160	0.3 0.4 0.5 0.6	0.38 (0.32 to 0.44)
laminar	12.0	1.2	160	0.3 0.4 0.5 0.6	0.45 (0.42 to 0.48)
turbulent	<0.2	open	158	0.3 0.4 0.5 0.6	0.52 (0.48 to 0.54)
turbulent	6.5	5.0	160	0.3 0.4 0.5 0.6	0.46 (0.41 to 0.51)
turbulent	12.0	2.6	160	0.3 0.4 0.5 0.6	0.46 (0.43 to 0.49)

It is perhaps of interest to note here in passing that the rate of abdominal gill beats of nymphs of the related Choroterpes (Choroterpes) ndebele Agnew, also from the Pienaars River, was observed incidentally to the present study to decrease very evenly with decrease in dissolved oxygen concen-

tration. At a concentration in the neighbourhood of 1 mg/l, which the nymphs were able to survive for more than 250 minutes, the gills stopped beating entirely. When the dissolved oxygen concentration was raised slightly the gills immediately restarted and the animals recovered. The dissolved oxygen concentration at which the gills were just stopped presumably corresponded to the "level of no excess activity" defined by Fry (1947). No clearcut "level of no excess activity" was observed for Choroterpes bugandensis.

BAETIS

Preliminary experiments with Baetis harrisoni soon indicated that for this species water current speed and the lethal low dissolved oxygen concentration were very closely interrelated. Also, many of the nymphs of this species found dead after exposure to low dissolved oxygen concentrations were found to have died during ecdysis. They showed the same symptoms, looseness and thoracic splitting of the skin, flexion and so forth, as had nymphs exposed to high temperatures and described in an earlier section. For this reason, the statistics of mortality during and out of ecdysis had to be computed separately, as had been done for high temperature studies. This added a complication to experimental design. Different oxygen levels had to be used for nymphs in and out of ecdysis. Extensive preliminary tests had to be carried out in order to find out which oxygen levels were to be used in the final experiment. Because the ecdysis rate at $20^{\circ}C$ was fairly low, relatively large numbers of animals had to be used in order to have enough moulting during the experiment.

The final experiment to estimate median lethal oxygen concentrations at different rates of water flow could not all be carried out on one day. For this reason, the experiment was divided at random into eight parts, each of which was carried out on a different day.

Median lethal low dissolved oxygen concentrations estimated from these experiments for Baetis harrisoni nymphs attempting ecdysis during the experiment (1000 minutes) are shown in table 35. These are the concentrations estimated to be lethal for 50 per cent of those nymphs that attempt ecdysis during the experiment. These lethal concentrations should be the same for any exposure time, but the number of nymphs that attempt ecdysis will obviously be proportional to the exposure time, so that the lethal concentration for nymphs in ecdysis is equivalent to the "incipient lethal limit" (Fry 1947) for the whole population for infinite exposure, during which all the nymphs would attempt ecdysis. Too few nymphs were found to die during the shorter exposure time used (250 minutes) to be able to estimate lethal oxygen levels for nymphs in ecdysis and to see whether or not the lethal concentrations for two exposure times did differ.

The results shown in table 35 show that the lethal dissolved oxygen concentration decreased very markedly with increase in water current speed. In completely stagnant water all nymphs attempting ecdysis died even in water supersaturated with oxygen (10 mg/l).

TABLE 35

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS ATTEMPTING ECDYSIS AT 20°C AND IN DIFFERENT WATER CURRENT SPEEDS

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	< 0.2	open	121	8.0 9.0 10.0 11.0	> 10
laminar	1.0	2.6	94	8.0 9.0 10.0 11.0	9.3 (7.4 to 11.1)
laminar	2.0	2.6	158	7.0 8.0 9.0 10.0	8.6 (7.8 to 9.4)
laminar	4.0	2.6	137	5.0 6.0 7.0 8.0	6.6 (6.0 to 7.2)
turbulent	6.0	5.0	106	3.5 4.0 5.0 6.0	4.2 (3.4 to 4.9)
laminar	7.0	1.6	108	3.0 3.5 4.0 5.0	4.5 (4.1 to 5.0)
turbulent	8.0	5.0	143	3.0 3.5 4.0 5.0	3.8 (3.6 to 4.1)
laminar	12.5	1.2	163	2.5 3.0 3.5 4.0	3.4 (3.1 to 3.8)

TABLE 35 (cont)

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	14.8	2.6	161	2.0 2.5 3.0 3.5	2.6 (2.3 to 2.9)
laminar	22.2	0.9	162	1.5 2.0 2.5 3.0	2.9 (2.1 to 3.7)
turbulent	22.2	2.6	123	1.5 2.0 2.5 3.0	2.4 (1.9 to 2.8)
turbulent	39.1	1.6	107	1.0 1.5 2.0 2.5	1.6 (1.4 to 1.7)
turbulent	58.6	1.6	147	1.0 1.5 2.0 2.5	2.0 (1.4 to 2.6)

In table 36 are shown median lethal dissolved oxygen concentrations for Baetis nymphs exposed to these concentrations for only 250 minutes and which have not attempted in this period to moult. Allanson (1961) observed marked diurnal fluctuations in dissolved oxygen concentrations in the polluted Jukskei River in which Baetis harrisoni occurs, with minima of the order of 3 mg/l lasting around 4 hours at night apparently associated with the intermittent release of polluted water upstream. It was intended by exposing nymphs to low oxygen concentrations for 250 minutes to approximate to exposures of this sort which might be expected in the field.

TABLE 36

250-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT AT 20°C AND IN DIFFERENT WATER CURRENT SPEEDS

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals not moulting	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	< 0.2	open	214	8.0 9.0 10.0 11.0	> 10 (ambiguous)
laminar	1.0	2.6	187	2.5 3.0 3.5 4.0	3.1 (1.7 to 3.4)
laminar	2.0	2.6	143	1.5 2.0 2.5 3.0	1.8 (1.5 to 2.1)
laminar	4.0	2.6	201	1.0 1.5 2.0 2.5	1.2 (0.8 to 1.6)
turbulent	6.0	5.0	156	0.6 1.0 1.5 2.0	0.8 (0.5 to 1.2)
laminar	7.0	1.6	173	0.6 1.0 1.5 2.0	1.1 (0.8 to 1.3)
turbulent	8.0	5.0	168	0.6 1.0 1.5 2.0	0.9 (0.6 to 1.1)
laminar	12.5	1.2	171	0.4 0.6 1.0 1.5	0.9 (0.6 to 1.1)

TABLE 36 (cont)

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals not moulting	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	14.8	2.6	186	0.4 0.6 1.0 1.5	0.8 (0.6 to 1.0)
laminar	22.2	0.9	195	0.4 0.6 1.0 1.5	0.6 (0.5 to 0.8)
turbulent	22.2	2.6	159	0.4 0.6 1.0 1.5	0.6 (0.4 to 0.9)
turbulent	39.1	1.6	155	0.4 0.6 1.0 1.5	0.5 (0.2 to 0.8)
turbulent	58.6	1.6	139	0.4 0.6 1.0 1.5	0.6 (0.2 to 0.9)

As may be seen from table 36, nymphs not attempting ecdysis were found to be relatively tolerant of 250 minutes' exposure to low oxygen concentrations, especially in water flows exceeding 10 cm/sec. With decrease in water current speed below 10 cm/sec they were found to be increasingly much less tolerant of low oxygen concentrations. Several individuals survived 250 minutes in various concentrations in completely stagnant water, but a majority died even in the presence of 10 mg/l dissolved oxygen.

Median lethal low dissolved oxygen concentrations found for Baetis harrisoni nymphs not attempting ecdysis during a 1000 minute exposure are shown in table 37. These lethal concentrations were uniformly slightly higher than those for 250 minutes' exposure and were similarly related to water flow rate. As was the case for 250 minutes' exposure, no apparent differences in lethal dissolved oxygen concentrations at equivalent water current speeds in laminar and in turbulent flow were found.

TABLE 37

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT AT 20°C AND IN DIFFERENT WATER CURRENT SPEEDS

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals not moulting	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	<0.2	open	188	8.0 9.0 10.0 11.0	>10 (ambiguous)
laminar	1.0	2.6	179	3.0 3.5 4.0 5.0	4.0 (3.6 to 4.3)
laminar	2.0	2.6	195	2.0 2.5 3.0 3.5	2.6 (2.2 to 3.0)
laminar	4.0	2.6	153	1.5 2.0 2.5 3.0	2.1 (1.9 to 2.3)

TABLE 37 (cont)

Nature of flow	Current speed (cm/sec)	Tube diameter (cm)	Animals not moulting	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
turbulent	6.0	5.0	167	1.0 1.5 2.0 2.5	1.3 (0.8 to 1.8)
laminar	7.0	1.6	164	1.0 1.5 2.0 2.5	1.5 (1.3 to 1.8)
turbulent	8.0	5.0	178	0.6 1.0 1.5 2.0	1.3 (0.9 to 1.7)
laminar	12.5	1.2	165	0.6 1.0 1.5 2.0	1.2 (1.0 to 1.3)
turbulent	14.8	2.6	173	0.6 1.0 1.5 2.0	0.8 (0.5 to 1.2)
laminar	22.2	0.9	168	0.6 1.0 1.5 2.0	1.1 (0.7 to 1.4)
turbulent	22.2	2.6	178	0.6 1.0 1.5 2.0	0.8 (0.6 to 1.1)
turbulent	39.1	1.6	152	0.6 1.0 1.5 2.0	0.8 (0.6 to 1.0)
turbulent	58.6	1.6	176	0.6 1.0 1.5 2.0	0.9 (0.7 to 1.1)

NYMPHS OF DIFFERENT SIZES

In two further experiments, nymphs of different sizes were exposed for 1000 minutes to different low dissolved oxygen concentrations in the lethal range and the median lethal concentrations estimated as before for each. At the end of each experiment the length of each individual, excluding the antennae and cerci, was measured under a microscope. Nymphs of the larger of the three size groups distinguished were similar in size to those used in all other experiments. The results are shown in tables 38 and 39. The smallest Choroterpes bugandensis nymphs tested were found to be able to tolerate lower concentrations of dissolved oxygen than were the larger nymphs ($p \quad 0.025$). The smallest Baetis harrisoni nymphs in ecdysis were found to be significantly more tolerant of low oxygen concentrations than were the larger nymphs in a similar state. The largest Baetis nymphs in ecdysis were the least tolerant of all the nymphs tested. The median lethal low oxygen concentrations of Baetis nymphs of different sizes not attempting ecdysis during the experiment, on the other hand, were not found to differ significantly from one another.

ADAPTATION TO LOW OXYGEN

In two further experiments, the susceptibilities to low dissolved oxygen concentrations of nymphs were compared after they had previously been held in the laboratory in water containing only 4 mg/l oxygen and in water almost saturated with respect to oxygen. For each species, nymphs were collected and divided at random into two groups. Each group was held for 24 hours in a gauze tray suspended in an aquarium.

TABLE 38

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS OF DIFFERENT SIZES, ALL AT 20°C AND IN WATER FLOWING AT 10 cm/sec (LAMINAR FLOW, 1.6 cm DIAMETER TUBE)

Body length (mm)	Number of nymphs	Oxygen test concentration (mg/l)	Lethal oxygen concentration (mg/l, 95% confidence limits in brackets
1.6 to 2.9	180	0.3 0.4 0.5 0.6 0.7 0.8	0.48 (0.42 to 0.54)
3.0 to 4.4	180	0.3 0.4 0.5 0.6 0.7 0.8	0.54 (0.49 to 0.59)
4.5 to 6.0	180	0.3 0.4 0.5 0.6 0.7 0.8	0.55 (0.52 to 0.59)

In one of these aquaria the dissolved oxygen concentration was maintained at 4 mg/l, as has been described, by aeration with a suitable mixture of air and nitrogen. In the other the water was strongly aerated with air alone. Nymphs of each group were then exposed for 1000 minutes to low dissolved oxygen concentrations and median lethal levels estimated as before for each.

TABLE 39

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES, ATTEMPTING AND NOT ATTEMPTING ECDYSIS, ALL AT 20°C AND IN WATER FLOWING AT 10 cm/sec (LAMINAR FLOW, 1.6 cm DIAMETER TUBE)

Body length (mm)	Ecdysis	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
2.0 to 3.4	attempted	164	2.0 2.5 3.0 3.5	2.3 (1.8 to 2.8)
	not attempted	169	0.6 1.0 1.5 2.0	1.1 (0.6 to 1.5)
3.5 to 4.9	attempted	159	2.0 2.5 3.0 3.5	2.7 (2.3 to 3.0)
	not attempted	134	0.6 1.0 1.5 2.0	1.0 (0.7 to 1.4)
5.0 to 6.5	attempted	154	2.5 3.0 3.5 4.0	3.5 (3.0 to 4.0)
	not attempted	137	0.6 1.0 1.5 2.0	1.2 (1.0 to 1.4)

The results are shown in tables 40 and 41. None of this evidence suggests that previous exposure to low oxygen might have made nymphs of either species either more or less tolerant of low dissolved oxygen concentration.

TABLE 40

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS HELD IN EITHER 4 mg/l OR IN 7.5 mg/l DISSOLVED OXYGEN FOR 24 HOURS BEFORE THE EXPERIMENT, ALL AT 20°C AND 10 cm/sec WATER FLOW (LAMINAR FLOW, 1.6 cm DIAMETER TUBE)

Oxygen concentration before experiment (mg/l)	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
7.5	160	0.4 0.5 0.6 0.7	0.53 (0.50 to 0.56)
4	160	0.4 0.5 0.6 0.7	0.55 (0.50 to 0.60)

TABLE 41

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS HELD IN EITHER 4 mg/l OR IN 7.5 mg/l DISSOLVED OXYGEN FOR 24 HOURS BEFORE THE EXPERIMENT, ATTEMPTING AND NOT ATTEMPTING ECDYSIS DURING THE EXPERIMENT, ALL AT 20°C AND 10 cm/sec WATER FLOW (LAMINAR FLOW, 1.6 cm DIAMETER TUBE)

Oxygen concentration before experiment (mg/l)	Ecdysis	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
7.5	attempted	189	2.5 3.0 3.5 4.0	3.7 (3.4 to 4.1)
	not attempted	122	0.6 1.0 1.5 2.0	1.2 (1.0 to 1.5)

TABLE 41 (cont)

Oxygen concentration before experiment (mg/l)	Ecdysis	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
4	attempted	192	2.5 3.0 3.5 4.0	3.3 (3.1 to 3.6)
	not attempted	124	0.6 1.0 1.5 2.0	1.3 (1.0 to 1.5)

TIME OF YEAR

The experiments described so far were all carried out during winter. For comparison, nymphs of each species were also exposed to low concentrations of dissolved oxygen during summer. Median lethal concentrations estimated from these observations are shown in table 42, and may be seen to be of the same order as those recorded earlier. Neither species appeared from these data to differ in their oxygen tolerances at different times of the year. However, this is perhaps an over-simplification. It is conceivable that more detailed study taking into consideration the rates of oxygen uptake by summer and winter nymphs at different temperatures might reveal seasonal differences in dissolved oxygen requirements of these nymphs.

TABLE 42

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR SUMMER NYMPHS OF CHOROTERPES BUGANDENSIS AND BAETIS HARRISONI, ALL AT 20°C AND IN WATER FLOWING AT 10 cm/sec (LAMINAR FLOW, 1.6 cm DIAMETER TUBE)

Species	Ecdysis	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
Choroterpes bugandensis	mixed	160	0.4 0.5 0.6 0.7	0.57 (0.54 to 0.60)
Baetis harrisoni	attempted	160	2.5 3.0 3.5 4.0	3.7 (3.3 to 4.1)
	not attempted	148	0.6 1.0 1.5 2.0	1.3 (1.0 to 1.6)

OBSERVATIONS OF BAETIS IN THE FIELD

Baetis harrisoni have been found in South Africa to occur quite commonly and in large numbers in polluted rivers. This was first noted by Harrison (1958b). As has been mentioned, Allanson (1961) found dissolved oxygen concentrations to drop quite markedly at night in a river where nymphs of this species were common. Table 36 shows that oxygen concentrations of this order (3 mg/l) would be lethal for all nymphs, irrespective of physiological state, in stagnant water or in water of current speed less than 2 cm/sec. Table 35 shows that 3 mg/l oxygen would

also be lethal for nymphs attempting ecdysis during this period in water flows slower than about 12 cm/sec.

Field work conducted as part of the present study did not reveal any situation where Baetis harrisoni occured in lower concentrations of dissolved oxygen than those reported by Allanson. In fact, dissolved oxygen concentrations measured at various points in the Jukskei River during this study were generally higher than he reported, possibly because the river was no longer as badly polluted. A number of other streams was also visited during this study. Those in which lower dissolved oxygen concentrations were found were all both very slow flowing and relatively heavily polluted. None contained Baetis harrisoni. Since the cause of low dissolved oxygen concentrations in streams of reasonable flow might be expected almost invariably to be organic pollution, ammonia and other toxic substances associated with pollution of this sort are likely to be present. Survival of nymphs in these rivers might be influenced by these factors as well as by dissolved oxygen concentration.

On two occasions, one when relatively large numbers of Baetis harrisoni were present and another when a far smaller population was found, intensive counts of nymphs were made at a station in the Braamfontein Spruit. In a stretch of stream about 10 metres long, the stream being about 3 metres wide at this point, 312 suitable sampling points were numbered and classified according to nature of substratum (bare rock face, above and below large submerged and exposed rocks, on and among smaller stones, pebbles and gravel, and sand) and according to water current speed measured 5 cm above the substratum. Of available sites in each category the number required was

selected at random, as was a further number held in reserve in case actual water current speeds at any of the selected sites, taken after each count, differed too much from the speeds measured when the sites were originally selected. Sampling points were visited in turn starting at the lowest point downstream.

Counts were made using a Surber sampler (Surber 1936). This was described in an earlier section and is designed to collect the animals in one square foot (929 sq. cm) of stream bed. In practice it was found that the numbers enclosed within the one foot square frame could often be seen and could be counted directly. Where the animals could not all be seen easily they were dislodged and washed gently into the net.

The counts obtained are shown in tables 43 and 44. As might perhaps be expected, they were found to be very variable. However, they show very clearly that most nymphs were found on the upper surfaces of large rocks exposed to water flow. In this position the animals would not only have been exposed to the fastest flowing water but would also best have been able to extract oxygen from the water at low concentrations of dissolved oxygen.

OBSERVATIONS OF CHOROTERPES IN THE FIELD

The results of this study have shown that nymphs of Choroterpes bugandensis to be able to tolerate relatively low concentrations of dissolved oxygen under widely differing conditions of water flow. Several measurements of dissolved oxygen were made at different times in the Pienaars River and in other streams in which Choroterpes bugandensis occured. Low oxygen concentrations were encountered only very rarely. The lowest of these (2.2 mg/l)

TABLE 43

NUMBERS OF BAETIS HARRISONI NYMPHS COUNTED IN RANDOM ONE-FOOT (30.48 cm) SQUARES IN THE BRAAMFONTEIN SPRUIT AT A TIME WHEN NYMPHS WERE GENERALLY ABUNDANT

Current speed	Substratum					
	rock face	on rocks	under rocks	small stones	coarse gravel	sand
fast (40 to 100) cm/sec)	71	23	0			
	62	77	0			
	114	42	4			
	106	3	2			
	25	72	0	-	-	-
	77	34	6			
	51	43	1			
	60	10	0			
	506	299	13			
median (10 to 20 cm/sec)	98	61	0	12		
	56	24	0	26		
	48	24	1	25		
	46	75	17	6		
	9	71	2	48	-	-
	54	39	8	3		
	40	13	6	11		
	107	74	1	39		
	458	381	35	170		
slow (2 to 5 cm/sec)	20	10	2	39	20	4
	7	28	10	8	12	26
	37	34	0	8	37	12
	3	11	0	37	37	5
	14	11	0	15	22	16
	26	1	4	9	14	7
	4	36	7	16	9	7
	21	7	1	43	2	20
	132	138	24	175	124	97

TABLE 44

NUMBERS OF BAETIS HARRISONI NYMPHS COUNTED IN RANDOM ONE-FOOT (30.48 cm) SQUARES IN THE BRAAMFONTEIN SPRUIT AT A TIME WHEN RELATIVELY LOW NUMBERS OF NYMPHS WERE PRESENT

Current speed	Substratum					
	rock face	on rocks	under rocks	small stones	coarse gravel	sand
fast (40 to 100 cm/sec)	12	34	1			
	29	16	2			
	42	6	0			
	13	24	1			
	8	4	6	-	-	-
	1	22	0			
	34	4	6			
	24	35	5			
	163	145	21			
median (10 to 20 cm/sec)	0	9	0	0		
	3	0	0	3		
	0	1	0	1		
	2	1	0	0		
	9	24	0	0	-	-
	6	2	4	19		
	6	0	0	2		
	10	10	1	0		
	36	48	5	25		
slow (2 to 5 cm/sec)	0	17	0	4	0	0
	0	1	0	0	0	0
	1	5	0	0	0	0
	3	0	1	0	0	0
	1	0	0	1	0	0
	0	6	0	2	0	7
	0	2	0	0	0	0
	1	3	0	0	0	0
	6	34	1	7	0	7

was recorded at dawn in a stagnant pool isolated at a time of low stream flow. The bottom of this pool was fairly thickly blanketed with diatoms. Although low, however, this concentration was still well within the range tolerated by Choroterpes bugandensis.

DISCUSSION

The observation made here that the lethal low dissolved concentration was greatly influenced by the water flow rate in the case of Baetis harrisoni and that it was scarcely, if at all, influenced by water rate in the case of Choroterpes bugandensis is in general agreement with the findings of other authors. Effects of water current speed on both the lethal dissolved oxygen concentration and the rate of oxygen uptake of aquatic insects have been studied in some detail by Ambühl (1959). He found the minimal dissolved oxygen concentration for nymphs of a species of Baetis and for larvae of three Trichoptera to decrease with increasing water current speed, as was found here for Baetis harrisoni. The animals he found to be physiologically dependent upon water flow for their oxygen supply either had no gills or, like Baetis harrisoni, had gills that did not beat. The gills of Choroterpes, on the other hand, beat continuously and create quite swift currents around the animals, as has been described by Eastham (1937) for Ecdyonurus nymphs. The water currents created by the gills evidently cause the water around the nymphs to be replaced continually and in so doing to make oxygen more readily available to them in stagnant water.

Further evidence of the respiratory importance of ephemeropteran gills used for ventilation has been provided by Wingfield (1939). The gills

of Baetis nymphs do not beat and he found their removal not to affect the oxygen consumption of nymphs at any level of dissolved oxygen. The gills of Ephemera nymphs, in contrast, do beat and Wingfield found their removal to result in marked respiratory depression at all dissolved oxygen levels. The slightly increased lethal low dissolved oxygen concentration found for Choroterpes nymphs in stagnant water might possibly be ascribed to the fact that the gills only beat intermittently at low concentrations of dissolved oxygen in the lethal range.

Ambühl (1959) has argued that dependence by nymphs upon water currents for the renewal of oxygen in the water surrounding them might be expected to increase in proportion to their metabolic rates. His work confirmed the earlier observations of other authors (Berg 1952 has found exceptions) that most invertebrates from swift flowing water have significantly higher metabolic rates than do animals from slow flowing water. Thus Fox and Simmonds (1933) and Fox, Simmonds and Washbourn (1935) found nymphs of a species of Baetis from fast flowing water to take up oxygen at 3 to 4 times the rate at which did Cloeon nymphs from slow flowing water, and also to be much less tolerant of low dissolved oxygen concentrations.

Macan (1961c) has pointed out how little is really known of the oxygen requirements of aquatic insects. There is some evidence to suggest, however, that those insects that live in situations where they are exposed to fast water flows expend a great deal of energy in hanging on and moving about in the current. Zahner (1959) found, for instance, that nymphs of a species of the dragonfly genus Agrion were able to move about in oxygenated water flowing at 50 to 60 cm/sec, but were restricted to slower flowing

water at lower concentrations of dissolved oxygen. Within the range of current speeds from 0 to 3 cm/sec, Ambühl (1959) found the oxygen uptake rates of Baetis nymphs to increase apparently linearly in proportion to the current speed.

Work on fish has shown that where active metabolic rates have been reduced by low levels of dissolved oxygen, it is the amount of oxygen available to the animal for locomotion and other activities, the "scope for activity", which is reduced (Fry and Hart 1948, Graham 1949, Fry 1957). That the scope for activity of Choroterpes nymphs was reduced at dissolved oxygen concentrations just above the lethal level was illustrated by the notable reduction in gill movements under these circumstances. While animals will survive temporary exposures to oxygen levels at which their activity is restricted, they might be adversely affected by permanent conditions of this sort. Reduced scope for activity has been held to explain observations that at low dissolved oxygen concentrations the rate of development of fish was reduced (Garside 1959, Kinne and Kinne 1962), that some fish lost weight (Davison et al 1959) and that feeding and a number of other activities of a copepod were restricted (Malovitskaya 1961).

A notable feature of mortality of Baetis harrisoni nymphs at low oxygen levels in this study was the sensitivity to oxygen lack of nymphs in ecdysis. Why this should not also have applied to Choroterpes nymphs is not clear, but the increased sensitivity of Baetis presumably had something to do with increased oxygen requirements during ecdysis. Greatly increased metabolic rates during ecdysis have been demonstrated in insects, for instance by Zwicky and Wigglesworth (1956).

There seems every reason to believe the mortality or survival of Baetis harrisoni nymphs at different dissolved oxygen concentrations and rates of water flow to have been determined primarily by their metabolic oxygen requirements and by the rates of diffusion of oxygen to the nymphs under different conditions. It seems clear that their oxygen requirements were increased both at higher water current speeds and during ecdysis. The inverse relation found here between current speed and lethal oxygen concentration indicates that diffusion of oxygen to the nymphs increased sharply with increase in current speed. Regression analysis of the data shown in tables 35 and 37 has been undertaken with these facts in mind. 1000-minute minimal oxygen concentrations at different water current speeds for nymphs both in and out of ecdysis and in both laminar and turbulent flow are shown in figure 36. Regression lines of the form:

$$Y = a + bX - c\,(X)^{\frac{1}{2}}$$

where: Y represents the lethal oxygen concentration estimated from the regression line,

X represents the average current speed in the tube ($\bar{u}$),

and a, b and c are regression constants,

are shown in the figure for nymphs attempting ecdysis in both laminar and turbulent flow and for nymphs in laminar flow not attempting ecdysis.

These regression lines were estimated by a least-squares method described by Guest (1961). Each lethal oxygen value was assigned as a weighting coefficient the reciprocal of its variance. The lethal oxygen values estimated in stagnant water were ignored, since their variances

were not known. For reasons which will be explained in the next paragraph, and because this was found to be feasible, the estimates of the constants a and b in the regression equations for nymphs in ecdysis in laminar and in turbulent flow were pooled. It was not found to be possible to use pooled estimated for a and b in the regression equation for nymphs not in ecdysis. No regression analysis was undertaken for nymphs in turbulent flow not attempting ecdysis, since all except the first two lethal dissolved oxygen concentrations were virtually identical. The regression equations obtained were:

$$Y = 11.86 + 0.34\ X - 3.66\ (X)^{\frac{1}{2}}$$

for nymphs in laminar flow attempting ecdysis during the experiment,

$$Y = 11.86 + 0.34\ X - 3.56\ (X)^{\frac{1}{2}}$$

for nymphs in turbulent flow attempting ecdysis during the experiment and

$$Y = 6.69 + 0.24\ X - 2.04\ (X)^{\frac{1}{2}}$$

for nymphs in laminar flow not attempting ecdysis. χ^2 tests showed the deviations of observed values from the first two lines not to be significant, but revealed the third line to be a rather poor fit of the observed data ($p < 0.025$). Even better visual fits were obtained when the estimates of a and b in the first two equations were not pooled. This produced a rather more strongly curved first line and a rather more flattened second line than those figured.

The fact that the first two of these lines fitted the observed data is consistent with the theory that the minimal dissolved oxygen concentration required by Baetis nymphs was increased by one factor acting in proportion to the water flow rate and was simultaneously decreased by a

Figure 36

1000-minute median lethal low dissolved oxygen concentrations for Baetis harrisoni nymphs at different rates of water flow:

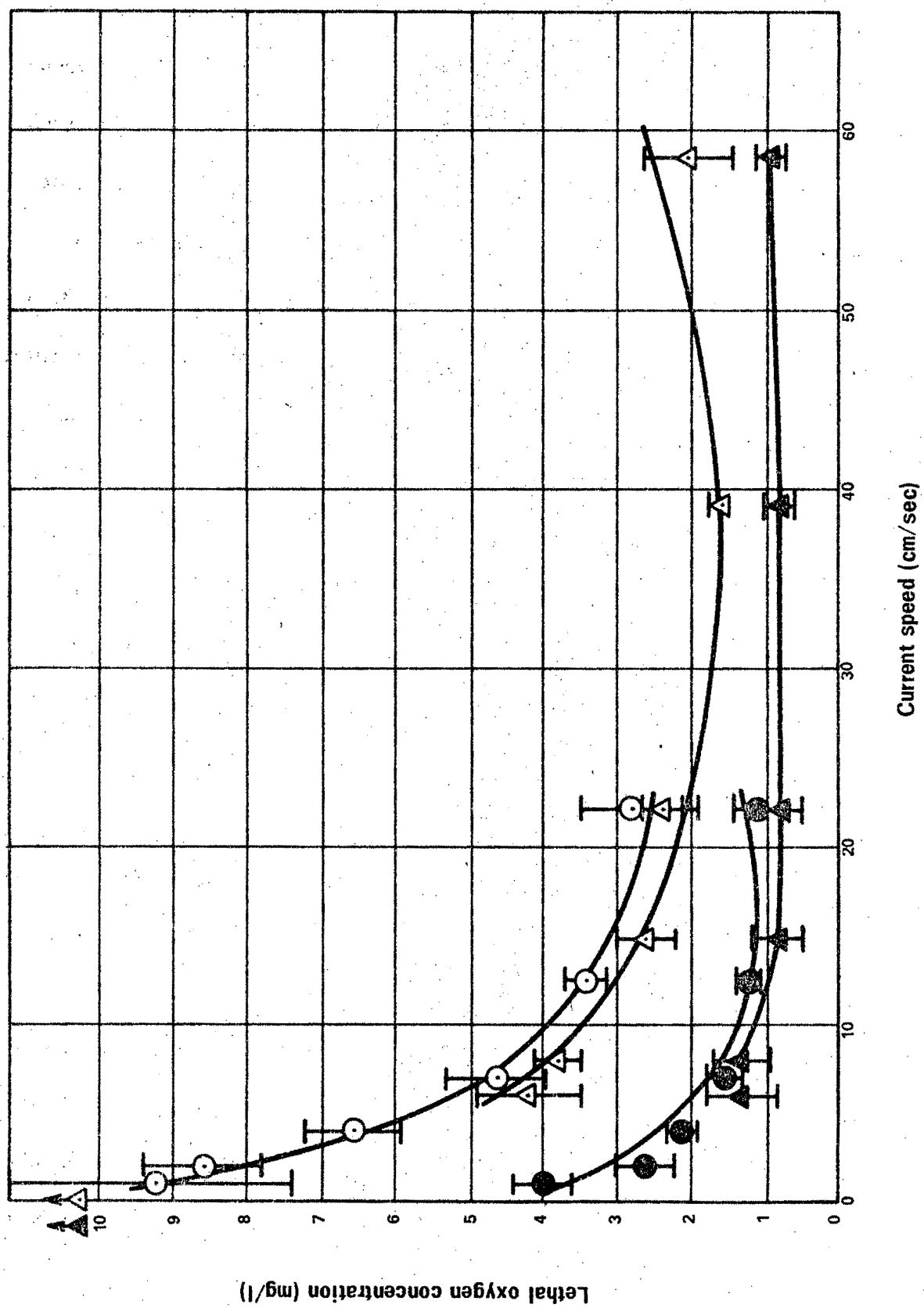
open circles - animals in laminar flow attempting ecdysis during the experiment, regression line $Y = 11.86+0.34X-3.66(X)^{\frac{1}{2}}$

open triangles - animals in turbulent flow attempting ecdysis during the experiment, regression line $Y = 11.86+0.34X-3.56(X)^{\frac{1}{2}}$

closed circles - animals in laminar flow not attempting ecdysis, regression line $Y = 6.69 + 0.24\ X - 2.04\ (X)^{\frac{1}{2}}$

closed triangles- animals in turbulent flow not attempting ecdysis, points joined by straight lines.

95% confidence limits shown about each median value.


Lethal oxygen concentration (mg/l)
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60
Current speed (cm/sec)

second factor acting in proportion to the square root of the water flow rate. The first factor is likely to have been the increase in metabolic oxygen requirements of nymphs with increase in current speed (Ambühl 1959), although this linear relation is probably a gross over-simplification of the true situation. The second factor is likely to have been the increase in diffusion of oxygen to the animals with increase in current speed. The diffusion of dissolved oxygen from flowing water to a stationary body in the stream flow depends on a large number of factors, among then the Reynolds number (Re), the shape of the object and the configuration of the boundary layer around it (Bird, Stewart and Lightfoot 1960). For a wide range of flow conditions, however, the rate of diffusion has been found to be proportional to the square root of the flow rate (Štráfelda 1960). The fact that the two lines could be fitted with pooled estimates for the constants a and b is consistent with the view that the lines differed only in the constant c, relating oxygen diffusion rate to water current speed.

Examination of these regression curves suggests that the minimal dissolved oxygen concentration required by Baetis nymphs might be increased not only at low rates of water flow but at very high rates of flow as well. The regression curve for nymphs in ecdysis suggests, for instance, that these nymphs might have been most tolerant of low dissolved oxygen concentrations at a current speed of around 35 to 40 cm/sec. However, the data shown in figure 36 is inconclusive on this point. If the lethal dissolved oxygen concentrations were increased they were certainly not increased by much.

Evidence will be presented in a later section of this report which suggests strongly that both Baetis and Choroterpes nymphs were able to distinguish between streams of water differing in dissolved oxygen content and to orientate themselves accordingly. If the gauze screens at the ends of each tube or perhaps the other nymphs in the tube disturbed the pattern of flow it is not altogether inconceivable that at least some of the active Baetis nymphs not in ecdysis might have been able to position themselves where flow conditions were slightly more favourable than they were elsewhere in the tube. Although there was no evidence in the present experiments to suggest this, the slight possibility should be borne in mind that the lethal dissolved oxygen concentrations estimated for nymphs in ecdysis might have been consistently low and might have appeared to be less influenced by water flow rate than they really were.

As has been found by other authors, smaller nymphs of both species were less tolerant of low dissolved oxygen levels than were larger nymphs. This is at first sight a little surprising, since the oxygen uptake rates of smaller mayfly nymphs are known to be higher in proportion to weight than are those of larger nymphs (Hilmy 1962). Only rather detailed analysis of the oxygen requirements during development would clarify this question.

Last instar nymphs were not included in any of the experiments reported here. These were found invariably to die in the experimental tubes soon after the start of the final moult. This applied to both species and has not been explained. During some of the experiments described here small numbers of final instar nymphs were held in open trays in the test tanks, where they were exposed to low oxygen concentrations.

Observations of their mortality appeared to indicate that the final instar nymphs were more sensitive to oxygen lack than were younger nymphs, but not very greatly so. All of the final instar nymphs seen to die at low oxygen levels appeared to have died during ecdysis. Once again it seems to be the increased oxygen needed for the final moult which makes these animals more susceptible.

The results described here have revealed Choroterpes nymphs which have been found mostly in relatively unpolluted streams and only occasionally in mildly polluted streams (see general introduction), to be significantly more tolerant of low oxygen concentrations than those of Baetis harrisoni, a species known to be tolerant of pollution (Harrison 1958b, Allanson 1961). However, an examination of the habitat of each has revealed that Choroterpes nymphs beneath stones in unpolluted streams might be exposed to lower dissolved oxygen concentrations than are Baetis nymphs on stones in swift flowing water in polluted streams.

In the course of a study of the ecology of certain mayfly nymphs which also live on the undersurfaces of stones in streams, Madsen (1968) measured both variations in dissolved oxygen concentration in the dead water among these stones, and rates of diffusion of oxygen to this dead water. He found low oxygen concentrations to occur quite commonly in the dead water as a result of slow diffusion of oxygen from the main stream. He concluded from his observations that only species which were fairly tolerant of occasional low dissolved oxygen concentrations would survive in these stream beds. Both Brundin (1951) and Moore and Burn (1968) have shown

that dissolved oxygen concentrations can vary tremendously from place to place within a body of water. They have also shown that these features in the micro-distribution of dissolved oxygen in relatively stagnant water are not only reflected in the patterns of distribution of benthic organisms, but are also of great importance in determining whether or not different species will survive times of general deoxygenation.

As important, perhaps, as these differences from place to place in a stream bed are variations in dissolved oxygen concentration which are known to occur with time. Hubault (1927), Butcher, Pentelow and Woodley (1930) and Allanson (1961), in particular, have described daily variations in dissolved oxygen in streams apparently arising as a result either of algal respiration or of intermittent discharge of organic effluents. Gunnerson (1964) has shown that oxygen sags of this sort can occur in streams at almost any time of day or night, a mass of poorly oxygenated water taking some time to travel downstream.

In the Jukskei River, at least at the time when the observations of Allanson (1961) were made, large numbers of Baetis harrisoni nymphs apparently lived at places where they were exposed during most nights to low dissolved oxygen concentrations. In fact, these low concentrations appear from the results of the present study to have been within the lethal range for nymphs undergoing ecdysis and for nymphs in relatively slow flowing water. No evidence was found in this study to suggest that ecdysis might be delayed under adverse conditions in order to provide nymphs with some protection against temporarily low oxygen levels, but only those

nymphs that happened to attempt ecdysis during this period would have been affected by the abnormally severe oxygen lack. The rest would have survived even quite severe deoxygenation if this was of short duration.

It is well known that the deoxygenating effects of organic matter are responsible in quite large measure for faunal changes observed to have been brought about by pollution in streams (Liebmann 1951, Hynes 1960). Presumed tolerances of low dissolved oxygen levels by different species have in fact frequently been equated with tolerances of polluted conditions in general in the assessment of pollution using faunal data (Bick 1963, Beak 1965). The complexities which can arise in such assessment in conditions of different water flow have been very neatly illustrated by Zimmermann (1961). He introduced controlled quantities of polluted water into long experimental channels inclined at different angles and found quite different distributions of invertebrate species in each. "Sensitive" species were able to colonize places in the swifter flowing water where the water quality was relatively poor in terms, for instance, of dissolved organic matter both because oxygenation of the water was improved and because dissolved oxygen was more readily available to the animals at faster current speeds.

The ability of Choroterpes bugandensis nymphs to tolerate low dissolved oxygen levels has been reasoned here to be a necessary adaptation to life under stones where they might be exposed to water of low oxygen content. However, their tolerance of low dissolved oxygen does not necessarily equip them for life in polluted streams. Here they might be exposed to water of even lower oxygen content, resulting perhaps from the accumulation of decomposable material between the stones in the stream bed.

Chutter (1967) has in fact shown that the presence of quite low levels of organic pollution can be revealed by the appearance in the fauna of stony runs of Tubificidae, Chironomidae and other animals normally found among accumulated organic matter in other biotopes.

Although Baetis harrisoni nymphs were really less tolerant of reduced dissolved oxygen than were nymphs of Choroterpes, they appear from the results of this study to be able to live in suitable situations on stones in swiftly flowing water in quite severely polluted streams in which quite low dissolved oxygen concentrations can occur.

SUMMARY

1. Choroterpes nymphs tolerated much lower concentrations of dissolved oxygen (the lethal limit for 1000 minutes' exposure being about 0.5 mg/l) than did Baetis nymphs (the lethal limit varying from above saturation to about 0.8 mg/l depending on water flow and the physiological state of the nymphs).

2. The lethal low dissolved oxygen concentration for Choroterpes nymphs was not notably affected by the rate or nature of water flow, but increased in stagnant water to 0.75 mg/l (for 1000 minutes' exposure).

3. Baetis nymphs all died in stagnant water but were increasingly tolerant of low oxygen concentrations with increase in the rate of water flow, more so in turbulent than in laminar flow (see figure 36).

4. Baetis nymphs were much less tolerant of low oxygen concentrations during ecdysis than at other times (see figure 36). Choroterpes nymphs were not found to be less tolerant during ecdysis.

5. Small Choroterpes nymphs were slightly more tolerant of low dissolved oxygen concentrations than were large nymphs (lethal limit 0.48 mg/l as opposed to 0.55 mg/l) Small Baetis nymphs in ecdysis were also more tolerant than were large nymphs in ecdysis (lethal limit in 10 cm/sec water flow 2.3 mg/l as opposed to 3.5 mg/l).
6. Previous exposure to low oxygen did not appear materially to affect the tolerance of either species.
7. Nymphs of both species at different times of year were found to be similarly tolerant of low dissolved oxygen.
8. The significance of this information in relation to the distribution of these nymphs is discussed.

MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT COMBINATIONS OF TEMPERATURE, DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

INTRODUCTION

Results reported in previous sections have shown nymphs of Choroterpes bugandensis and Baetis harrisoni to differ quite markedly in their tolerances of high temperatures and low dissolved oxygen concentrations. Choroterpes nymphs were found to be fairly tolerant of both factors and were seen to live in sluggish streams in which extremes of these factors might occur from time to time. Baetis harrisoni nymphs, on the other hand, were found not be able to tolerate either very high temperatures or very low dissolved oxygen concentrations. However, Baetis nymphs were found to be much more tolerant of these factors in faster flowing water than they were in very slow flowing water. Oxygen appeared to be more readily available to them in faster flowing water. It was reasoned that dependence upon water flow for an adequate supply of oxygen was an aspect of the physiological adaptation of this species to its habitat in swift flowing streams as has been found by Ambühl (1959) for other species of Baetis.

There is other published evidence to suggest that the effects of temperature, dissolved oxygen and water flow rate on the survival of mayfly nymphs might be interrelated. Pleskot (1953), for instance, has suggested on the basis of field observations that death of certain Ephemeroptera at high temperatures might be brought about by asphyxiation. In the experiments described here, nymphs of both Baetis harrisoni and Choroterpes bugandensis were exposed to different combinations of these three factors

and their combined effects on mortality and survival of each species observed.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected in the Braamfontein Spruit and Pienaars River as has been described elsewhere. Before being exposed to different combinations of the factors being investigated they were held for 24 hours in the laboratory in open gauze trays. The trays in turn were suspended in well aerated water either at the same temperature at which the experiments were to be carried out or, if the experiments were to be carried out at temperatures in the lethal range, at 20°C. The water in these gauze trays was kept in motion at all times.

The apparatus in which animals in 15 cm perspex experimental tubes were exposed in aquaria of water to controlled test conditions has also been described. The experiments described here were of factorial design. In the first series of experiments, groups of animals in each possible combination of three temperatures and four different flow conditions were each exposed to a number of suitable low oxygen concentrations. After 1000 minutes' exposure numbers of nymphs found not to recover were noted. The lethal low oxygen concentration for each combination of temperature and water current speed was estimated from these observations by probit analysis (Finney 1952). In the second series of experiments, groups of nymphs at each possible combination of these different dissolved oxygen concentrations and six different water flow conditions were each exposed to a number of suitable temperatures in the upper lethal range. From mortality observed after 1000 minutes the upper lethal temperature was calculated for each combination of dissolved

oxygen concentration and water current speed.

In order to be able to decide on the precise levels of each factor to be tested a number of preliminary experiments had first to be carried out. To make possible the calculation of median lethal oxygen or temperature levels, conditions had to be created in each test which would kill some but not all of the test animals.

In each experiment a large number of combinations of temperature, dissolved oxygen and water current speed had to be tested. Even when different current speeds were combined in the same tank by use of tubes of different diameters the number of combinations required greatly exceeded the number of the test tanks available. For this reason each experiment was carried out piecemeal on different days over several weeks. The combinations of temperature, oxygen and flow to be tested on each day were selected at random. Animals were collected separately for each day. Each combination of factors was tested at least twice on different days.

CHOROTERPES AND OXYGEN

Median lethal concentrations of dissolved oxygen for summer *Choroterpes bugandensis* nymphs at different combinations of temperature and water current speed are shown in table 45. As may be seen, nymphs of this species were found to be less tolerant of low oxygen concentrations at higher temperatures. However, although the differences between lethal low oxygen concentrations estimated at different temperatures were statistically significant they only amounted to about $0.1^{\circ}C$ to $0.3^{\circ}C$.

TABLE 45

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT COMBINATIONS OF TEMPERATURE AND WATER CURRENT SPEED

Temperature	Current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
15°C	<0.2	turbulent	open	160	0.3 0.4 0.5 0.6	0.53 (0.49 to 0.57)
	2.6	laminar	2.6	160	0.3 0.4 0.5 0.6	0.47 (0.45 to 0.49)
	12.0	laminar	1.2	160	0.3 0.4 0.5 0.6	0.48 (0.45 to 0.51)
	12.0	turbulent	2.6	160	0.3 0.4 0.5 0.6	0.44 (0.40 to 0.48)
20°C	< 0.2	turbulent	open	157	0.4 0.5 0.6 0.7	0.60 (0.56 to 0.64)
	2.6	laminar	2.6	160	0.4 0.5 0.6 0.7	0.53 (0.50 to 0.56)
	12.0	laminar	1.2	160	0.4 0.5 0.6 0.7	0.55 (0.53 to 0.57)
	12.0	turbulent	2.6	160	0.4 0.5 0.6 0.7	0.55 (0.53 to 0.57)

TABLE 45 (cont)

Temperature	Current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
25°C	< 0.2	turbulent	open	160	0.5 0.6 0.7 0.8	0.72 (0.69 to 0.75)
	2.6	laminar	2.6	159	0.5 0.6 0.7 0.8	0.69 (0.66 to 0.72)
	12.0	laminar	1.2	160	0.5 0.6 0.7 0.8	0.71 (0.68 to 0.74)
	12.0	turbulent	2.6	160	0.5 0.6 0.7 0.8	0.70 (0.67 to 0.73)

BAETIS AND OXYGEN

In the experiments involving Baetis harrisoni, many of the animals found dead at the end of each exposure to a combination of temperature and oxygen concentration were found to have died during ecdysis, as has been described in previous sections of this report. The symptoms these individuals showed, such as a loose outer skin, a split between the wingbuds, thoracic flexion, incomplete ecdysis and so forth, have been described in the section dealing with high temperature tolerance. As before, it was established at the end of each experiment which of the dead and living animals had either moulted or had attempted ecdysis during exposure to the

test conditions and which had not. Numbers dead and alive in these two groups were counted and lethal oxygen concentrations calculated separately for animals in and out of ecdysis. The experiment described here was carried out during summer.

Some mortality of nymphs attempting ecdysis was also observed among nymphs held at non-lethal oxygen concentrations while these experiments were being carried out. In a previous section this mortality of ecdysing nymphs not caused by the lethal factor being tested was reasoned possibly to have been caused by handling of nymphs which had already started to moult. This mortality was treated statistically as "natural" mortality and the calculation of median lethal levels for nymphs in ecdysis balanced and compensated for this mortality in each instance (Finney 1952).

Median lethal low dissolved oxygen concentrations, respectively for animals in and out of ecdysis, are shown in tables 46 and 47 for different combinations of temperature and water current speed. These results are further illustrated in figure 37 for animals in ecdysis in laminar flow, figure 38 for animals in ecdysis in turbulent flow, figure 39 for animals in laminar flow not attempting ecdysis and figure 40 for animals in turbulent flow not attempting ecdysis. As was shown in the previous section, the dissolved oxygen requirements of Baetis nymphs were influenced most strikingly by the water current speed. In addition, minimal dissolved oxygen requirements of nymphs were found in almost all instances to be reduced at higher temperatures.

TABLE 46

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS AT DIFFERENT COMBINATIONS OF TEMPERATURE AND WATER CURRENT SPEED FOR SUMMER BAETIS HARRISONI NYMPHS IN ECDYSIS

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
15°C	2.7	laminar	2.6	124	5.0 6.0 7.0 8.0	6.3 (5.8 to 6.8)
	7.0	laminar	1.6	135	3.0 3.5 4.0 5.0	4.4 (4.0 to 4.7)
	22.2	laminar	0.9	157	2.0 2.5 3.0 3.5	2.3 (2.1 to 2.6)
	8.0	turbulent	5.0	142	3.0 3.5 4.0 5.0	3.2 (2.9 to 3.6)
	14.8	turbulent	2.6	161	1.5 2.0 2.5 3.0	2.0 (1.8 to 2.2)
	39.1	turbulent	1.6	142	1.0 1.5 2.0 2.5	1.4 (1.3 to 1.6)
20°C	2.7	laminar	2.6	178	5.0 6.0 7.0 8.0	7.0 (6.5 to 7.6)
	7.0	laminar	1.6	152	3.5 4.0 5.0 6.0	4.4 (4.0 to 4.9)

TABLE 46 (cont)

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
20°C	22.2	laminar	0.9	157	2.0 2.5 3.0 3.5	2.3 (2.0 to 2.6)
	8.0	turbulent	5.0	158	2.5 3.0 3.5 4.0	3.6 (3.2 to 3.9)
	14.8	turbulent	2.6	169	1.5 2.0 2.5 3.0	2.7 (2.4 to 3.0)
	39.1	turbulent	1.6	160	1.0 1.5 2.0 2.5	1.5 (1.4 to 1.6)
25°C	2.7	laminar	2.6	243	5.0 6.0 7.0 8.0	> 8
	7.0	laminar	1.6	280	4.0 5.0 6.0 7.0	5.4 (5.0 to 5.7)
	22.2	laminar	0.9	239	2.0 2.5 3.0 3.5	2.8 (2.5 to 3.1)
	8.0	turbulent	5.0	265	3.0 3.5 4.0 5.0	4.3 (4.0 to 4.6)
	14.8	turbulent	2.6	234	2.0 2.5 3.0 3.5	2.9 (2.7 to 3.1)

TABLE 46 (cont)

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
25°C	39.1	turbulent	1.6	271	1.0 1.5 2.0 2.5	1.9 (1.7 to 2.1)

TABLE 47

1000-MINUTE MEDIAN LETHAL LOW DISSOLVED OXYGEN CONCENTRATIONS AT DIFFERENT COMBINATIONS OF TEMPERATURE AND WATER CURRENT SPEED FOR SUMMER BAETIS HARRISONI NYMPHS NOT ATTEMPTING ECDYSIS

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
15°C	2.7	laminar	2.6	138	0.6 1.0 1.5 2.0	1.1 (0.9 to 1.2)
	7.0	laminar	1.6	164	0.4 0.6 1.0 1.5	0.7 (0.6 to 0.8)
	22.0	laminar	0.9	143	0.4 0.6 1.0 1.5	0.6 (0.5 to 0.8)
	8.0	turbulent	5.0	159	0.4 0.6 1.0 1.5	0.9 (0.8 to 1.0)

TABLE 47 (cont)

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
15°C	14.8	turbulent	2.6	148	0.4 0.6 1.0 1.5	0.7 (0.6 to 0.9)
	39.1	turbulent	1.6	148	0.4 0.6 1.0 1.5	0.6 (0.6 to 0.7)
20°C	2.7	laminar	2.6	177	1.0 1.5 2.0 2.5	1.6 (1.4 to 1.7)
	7.0	laminar	1.6	137	0.6 1.0 1.5 2.0	1.1 (0.9 to 1.3)
	22.2	laminar	0.9	168	0.4 0.6 1.0 1.5	0.8 (0.7 to 0.9)
	8.0	turbulent	5.0	157	0.4 0.6 1.0 1.5	0.9 (0.8 to 0.9)
	14.8	turbulent	2.6	141	0.4 0.6 1.0 1.5	0.8 (0.7 to 1.0)
	39.1	turbulent	1.6	167	0.4 0.6 1.0 1.5	0.6 (0.5 to 0.7)

TABLE 47 (cont)

Temperature	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Animals attempting ecdysis	Oxygen test concentrations (mg/l)	Lethal oxygen concentration (mg/l), 95% confidence limits in brackets
25°C	2.7	laminar	2.6	132	1.5 2.0 2.5 3.0	2.3 (2.1 to 2.4)
	7.0	laminar	1.6	144	0.6 1.0 1.5 2.0	1.4 (1.3 to 1.5)
	22.2	laminar	0.9	140	0.6 1.0 1.5 2.0	1.0 (0.9 to 1.1)
	8.0	turbulent	5.0	108	0.4 0.6 1.0 1.5	0.9 (0.8 to 0.9)
	14.8	turbulent	2.6	125	0.4 0.6 1.0 1.5	1.0 (0.9 to 1.1)
	39.1	turbulent	1.6	123	0.4 0.6 1.0 1.5	0.9 (0.8 to 1.0)

It was shown in the previous section that regression lines of the form:

$$Y = a + b X - c(X)^{\frac{1}{2}}$$

where : Y represents the "expected" lethal low dissolved oxygen concentration,

X represents the water current speed, and

a, b and c are regression coefficients

Figure 37

1000-minute median lethal low dissolved oxygen concentrations for summer Baetis harrisoni nymphs in laminar flow attempting ecdysis, in different water current speeds and at the following temperatures:

squares - $25^{o}C$

regression line $Y = 13.48 + 0.45 X - 4.34 (X)^{\frac{1}{2}}$

circles - $20^{o}C$

regression line $Y = 12.79 + 0.45 X - 4.34 (X)^{\frac{1}{2}}$

triangles $15^{o}C$

regression line $Y = 12.55 + 0.45 X - 4.34 (X)^{\frac{1}{2}}$.

95% confidence limits shown for each median value.

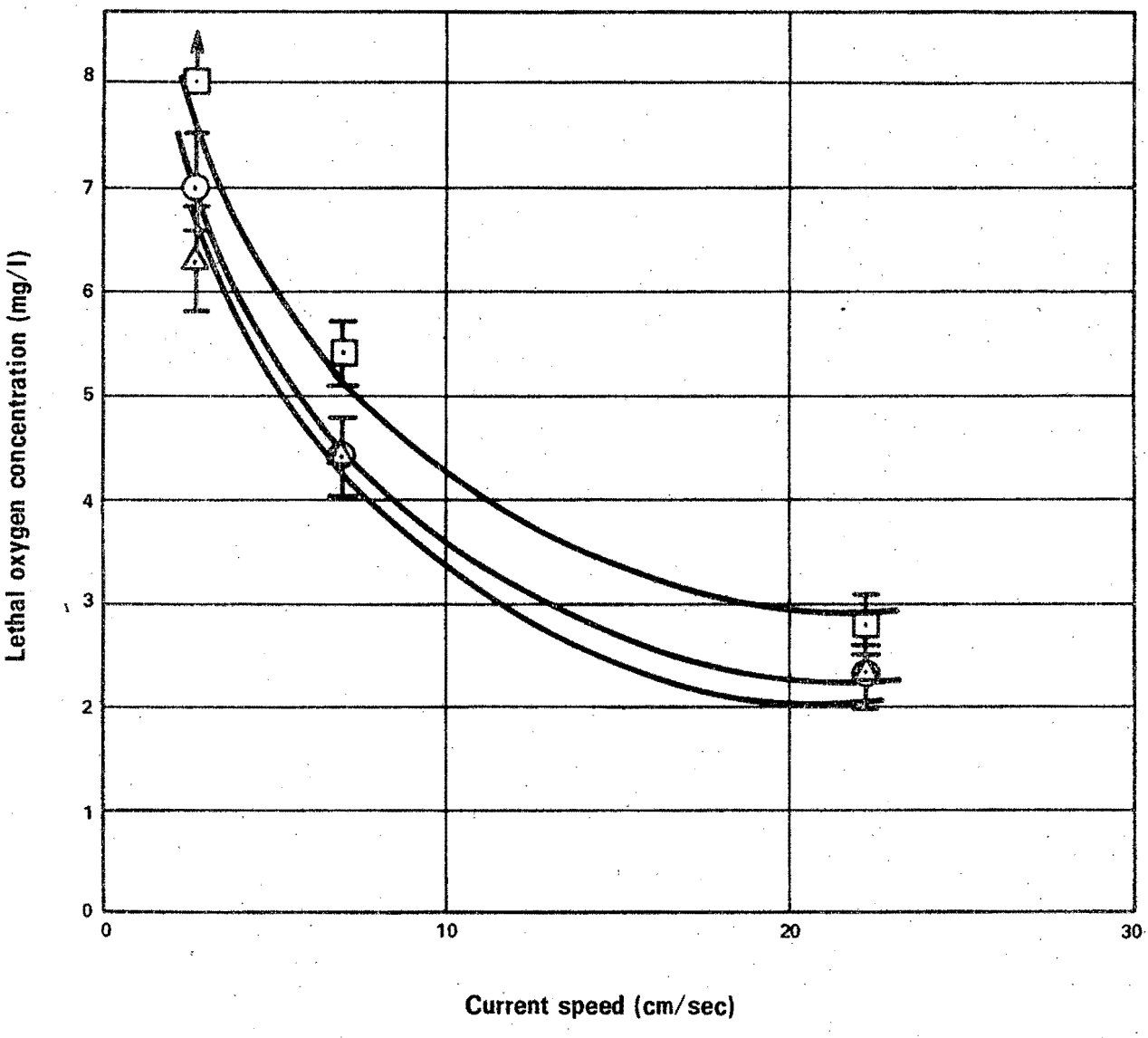

Lethal oxygen concentration (mg/l)
0
1
2
3
4
5
6
7
8
0
10
20
30
Current speed (cm/sec)

Figure 38

1000-minute median lethal low dissolved oxygen concentrations for summer Baetis harrisoni nymphs in turbulent flow attempting ecdysis, in different water current speeds and at the following temperatures:

squares - 25°C

circles - 20°C

triangles - 15°C.

95% confidence limits shown about each median value.

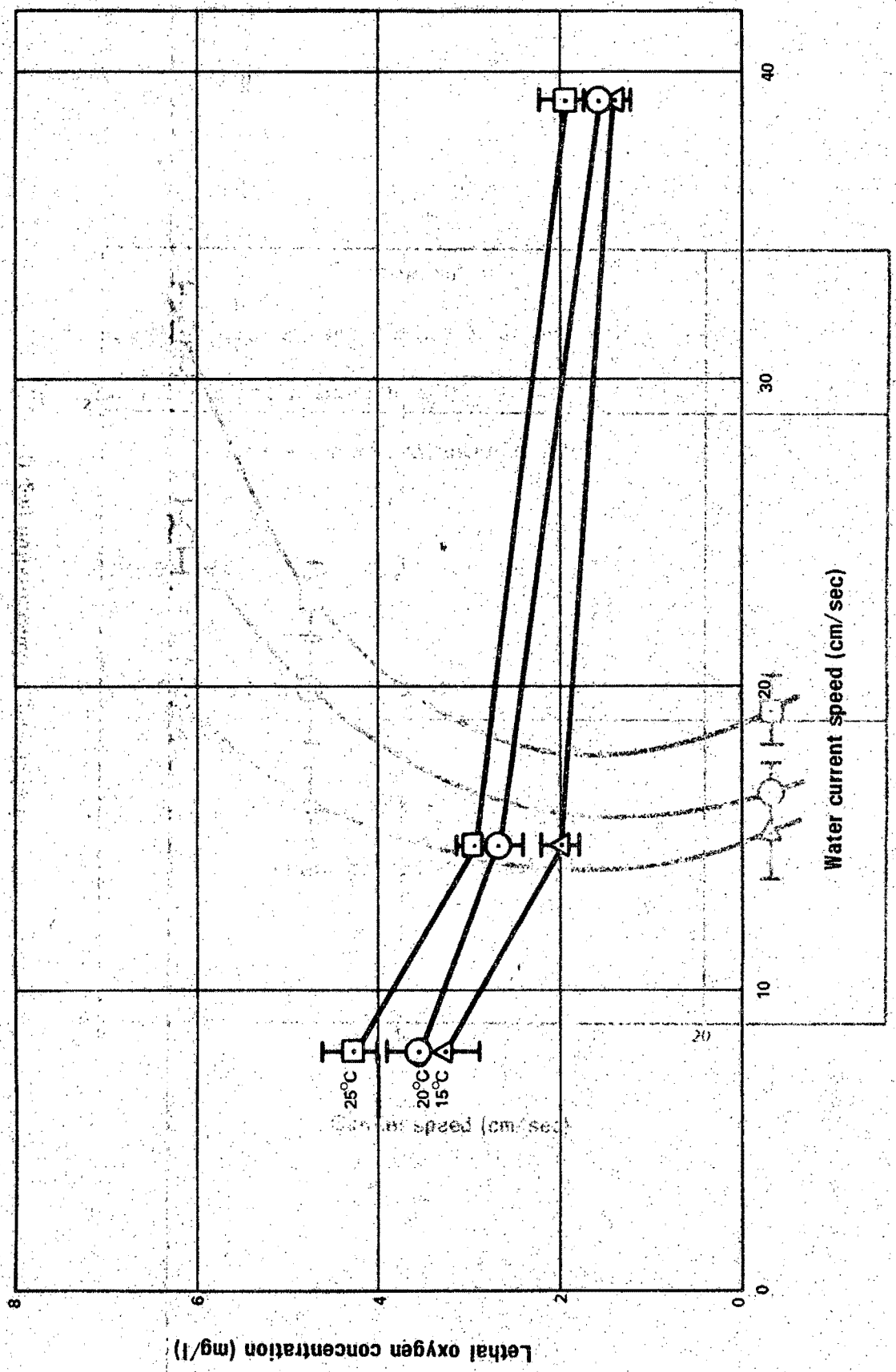

Lethal oxygen concentration (mg/l)
0
2
4
6
8
10
20
30
40
Water current speed (cm/sec)
25°C
20°C
15°C

Figure 39

1000-minute median lethal low dissolved oxygen concentrations for summer Baetis harrisoni nymphs in laminar flow not attempting ecdysis, in different water current speeds and at the following temperatures:

squares - $25^{o}C$

fitted line $Y = 4.74 + 0.22\ X - 1.85\ (X)^{\frac{1}{2}}$

circles - $20^{o}C$

fitted line $Y = 2.80 + 0.16\ X - 1.00\ (X)^{\frac{1}{2}}$

triangles - $15^{o}C$

fitted line $Y = 2.26 + 0.11\ X - 0.89\ (X)^{\frac{1}{2}}$.

95% confidence limits shown for each median value.

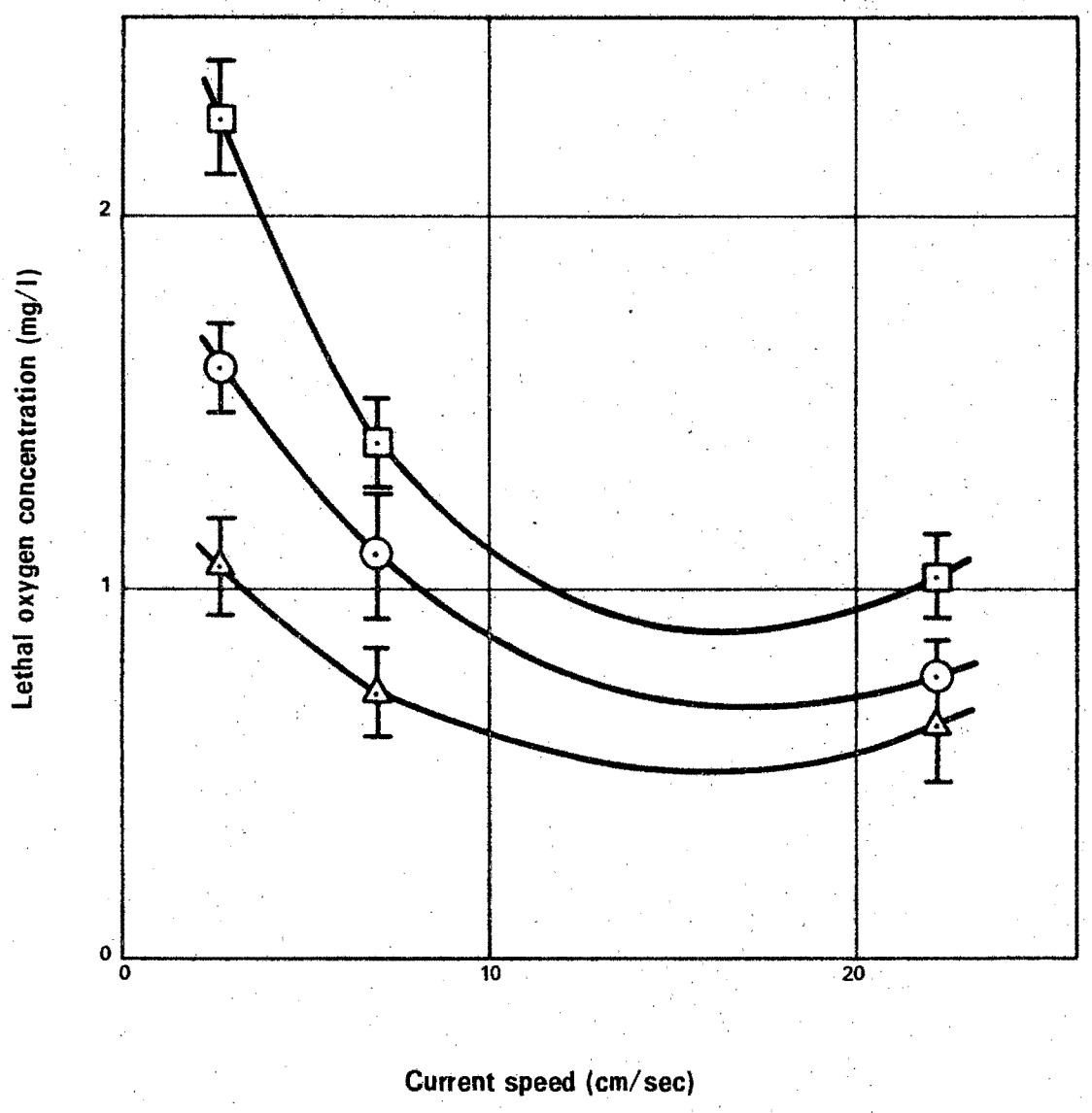

Lethal oxygen concentration (mg/l)
2
1
0
0
10
20
Current speed (cm/sec)

Figure 40

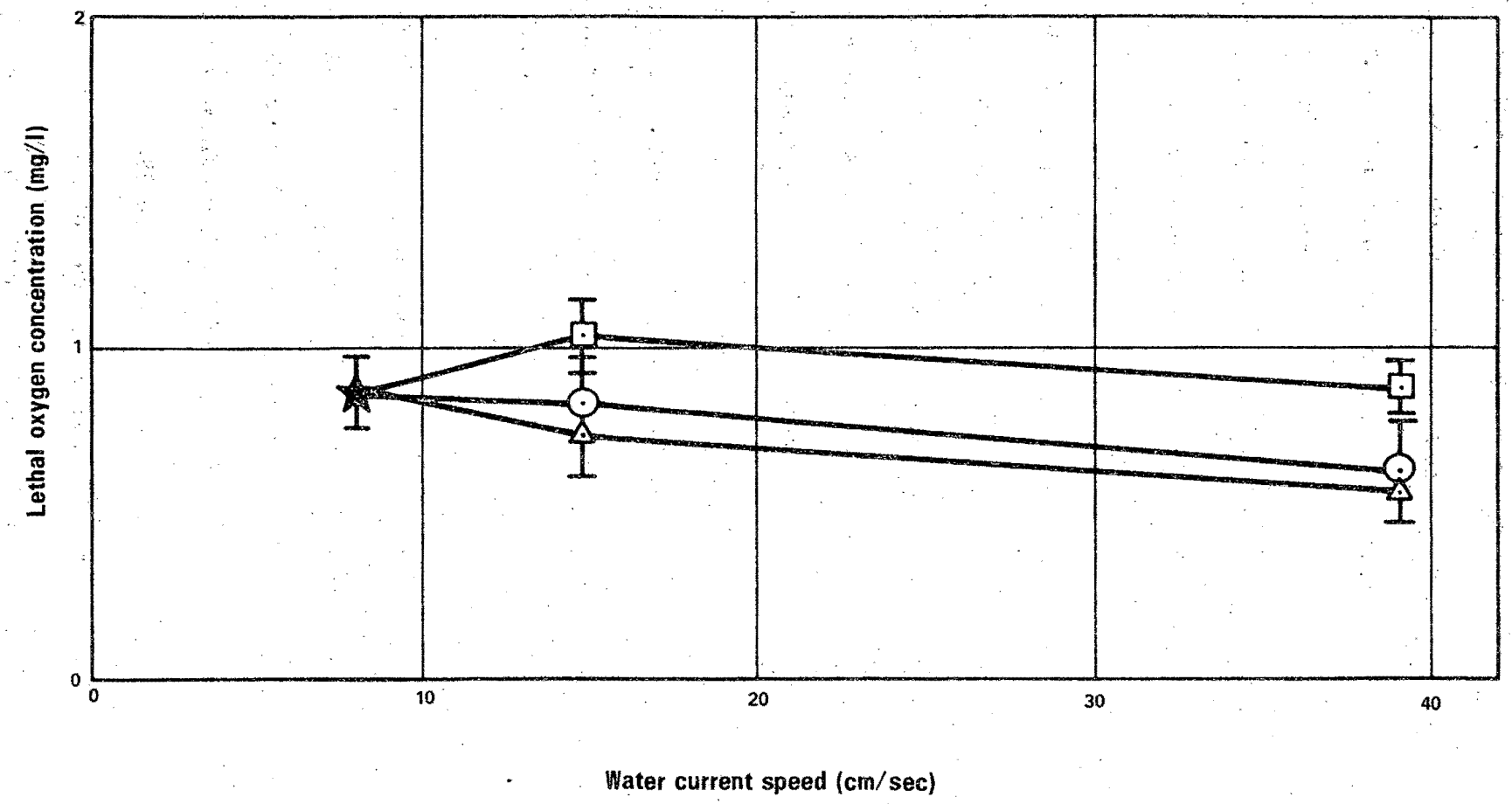
1000-minute median lethal low dissolved oxygen concentrations for summer Baetis harrisoni nymphs in turbulent flow not attempting ecdysis, in different water current speeds and at the following temperatures:

squares - 25°C

circles - 20°C

triangles - 15°C.

95% confidence limits shown about each median value.


Lethal oxygen concentration (mg/l)
2
1
0
0
10
20
30
40
Water current speed (cm/sec)

in most instances provided a satisfactory description of the observed relation between water current speed and the minimal dissolved oxygen requirements of Baetis nymphs. Lines of this form have been fitted to the data shown in figure 37, the estimates of b and c being pooled in the three equations. The lethal dissolved oxygen concentrations for nymphs in laminar flow attempting ecdysis shown in figure 37 were significantly higher at 25°C than they were at either 15°C or 20°C. At only one of the current speeds tested was the lethal dissolved oxygen concentration at 20°C higher than that at 15°C ($p < 0.05$). The value of a in the three regression equations increased with increasing temperature. No realistic analysis of the mathematical relation between experimental temperature and minimal dissolved oxygen requirements of the nymphs is possible from the data available without detailed knowledge of the metabolic oxygen requirements of nymphs, which is not available. These requirements must presumably be greatly influenced both by the temperatures at which the nymphs had been living and by recent changes in temperature to which they had been exposed (Fry 1964).

Regression equations of the form shown in figure 37 can be fitted individually to each set of three points at the same temperature in figures 38 and 39. However, the fit of these lines is extremely poor when pooled estimates of the regression constants are used. Lines fitted to each set of three points are shown in figure 39 but not in figure 38. Reasonable approximations to the data shown in figure 40 were not obtained by regression analysis. The lines shown in figure 39 are unacceptable because they suggest that lowest lethal dissolved oxygen concentrations might be found at

a current speed of around 16 cm/sec and that the dissolved oxygen requirements of nymphs might increase rapidly with increase in current speed over about 20 cm/sec, for which there is no direct evidence. These data do not differ greatly from those shown in figure 36 of the previous section. In the discussion of figure 36 in the previous section it was suggested that the points comparable to those shown in figure 37 might possibly have been uniformly lower than they ought to have been. This makes interpretation of the regression lines difficult.

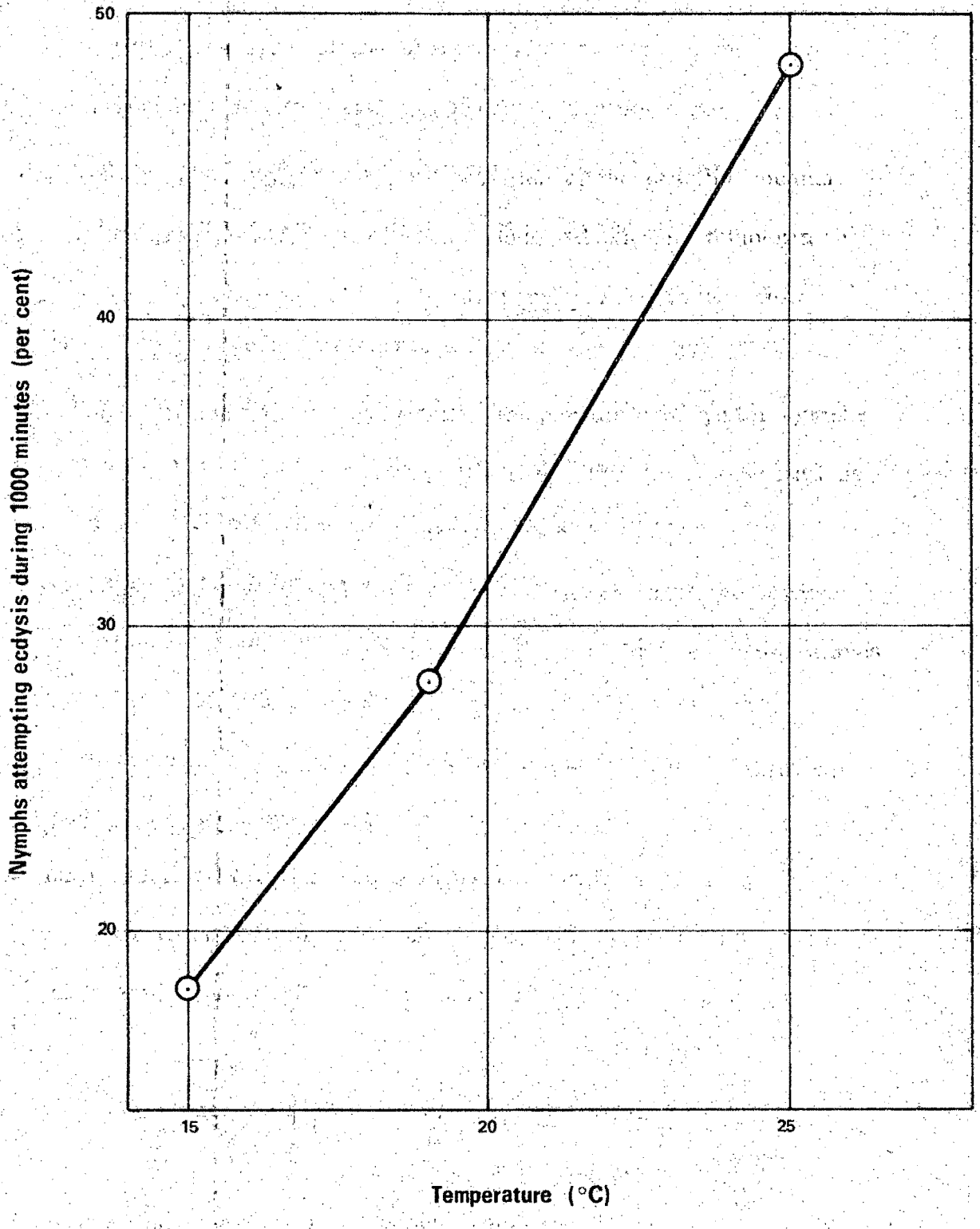
No other acceptable regression lines could be found to fit the data shown in figures 38, 39 and 40. In all, the lethal dissolved oxygen concentrations estimated were highest at 25°C and lowest at 15°C, presumably because the metabolic oxygen requirements of nymphs increased with increasing temperature. Figures 38 and 39 suggest that the influence of water temperature on the lethal dissolved oxygen concentration was greatest in slow flowing water.

One result of the experiments described here is not reflected either in the tables or in figures 37, 38, 39 and 40. Although the median lethal low dissolved oxygen concentrations estimated at different temperatures did not differ very greatly, many more nymphs were found to moult at higher temperatures. Because nymphs attempting ecdysis were less tolerant of low dissolved oxygen levels than were nymphs not attempting ecdysis, the total mortality rate increased very markedly with increase in temperature.

The influence of temperature on the relative numbers of summer Baetis harrisoni nymphs attempting ecdysis is illustrated in figure 41. Numbers of nymphs were collected and distributed at random among gauze

Figure 41

Numbers of summer Baetis harrisoni nymphs attempting ecdysis during 1000 minutes in the laboratory at different temperatures (the results expressed per cent)


50
40
30
20
Nymphs attempting ecdysis during 1000 minutes (per cent)
15
20
25
Temperature (°C)

trays suspended in aquaria in which the water temperature was maintained at 15°C, 20°C and 25°C. Algal food was supplied and the water kept in motion. After a day the nymphs were transferred to clean gauze trays in water at the same temperature. The water was again kept in motion but food was not supplied. After 1000 minutes the animals were removed and the number of nymphs that had either moulted or had begun to moult in each tray was established.

The fact that differences both in the lethal low dissolved oxygen concentration for nymphs in and out of ecdysis and in the relative numbers of nymphs attempting ecdysis influenced the total mortality rate in low dissolved oxygen concentrations at 15°C, 20°C and 25°C is illustrated in figures 42, 43, 44 and 45. Figures 42, 43 and 44 show mortality (in ecdysis, out of ecdysis and total) for experimental groups held at different low dissolved oxygen concentrations in a laminar flow of 7 cm/sec at 15°, 20°C and 25°C. Each of these three figures is plotted on probability paper, with ordinate values shown per cent but the ordinate in fact being a probit scale. The straight lines A and B in each figure are fitted probit mortality lines, respectively for nymphs out of ecdysis and in ecdysis, from which the median lethal values shown in tables 46 and 47 were calculated. The sinuate line C in each figure was calculated as:

$$Y_C \text{ (probit)} = \text{probit}\left[Y_A \text{(per cent)} + EY_B \text{ (per cent)}\right]$$

for each dissolved oxygen concentration (X), where:

Y_C is the "expected" total mortality, here plotted on a probit scale,

Y_A and Y_B represent "expected" mortalities respectively out of

ecdysis and in ecdysis, calculated in probits from the probit lines A and B and here converted to values per cent,

E is the proportion of nymphs expected to attempt ecdysis at the test temperature (from figure 41).

A χ^2 test has shown the agreement between observed total mortality and "expected" Y_C to be surprisingly good in each instance. Similar figures drawn for the other tests summarized in tables 46 and 47 but not shown here also showed a reasonable fit between observed total mortality and Y_C.

Empirical estimates of the low dissolved oxygen concentrations expected to cause a total of 50 per cent of animals to die after 1000 minutes' exposure may be read off each line C in figures 42, 43 and 44. In figure 45 these are compared with the median lethal values for nymphs in and out of ecdysis. As may be seen, the median lethal levels for the total population increase more rapidly with increasing temperature than do the median lethal levels for nymphs either in or out of ecdysis. Comparison with figures 42, 43 and 44 shows the increased total mortality at high temperatures to have resulted from the fact that more nymphs attempted ecdysis.

CHOROTERPES AND TEMPERATURE

Median upper lethal temperatures for summer Choroterpes buganden-sis at three different dissolved oxygen concentrations are shown in table 48. This experiment was also carried out piecemeal over several days. Tubes of internal diameter 2.6 cm were used, through which a water current speed of 15 cm/sec was maintained, at which speed the flow was turbulent. As

Figure 42

Mortality of summer Baetis harrisoni nymphs at different low dissolved oxygen concentrations at 15°C in laminar water flow of 7 cm/sec:

open circles and line A - nymphs not in ecdysis

closed circles and line B - nymphs in ecdysis

shaded circles and line C- total.

For explanation see text.

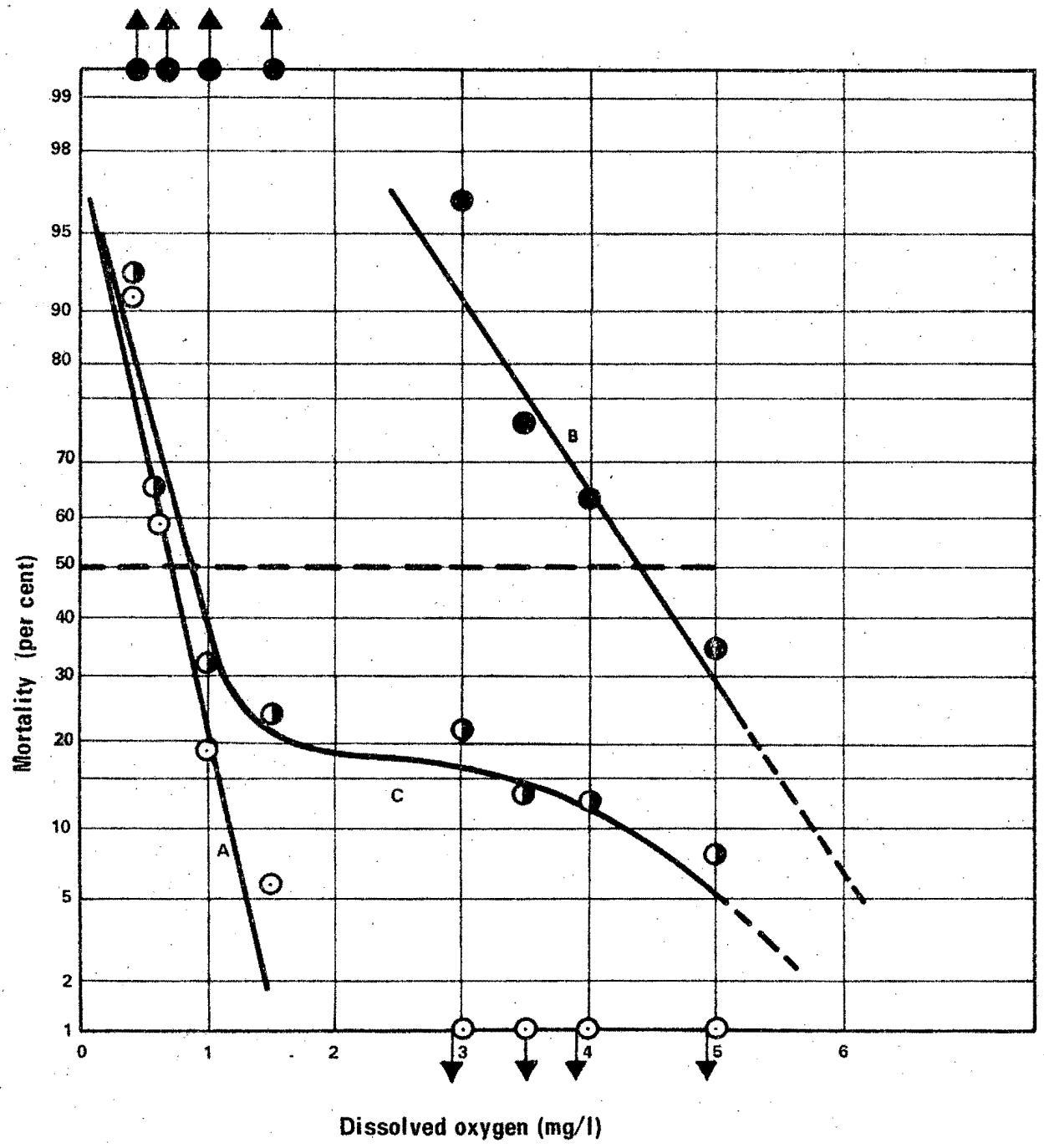

99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
Mortality (per cent)
0
1
2
3
4
5
6
A
B
C
Dissolved oxygen (mg/l)

Figure 43

Mortality of summer Baetis harrisoni nymphs at different low dissolved oxygen concentrations at 20°C in laminar water flow of 7 cm/sec:

open circles and line A - nymphs not in ecdysis

closed circles and line B - nymphs in ecdysis

shaded circles and line C - total.

For explanation see text.

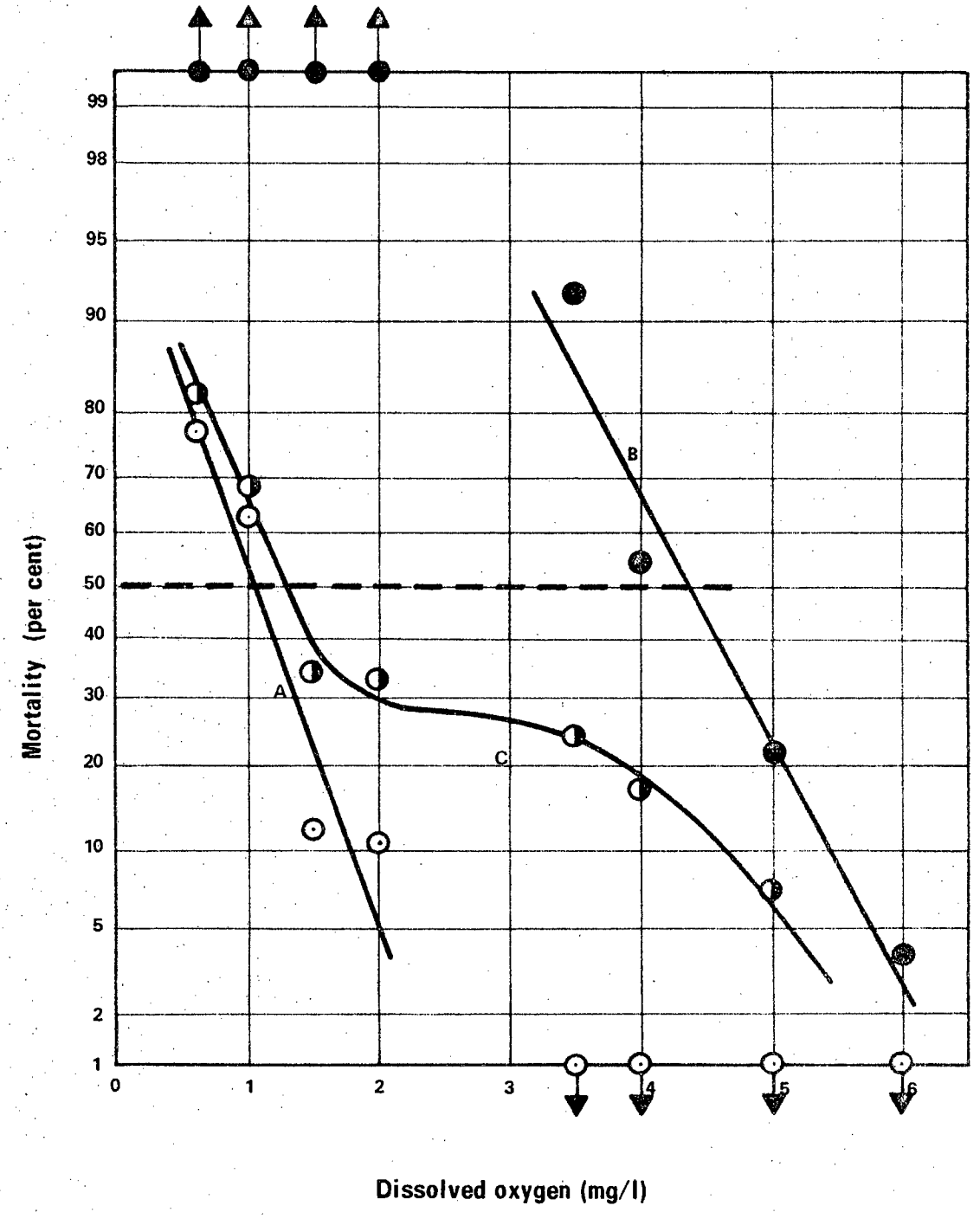

Mortality (per cent)
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
1
2
3
4
5
6
A
B
C
Dissolved oxygen (mg/l)

Figure 44

Mortality of summer Baetis harrisoni nymphs at different low dissolved oxygen concentrations at 25°C in laminar water flow of 7 cm/sec:

open circles and line A - nymphs not in ecdysis

closed circles and line B - nymphs in ecdysis

shaded circles and line C - total.

For explanation see text.

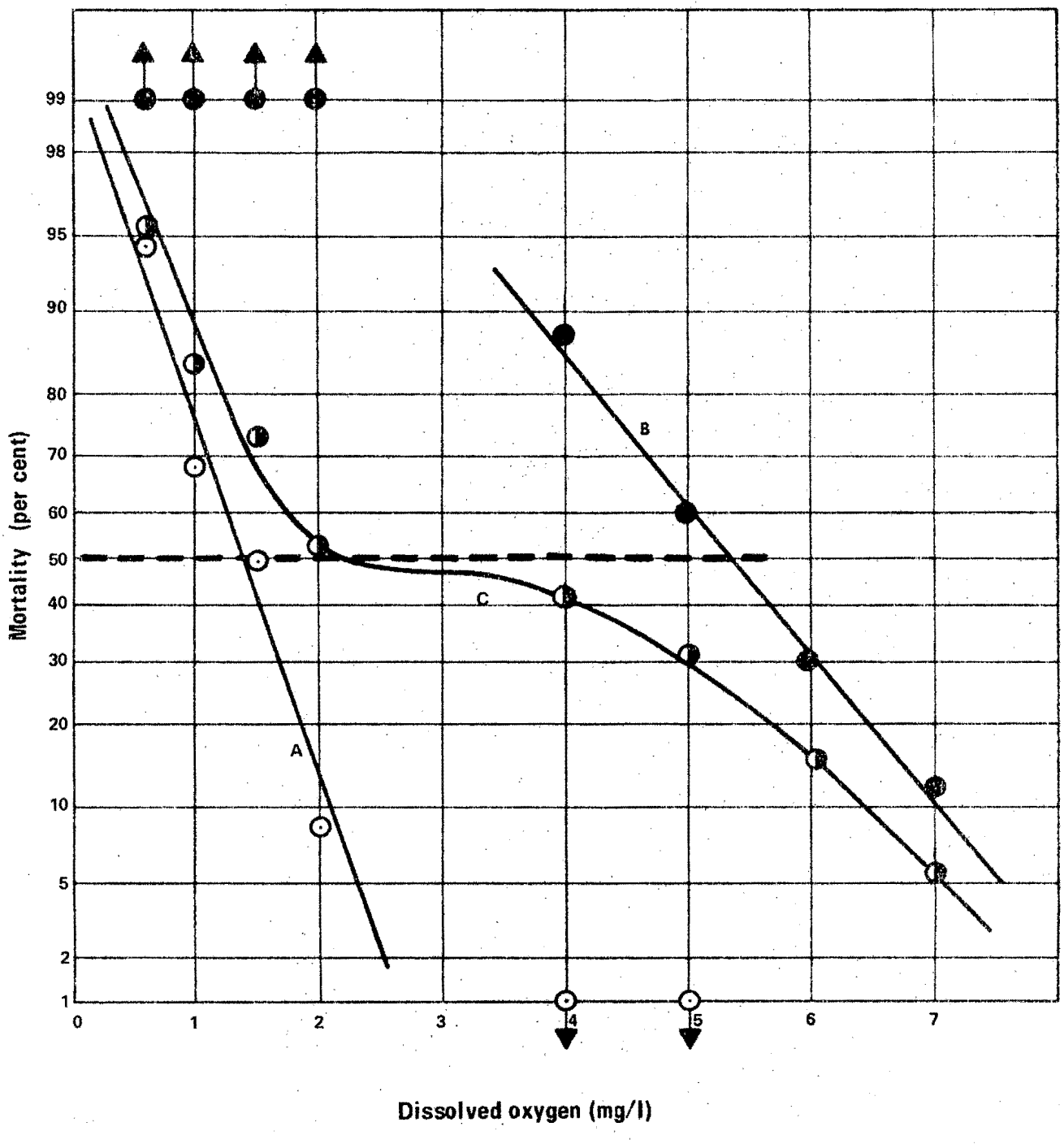

Mortality (per cent)
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
1
2
3
4
5
6
7
A
B
C
Dissolved oxygen (mg/l)

Figure 45

Median lethal low dissolved oxygen concentrations for summer *Baetis harrisoni* nymphs in laminar water flow of 7 cm/sec at 15°C, 20°C and 25°C:

open circles -- animals not in ecdysis

closed circles -- animals in ecdysis

shaded circles -- total

For explanation see text.

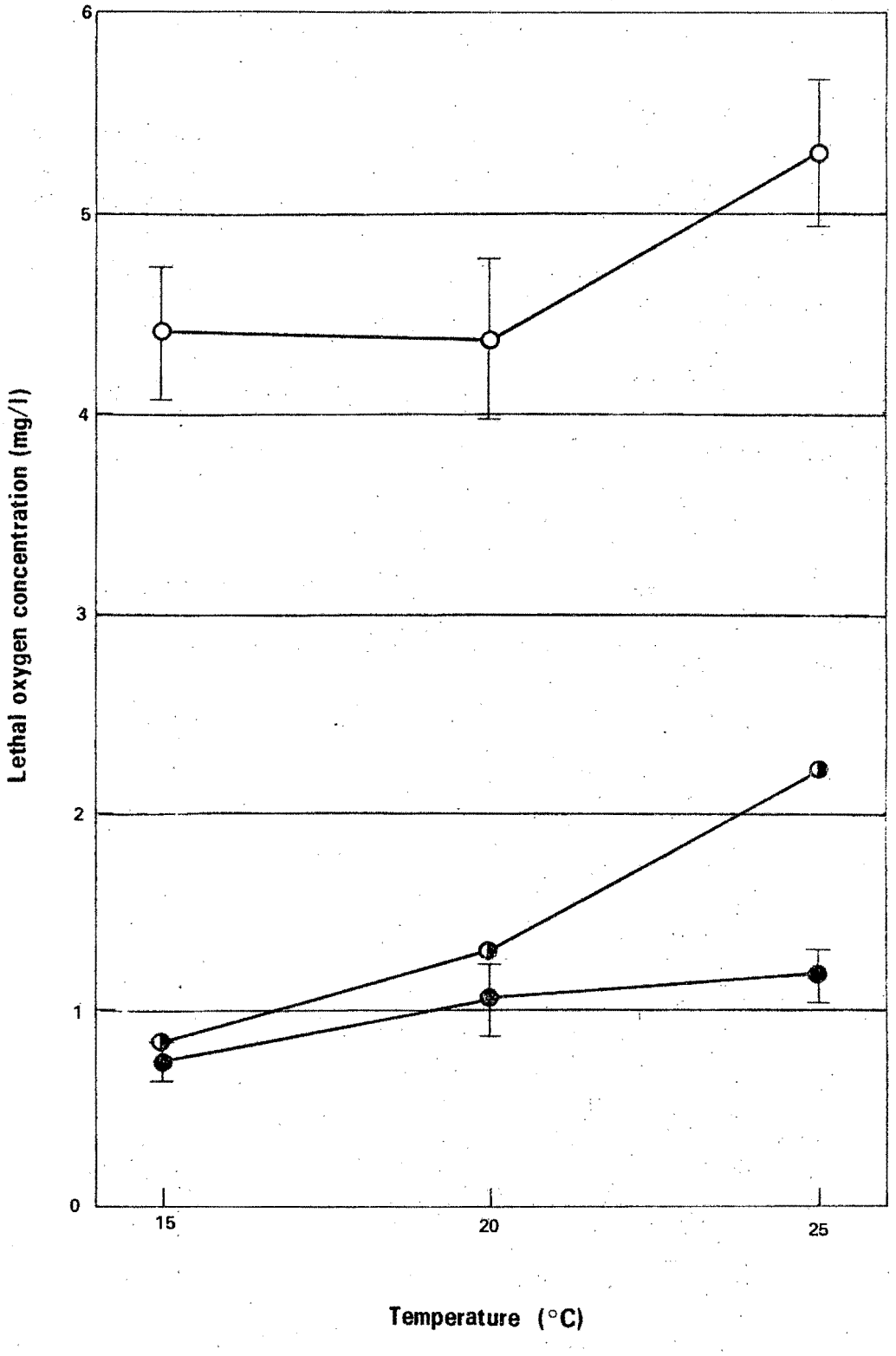

Lethal oxygen concentration (mg/l)
6
5
4
3
2
1
0
15
20
25
Temperature (°C)

TABLE 48

1000-MINUTE MEDIAN LETHAL UPPER LETHAL TEMPERATURES FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT CONCENTRATIONS OF DISSOLVED OXYGEN

Dissolved oxygen (mg/l)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
8	160	35.6 35.8 36.0 36.2	36.0 (35.8 to 36.2)
4	160	35.4 35.6 35.8 36.0	35.6 (35.4 to 35.9)
1	160	34.6 34.8 35.0 35.2	34.9 (34.7 to 35.2)

may be seen, the upper lethal temperatures of these nymphs were reduced significantly at lower concentrations of dissolved oxygen. As in the case of other experiments involving this species, the nymphs attempting ecdysis during the experiment were not found to be less tolerant than those not in ecdysis.

BAETIS AND TEMPERATURE

The results of the present study have revealed striking resemblances between circumstances and symptoms of mortality of Baetis nymphs at high temperatures and in low dissolved oxygen concentrations. In both cases nymphs in ecdysis were found to be significantly less tolerant than were nymphs out of ecdysis. In both instances mortality was markedly influenced by the

rate of water flow. These resemblances were further investigated in an experiment carried out during winter in which high temperature tolerances of Baetis harrisoni nymphs were compared at different dissolved oxygen concentrations.

The results of this experiment, again carried out piecemeal on different days over several weeks, are summarized in tables 49 and 50. For

TABLE 49

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES AT DIFFERENT WATER CURRENT SPEEDS AND DISSOLVED OXYGEN CONCENTRATIONS FOR WINTER BAETIS HARRISONI NYMPHS ATTEMPTING ECDYSIS

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
8	2.7	laminar	2.6	152	23.5 24.0 24.5 25.0	24.0 (23.6 to 24.4)
	7.0	laminar	1.6	163	24.5 25.0 25.5 26.0	25.5 (25.3 to 25.8)
	22.2	laminar	0.9	171	25.5 26.0 26.5 27.0	26.4 (26.3 to 26.6)
	8.0	turbulent	5.0	167	25.0 25.5 26.0 26.5	26.2 (26.0 to 26.3)
	14.8	turbulent	2.6	169	25.5 26.0 26.5 27.0	26.8 (26.7 to 27.0)

TABLE 49

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% condifence limits in brackets
	39.1	turbulent	1.6	171	25.5 26.0 26.5 27.0	25.9 (25.7 to 26.1)
6	2.7	laminar	2.6	148	20.5 21.0 21.5 22.0	20.9 (20.7 to 21.1)
	7.0	laminar	1.6	151	23.5 24.0 24.5 25.0	24.9 (24.7 to 25.1)
	22.2	laminar	0.9	151	25.0 25.5 26.0 26.5	25.8 (25.6 to 26.0)
	8.0	turbulent	5.0	161	24.0 24.5 25.0 25.5	25.4 (25.2 to 25.6)
	14.8	turbulent	2.6	148	24.5 25.0 25.5 26.0	25.8 (25.5 to 26.0)
	39.1	turbulent	1.6	164	24.5 25.0 25.5 26.0	24.6 (24.3 to 24.7)
4	2.7	laminar	2.6	156	17.0 18.0 19.0 20.0	lethal at all temperatures

TABLE 49 (cont)

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C) 95% confidence limits in brackets
4	7.0	laminar	1.6	176	22.5 23.0 23.5 24.0	23.3 (23.1 to 23.5)
	22.2	laminar	0.9	156	24.0 24.5 25.0 25.5	24.5 (24.3 to 24.7)
	8.0	turbulent	5.0	148	23.0 23.5 24.0 24.5	23.9 (23.7 to 24.0)
	14.8	turbulent	2.6	141	23.5 24.0 24.5 25.0	24.5 (24.3 to 24.8)
	39.1	turbulent	1.6	140	23.5 24.0 24.5 25.0	24.3 (24.1 to 24.5)

TABLE 50

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES AT DIFFERENT WATER CURRENT SPEEDS AND DISSOLVED OXYGEN CONCENTRATIONS FOR WINTER BAETIS HARRISONI NYMPHS NOT ATTEMPTING ECDYSIS

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature (°C), 95% confidence limits in brackets
8	2.7	laminar	2.6	139	28.0 28.5 29.0 29.5	28.6 (28.4 to 28.8)

TABLE 50 (cont)

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature(°C), 95% confidence limits in brackets
8	7.0	laminar	1.6	135	28.5 29.0 29.5 30.0	29.2 (29.0 to 29.3)
	22.2	laminar	0.9	128	28.5 29.0 29.5 30.0	29.3 (29.1 to 29.5)
	8.0	turbulent	5.0	122	28.0 28.5 29.0 29.5	29.0 (28.9 to 29.1)
	14.8	turbulent	2.6	130	28.5 29.0 29.5 30.0	29.4 (29.2 to 29.5)
	39.1	turbulent	1.6	127	28.5 29.0 29.5 30.0	29.4 (29.2 to 29.6)
6	2.7	laminar	2.6	146	27.0 27.5 28.0 28.5	27.2 (26.9 to 27.5)
	7.0	laminar	1.6	147	28.0 28.5 29.0 29.5	28.7 (28.6 to 28.9)
	22.2	laminar	0.9	127	28.0 28.5 29.0 29.5	28.7 (28.4 to 28.9)
	8.0	turbulent	5.0	129	27.5 28.0 28.5 29.0	28.4 (28.2 to 28.6)

TABLE 50 (cont)

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Test temperatures (°C)	Lethal temperature(°C), 95% confidence limits in brackets
6	14.8	turbulent	2.6	124	28.0 28.5 29.0 30.0	28.7 (28.5 to 29.0)
	39.1	turbulent	1.6	126	28.0 28.5 29.0 29.5	28.8 (28.6 to 28.9)
4	2.7	laminar	2.6	136	25.5 26.0 26.5 27.0	25.6 (25.5 to 25.8)
	7.0	laminar	1.6	124	26.5 27.0 27.5 28.0	26.9 (26.8 to 27.1)
	22.2	laminar	0.9	136	26.5 27.0 27.5 28.0	27.1 (26.9 to 27.3)
	8.0	turbulent	5.0	117	26.0 26.5 27.0 27.5	27.2 (27.0 to 27.4)
	14.8	turbulent	2.6	137	26.5 27.0 27.5 28.0	27.4 (27.2 to 27.5)
	39.1	turbulent	1.6	127	26.5 27.0 27.5 28.0	27.5 (27.3 to 27.7)

this experiment, Baetis harrisoni nymphs after 24 hours in the laboratory at 20°C in oxygen saturated water were exposed to different combinations of temperature, dissolved oxygen and water current speed. Mortality at each combination of these factors was noted both for nymphs attempting and not attempting ecdysis. In the previous experiment it had been found that the dissolved oxygen concentrations which were lethal for nymphs in and out of ecdysis were sufficiently different from one another to make it necessary to use different test levels of dissolved oxygen for each. The same difficulty was experienced in this experiment. Different test temperatures had to be used for nymphs in and out of ecdysis. These results confirm that both dissolved oxygen concentration and water current speed influenced the mortality of Baetis harrisoni nymphs at high temperatures.

DISCUSSION

From the results presented here it seems clear that temperature, dissolved oxygen concentration and water current speed jointly affect the survival of Baetis harrisoni nymphs in a somewhat complex manner. However, efforts to describe these joint effects in simple methamatical terms, and in so doing to draw inference as to the mechanism of their interaction, have been unsuccessful. The equation describing the current speed/low oxygen/high temperature tolerance zone for nymphs of this species should include functions for such factors as

(a) the rate of diffusion of oxygen to the nymphs, as influenced by water flow conditions, which can not be predicted for the circumstances of these experiments

(b) the metabolic oxygen requirements of nymphs at different stages in their life history, which are not known

(c) the metabolic oxygen requirements of nymphs maintaining their position in different current speeds, also unknown

(d) the metabolic oxygen requirements of nymphs at different temperatures and during acclimation to changed temperature, also unknown and shown by Fry and Hart (1948) and Woynarovich (1961) often to be complex.

It is clear from the complexity of the situation that observed data might be difficult to represent with a simple equation. On the basis of field observations and laboratory rearing of the number of stenothermal Leptophlebiidae, Pleskot (1953) has in fact suggested that death of these animals at higher temperature was caused by asphyxiation. She also found nymphs to be most susceptible to high temperature and oxygen lack during ecdysis and as last instar nymphs.

Somewhat similar observations were made by Zahner (1959). He found nymphs of a species of the dragonfly genus Agrion to be restricted to cooler water by oxygen lack at higher temperatures. If relatively widespread species such as Baetis harrisoni are limited in this way by oxygen lack at high temperatures the same might apply to even greater degree to the many insects whose distribution is limited to cold and torrential mountain streams. These species are mostly revealed by this distribution to be stenothermal and typically only survive in well oxygenated and swift flowing water (Illies and Botosaneau 1963). The South African forms include several species of Baetis (Harrison 1965b). Studies of the oxygen requirements and temperature tolerances of these animals would be of great interest.

Comparison of tables 46 and 47 with tables 49 and 50 reveals that summer Baetis harrisoni nymphs were able to tolerate oxygen-temperature combinations which would have killed all the winter nymphs. The discrepancies between the results of these two experiments are illustrated in figures 46 and 47, two conjectural three-dimensional representations of the possible current speed/ dissolved oxygen/ temperature tolerance zones for Baetis nymphs in ecdysis, respectively in laminar and in turbulent flow, as suggested by the results shown in tables 46, 47, 49 and 50.

The discrepancies between tables 46 and 47 that are illustrated in figures 46 and 47 were due mainly to the observation that summer Baetis harrisoni nymphs at 25°C were able to tolerate fairly low concentrations of dissolved oxygen. During winter, and on several occasions in summer as well, nymphs of this species were found to die at 25°C in water saturated with oxygen, even if the temperature was raised very slowly. This suggests that the summer nymphs found in the experiment described here to be tolerant of low dissolved oxygen concentrations at 25°C were more than usually tolerant of high temperatures as well and were enabled by some adjustment to temperature to tolerate these conditions.

As was shown in an earlier section, Baetis harrisoni nymphs collected at certain times of year were found to be far more tolerant of high temperatures than were nymphs collected at other times. Nymphs were not ever found to become as tolerant through acclimation over a few days to high temperatures in the laboratory and adjustments to temperature that take part over much longer periods of time in the field are evidently of great importance in determining the temperature tolerances of nymphs.

The heavy black lines in figures 46 and 47 connect observed points taken from tables 46 and 49. The broken lines are conjectural and illustrate that even the normal increase in upper lethal temperatures which would be expected in summer could explain the differences between tables 46 and 49.

SUMMARY

1. Lethal low dissolved oxygen concentrations for summer Choroterpes nymphs were not influenced by differences in water current speed but were increased by 0.02 to 0.06 mg/l in stagnant water.
2. Lethal low dissolved oxygen concentrations for summer Choroterpes nymphs were relatively low at 25°C (average 0.46 mg/l in flowing water), higher were at 20°C (average 0.54 mg/l in flowing water) and even higher at 25°C (average 0.70 mg/l in flowing water).
3. Lethal low dissolved oxygen concentrations for summer Baetis nymphs were relatively high in water flowing at 2.7 cm/sec (from 6.3 mg/l to well above saturation for nymphs in ecdysis and from 1.1 to 2.2 mg/l for nymphs not in ecdysis, depending on temperature) and relatively low in water flowing at 14.8 to 39.1 cm/sec (from 1.9 to 2.3 mg/l for nymphs in ecdysis and from 0.6 to 1.0 mg/l for nymphs not in ecdysis in 14.8 to 39.1 cm/sec water flow, depending on the temperature and the speed and nature of the flow).
4. Lethal low dissolved oxygen concentrations for summer Baetis nymphs were lower at 15°C (from 1.4 to 6.3 mg/l for nymphs in ecdysis and from 0.6 to 1.1 mg/l for nymphs not in ecdysis, depending on current speed) than they were at 20°C (from 1.5 to 7.0 mg/l for nymphs in ecdysis and from 0.6 to 1.6 mg/l for nymphs not in ecdysis, depending

Figure 46

Conjectural three-dimensional representation of the possible median limits of tolerance of combinations of dissolved oxygen, temperature and current speed by nymphs of Baetis harrisoni in laminar flow attempting ecdysis, as suggested by:

open circles - upper lethal temperatures of winter nymphs at different dissolved oxygen concentrations and current speeds

closed circles - lethal low dissolved oxygen concentrations of summer nymphs at different temperatures and current speeds.

Solid lines connect observed points. Conjectural lines are broken. For explanation see text.

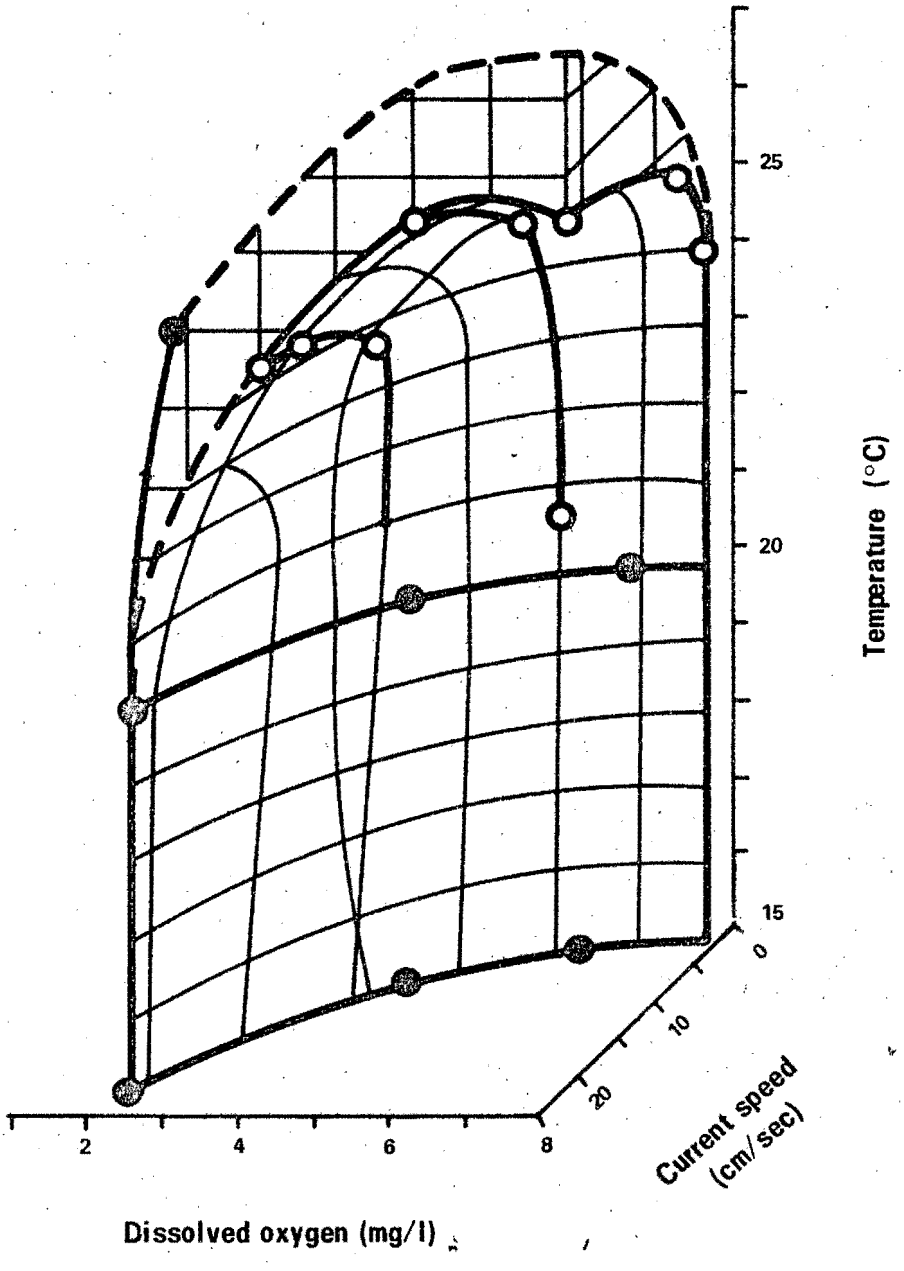

25
20
15
Temperature (°C)
0
10
20
Current speed (cm/sec)
2
4
6
8
Dissolved oxygen (mg/l)

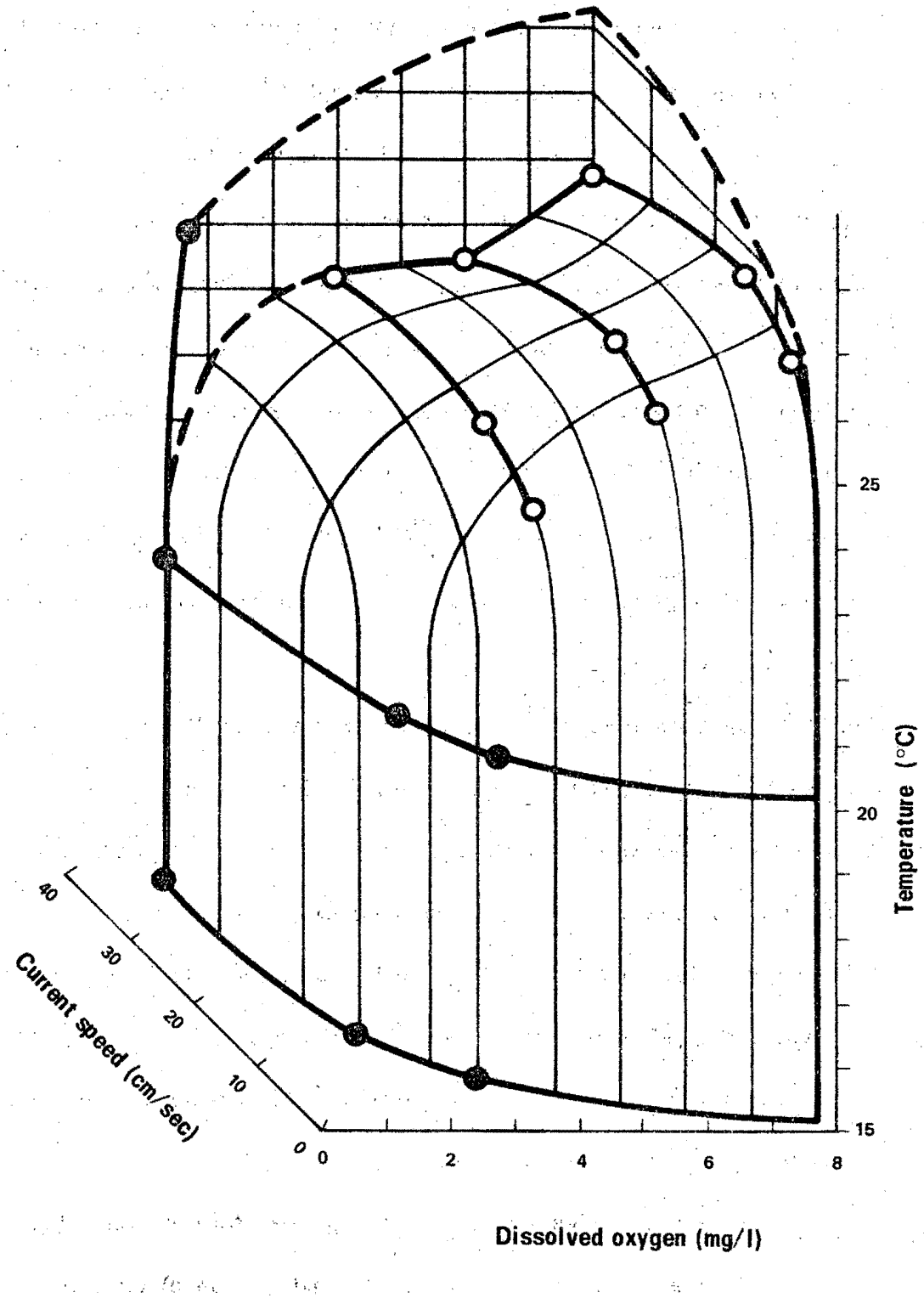
Figure 47

Conjectural three-dimensional representation of the possible median limits of tolerance of combinations of dissolved oxygen, temperature and current speed by nymphs of Baetis harrisoni in turbulent flow attempting ecdysis, as suggested by:

open circles - upper lethal temperatures of winter nymphs at different dissolved oxygen concentrations and current speeds

closed circles - lethal low dissolved oxygen concentrations of summer nymphs at different temperatures and current speeds.

Solid lines connect observed points. Conjectural lines are broken. For explanation see text.


Temperature (°C)
25
20
15
Current speed (cm/sec)
40
30
20
10
0
0
2
4
6
8
Dissolved oxygen (mg/l)

on current speed) and highest at 25°C (from 1.9 to well above saturation for nymphs in ecdysis and from 0.9 to 2.3 mg/l for nymphs not in ecdysis, depending on current speed).

5. Upper lethal temperatures for summer Choroterpes were highest in water containing 8 mg/l dissolved oxygen (36.0°C), lower in 4 mg/l dissolved oxygen (35.6°C) and even lower in 1 mg/l dissolved oxygen (34.9°C).

6. Upper lethal temperatures for winter Baetis nymphs were relatively low in water flowing at 2.7 cm/sec (from less than 17°C to 24.0°C for nymphs in ecdysis and from 25.6°C to 28.6°C for nymphs not in ecdysis, depending on dissolved oxygen concentration) and higher in water flowing at 14.8 to 39.1 cm/sec (from 24.3°C to 26.8°C for nymphs in ecdysis and from 27.1°C to 29.4°C for nymphs not in ecdysis, depending on dissolved oxygen concentration and on water current speed).

7. Upper lethal temperatures for winter Baetis were highest in 8 mg/l dissolved oxygen (from 24.0°C to 26.8°C for nymphs in ecdysis and from 28.6°C to 29.4°C for nymphs not in ecdysis, depending on current speed), lower in 6 mg/l dissolved oxygen (from 20.9°C to 25.8°C for nymphs in ecdysis and from 27.2°C to 28.8°C for nymphs not in ecdysis, depending on current speed) and lower still in 4 mg/l dissolved oxygen (from less than 17°C to 24.5°C for nymphs in ecdysis, depending on current speed).

8. Baetis nymphs in ecdysis were invariably less tolerant of either low dissolved oxygen concentrations (see 3. and 4. above) or high tempe-

ratures (see 6. and 7. above) than were nymphs not in ecdysis. Choroterpes nymphs in ecdysis were not less tolerant than those not in ecdysis.

9. Summer Baetis nymphs tolerated combinations of low dissolved oxygen and high temperature in which winter nymphs all died. It is suggested that differences in oxygen-temperature tolerance between summer and winter nymphs resulted from acclimatization to the temperature regimes of the streams in which they had been living.

BEHAVIOUR OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS UNDER DIFFERENT CONDITIONS OF LIGHT, TEMPERATURE, DISSOLVED OXYGEN AND WATER FLOW

INTRODUCTION

In previous sections experiments are described in which the water current speed, temperature and low dissolved oxygen tolerances of nymphs of Baetis harrisoni and Choroterpes bugandensis were investigated. In the course of these investigations a number of quite casual observations were made of behaviour of these nymphs in different situations. Some of these observations relating to the reactions of nymphs to different conditions of light, water current speed and dissolved concentration are described here. The observations are also discussed in relation to what is now known of the environmental tolerances of the two species.

Verrier (1945, 1954) has said that nymphs of different Ephemeroptera react variously and characteristically to light, water currents, temperatures and dissolved oxygen concentrations. She has described ways in which these reactions are reflected in the distribution of different species. Ambühl (1959) has discussed behavioural adaptations of a number of Ephemeroptera and Trichoptera to life in flowing water. He has shown that these animals have available to them a wide range of flow conditions and are able each to find on and among stones on the stream bed the conditions they require. Aggregations of Ephemeroptera and other animals on particular stones in a stream often appear to be the result of their reactions to flow conditions. Simuliidae in particular tend to aggregate to a marked degree, because they apparently require particular

flow conditions for the collection of food from the flowing water (Phillipson 1958).

Hughes (1966a, b) has studied in some detail the reactions to light of nymphs of Baetis harrisoni and Neurocaenis discolor. He concludes that light is a factor of some importance in determining their distribution in stream beds.

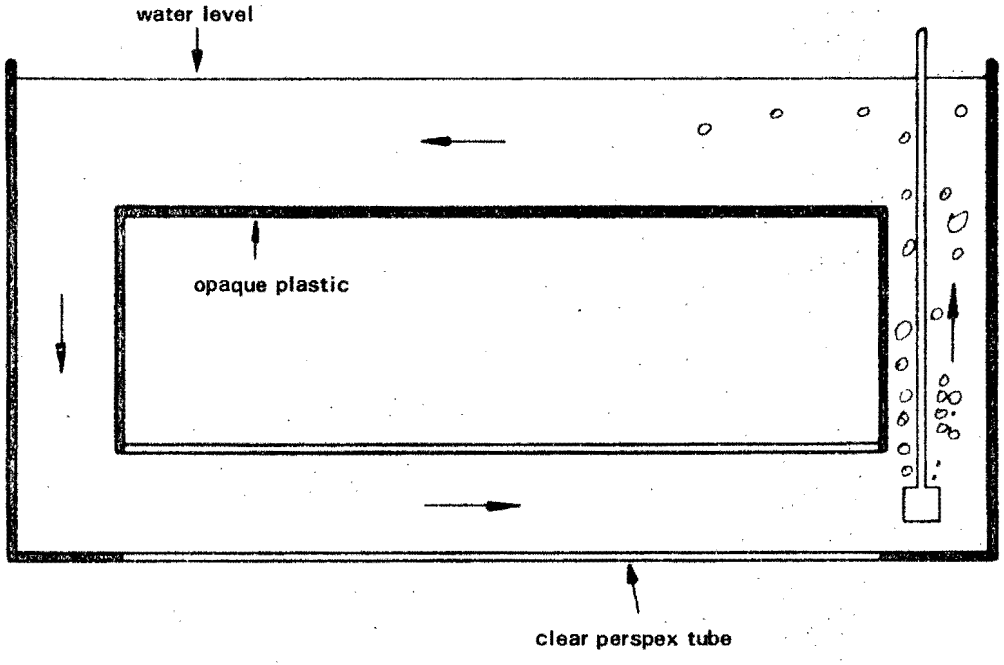
Some freshwater invertebrates have been found to be able to detect and to avoid water low in dissolved oxygen. Phillipson (1956) found Simulium larvae in water containing less oxygen than 25 per cent of saturation to detect and to move into aerated water. Costa (1967) has shown that Gammarus are able to detect low concentrations of oxygen in water and and also to move into aerated water. Moore and Burn (1968) found they could only explain the survival of invertebrates in a deoxygenated pond if it was assumed that these animals had been able to seek out pockets of less deoxygenated water.

MATERIAL AND APPARATUS

Baetis harrisoni and Choroterpes bugandensis nymphs, respectively, were collected and observed in the Braamfontein Spruit near Johannesburg and the Pienaars River near Pretoria. Several of the laboratory observations of behaviour of these nymphs were carried out in a perspex tube about 40 cm long with an internal diameter of about 6 cm. This apparatus is illustrated in figure 48. Water could be recirculated through the perspex tube via a plastic open channel connected to each end of the perspex tube by means of a plastic vertical pipe. A slow flow of water through the

Figure 48

Perspex tube in which the behaviour of nymphs was observed, showing circulation of water.


water level
opaque plastic
clear perspex tube

perspex tube was obtained by bubbling air fairly vigorously from a diffuser in one upright section. Water temperature could be controlled by immersing the apparatus in a tank of water. Faster flows of water could be obtained when the apparatus was immersed by leading water driven by the circulation pump of the thermostat-heater into that vertical section of the apparatus which was not being aerated. Flat stones could be placed as required in the perspex tube and animals introduced for observation. When the apparatus was not immersed in a tank a fluorescent light tube could be placed along the upper or lower margin of the tube, separated from the tube in each instance by a piece of sand-blasted glass to diffuse the light.

RHEOTAXIS

As Hughes (1966 b) has reported, Baetis harrisoni nymphs in flowing water invariably turned to face the current (rheotaxis). This reaction was usually only shown when the animals were attached to the substratum. Choroterpes bugandensis nymphs have been observed here to do the same, as all other mayfly nymphs for which any information is available have been found to do (Verrier 1953, Ambühl 1959). The mechanism whereby this is achieved is not known. It has been suggested that the animals orientate to the line of least resistance (Schwartzkopff 1964). It was noted here that neither Baetis nor Choroterpes nymphs responded to jets of water played onto their legs or abdominal regions in a direction at right angles to the body axis. However, they instantly turned if the water was played on their head regions. The reaction was perhaps a little too positive to be merely adoption of the line of least resistance.

Nymphs in the field, or in any situation where food was available on the surface of the rocks on which they were living, were seen to move about feeding on the rock surface apparently haphazardly. They kept facing the general direction of the source of the current. If kept in a trough or tube Baetis harrisoni nymphs tended to collect at the upstream end, as Hughes (1966 b) found. Although they moved about apparently haphazardly they seem to have moved upstream rather more than they moved downstream, presumably because they were facing in that direction. Choroterpes bugandensis nymphs, on the other hand, tended if anything to collect at the downstream ends. While they kept facing the current they tended to move sideways and backwards rather more than they moved forward. This was part of another behaviour pattern and will be discussed separately.

SWIMMING

Baetis harrisoni nymphs were seen in clear streams to move around quite freely and actively over the stones in current. From time to time individuals were seen to swim with a characteristic darting motion from one stone to another. They moved by means of rapidly repeated undulations as has been described by Nachtigall (1965), often for a distance of 20 cm or more. They did not seem to eject a jet of water from the hindgut during swimming, as was described by Tonner (1936) for Cloeon nymphs.

Baetis nymphs were seen to swim in this way in almost any direction relative to the direction of water flow. However, they normally swam in directions which took them at least a little way upstream. From the fact that they took off in different directions it might be supposed that the

primary orientation of the swimming trajectory was not rheotactic. It is a little difficult to speculate to what extent nymphs while swimming were influenced by the direction of water flow. While they were swimming they would have been subjected to water pressures caused by their forward motion through the water. These pressures might have negated the effect of the stream flow. On occasion, nymphs were seen to falter for some reason in their swim. Almost invariably they started swimming again, but in a direction pointing somewhat more into the current than before.

Choroterpes bugandensis nymphs were also found to be capable of swimming in a similar but more sluggish manner. However, this was only seen when the nymphs were disturbed and found themselves floating free in the water. These nymphs either swam strongly downward to the stream bottom at almost any angle to the stream flow or else swam weakly and jerkily with head uppermost, body almost vertical and legs outstretched. The strong downward swimming action seemed equivalent to the swimming of Baetis. The weak upward swimming seemed more like passive drifting in Baetis and is discussed further below.

Some Choroterpes nymphs, if further disturbed while swimming weakly upwards quickly turned and swam downwards. This seems not only to confirm that the two swimming motions were essentially different, but also suggests that strong downward swimming is an alarm reaction. This might also be true to some extent for Baetis harrisoni since physical disturbance or a shadow passing over them very often caused nymphs of this species to dart away to a new position.

PASSIVE DRIFTING

Hughes (1966 a) has described passive drifting of Baetis harrisoni nymphs. Alverdes (1927) has described the same thing for Cloeon nymphs. Nymphs with arched backs and outstretched legs were seen fairly frequently in the Braamfontein Spruit being carried passively with the stream. They could interrupt their drifting motion suddenly and swim vigorously to settle on a rock or might swim a centimetre or two and again revert to drifting. Drifting nymphs were seen to settle on rocks if the current carried them onto the rocks. Drift of Baetis nymphs will also be discussed later in relation to the dorsal light response of these nymphs.

Whether or not weak upward swimming of Choroterpes bugandensis nymphs was equivalent to the drift behaviour of Baetis it seems to have achieved the same effect. Animals were seen to be carried passively downstream and to settle on rocks past which they drifted.

PHOTOTAXIS

Hughes (1966 b) has described positive phototaxis of Baetis harrisoni nymphs. This was seen particularly clearly in the present study under the following circumstances. Nymphs were collected and transferred in the field to a green plastic bucket containing about 5 cm of water. When the water was stirred fairly vigorously those nymphs sitting on the sides and bottom of the bucket turned to face the current. Some of the nymphs suspended in the water swam in different directions, many were swirled around with the water. The bucket, up to this moment entirely in the shade, was then moved so that a shaft of sunlight fell onto a segment of the outside of the

bucket some 7 cm wide illuminating a small area inside the bucket through the translucent plastic. Immediately all of the nymphs suspended in the water in the vicinity of the illuminated area turned and swam towards the source of light. None of these nymphs appeared to be influenced at all by the water crossing at right angles to their swim trajectory, although the speed of the water exceeded their swimming speed. They all collected in close formation, still swimming towards the light and at right angles to the current, at the illuminated side of the bucket. Meanwhile, the now small number of nymphs still attached, even those on the bottom and illuminated from above and those few on the sunlit side illuminated from below through the plastic, still faced the current and showed no sign of being influenced by the light.

These observations seem to show that attached nymphs orientated primarily by rheotaxis while the swimming nymphs orientated primarily by positive phototaxis. The attached nymphs appeared not be influenced by light and the swimming nymphs not to be influenced by water flow. A factor which might have contributed to this difference in behaviour between attached and swimming nymphs was possible inhibition of phototaxis in attached nymphs by contact with the bucket (thigmotaxis). However, why attached nymphs should have shown rheotaxis and swimming nymphs have shown no reaction to current is not at all clear. A few of the swimming nymphs were seen to settle on the walls of the bucket while the water was still in motion. They immediately turned to face the current and showed no sign of being influenced by the light.

Choroterpes bugandensis nymphs similarly placed in a bucket and exposed simultaneously to light and water flow behaved quite differently. The attached nymphs all turned to face the water current as had the Baetis nymphs, but when sunlight was allowed to fall on part of the outside of the bucket most of the nymphs, both attached and swimming moved away from the illuminated area. A few of each remained, as though unaffected by the light. The stimulus to move seems possibly to have been provided by an orthokinetic response to illumination similar to that described for Neurocaenis nymphs by Hughes (1966 b). Orientation of animals so moved was perhaps then determined by negative phototaxis.

DORSAL LIGHT RESPONSE

Hughes (1966 a) has described the dorsal light response of Baetis harrisoni nymphs. This resembles that of Cloeon nymphs (Alverdes 1927). Hughes attributed reception of dorsal light by Baetis to the ocelli, but Alverdes found the response of Cloeon to be eliminated by destruction of the compound eyes, not by destruction of the ocelli. In this study the dorsal light response of Baetis harrisoni nymphs was best shown in the apparatus illustrated in figure 48. Nymphs were introduced, as far as possible on their sides, into the water flowing through the perspex tube and their swimming and drift orientation and settlement observed.

In these experiments, when the fluorescent tube above the perspex tube was illuminated and a sheet of black paper placed below the perspex tube, Baetis nymphs invariably swam with their dorsal surfaces uppermost. They also drifted with their dorsal surfaces more or less uppermost.

Drifting nymphs tended to roll and yaw with the current, but normally corrected their position with an occasional short burst of swimming. When the stiffly spread legs of these drifting animals came into contact with a solid object the animals almost invariably alighted, even if only temporarily. When any other part of the head or body touched a solid object but the legs did not, the animals showed no reaction and consequently drifted further. Numbers of nymphs reacting in these ways in an experiment are shown in table 51. Those that alternately swam and drifted are grouped according to which they did most.

When the fluorescent tube below was illuminated and a sheet of black paper placed above the perspex tube Baetis nymphs introduced into the tube clearly swam and drifted upside down (i.e. with their ventral surfaces uppermost). Swimming nymphs most often swam upwards and often alighted either on the upper margin of the tube or on the undersurfaces of stones placed in the stream near to the upper margin of the tubes. Nymphs alighting at these places often stayed there for some time. Drifting nymphs, on the other hand, very slowly sank in the water as they travelled downstream. Occasionally nymphs which happened to drift into the leading edges of stones in the flow were seen to alight there, apparently because their legs had come in contact with the stone. Generally, however, these drifting nymphs were found to sink slowly to the bottom where they lay motionless with their ventral surfaces uppermost, their legs still stiffly spread and their backs still arched. Here they would have lain apparently indefinitely if allowed to do so. Numbers of nymphs behaving in different ways when illuminated from below are shown in table 51, where the nymphs that ended up uside-down on the bottom are termed "settled".

TABLE 51

BEHAVIOUR OF BAETIS HARRISONI NYMPHS ILLUMINATED FROM ABOVE AND FROM BELOW

Illumination	From above				From below			
Orientation	Dorsum up	Venter up	On side	Total	Dorsum up	Venter up	On side	Total
Swam up and alighted	0	0	0	0	0	2	0	2
Swam down and alighted	5	0	0	5	0	0	0	0
Swam to side and alighted	14	0	0	14	0	11	0	11
Drifted and alighted	15	0	6	21	0	3	5	8
Drifted and settled	0	0	0	0	0	19	0	19

Choroterpes bugandensis nymphs, in contrast to those of Baetis, showed no dorsal light response. Numbers behaving in different ways when illuminated from above and from below are shown in table 52. Nymphs introduced into the perspex tube either swam with their dorsal surfaces uppermost or drifted swimming weakly upwards in the position described earlier (listed as "drifting" in table 52)whether illuminated from above or below.

SKOTOTAXIS

Hughes (1966 b) found that swimming Neurocaenis nymphs orientated to face dark objects or dark areas with defined margins (skototaxis or skototelotaxis). Many of them then swam towards these dark patches and alighted on them. He found no evidence of skototaxis in Baetis nymphs.

TABLE 52

BEHAVIOUR OF CHOROTERPES BUGANDENSIS NYMPHS ILLUMINATED FROM ABOVE AND FROM BELOW

Illumination	From above				From below			
Orientation	Dorsum up	Venter up	On side	Total	Dorsum up	Venter up	On side	Total
Swam up and alighted	0	0	0	0	9	0	7	16
Swam down and alighted	19	0	2	21	0	0	0	0
Swam to side and alighted	2	0	0	2	3	0	2	5
Drifted and alighted	7	0	0	7	9	0	0	9
Drifted and settled	0	0	0	0	0	0	0	0

Observations were carried out in the apparatus shown in figure 48 with a few small patches of black paper stuck onto the inner surface of the perspex tube. White paper was placed on the outside of the tube behind the patches so that the patches would stand out in contrast. The same number of small patches of white paper were also stuck onto the inner surface of the tube along the lower midline to contrast with the black paper below and outside the tube. The area covered by the white backing was the same as that covered by the black backing.

As shown in table 53, when Baetis harrisoni nymphs were introduced into the tubes they either drifted with the current or swam to the side and alighted. A number of those that swam to the side swam straight for one

TABLE 53

NUMBERS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS ALIGHTING ON BLACK AND WHITE SURFACES WHEN ILLUMINATED FROM ABOVE

	Baetis harrisoni	Choroterpes bugandensis
Swam to black patch on side	4	3
Swam to white patch on bottom	7	2
Swam to black backing on bottom	12	19
Swam to white backing on side	5	0
Swam to unbacked perspex	0	0
Drifted	12	6
Total	40	30

of the patches of black or white paper and alighted on them. A larger number alighted on the perspex both at places where it was backed with white paper and where it was backed with black paper. Since from Hughes's results orientation towards the patches was not skototaxis, and since they swam to both black and white patches, it is suggested that these Baetis nymphs were able to perceive surface qualities of the paper and possibly to elect to land on this in preference to the perspex.

When Choroterpes bugandensis nymphs were introduced into the perspex tube with black and white patches in the positions described a few swam straight for the black patches and alighted on them, but a majority of those that alighted swam downwards and alighted on the bottom, almost invariably

where the perspex was backed with black paper. As shown in table 53, the orientation of these nymphs with respect to the patches of paper was clearly different from that of Baetis nymphs.

Since it seemed that both skototaxis and negative phototaxis might have been involved here, the observations dealing with Choroterpes nymphs were repeated with the perspex tube illuminated from below, backed above with black paper and with odd white patches above and odd black patches backed with white along the sides. The fluorescent tube providing the illumination was placed on the floor and the perspex tube placed at bench height, so that illumination provided was not too intense. A few of the nymphs introduced into the tube so set up swam straight to the black patches along the side of the tube. A greater number swam for the black-backed section above and alighted there, as shown in table 54. None was seen to alight either on the perspex in the lower half of the tube or on the white patches in the upper half.

TABLE 54

NUMBERS OF CHOROTERPES BUGANDENSIS NYMPHS ALIGHTED ON BLACK AND WHITE SURFACES WHEN ILLUMINATED FROM BELOW

Swam to black patch to one side	7
Swam to white patch above	0
Swam to black backing above	15
Swam to white backing to one side	0
Swam to unbacked perspex	0
Drifted	8
Total	30

Although the distinctions between skototaxis, phototaxis and telotactic response to visual characteristics of the surface of possible places to alight were perhaps not always clear in these experiments, it seems that Choroterpes nymphs showed a skototactic response to defined dark patches where-Baetis nymphs did not. As in the case of Neurocaenis this skototactic orientation was presumably involved in the behaviour of Choroterpes which resulted in their being found on the undersurfaces of rocks in the field (Hughes 1966 b).

THIGMOTAXIS

Hughes (1966 b) has shown light reactions of both Baetis and Neurocaenis nymphs to be inhibited by contact with the substratum. In the present study thigmotaxis as he has described was seen to influence the light responses of Baetis harrisoni and Choroterpes bugandensis nymphs particularly clearly in the crude experiments in which they were exposed to water currents and to light in a green bucket. Thigmotaxis was suggested as a possible reason for the different reactions to light and to water currents shown by attached and by swimming nymphs.

Casual observation of their behaviour on rocks both in streams and in the laboratory suggest both that the alarm reaction of Baetis harrisoni and Choroterpes bugandensis nymphs differed and that thigmotaxis was involved in the alarm reactions of Choroterpes but not those of Baetis. Baetis nymphs when alarmed either ran to a different position on the rock or swam to a different rock. Choroterpes nymphs appeared to be very easily alarmed while walking around on rocks either by physical disturbance

of the rock or water or by shadows passing over them. Choroterpes disturbed under these circumstances quickly scuttled away either to the undersurface of the rock or to a depression in the rock surface, however shallow. Once settled in a depression they did not readily move again, even if prodded. Inhibition of further flight was apparently thigmotactic.

POSITION OF ROCKS

Both Hughes (1966 b) and Agnew (1967) have discussed possible behaviour patterns which might explain the common observation that Baetis harrisoni nymphs are normally found on top of stones in current while Neurocaenis discolor nymphs, like those of Choroterpes bugandensis, are most usually found on the undersurfaces of stones in streams. Hughes observed more than 50 per cent of Baetis nymphs in a shallow artificial stream in bright sunlight to crawl under objects resembling stones but most nymphs in less intense light to be found on the upper surfaces of these objects. He explains their behaviour in bright sunlight to be due to orthokinetic activity caused by the intense light. He reasoned that nymphs landing up beneath stones became less active and tended to stay there. The movement of nymphs to the upper surfaces of rocks in less intense sunlight he explained in terms both of their positive phototaxis and presumed reactions to water flow conditions. Agnew has suggested that Baetis harrisoni nymphs in nature keep to the upper surfaces of rocks because the undersurfaces are inhabited by other species which compete with the Baetis nymphs for algal food.

In the present study Baetis harrisoni nymphs were introduced into the apparatus illustrated in figure 48 and provided both with light from above

and with a few flat stones placed in the perspex tube end on to the current. The stones had short growths of algae on both their upper and lower surfaces. These nymphs settled on the perspex and on the stones and were observed both during the night and the day for several days. At night all lights were switched off and a torch used quickly to see how the nymphs were distributed on the stones at odd times. Table 55 shows totals of counts made on eight occasions during the day and eight occasions at night.

TABLE 55

NUMBERS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS COUNTED ON UPPER AND LOWER SURFACES OF STONES IN THE LABORATORY. 12 NYMPHS OF EACH SPECIES WERE USED AND NUMBERS SHOWN ARE TOTALS FOR EIGHT COUNTS MADE DURING THE DAY AND EIGHT MADE AT NIGHT

Species	Baetis harrisoni			Choroterpes bugandensis		
Position	Above stones	Below stones	Elsewhere	Above stones	Below stones	Elsewhere
Day (illuminated from above)	52	17	27	11	74	11
Night	39	28	29	36	44	16
Day (illuminated from below)	-	-	-	62	14	20
Night	-	-	-	38	35	23

During the day the nymphs were seen to move freely over the rock surfaces and to feed freely on both the upper and lower surfaces. However, on each occasion most nymphs were to be seen on top of the stones. At night a majority of nymphs was still to be seen on top of the stones but a greater proportion of nymphs was found under the stones than had been

found there during the day.

The difference in numbers of Baetis nymphs found here on top of the stones at night and during the day could equally well be attributed to positive phototaxis or to the dorsal light response. Phototaxis might be presumed to have caused at least some nymphs on the sides of stones to turn upwards. They would then have been more likely to move upwards than to move downwards. The dorsal light response has been shown to orientate swimming and drifting animals. When illuminated from above swimming and drifting animals apparently only settle on the upper surfaces of stones.

Choroterpes bugandensis nymphs were introduced into this apparatus under similar circumstances and their distribution on the stones observed. Totals of counts made during the day and at night are shown in table 55. During the day many more nymphs were found on the undersurfaces of the stones than were found on top of the stones. Those nymphs found on top of the stones quickly took fright at any movement in their field of vision. Their alarm reaction, involving both skototactic orientation to shaded areas and recesses and thigmostatic inhibition of activity once lodged in a hollow of the rock, has already been described. At night very roughly equal numbers seemed to be distributed above and below stones.

These observations with Choroterpes nymphs were repeated in a darkened room with illumination from below and the distribution of nymphs above and below the stones compared (table 55). Under these circumstances many more nymphs were found on the upper surfaces of the stones. Nymphs in illuminated sections of the tube, including those on the undersurfaces of stones, showed the same alarm reaction when disturbed as had

those under illumination from above. However, instead of escaping to the undersurface of the rocks they escaped to the shaded upper surfaces.

TEMPERATURE REACTIONS

In order to be able to observe the effects on Baetis harrisoni and Choroterpes bugandensis nymphs of varied temperature conditions the apparatus illustrated in figure 48 was immersed in a tank of water. The water temperature in the tank was set and maintained at 20°C by means of a thermostat-heater and nymphs were introduced into the perspex tube. A number of small stones with algal growth over them were also placed in the tube. A second tank was then set up on a metal frame about one metre higher than the first. At different times the water in the second tank was set at different temperatures. Water could be led out to the second tank through a plastic tube of 1 cm internal diameter and the volume of this outflow controlled by means of a screw clamp. The outflow was introduced into the perspex tube containing the animals through a glass tube bent at right angles and held in the hand. Water lost from the second tank was replaced through a pipe connected to the tap. The first tank had an overflow pipe which ran to waste.

Water led into the perspex tube from the second tank could be seen in the perspex tank. By manipulating the glass tube in the hand a stream of this water could be played onto nymphs as required. The reactions of the nymphs to this water were then noted and have been summarized in table 56.

TABLE 56

REACTIONS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS TO WATER OF DIFFERENT TEMPERATURES

Temperature of test stream(°C)	Baetis		Choroterpes	
	Reacting	Not reacting	Reacting	Not reacting
10	9	1	10	0
15	1	9	3	7
20	0	10	0	10
25	10	0	2	8
30	10	0	7	3

Nymphs reacting to sudden exposure to a stream of hot or cold water did so by jerky movements of the legs and sweeping movements of the abdomen. Frequently the animals moved out of the flow. Most especially, Baetis tended to avoid warmer water. Some nymphs repeatedly moved away from water played on them. The reactions of these nymphs to water of different temperature appeared in each case to be orthokinetic.

These observations show that nymphs of both species were able to detect differences in temperature of water to which they were exposed. Their reactions appeared to be orthokinetic (i.e. undirected). More would really need to be known of this reaction to be able to discuss its ecological importance.

Observations made in the Braamfontein Spruit and Pienaars River suggested that temperature differences of the variations in temperature of the order of 0.3 to 0.7°C could be expected from place to place across a stream.

On one occasion described in an earlier section Baetis harrisoni nymphs were observed in the Braamfontein Spruit at a time of abnormally high temperatures. Temperatures at different places in the river varied from 27.8°C to 28.2°C. Since these temperatures were very close to the lethal limits for this species it would presumably have been to their advantage to have sought out the cooler water. However, on this occasion nymphs seemed to be distributed in the river quite independently of the small temperature differences present. On another occasion in the Pienaars River, also described in an earlier section, Choroterpes bugandensis nymphs were found at a time of abnormally high temperatures not to be confined to the cooler parts of the river.

REACTIONS TO DISSOLVED OXYGEN

In order to observe the reactions to water of different dissolved oxygen content of Baetis and Choroterpes nymphs the same two tanks were set up. The temperature in each was set at 20°C. In the first series of observations the water in the first tank containing the perspex tube and animals was strongly aerated while the dissolved oxygen concentration in the second tank was reduced and maintained at a selected level by aeration with a suitable mixture of air and nitrogen. Where water of low dissolved oxygen content was to be supplied the replacement water supply to the second tank was led first to a third tank which was covered and strongly aerated with pure nitrogen and which was placed on top of the second tank. Water travelled from this third tank through an overflow pipe into the second tank which was also covered. The water in the second tank was coloured using a dye to make it visible in the perspex tube in which it was to be played onto the animals.

The concentration of dissolved oxygen in the first tank was measured from time to time to see whether or not it had been reduced by the inflow of deoxygenated water. In no case was a concentration less than 6.5 mg/l recorded.

The observed reactions of Baetis harrisoni and Choroterpes buganden-sis nymphs in this first series of experiments are summarized in table 57.

TABLE 57

REACTIONS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS TO WATER OF LOW DISSOLVED OXYGEN CONTENT

Dissolved oxygen in test stream (mg/l)	Baetis		Choroterpes	
	reacting	not reacting	reacting	not reacting
0.5	10	0	10	0
2	10	0	2	8
4	7	3	0	10
7	0	10	0	10

It was found that nymphs reacted sharply to deoxygenated water by rapid and apparently orthokinetic evasive action. Even if repeatedly exposed to this deoxygenated water they continued to avoid it.

A second series of observations was then carried out in which the dissolved concentration in the first tank containing the animals was reduced and their reactions to oxygenated water compared. These observations are summarized in table 58.

TABLE 58

REACTIONS TO OXYGENATED WATER OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN WATER OF REDUCED DISSOLVED OXYGEN CONTENT

Dissolved oxygen in tank (mg/l)	Baetis		Choroterpes	
	reacting	not reacting	reacting	not reacting
1	-	-	10	0
4	10	0	0	10
7	0	10	0	10

As may be seen from table 58 both species reacted to oxygenated water. Once again, their reactions consisted of jerky locomotory movements. No evidence of any directed response was seen and the response of both species to oxygenated water is assumed to be orthokinetic.

There seems to be no doubt from the observations reported here that both Baetis harrisoni and Choroterpes bugandensis nymphs were able to detect differences in dissolved oxygen content of water reaching them. In all cases they appeared to react to both favourable and unfavourable but changed oxygen conditions with undirected orthokinetic movements. These are the sort of reactions that often enable animals to seek out more favourable conditions (Fraenkel and Gunn 1961). In the present case, however, it is not at all clear how this might have been achieved. If reactions to both favourable and unfavourable conditions consisted of undirected haphazard movements it is difficult to see how the animal could land up in conditions favourable to it. In field observations made at different times in the Braamfontein Spruit and Pienaars River no great differences in dissolved oxygen were found, but Madsen (1968) has reported quite important differences between dissolved oxygen concentrations at different places in a stony

stream. Clearly, if such differences exist then the behavioural reactions of nymphs are evidently capable or may be expected to offer them a great deal of protection against exposure to deoxygenated water.

SUMMARY

1. Baetis and Choroterpes nymphs reacted in a number of characteristic ways to light and water current speed conditions.

2. The possible role of these reactions in determining observed patterns in the distribution of these nymphs on and among stones in stream beds is discussed.

3. Nymphs of both species reacted to sudden exposure to water of a different temperature with undirected orthokinetic movements.

4. Nymphs of both species in oxygenated water reacted to water of low dissolved oxygen content. Those in low dissolved oxygen concentrations reacted to oxygenated water.

BEHAVIOUR OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN THE PRESENCE OF SILT

INTRODUCTION

It has been observed that faunal collections made in stretches of South African rivers where silt is deposited or where the water has a high suspended solids content contain relatively low numbers of certain invertebrate groups (Chutter 1967). Similar observations have been made in rivers elsewhere in the world (Sprules 1947, Tarzwell 1957, Herbert et al. 1961). It has been suggested that in most instances this reduction in the diversity of the fauna has been due to the physical obliteration of habitats of certain species. For instance, Ellis (1936) found a number of bivalves to be killed by deposition of 0.5 cm or so of silt on the sand in which they were living, but that they all thrived in cages in the free water above. It has also been suggested that behavioural reactions of certain species might cause them to move away from places where silt is being deposited. A number of invertebrates of mountain streams are only found on clean stones (Illies and Botoseneanu 1963). Harrison (1958a) has reported observations of the distribution and behaviour of certain mayfly nymphs in the depositing zone of the Great Berg River. He found that these species avoided deposits of silt.

Considerable published evidence is available of the deleterious effects of silt on the fish fauna of rivers (Ellis 1937, Griffin 1943). These effects have most often been attributed to reduction in the food available for fish (Doudoroff 1957, Hynes 1960). However, although most fish survive in high concentrations of suspended material this material can cause considerable damage to fish gills and in certain circumstances can also cause mortality

of fish (Hoak 1959, Herbert and Merkens 1961, Herbert and Richards 1963).

Nymphs of both Baetis harrisoni and Choroterpes bugandensis inhabit stones in flowing water and are known from both clear and turbid rivers in the Transvaal. Observations are reported here of the behaviour of these nymphs in the presence of suspended and depositing silt. It seems from these that silt is a factor influencing the distribution of both species, at least within streams in which they occur. Observations of mortality of nymphs of both species at 20°C and at lethal high temperatures have also been made in order to see whether or not the presence of silt affected the tolerances by these nymphs of other factors.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected as before in the Braamfontein Spruit and Pienaars River. Laboratory experiments were carried out, as before, in apparatus consisting of a number of aquaria, each containing an impeller which could drive a controlled current of water through a number of 15 cm perspex experimental tubes in which the animals were contained. Experimental animals were prevented from escaping from the tubes by a disc of stainless steel gauze inserted at either end of each tube.

Very turbid water with a suspended solids content of 1.5 to 2.5 g/l was collected from a muddy rainwater pool. It was diluted with borehole water to give a calculated suspended solids content of 1.5 g/l. The silt was kept in suspension, as far as was possible, by vigorous aeration. The suspended material appeared to consist of clay.

BEHAVIOUR

Neither Baetis harrisoni nor Choroterpes bugandensis nymphs in the laboratory appeared to be affected at all by the presence of suspended material. However, they all avoided patches of silt deposited in the experimental tubes. Nymphs coming into contact even with a very light deposit of silt immediately either darted or crawled away, apparently preferring a firm substratum.

The previous section contains descriptions of various behavioural reactions of Baetis harrisoni nymphs to such factors as light and current speed. A simple piece of apparatus was used in which the behaviour of nymphs could be observed (figure 48) and it was concluded that reactions to these factors were important in determining where nymphs were to be found in streams. Using this apparatus Baetis nymphs were introduced into the perspex observation tube in which a number of flat stones from the Pienaars River had carefully been positioned. Their distribution on these stones was noted at intervals of time. The stones were all covered on one side with a layer of mixed silt and algae about 1 to 3 mm thick. Some of the stones were positioned with the aufwuchs layer uppermost and other were placed with the clean side above and the aufwuchs layer below. During the day the tube was illuminated from above and a piece of black paper placed underneath the tube. This was necessary since the animals orientate while swimming and drifting by means of a dorsal light response (Hughes 1966 a).

Observations made of the distribution of Baetis nymphs on stones covered on one side with a layer of aufwuchs are summarized in table 59 and show clearly that the nymphs avoided the aufwuchs. Most of the nymphs were found on each occasion on the clean upper surfaces of those stones positioned with the side covered with aufwuchs below. A much smaller number of nymphs was found on the clean lower surfaces of those stones positioned with their aufwuchs layer uppermost. On no occasion was a nymph seen on the aufwuchs itself.

TABLE 59

NUMBERS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS COUNTED IN THE LABORATORY ON STONE SURFACES WITH AND WITHOUT AUFWUCHS. 12 NYMPHS OF EACH SPECIES WERE USED AND NUMBERS SHOWN ARE TOTALS FOR EIGHT COUNTS.

Species	Baetis harrisoni	Choroterpes bugandensis
Clean stone upper surface	69	7
Upper surface with aufwuchs	0	0
Clean stone undersurface	12	82
Undersurface with aufwuchs	0	0
Elsewhere	15	7

Nymphs of Baetis harrisoni in the field were characteristically found on top of larger rocks in flowing water. They were also found in places on vegetation trailing in the water or even on sand when large numbers were present in the river. In the Braamfontein Spruit in relatively clear water nymphs were always observed to avoid patches of silt deposited on leaves,

on rock surfaces and on the stream bottom. Nymphs released upstream almost invariably attached themselves to the first solid surface they touched. However, nymphs coming to rest on a surface covered with silt immediately took off again.

At one spot in the Braamfontein Spruit a stretch of deposited silt and fine sand was found on whose surface were scattered a number of larger sand grains and tiny pebbles. When large numbers of Baetis harrisoni nymphs were present in the river there seemed to be hardly a pebble or larger sand grain which did not have a nymph clinging to it.

To judge from the appearance of the water, floods in the Braamfontein Spruit must carry a great deal of silt downstream. It is not known to what extent silt deposited during floods might cause Baetis harrisoni nymphs to dislodge and to be swept downstream. However, the drastic effects of floods on populations of this species have been described in an earlier section.

Nymphs of Choroterpes bugandensis were characteristically found in the Pienaars River on the undersurfaces of rocks over which water was flowing. In some spots in this river the stones were covered with a thick layer of aufwuchs consisting principally of a mixture of silt and diatoms. The undersurfaces of these stones, in contrast, were clean. Elsewhere in the same river stones were found with relatively clean upper surfaces and occasional Choroterpes bugandensis were seen in daytime venturing out onto these upper surfaces. As for Baetis, observations were made in the laboratory of the distribution of Choroterpes bugandensis nymphs on stones

clean on one side and covered with aufwuchs on the other, all of which were collected in the Pienaars River. As before, the perspex observation tube described in the previous section and shown in figure 48 was used. Again, some of the stones were positioned with the side covered with aufwuchs uppermost and some were positioned with their clean side above. As before, the tube was illuminated from above and a piece of black paper placed underneath it. The counts made are summarized in table 59 . At all times, most of the Choroterpes nymphs introduced into the tube were found on the clean undersurfaces of those stones positioned with their aufwuchs layer uppermost. A few were found on the upper surfaces of stones positioned with their aufwuchs-covered side below. No nymphs were seen on any occasion on the aufwuchs itself. As was found for Baetis harrisoni, these nymphs clearly avoided the aufwuchs.

Choroterpes bugandensis nymphs made in the river to drift downstream with the current were generally seen to attach themselves to the first solid object with which they came into contact. Once attached they moved about and if they found either a crevice or access to the undersurface they moved downwards out of the current and out of the light. Nymphs coming to rest on a surface covered with silt generally shot off into the current again and drifted further downstream, as nymphs of Baetis harrisoni had been seen to do.

MORTALITY AND HIGH TEMPERATURE TOLERANCE

Choroterpes bugandensis nymphs kept in the laboratory at 20°C in water containing 1.5 g/l suspended material all survived the 1000 minutes' exposure. Some mortality of Baetis harrisoni nymphs was recorded at 20°C

in the presence of suspended material (13 out of 400 individuals) but this was lower than that recorded in clean borehole water (16 out of 400).

In table 60 estimates are shown of 1000-minute median upper lethal temperatures for Baetis harrisoni and Choroterpes bugandensis nymphs in clean borehole water and in the presence of 1.5 g/l suspended material. In a previous section it was shown that Baetis harrisoni nymphs in ecdysis were less tolerant of high temperatures. The results shown in table 60 refer only to nymphs in ecdysis. Numbers of nymphs attempting ecdysis in each exposure were determined as has been described. As may be seen, no evidence has emerged from these results to suggest that silt might have had a harmful effect on these nymphs.

TABLE 60

1000-MINUTE MEDIAN UPPER LETHAL TEMPERATURES IN THE PRESENCE AND ABSENCE OF SILT FOR BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

Mayfly	Silt concentration (g/l)	Number of nymphs	Temperature levels	Lethal temperature (^{o}C), 95% confidence limits in brackets
Baetis (in ecdysis only)	0	158	25.5 26.0 26.5 27.0	26.4 (26.1 to 26.7)
	1.5	142	25.5 26.0 26.5 27.0	26.2 (25.9 to 26.5)
Choroterpes	0	160	35.5 36.0 36.5 37.0	36.1 (35.8 to 36.4)
	1.5	160	35.5 36.0 36.5 37.0	36.0 (35.8 to 36.2)

SUMMARY

1. Nymphs of both species avoided deposited silt, both in the laboratory and in the field.

2. Neither suspended nor deposited silt was observed either to cause mortality of Baetis or Choroterpes nymphs or to alter their upper lethal temperatures.

MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN ACID SOLUTIONS

INTRODUCTION

Widely differing views have been expressed about the possible importance of hydrogen ion concentration in water as a factor limiting the distribution of aquatic animals (Macan 1963). Much of this confusion arises in the interpretation of observed occurrences of species in waters of particular pH values and their apparent absence from waters of different pH values. In South Africa, the invertebrates of a number of acid standing and flowing waters which either drain mountains composed of Table Mountain Sandstone, or which receive acid mine drainage, have been studied in some detail (Harrison and Elsworth 1958, Harrison 1958c, Harrison and Agnew 1962). These waters remain acid at all times. Their invertebrate faunas differ quite strikingly from those of non-acid standing and flowing waters elsewhere in the country. Similar rivers differing apparently only in pH value have consistently been found to contain different diatoms and macro-invertebrates (Cholnoky 1968, Harrison and Agnew 1962). Results are reported here of experiments in which the toxicity of acid waters to Baetis harrisoni and Choroterpes bugandensis nymphs has been investigated. The results are discussed in the light of what is known of the distribution of these species in acid streams.

Baetis harrisoni is a common mayfly in both acid and alkaline South African rivers. Choroterpes bugandensis appears not be as widely distributed in South Africa but is known from alkaline and from mildly acid rivers in the Transvaal. Nymphs of both species have been exposed to water made acid using hydrochloric, sulphuric and nitric acids and carbon dioxide.

Median lethal low pH values have been estimated in different chloride, sulphate and free carbon dioxide concentrations.

Both species have been found in the Olifants River system in the eastern Transvaal in which several tributaries and a considerable stretch of the Olifants River itself have been made acid by mine drainage (Harrison 1958c). Observations have been made of the distribution of each species in various tributaries and of mortality and survival of nymphs at different pH values in these rivers.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected for tolerance experiments in the Braamfontein Spruit and Pienaars River, as has been described previously. Before being exposed in experiments to acid water they were kept in the laboratory for 24 hours in well aerated water at 20°C and at controlled pH values. Food was supplied during this period and, as usual, the animals appeared to be in good condition.

Experiments were carried out in the same apparatus described in earlier sections. The animals were placed in a number of 15 cm perspex experimental tubes which were then transferred to 40 l experimental tanks in which controlled levels of pH in the lethal range were maintained. Temperatures in the experimental tanks were maintained at 20°C in all of the experiments described here. A water current rate of 10 cm/sec was driven through each experimental tube by means of a perspex impeller suspended in the tank. Experimental tubes of 1.6 cm internal diameter were used in all of these experiments. At this flow rate the flow through these tubes

was laminar throughout.

Desired pH levels were obtained by addition to borehole water of dilute acid. Frequent measurements of pH were made during each experiment and very small amounts either of hydrochloric acid or of sodium hydroxide were added whenever necessary. At no time during any experiment did any pH value differ from the desired pH by more than 0.1 of a pH unit.

Some manipulation was required to obtain the concentration of free carbon dioxide required in each experimental tank. The borehole water used had been aerated for 48 hours to allow its carbon dioxide content to reach equilibrium with that of air. The pH of this water varied between pH 6.9 and 7.2. Before addition of acid the bicarbonate alkalinity of the water was measured. An amount of sodium bicarbonate was then added, calculated to bring the total concentration of carbonate, bicarbonate and carbon dioxide up to a level which would yield whatever concentration of free carbon dioxide was required at the experimental pH value. The proportion of carbon dioxide in the air to be brought in contact with the water at the experimental pH value which would be in equilibrium with the free carbon dioxide in this water was also calculated. This was generally of the order of between five times and fifty times the carbon dioxide content of atmospheric air for free dissolved carbon dioxide concentrations of from 10 mg/l to 100 mg/l. After addition of acid the borehole water was aerated with a mixture of air and carbon dioxide in the proportions calculated, and the tank covered tightly so that only this mixture came into contact with the water. Further determinations of pH and of bicarbonate alkalinity were made in order to confirm that the correct values were maintained.

In order to get water at a low pH with less than 1 mg/l dissolved carbon dioxide, the borehole water had first to be rendered bicarbonate-free. This was achieved by reducing the pH to 4.0 and by aerating for two days with air which had been passed over potassium hydroxide.

The tolerance experiments described here were carried out along the same lines as previous experiments. Nymphs were exposed to test solutions for 1000 minutes and the numbers of nymphs dying and recovering noted. From these counts median lethal pH values were calculated in each instance by probit analysis (Finney 1952).

pH AND CARBON DIOXIDE TOLERANCE

Median lethal low pH values for Baetis harrisoni nymphs in water made acid using different acids are shown in tables 61, 62 and 63. These lethal values have been estimated separately for free carbon dioxide concentrations of 0, 10 and 100 mg/l. From tables 61, 62 and 63 it will be seen that 100 mg/l free carbon dioxide reduced the acid tolerance of Baetis nymphs ($p < 0.05$ for each separately, $p < 0.01$ when taken together). Carbon dioxide concentrations of this order are found only exceptionally in natural waters (Hynes 1960). The lethal low pH values found at 0 and 10 mg/l free carbon dioxide concentrations did not differ greatly from one another.

Tables 61, 62 and 63 also show that water made acid using hydrochlric and sulphuric acids was about equally toxic to Baetis nymphs, while water made acid using nitric acid was rather more toxic, presumably because nitrate ions were more toxic than were either chloride or sulphate ions.

TABLE 61

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR BAETIS HARRISONI NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF HYDROCHLORIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
0	242	3.6 3.8 4.0 4.2 4.4 4.6	4.1 (4.0 to 4.2)
10	240	3.6 3.8 4.0 4.2 4.4 4.6	4.2 (4.0 to 4.3)
100	240	3.6 3.8 4.0 4.2 4.4 4.6	4.4 (4.2 to 4.6)

TABLE 62

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR BAETIS HARRISONI NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF SULPHURIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
0	240	3.6 3.8 4.0 4.2 4.4 4.6	4.1 (4.0 to 4.3)

TABLE 62 (cont)

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
10	275	3.6 3.8 4.0 4.2 4.4 4.6	4.2 (4.1 to 4.3)
100	239	3.6 3.8 4.0 4.2 4.4 4.6	4.5 (4.4 to 4.6)

TABLE 63

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOE BAETIS HARRISONI NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF NITRIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
0	240	4.0 4.2 4.4 4.6 4.8 5.0	4.6 (4.5 to 4.7)
10	240	4.0 4.2 4.4 4.8 5.0 5.2	4.6 (4.5 to 4.7)
100	240	4.0 4.2 4.4 4.6 4.8 5.0	4.8 (4.7 to 5.0)

Median lethal concentrations for Baetis harrisoni of the sodium salts of these anions, (in this case at pH 7.0) are shown in table 64. The lethal concentrations of the anions at pH 7 greatly exceeded their concentrations in the experimental acid solutions. Mortality of Baetis nymphs in the acid solutions may therefore be assumed to have been due primarily to the hydrogen ion concentration.

TABLE 64

1000-MINUTE MEDIAN LETHAL CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS OF SODIUM CHLORIDE, SODIUM SULPHATE AND SODIUM NITRATE AT 20°C AND pH 7

Salt	Number of nymphs	Test salinities (eq/l)	Lethal concentration (eq/l as anion), 95% confidence limits in brackets
NaCl	238	0.20 0.21 0.22 0.23 0.24 0.25 0.26 0.28	0.23 (0.22 to 0.24)
Na_2SO_4	232	0.16 0.17 0.18 0.19 0.20 0.21 0.22 0.23	0.19 (0.18 to 0.21)
$NaNO_3$	240	0.10 0.11 0.12 0.13 0.14 0.15 0.16 0.17	0.13 (0.12 to 0.13)

Equivalent median lethal pH values for Choroterpes bugandensis in solutions made acid using hydrochloric, sulphuric and nitric acids are shown in tables 65, 66 and 67. As may be seen, nymphs of this species were rather less tolerant of acid solutions than were nymphs of the previous species. Carbon dioxide was again not observed either to affect the lethal pH value greatly or to be toxic on its own in the range in which it might be expected to occur in natural waters.

TABLE 65

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF HYDROCHLORIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
0	240	4.8 5.0 5.2 5.4 5.6 5.8	5.2 (5.2 to 5.3)
10	240	4.8 5.0 5.2 5.4 5.6 5.8	5.2 (5.1 to 5.3)
100	240	4.8 5.0 5.2 5.4 5.6 5.8	5.4 (5.3 to 5.5)

TABLE 66

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF SULPHURIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
0	240	4.8 5.0 5.2 5.4 5.6 5.8	5.2 (5.1 to 5.3)
10	240	4.8 5.0 5.2 5.4 5.6 5.8	5.3 (5.2 to 5.4)
100	240	4.8 5.0 5.2 5.4 5.6 5.8	5.3 (5.1 to 5.4)

TABLE 67

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF NITRIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	Test pH levels	Lethal pH, 95% confidence limits in brackets
0	240	5.0 5.2 5.4 5.6 5.8 6.0	5.4 (5.3 to 5.6)

TABLE 67 (cont)

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF FREE CARBON DIOXIDE. pH VALUES OBTAINED BY ADDITION OF NITRIC ACID

Free carbon dioxide (mg/l)	Number of nymphs	Test pH levels	Lethal pH, 95% confidence limits in brackets
10	240	5.0 5.2 5.4 5.6 5.8 6.0	5.4 (5.3 to 5.5)
100	240	5.0 5.2 5.4 5.6 5.8 6.0	5.6 (5.5 to 5.8)

The highest concentrations of chloride, sulphate and nitrate ions found in any tanks at the end of these experiments were 11, 14 and 21 meq/l, in that order. As may be seen from table 68, these concentrations were well below those concentrations of these ions which were found to be lethal for Choroterpes nymphs at pH 7.

NYMPHS OF DIFFERENT SIZES

Median lethal low pH values for Baetis harrisoni and Choroterpes bugandensis nymphs of different sizes are shown in tables 69 and 70. These nymphs were grouped according to body length, excluding antennae and cerci, measured under a microscope at the end of the experiment. The nymphs of different sizes did not differ significantly from one another in their tolerances of acid water.

TABLE 68

1000-MINUTE MEDIAN LETHAL CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS OF SODIUM CHLORIDE, SODIUM SULPHATE AND SODIUM NITRATE AT 20°C AND pH 7

Salt	Number of nymphs	Test salinities (eq/l)	Lethal concentration (eq/l as anion) 95% confidence limits in brackets
NaCl	240	0.16 0.17 0.18 0.19 0.20 0.21 0.22	0.20 (0.19 to 0.20)
Na_2SO_4	240	0.13 0.14 0.15 0.16 0.17 0.18 0.19 0.20	0.16 (0.16 to 0.17)
$NaNO_3$	240	0.08 0.085 0.09 0.095 0.10 0.11 0.12 0.13	0.10 (0.10 to 0.11)

PREVIOUS EXPOSURE TO ACID WATER

Median lethal low pH values for Baetis harrisoni and Choroterpes bugandensis nymphs held in the laboratory for 24 hours at pH 6.0 and at pH 8.0 before the start of the experiment are shown in tables 71 and 72. As may be seen, the pH tolerances of nymphs previously held at pH 6 and pH 8 did not differ significantly.

TABLE 69

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES

Body length (mm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
2.0 to 3.4	172	4.0 4.2 4.4 4.6	4.3 (4.0 to 4.5)
3.5 to 4.9	153	4.0 4.2 4.4 4.6	4.4 (4.2 to 4.7)
5.0 to 6.0	158	4.0 4.2 4.4 4.6	4.3 (4.2 to 4.5)

TABLE 70

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS OF DIFFERENT SIZES

Body length (mm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
1.5 to 2.9	170	5.0 5.2 5.4 5.6	5.3 (5.1 to 5.4)
3.0 to 4.4	160	5.0 5.2 5.4 5.6	5.2 (5.0 to 5.3)
4.5 to 6.0	160	5.0 5.2 5.4 5.6	5.2 (5.1 to 5.4)

TABLE 71

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR BAETIS HARRISONI NYMPHS PREVIOUSLY HELD AT pH 6 AND AT pH 8.

Previous pH	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
8	128	4.0 4.2 4.4 4.6	4.2 (4.0 to 4.4)
6	137	4.0 4.2 4.4 4.6	4.3 (4.0 to 4.6)

TABLE 72

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS PREVIOUSLY HELD AT pH 6 AND pH 8

Previous pH	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
8	160	5.0 5.2 5.4 5.6	5.4 (5.2 to 5.6)
6	160	5.0 5.2 5.4 5.6	5.3 (5.1 to 5.5)

TIME MORTALITY

Populations of each species were held in a specially constructed shallow dish which in turn was suspended in an experimental tank. A small stream of water from the tank was led into the dish. By means of a thin

wire passing under the glass cover of the tank it was possible at set intervals of time to wipe away any condensation water on the glass cover, to examine each individual and to prod individuals in order to establish whether they were alive or dead, if this was in doubt.

From the observed numbers dead and alive at intervals of time, median times of survival of each population were computed by probit analysis (Bliss 1937). These median times of survival for both Baetis harrisoni and Choroterpes bugandensis at different pH values are shown in figures 49 and 50. Here it may be seen that for each species the median time of survival increased with increase in pH value to an apparent asymptotic threshold value. The median times of survival appeared either to be less than 1000 minutes or to be infinite. Almost all of the mortality occurring at a particular pH took place within the standardized 1000-minute exposure time used in the dosage-mortality experiments reported at the beginning of this section. The 1000-minute median lethal pH values obtained from these experiments will therefore be close to the threshold median lethal values for prolonged exposure.

FIELD OBSERVATIONS

Harrison and Elsworth (1958) collected Baetis harrisoni nymphs in acid headwaters of the Great Berg River in the southern Cape Province. They found that the most acid water in which they collected this species had a pH value of 4.3 which corresponds almost exactly to the median lethal limit indicated by the present study. In fact considerable mortality of nymphs in most batches tested here would have been expected at this

pH value. Choroterpes bugandensis is not known from the Cape Province, but the related Choroterpes (Euthraulus) elegans has been found quite commonly in streams that are not acid (Harrison and Agnew 1962). Neither Baetis harrisoni nor Choroterpes (Euthraulus) has been collected in the course of any other published study of acid streams in this country (Harrison 1958c, Harrison, Keller and Dimovič 1960, Kemp 1967), suitable stony run biotopes being either not present or not sampled. In the catchment of the Olifants River in the eastern Transvaal, parts of which receive acid mine drainage, G.E. Venter (unpublished data) found Baetis harrisoni nymphs in slightly acid water (pH 5.5 minimum) but not in more acid stretches. He found Choroterpes (Euthraulus) nymphs in less acid water, the lowest value recorded at a place where they were found being pH 6.0. H.P. Hofmeyr (unpublished data) has made similar observations in this area.

Visits were also paid during the present study to certain of the acid polluted streams of the Olifants River catchment. Baetis harrisoni and Choroterpes bugandensis nymphs were found during each visit wherever they had previously been reported by Venter and Hofmeyr. The most severely acid stream visited was the Klipspruit in which G.E. Venter had found pH values over a period of ten months to remain between pH 2.7 and pH 3.1. Mine drainage pollution had evidently since been reduced in this stream and values measured during the present study varied between pH 3.8 and pH 4.5. The water here was clear and fairly deep flowing. Abundant vegetation was present, especially swamp reeds (Phragmites communis) and bog moss (Sphagnum sp.). No Baetis harrisoni or Choroterpes bugandensis nymphs were found here but, from the topography of the river, none would have

Figure 49

Median times of survival of Baetis harrisoni nymphs in acid solutions. 95% confidence limits shown about each median value.

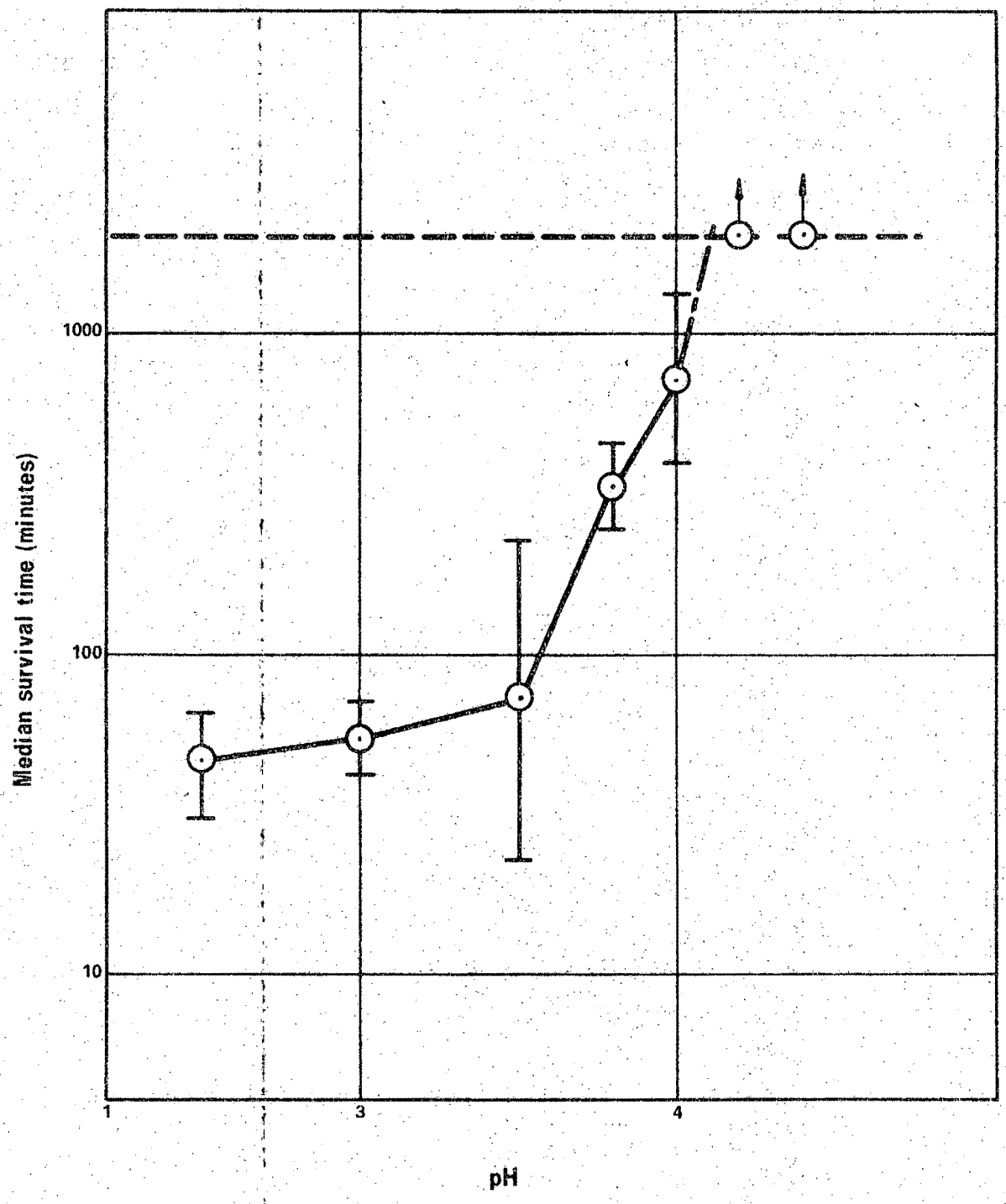
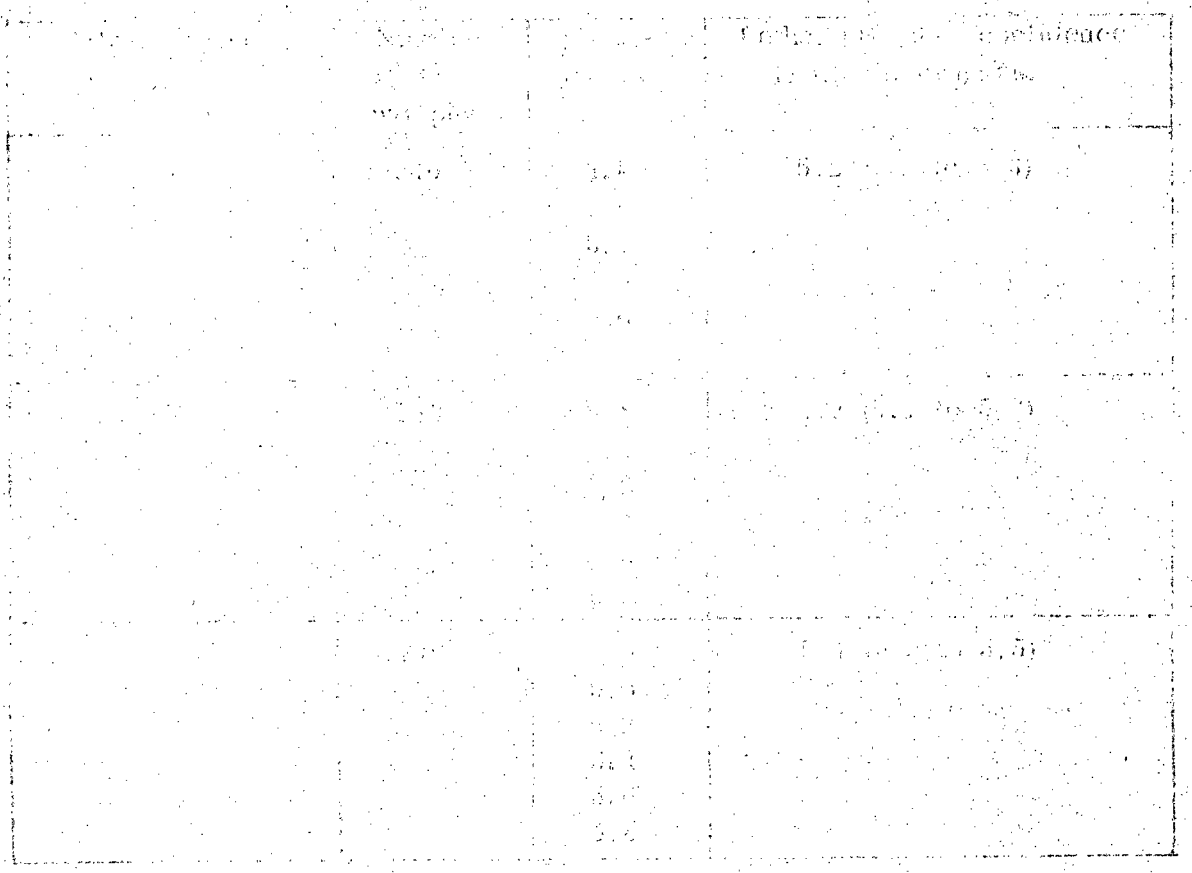
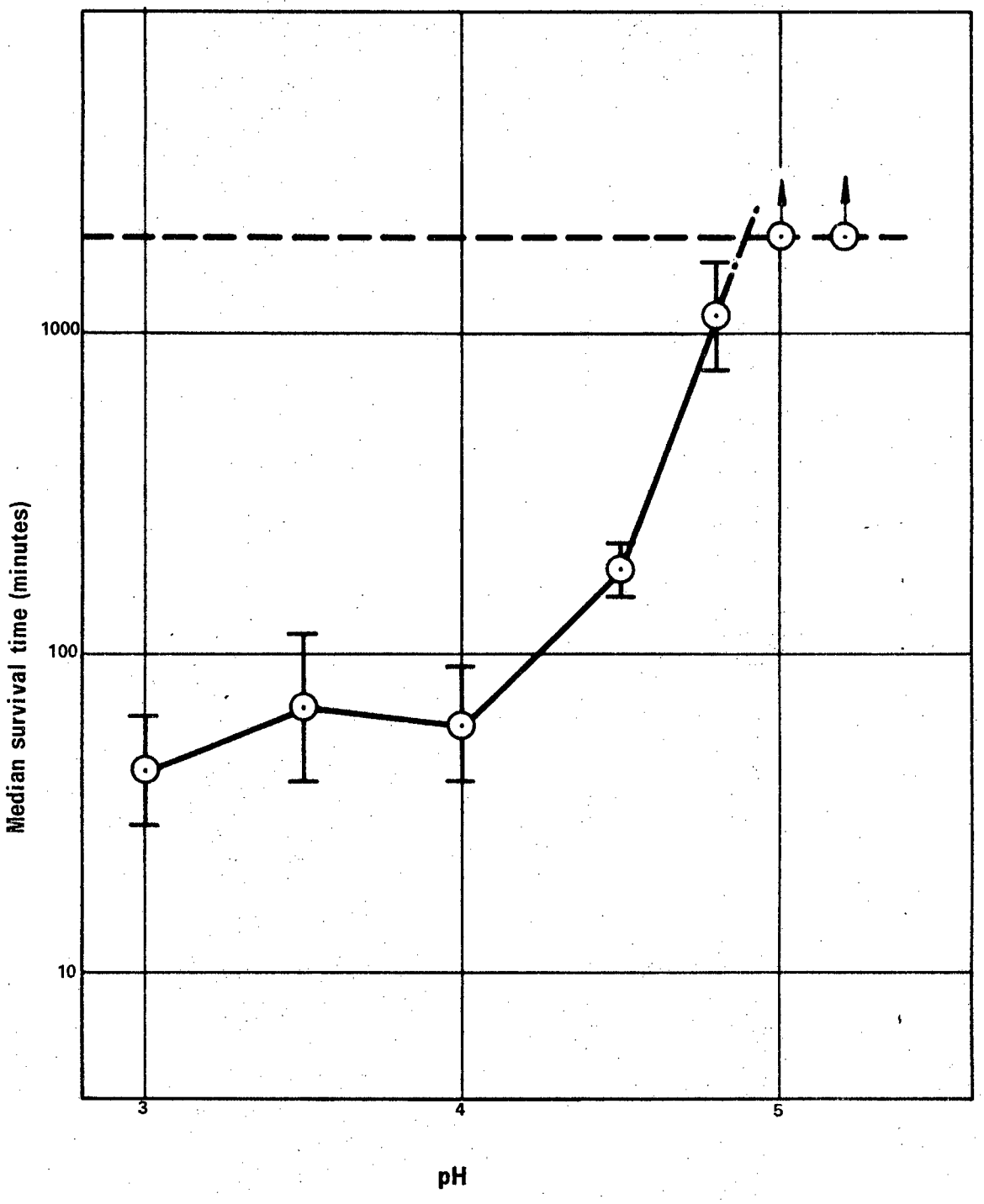

Median survival time (minutes)
1000
100
10
1
3
4
pH

Figure 50

Median times of survival of Choroterpes bugandensis nymphs in acid solutions. 95% confidence limits shown about each median value.




Median survival time (minutes)
1000
100
10
3
4
5
pH

been expected. No stony runs, the biotope in which they are typically found, were found in the length of stream visited.

Another tributary of the Olifants River visited during the present study was the Wilge River. This was also reported by G.E. Venter to receive acid drainage water. When visited during the present study the water was found to be shallow, clear and slightly acid (pH 6.1 to 6.4). Both Baetis harrisoni and Choroterpes bugandensis nymphs were found, though neither in any abundance.

Visits were also paid to the Coronation Stream, a small tributary of the Wilge River. The water here was also found to be shallow, clear and acid (pH 4.5 to 5.0). This stream was sandy and really suitable places for the collection of Baetis and Choroterpes nymphs were not found. However, a few Baetis harrisoni nymphs were found on each occasion on trailing vegetation. No Choroterpes were found.

These observations indicated quite clearly that Baetis harrisoni and Choroterpes bugandensis nymphs were to be found quite commonly in water in these streams only a little less acid than water which had been found in the laboratory to be lethal. A number of simple field experiments were then carried out in order to confirm that these species could not survive in tributaries in the Olifants River catchment which were more acid than those in which they had been found. For this purpose four frames 30 cm x 30 cm x 30 cm were made out of steel wire and covered on all sides with coarse nylon gauze. On two separate occasions these frames, containing both Baetis harrisoni and Choroterpes bugandensis nymphs, were placed in the

Klipspruit and the Coronation Stream and left there overnight. On each occasion the containers were anchored in a shallow flowing stretch of the stream both by means of rocks and using wire attached to spikes driven into the stream bank. The nymphs were inspected in the morning and numbers dead and alive noted.

Both Baetis harrisoni and Choroterpes bugandensis nymphs held in the Klipspruit in this way were found all to die. Measurements of pH made at different points in the stream both during the afternoon before the nymphs were put into the river and during the morning after they had been removed fell within the range pH 3.9 to 4.1.

When nymphs were held overnight in the Coronation Stream all of the Choroterpes bugandensis nymphs died and altogether 14 of a total of 151 Baetis harrisoni nymphs were found dead. Here pH measurements made during the afternoon before and morning after the exposure ranged between pH 4.8 and 4.9.

When nymphs collected in the Wilge River were held overnight in the Klipspruit and Coronation Stream, a smaller number of nymphs taken to these streams were taken back to the Wilge River on the same afternoon and held in one of the containers in the Wilge River. These were also inspected in the morning in order that their mortality could be compared with those of nymphs in the more acid streams. None of the Choroterpes nymphs held in the Wilge River was found to die. Of a total of 42 Baetis nymphs held in the Wilge River, 6 died possibly as a result of damage received in collection or transport.

DISCUSSION

Both Baetis harrisoni and Choroterpes bugandensis nymphs have been found in the field living in water of a wide range of pH values. In the present study it has been seen that they both were able to tolerate moderately low pH values in the laboratory and in places to live in streams at pH values only very slightly higher than those found to be lethal in the laboratory. Moreover, both species were found to die if held in streams slightly more acid than the most acid in which they were found living. The conclusion seems inescapable that the distribution of Baetis and Choroterpes in at least certain streams of the Olifants River catchment was limited by their inability to survive the unusually low pH values found in streams receiving the worst acid drainage.

Acid mine drainage has been found to cause acid pollution of streams elsewhere in the world and it has also been commonly found that these streams contain far fewer invertebrate species than do unpolluted streams (Jewell 1922, Lackey 1938, 1939, Parsons 1968). The fact that numbers of species were reduced in these streams has suggested that the more sensitive species were eliminated by lethal acid conditions. However, the fact that unpolluted and acid polluted waters are also inhabited by different algal species (Cholnoky 1968) could conceivably also influence the fauna present. Acid waters are generally less productive than are alkaline waters, possibly because bicarbonate concentrations are low and carbon dioxide consequently less readily available to photosynthesizing plants at low pH values (Johnson, Michalski and Christie 1969). Nothing is known of possible ways in which these differences might be reflected in the invertebrate faunas of acid and alkaline streams.

The acid streams of the Cape Province differ in many respects from those in the Transvaal and Natal. In the Cape acid streams, which drain peaty sponges on mountains composed of Table Mountain Sandstone, the water has been found to be soft, to be poorly buffered and to be low in dissolved salts (Harrison and Agnew 1962). In the Natal and Transvaal acid streams, which all receive acid mine drainage, the water has been found most often to be hard and fairly well buffered and almost invariably to contain very high concentrations of dissolved salts, especially sulphate ions. The water used in the experiments described have (see the general introduction) superficially resembled that of the Cape acid streams and there seems no reason to suppose that the low pH values found to be lethal to nymphs in the laboratory would not be lethal in these streams as well.

Hutchinson (1941) has commented very unfavourably on conclusions drawn from field data regarding possible influences of pH on animal abundances. As Harnisch (1951) and Macan (1961 b, 1963) have pointed out, many of the apparent correlations revealed in the literature with pH might as well have applied to other, less easily measured factors. Cholnoky (1968) has shown that certain algae found in the field only in water of certain pH values may be grown in the laboratory at quite different pH values. Mosquito larvae once thought only to live at certain pH values were subsequently found to tolerate an exceptionally wide range of pH (Keilin 1927) and pH is obviously not as important a factor as early workers once suggested (Skadowsky 1923, Bresslau 1926). However, the present study has certainly shown that instances occur where animals are prevented by low pH values alone from living in certain streams.

The time-mortality experiments reported here suggested that animals surviving 1000 minutes in acid water might be able to survive indefinitely. This is of interest in relation to observations made by other authors. Krey (1937) found the internal pH of certain Trichoptera not to be affected by the pH of the water in which they were living. This suggests that pH tolerance of these animals might depend on the degree to which the alkali reserves of their bodies are able to maintain the pH of their internal fluids (Harnisch 1951). If they are able to do this they will presumably survive indefinitely.

Some fish have been found similarly to be able to maintain the pH of their internal fluids (Powers and Logan 1925, Jobes and Jewell 1927, Wiebe et al. 1934). The tolerance limit for most fish is of the order of pH 5 (Ellis 1937, Doudoroff and Katz 1950, Jones 1964). Mortality of fishes in acid water can extend over several days and the mechanism of death of fish appears to differ from that in aquatic insects. Where the anion is not toxic, death has been held to result from the production of mucus on the gill surfaces causing asphyxiation (Ellis 1937, Westfall 1945). Coagulation of mucus has also been observed in aquatic snails (Carpenter 1923). Lloyd and Jordan (1964) on the other hand, have attributed death of trout in acid water to lowering of the internal pH.

It is of interest to note that many fish grow much more slowly in acid (but non-lethal) water than they do in alkaline water (Jones 1964). The reasons for this are not understood and early suggestions that fish in acid water have less food to eat have in certain instances at least been shown not to be true (Frost 1939, 1942). More recently Lloyd and Jordan (1964)

have shown that increased free carbon dioxide, although it did not affect the resistance times of fish at very low pH values, reduced the tolerances by trout of pH values at which they took several days to die.

The results of this study indicated that the tolerances by Baetis and Choroterpes nymphs of acid water were unaffected by moderate concentrations of free carbon dioxide and only slightly reduced in exceptionally high concentrations of free carbon dioxide. Naumann (1933), in contrast, has reported the reactions of Daphnia to light and other factors to be affected by carbon dioxide concentrations as low as 20 mg/l.

Comparison of the toxicity to aquatic invertebrates and fish of solutions made acid using different acids has shown that a number of anions are themselves toxic to these animals (Gueyland and Duval 1922, Jones 1939, 1941, Anderson 1946). Results presented here have shown nitrate ions to be slightly more toxic than either chloride or sulphate ions. The last two are apparently the least toxic to aquatic animals of all anions (Harnisch 1951).

SUMMARY

1. Baetis nymphs tolerated lower pH values (pH 4.3) than did Choroterpes nymphs (pH 5.2) Lower pH values than these are known to occur both in Transvaal streams receiving acid mine drainage, and in streams draining Table Mountain Sandstone mountains in the south-western Cape Fold Belt.

2. Dissolved free carbon dioxide up to 100 mg/l did not cause mortality of either Baetis or Choroterpes nymphs at pH 6 but increased mortality of these nymphs at lower pH values. Ten mg/l free carbon dioxide did not affect their lethal pH values.

3. Borehole water was toxic to Baetis and to Choroterpes nymphs at the same pH value whether it was made acid using hydrochloric or sulphuric acid. Water made acid using nitric acid was found to be toxic at a higher pH value.

4. Nymphs of either species held in acid water for 24 hours were not found to be more tolerant of low pH than were nymphs held in alkaline water.

5. Nymphs of different sizes did not differ in their pH tolerances.

6. With very few exceptions all mortality of nymphs in acid water took place during the first 1000 minutes of exposure.

7. Both species were found living in acid water in tributaries of the Olifants River in the eastern Transvaal. Baetis nymphs were found in several localities where the pH at the time of collection ranged from 4.5 to 5.0. Choroterpes nymphs were found in several localities where the pH at the time of collection ranged from 5.8 to 6.5

8. Baetis nymphs held overnight in a stream where the pH was measured to be 4.0 all died, as did Choroterpes nymphs held overnight in a stream at pH 4.8.

INFLUENCE OF TEMPERATURE, DISSOLVED OXYGEN AND WATER CURRENT SPEED ON MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN ACID SOLUTIONS

INTRODUCTION

It was reasoned in the previous section that the distribution of nymphs of both Baetis harrisoni and Choroterpes bugandensis in acid streams of the Olifants River catchment in the eastern Transvaal was limited by lethal low pH values found in those streams that were most severely polluted by mine drainage. In confirmation of laboratory observations of their tolerances, the two species were found in streams where the pH was slightly higher than their lethal limit and were absent from other more acid streams. Low pH values that would have been lethal for these species have been found in the Transvaal and in Natal only in those streams receiving acid mine drainage (Harrison 1958 c, Kemp 1967). Low pH values that would be lethal to Baetis nymphs have been recorded in the Cape Province in the naturally acid streams of the Cape Fold Belt (Harrison and Agnew 1962).

The toxicity of acid solutions to certain fish has been found to be greatly increased at low concentrations of dissolved oxygen, presumably because the availability of oxygen to these fish had been reduced by the formation of a mucus coagulation film on the gills (Westfall 1945). In those invertebrates in which no coagulation film is formed dissolved oxygen might still influence the toxicity of acid solutions, since Hyman (1925) found respiration rates of a number of invertebrates to be depressed at low pH values. As Harnisch (1951) has pointed out, pH changes can affect proteins and, through them, the permeability of biological membranes to oxygen and other dissolved gases.

Temperature is also known to affect the toxicity to aquatic animals of a number of poisons (Wuhrmann and Woker 1955). As Lloyd (1961a) has pointed out for fish, increased oxygen uptake results in increased production of carbon dioxide and a reduction in pH in the water immediately around the animal. Results are presented here of experiments in which the mortality and survival of Baetis harrisoni and Choroterpes bugandensis nymphs have been compared at a number of different combinations of temperature and dissolved oxygen concentrations.

In the previous section the possible influence of free carbon dioxide on the acid tolerances of Baetis and Choroterpes was investigated. Whereas fish had been found to be killed by high concentration of free carbon dioxide even at moderately high pH values (Lloyd and Jordan 1964), the acid tolerance of the mayfly nymphs being studied here was unaffected by moderate concentrations of free carbon dioxide. Lloyd and Jordan also found the toxicity of acid solutions to be reduced at high concentrations of bicarbonate ion. The experiments described here were carried out in water from which both the free carbon dioxide and the bicarbonate had as far as possible been removed.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected, as for other studies, in the Braamfontein Spruit and Pienaars River and held in the laboratory in well-aerated water at 20°C for a day before exposure to test solutions. Temperatures and dissolved oxygen concentrations in each

tank were controlled as has been described. During each experiment the animals were held in experimental tubes either of 1.6 cm internal diameter or of different diameters as will be described, through which a controlled current of water was driven.

In preparation for all except the last experiment reported here the borehole water to be used was first made acid (pH 4) by addition of hydrochloric acid and was bubbled for 24 hours with air which had been passed over potassium hydroxide. In this way most of both the dissolved carbon dioxide and the bicarbonate ions were removed. The test pH values required were then obtained by addition of sodium hydroxide. Aeration with the relevant gas mixture, from which the carbon dioxide had been removed, was continued and each tank was allowed to stabilize for a further 24 hours. Both during this period of equilibration and during the subsequent experiment, frequent measurements were made of pH values in each tank. Wherever necessary the pH values were corrected by adding small amounts of dilute hydrochloric acid or sodium hydroxide solution. At no time during any experiment was the pH in any tank found to deviate from the desired pH value by more than 0.1 of a pH unit.

Each of the experiments described here was carried out in separate parts over several days, since to have carried out an experiment all on one day would have needed many more tanks than were actually available. In each experiment numbers of nymphs at each temperature, dissolved oxygen concentration or water current speed were exposed to several different low pH values in the lethal range. Numbers surviving and not surviving exposure of 1000 minutes were noted. From these numbers the median lethal

low pH value at each temperature, dissolved oxygen concentration or water current current speed was calculated by probit analysis (Finney 1952).

INFLUENCE OF TEMPERATURE

In the first two experiments Baetis harrisoni and Choroterpes bugandensis nymphs were exposed at each of four different temperatures to a number of different pH values in the lethal range. These experiments were carried out during summer in oxygen saturated water. The animals were enclosed in experimental tubes of 1.6 cm internal diameter through which a laminar flow of water of 10 cm/sec was driven. Median lethal pH values at different temperatures are shown in tables 73 and 74. As may be seen, the acid tolerances of Baetis nymphs were found to decrease significantly with increase in temperature. The acid tolerances of Choroterpes nymphs also decreased significantly with increase in temperature although median lethal pH values at $5^{o}C$ intervals were individually not significantly different from one another.

Nymphs of both species were observed at all temperatures to have moulted during these experiments. Comparisons were made of the numbers of Baetis dead and alive in and out of ecdysis. Only in the case of Baetis nymphs at $25^{o}C$ was any difference in susceptibility of nymphs in and out of ecdysis found. As may be seen in table 73, nymphs attempting ecdysis at this temperature were found to be less tolerant of acid solutions than were nymphs not attempting ecdysis ($p < 0.05$). This difference in susceptibility presumably indicates that $25^{o}C$ was close to the lethal high temperature for

TABLE 73

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT TEMPERATURES

Temperature	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
10°C	189	3.6 3.8 4.0 4.2	3.9 (3.7 to 4.1)
15°C	195	3.8 4.0 4.2 4.4	4.1 (3.9 to 4.3)
20°C	198	4.0 4.2 4.4 4.6	4.2 (4.0 to 4.4)
25°C	111 attempting ecdysis	4.4 4.6 4.8 5.0	4.9 (4.7 to 5.2)
	84 not attempting ecdysis		4.7 (4.4 to 4.9)

this species. Results reported in an earlier section have shown that nymphs in ecdysis were more sensitive both to high temperatures and to oxygen lack than they were at other times. Intolerance by Baetis of high temperature evidently contributed to this mortality of nymphs in ecdysis at 25°C.

INFLUENCE OF DISSOLVED OXYGEN

In two further experiments, nymphs of each species were exposed to acid water at a number of different dissolved oxygen concentrations.

TABLE 74

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT TEMPERATURES

Temperature	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
10°C	160	4.6 4.8 5.0 5.2	5.0 (4.9 to 5.2)
15°C	160	4.8 5.0 5.2 5.4	5.1 (4.9 to 5.2)
20°C	160	4.8 5.0 5.2 5.4	5.2 (5.1 to 5.4)
25°C	160	5.0 5.2 5.4 5.6	5.3 (5.1 to 5.4)

These experiments were carried out at 20°C in a laminar flow speed of 10 cm/sec driven through experimental tubes of 1.6 cm internal diameter.

Median lethal pH values found for Baetis harrisoni and Choroterpes bugandensis nymphs at these different oxygen concentrations are shown in tables 75 and 76. As may be seen, the acid tolerances of these nymphs appeared to be unaffected by quite large differences in oxygen concentration. Only Baetis nymphs at the lowest oxygen level (4 mg/l) were found to be less tolerant of low pH values than were both those not attempting ecdysis ($p < 0.05$) and those at higher concentrations of dissolved oxygen ($p < 0.05$).

TABLE 75

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT DISSOLVED OXYGEN CONCENTRATIONS

Dissolved oxygen (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
8	187	3.8 4.0 4.2 4.4 4.6 4.8	4.3 (4.2 to 4.4)
6	183	3.8 4.0 4.2 4.4 4.6 4.8	4.1 (4.0 to 4.2)
4	117 attempting ecdysis	3.8 4.0 4.2 4.4 4.6	4.5 (4.3 to 4.6)
	80 not attempting ecdysis	4.8	4.3 (4.2 to 4.5)

It seems likely that oxygen lack contributed to the mortality of nymphs in ecdysis at 4 mg/l dissolved oxygen.

INFLUENCE OF WATER FLOW

In another two experiments, summer Baetis harrisoni and Choroterpes bugandensis nymphs were exposed to acid water in different rates of water flow. These experiments were carried out at 20°C and in water containing about 7.5 to 7.7 mg/l dissolved oxygen, a concentration close to saturation.

TABLE 76

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT DISSOLVED OXYGEN CONCENTRATIONS

Dissolved oxygen (mg/l)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
8	240	4.6 4.8 5.0 5.2 5.4 5.6	5.2 (5.1 to 5.4)
4	240	4.6 4.8 5.0 5.2 5.4 5.6	5.1 (5.0 to 5.2)
2	240	4.6 4.8 5.0 5.2 5.4 5.6	5.3 (5.1 to 5.4)

Different controlled rates of water flow were obtained using experimental tubes of different diameters, as has been described in earlier sections.

Median lethal low pH values for Baetis harrisoni and for Choroterpes bugandensis nymphs at these different rates of water flow are shown in tables 77 and 78. Baetis nymphs at very slow water current speeds were found to be less tolerant of low pH values than were nymphs at other speeds ($p < 0.05$). In this case, individuals attempting ecdysis during the experiment were less tolerant than were those not attempting ecdysis ($p < 0.05$) and

the median lethal pH value was estimated seperately for nymphs in and out of ecdysis. In all other situations nymphs in and out of ecdysis and at different current speeds appeared to be equally tolerant, as did Choroterpes nymphs.

TABLE 77

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER BAETIS HARRISONI NYMPHS IN DIFFERENT RATES OF WATER FLOW

Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
2.7	laminar	2.6	67 attempting ecdysis	4.2 4.4 4.6 4.9	4.7 (4.5 to 4.9)
			107 not attempting ecdysis		4.4 (4.2 to 4.6)
7.0	laminar	1.6	163	4.0 4.2 4.4 4.6	4.1 (4.0 to 4.2)
22.2	laminar	0.9	162	4.0 4.2 4.4 4.6	4.2 (4.1 to 4.4)
8.0	turbulent	5.0	160	4.0 4.2 4.4 4.6	4.2 (4.1 to 4.4)
14.8	turbulent	2.6	178	4.0 4.2 4.4 4.6	4.1 (4.0 to 4.2)
39.1	turbulent	1.6	154	4.0 4.2 4.4 4.6	4.3 (4.1 to 4.5)

TABLE 78

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT RATES OF WATER FLOW

Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
2.7	laminar	2.6	160	5.0 5.2 5.4 5.6	5.3 (5.2 to 5.4)
7.0	laminar	1.6	158	5.0 5.2 5.4 5.6	5.2 (5.1 to 5.4)
22.2	laminar	0.9	159	5.0 5.2 5.4 5.6	5.3 (5.2 to 5.4)
8.0	turbulent	5.0	160	5.0 5.2 5.4 5.6	5.3 (5.1 to 5.4)
14.8	turbulent	2.6	160	5.0 5.2 5.4 5.6	5.3 (5.2 to 5.5)
39.1	turbulent	1.6	160	5.0 5.2 5.4 5.6	5.3 (5.2 to 5.4)

Findings have been reported in an earlier section which showed that Baetis harrisoni nymphs suffered from oxygen lack at slow water current speeds. Both higher mortality of Baetis nymphs at low water current speeds and increased sensitivity of ecdysing nymphs in this situation strongly

suggest an insufficiency of oxygen to have been the factor affecting the acid tolerance of these animals. The data shown in tables 75 and 77 are in general agreement in this respect.

Another alternative possible explanation for the reduced acid tolerance of Baetis at low water flows and at low oxygen concentrations suggests itself. It could be argued that a reduction in pH might have taken place in the immediate vicinity of the animal as a result of the production of respiratory carbon dioxide. Animals in ecdysis presumably produced carbon dioxide at a greater rate than at other times and would in turn have been subjected to lower pH values than were animals not in ecdysis. At faster water flows respiratory carbon dioxide would have been washed away and the effect of respiratory carbon dioxide would have disappeared.

In order further to investigate the suggestion that respiratory carbon dioxide might have influenced the tolerance by Baetis nymphs of low pH values a further experiment was conducted. Nymphs were exposed to acid water in the lethal pH range at each combination of two different concentrations of dissolved oxygen, two different concentrations of free carbon dioxide and two different water current speeds. The median lethal pH values found for each of these different situations are shown in table 79.

In many of the combinations tested in this experiment no difference could be detected between the sensitivities of nymphs in and out of ecdysis. However, lethal values were estimated separately for each.

The results of this experiment are at first sight somewhat curious. In the previous section results were reported showing that, at low pH levels, free carbon dioxide did not apparently influence acid tolerances of nymphs,

TABLE 79

1000-MINUTE MEDIAN LETHAL LOW pH VALUES FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION, FREE CARBON DIOXIDE CONCENTRATION AND WATER CURRENT SPEED (LAMINAR THROUGHOUT)

Dissolved oxygen (mg/l)	Free carbon dioxide (mg/l)	Water current speed (cm/sec)	Tube diameter (cm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
8	0	2	2.6	80 attempting ecdysis	4.2	4.7(4.4 to 4.9)
				132 not in ecdysis	4.4 4.6 4.8	4.4(4.3 to 4.6)
		5.3	1.6	74 attempting ecdysis	4.0 4.2	4.4(4.2 to 4.5)
				143 not in ecdysis	4.4 4.6	4.3(4.1 to 4.5)
	10	2	2.6	98 attempting ecdysis	4.2	4.4(4.2 to 4.6)
				123 not in ecdysis	4.4 4.6 4.8	4.4(4.2 to 4.6)
		5.3	1.6	92 attempting ecdysis	4.0 4.2	4.3(4.1 to 4.6)
				128 not in ecdysis	4.4 4.6	4.3(4.1 to 4.5)

TABLE 79 (cont)

Dissolved oxygen (mg/l)	Free carbon dioxide (mg/l)	Water current speed (cm/sec)	Tube diameter (cm)	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
4	0	2	2.6	78 attempting ecdysis	4.4 4.6	4.8(4.5 to 5.0)
				145 not in ecdysis	4.8 5.0	4.7(4.5 to 4.9)
		5.3	1.6	77 attempting ecdysis	4.0 4.2	4.6(4.5 to 4.7)
				139 not in ecdysis	4.4 4.6	4.3(4.2 to 4.5)
	10	2	2.6	63 attempting ecdysis	4.2 4.4	4.7(4.5 to 4.9)
				156 not in ecdysis	4.6 4.8	4.4(4.2 to 4.5)
		5.3	1.6	85 attempting ecdysis	4.0 4.2	4.4(4.2 to 4.6)
				124 not in ecdysis	4.4 4.6	4.5(4.4 to 4.6)

while exceptionally high free carbon dioxide concentrations made nymphs a little less tolerant of acid solutions. The results shown in table 79 appear to show the opposite. The median lethal low pH values in the presence of 10 mg/l free carbon dioxide were not ever significantly higher than those found in the

absence of carbon dioxide. In three instances (nymphs in ecdysis in 2 cm/sec flow and 8 mg/l oxygen, nymphs not in ecdysis in 2 cm/sec flow and 4 mg/l oxygen and nymphs in ecdysis in 5.3 cm/sec flow in 4 mg/l oxygen, for each of which $p < 0.05$) the nymphs in water free of carbon dioxide were less tolerant than those in 10 mg/l carbon dioxide. Only one possible explanation for the decreased tolerance in these instances of nymphs in the absence of carbon dioxide seems to suggest itself. At the lower current speed of this experiment it might perhaps have been possible for respiratory carbon dioxide in unbuffered water free of carbon dioxide to reduce the pH of water in the immediate vicinity of nymphs and so to increase the mortality of nymphs in this water. In the faster flows of the previous experiment, it is suggested this did not occur.

If these results show that respiratory carbon dioxide could cause increased mortality of Baetis nymphs at low water flows in acid solutions, they also show that this was not the only interaction between dissolved oxygen, carbon dioxide and water flow which occured. Lethal pH values were lower in faster than they were in slower water flows (pooled Student's t test, $p < 0.025$). Furthermore, lethal pH values were also lower at higher dissolved oxygen concentrations ($p < 0.025$). It is possible to link water current speed with the influence of respiratory carbon dioxide, but the influence of oxygen is clearly a separate factor influencing the toxicity of acid solutions to nymphs.

SUMMARY

1. Median lethal low pH values for Baetis nymphs increased with increase in temperature (from pH 3.9 at 10°C to pH 4.7 to 4.9 at 25°C) as did those for Choroterpes nymphs (from pH 5.0 at 10°C to pH 5.3 at 25°C).

2. Choroterpes nymphs were equally tolerant of acid solutions in a wide range of dissolved oxygen concentrations and water current speeds (lethal level pH 5.1 to 5.3)

3. Baetis nymphs were less tolerant of acid solutions at low dissolved oxygen concentrations near the lethal limit (4 mg/l) than they were at higher dissolved oxygen concentrations (lethal level pH 4.3 to 4.5 as opposed to pH 4.1 to 4.3).

4. Baetis nymphs were less tolerant of acid solutions at very slow water current speeds than they were in faster flowing water (lethal level pH 4.4 to 4.7 as opposed to pH 4.1 to 4.3).

5. Baetis nymphs were also found, at very slow water flow rates (2 cm/sec), to be more tolerant in some instances of acid solutions where 10 mg/l free carbon dioxide was present than they were in the absence of free carbon dioxide (lethal level pH 4.3 to 4.5 as opposed to pH 4.4 to 4.8).

MORTALITY AND SURVIVAL OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS IN ALKALINE SOLUTIONS

INTRODUCTION

During the present study of the tolerances of Baetis harrisoni and Choroterpes bugandensis nymphs several determinations were made of pH values in situations in the Transvaal where these species were found. On one occasion in the polluted Jukskei River, where the first species occurs, a diurnal rise in pH to pH 9.2 was observed. On another occasion in the Pienaars River, where the second species occurs, pH 9.0 was recorded. In both instances, these high pH values appeared to be associated with algal activity at low stream flows. It seems reasonable to suppose that even higher pH values might occur from time to time in these and other rivers. High pH values of this order approach those which are lethal for a wide range of aquatic invertebrates and fish (Doudoroff and Katz 1950). Results are reported here of experiments carried out in the laboratory to establish the lethal limits of high pH for Baetis and Choroterpes nymphs.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected as before in the Braamfontein Spruit and Pienaars River and held in the laboratory at 20°C and at pH 7 and other specified pH values in the laboratory for a day before being exposed for 1000 minutes to different high pH values in the lethal range. All the experiments described here were carried out at 20°C in water flowing at 10 cm/sec through tubes of 1.6 cm internal diameter, at which speed the flow in these tubes was laminar.

Experimental pH values were obtained by addition of sodium hydroxide or other alkaline solutions to borehole water. Frequent pH measurements were made both during the 24 hours set aside to allow the water to stabilize and during the subsequent experiment. The pH values were corrected as required. Deviations from desired values did not at any time during an experiment exceed 0.1 of a pH unit.

TOXICITY OF ALKALINE SOLUTIONS

Nymphs of Baetis harrisoni and Choroterpes bugandensis were exposed to solutions made alkaline by addition of sodium hydroxide, sodium bicarbonate and calcium hydroxide and mortality of nymphs exposed to these solutions for 1000 minutes was noted. Median lethal pH values calculated by probit analysis (Finney 1952) from these observations are shown in tables 80 and 81. These show that similar pH values were tolerated in all three solutions. Both species were able to tolerate pH values well in excess of any known to occur in South African rivers. Baetis nymphs were found to be significantly more tolerant of high pH values than were Choroterpes nymphs.

TIME MORTALITY

Diurnal rises in pH resulting from algal photosynthesis inevitably last only for a few hours. In order to predict whether or not exceptional pH rises of this sort might be lethal it is necessary to know what times of exposure to different high pH values would be necessary to cause mortality. For this purpose, nymphs of each species were placed in open containers made of gauze and suspended in the experimental tanks in which

different high pH values in the lethal range were maintained. Here they could be prodded from time to time to distinguish living from dead individuals. Numbers dead at different times were counted. From these counts median survival times at each pH were calculated by probit analysis (Bliss 1937).

TABLE 80

1000-MINUTE MEDIAN LETHAL HIGH pH VALUES FOR BAETIS HARRISONI NYMPHS IN DIFFERENT SALT SOLUTIONS

Alkali used	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
NaOH	240	10.2 10.4 10.6 10.8 11.0 11.2	10.7 (10.6 to 10.9)
$NaHCO_3$	240	10.2 10.4 10.6 10.8 11.0 11.2	10.8 (10.7 to 11.0)
$Ca(OH)_2$	235	10.2 10.4 10.6 10.8 11.0 11.2	10.7 (10.6 to 10.8)

Median times of survival of Baetis harrisoni and Choroterpes buganden-sis nymphs calculated from these observations are illustrated, respectively in figures 51 and 52. From these data it seems that exposures of 2-3 hours to pH 11 would be lethal for Baetis nymphs and similar exposure to pH 10.5 would be lethal for Choroterpes nymphs.

TABLE 81

1000-MINUTE MEDIAN LETHAL HIGH pH VALUES FOR CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT SALT SOLUTIONS

Alkali used	Number of nymphs	pH test levels	Lethal pH, 95% confidence limits in brackets
NaOH	240	9.8 10.0 10.2 10.4 10.6 10.8	10.2 (10.1 to 10.3)
$NaHCO_3$	242	9.8 10.0 10.2 10.4 10.6 10.8	10.3 (10.1 to 10.4)
$Ca(OH)_2$	240	9.8 10.0 10.2 10.4 10.6 10.8	10.2 (10.1 to 10.4)

DISCUSSION

The lethal values found here for Baetis and Choroterpes nymphs fall within the range found lethal for other animals. Reliable estimates of maximal pH values tolerated by fish and various invertebrates which have appeared in the literature have varied between about pH 9.5 and pH 11.5 (Anderson 1927, Bandt 1936, Ellis 1937, Eicher 1946, Doudoroff and Katz 1950, Ivasik 1959, Malovitskaya 1961, Bogatova 1962, Jones 1964, Jordan and Lloyd 1964). Lethal values outside of this range have also been reported. However, it is difficult to evaluate these findings. They all appear to conflict with the

Figure 51

Median times of survival of Baetis harrisoni nymphs in alkaline solutions. 95% confidence limits shown about each median value.

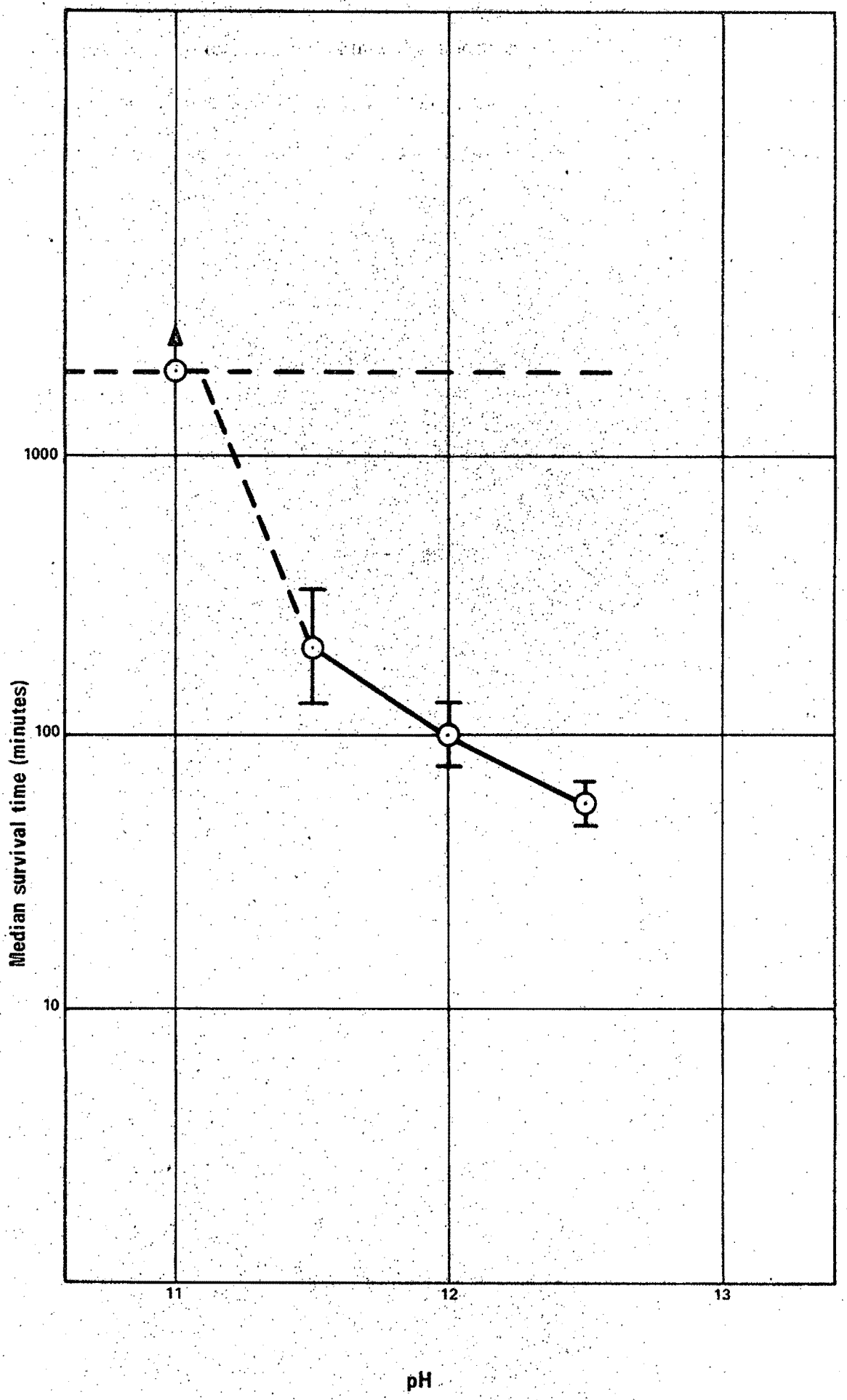
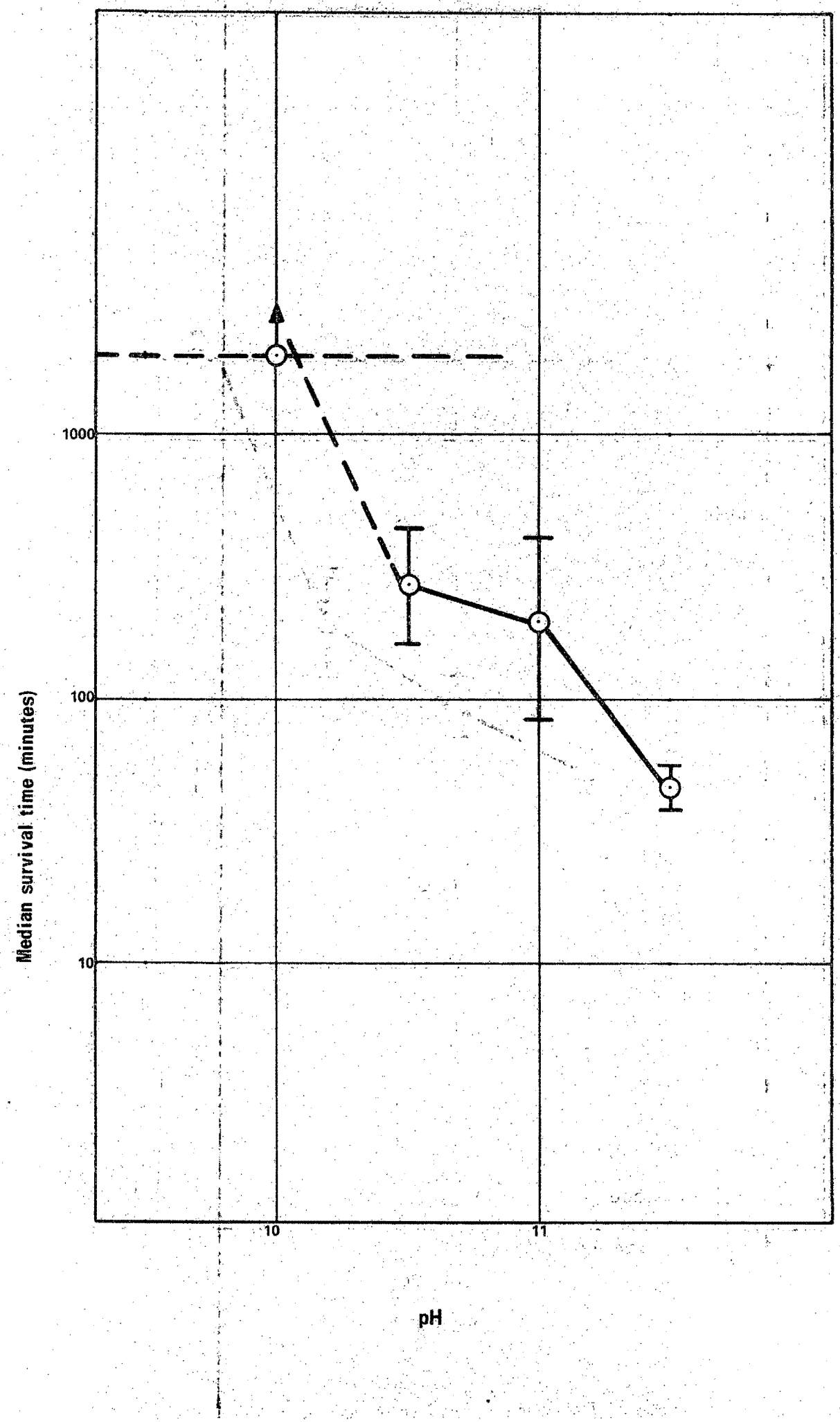

1000
100
10
Median survival time (minutes)
11
12
13
pH

Figure 52

Median times of survival of Choroterpes bugandensis nymphs in alkaline solutions. 95% confidence limits shown about each median value.


1000
100
10
Median survival time (minutes)
10
11
pH

results of later workers.

Little is known of the relative toxicities of those cations which might be associated in the field with highly alkaline natural waters and industrial effluents. These would include sodium, potassium, calcium and magnesium. Schröder (1924) found that rotifers he studied were more tolerant of high pH values when these were achieved by addition of calcium hydroxide than they were when sodium hydroxide was the alkali used. However, in reviewing the literature, Jones (1964) has concluded that possibly toxicity of the cations present is of only minor importance. In the present study solutions of sodium and calcium hydroxide appeared to be equally toxic at the same pH, as did solutions of sodium bicarbonate. The bicarbonate alkalinity of the water therefore also had no evident influence on the toxicity of alkaline solutions.

In relation to cation toxicity at high pH values it might perhaps be mentioned that increase in pH could under certain circumstances influence the solubility and hence the toxicity of cations other than those of the alkali added. This problem is discussed in a later section dealing with heavy metal toxicity.

SUMMARY

1. Baetis nymphs tolerated higher pH values (1000-minute median lethal value pH 10.7) than did Choroterpes nymphs (lethal value pH 10.2).
2. Both species were able to tolerate even higher pH values if these were maintained only for 2-3 hours. (Baetis pH 11.2, Choroterpes pH 10.4
3. Solutions made alkaline by the addition to borehole water of sodium hydroxide, sodium bicarbonate and calcium hydroxide were equally toxic to Baetis and Choroterpes nymphs at the same pH value.

TOXICITY OF AMMONIA TO BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

INTRODUCTION

In has been known for many years that the toxicity to aquatic animals of solutions of ammonium salts was greatly influenced by the pH of the solution (Ellis 1937). Wuhrmann, Zehender and Woker (1947) and Wuhrmann and Woker (1948) were the first to show that the toxicity to fish of solutions of ammonium salts depended on the concentrations of free "unionized" ammonia (NH_3 or NH_4OH) present rather than that of ammonium ions. Since the ammonia-ammonium ratio in these solutions increases very rapidly with increase in pH value around pH 8 (Bates and Pinching 1950), this explained why solutions which were apparently non-toxic at pH 7 became extremely toxic to fish when the pH was raised. Later work (Warren 1962) showed ammonia and ammonium to be equally toxic to living cells once they had penetrated the cell membrane. However, ammonia enters cells very rapidly while ammonium ions have been found to enter only very slowly (Milne, Scribner and Crawford 1958).

High concentrations of ammonia are common in polluted rivers and ammonia had been held to be the factor most likely to cause fish mortalities in rivers polluted by domestic sewage (Lloyd 1961a). Mayflies, in common with other aquatic insects, have been found to tolerate quite high concentrations of ammonia in laboratory experiments (Stammer 1953). However, field observations indicate that they are relatively less tolerant of stream pollution than are most other aquatic invertebrates. Mayfly nymphs

have been found to disappear even from fairly well-oxygenated streams receiving organic pollution (Harrison 1958b). Absence of mayflies in general and of certain species in particular has therefore been used as a measure of stream pollution (Beak 1965, Chutter in press). This study of the toxicity of ammonia to nymphs of Baetis harrisoni and Choroterpes bugandensis was undertaken in order to see at what concentrations these species would be eliminated from polluted Transvaal streams.

No observations of ammonia toxicity mean very much without reference to the pH of the medium. Other factors which should apparently also be taken into account are the free carbon dioxide concentration (Lloyd and Herbert 1960) and the bicarbonate alkalinity (Brown 1968). These have both been found to influence the toxicity of ammonia solutions to fish. Since both of these factors are also markedly affected by the pH of the water, their independent influence on the toxicity of ammonia to Baetis and Choroterpes nymphs has been investigated as part of the present study.

Lethal conditions for mayflies in streams containing ammonia-ammonium solutions are presumably most likely to be created when pH values in the river water rise during the day as a result of photosynthetic activity. Even a small rise in pH would cause the ratio of unionized ammonia to ammonium ions to rise sharply. Such rises in pH are very common in streams carrying pollution and were observed, for instance, by Allanson (1961) in the Jukskei River. In order to be able to predict possible lethal conditions in the field under such circumstances it was necessary to know something of the time-mortality relations of Baetis and Choroterpes nymphs in ammonia solutions. The

times to death of nymphs of the two species being studied were therefore investigated.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected in the Braamfontein Spruit and Pienaars River and, after being kept in the laboratory for a day in well-aerated water at 20°C, were exposed to ammonia-ammonium solutions under controlled conditions in the apparatus described in the introductory section.

For each tank, the amount of ammonium chloride required to give a desired concentration of ammonia at the temperature and pH of the test was calculated. Total ammonium concentrations were determined at the end of each test in order to ascertain whether or not there had been any significant loss of gaseous ammonia to the air. In no case was the loss over 1000 minutes greater than 2 per cent. In most cases it was much less.

Different concentrations of free carbon dioxide were obtained in the test tanks as required by mixing small controlled quantities of carbon dioxide gas with compressed air being bubbled through each tank. The amount of carbon dioxide required in each instance was calculated beforehand. In order to obtain water free of carbon dioxide the water was first made acid (pH 6) by addition of hydrochloric acid and aerated strongly with air which had been passed over potassium hydroxide for 24 hours. The pH was then re-adjusted using sodium hydroxide. The concentration of free carbon dioxide was estimated either from first principles (Saunders 1926) or using the

nomogram of Dye (1952) based on the temperature, pH and bicarbonate alkalinity.

Desired pH values were achieved by the addition of small quantities of dilute hydrochloric acid or sodium hydroxide. This was done after the bicarbonate had been added and the water bubbled for two hours with the gas mixture being used. After adjustment of the pH the water was bubbled for 48 hours and allowed to reach an equilibrium. pH measurements were made at frequent intervals during this period and throughout the course of each experiment. Droplets of acid or alkali were added as required. In no case was a deviation greater than 0.1 of a pH unit recorded, in spite of the very low buffering capacity of many of the solutions tested. The bicarbonate alkalinity was also determined from time to time. In no case did this differ much from desired values.

The experiments described here were all carried out at 20°C and at a water current speed of 10 cm/sec. Experimental tubes of 1.6 cm internal diameter were used throughout, so that flow in each tube was laminar. Groups of animals were exposed in the tubes to different ammonia solutions for 1000 minutes in each experiment and median lethal ammonia concentrations estimated by probit analysis (Finney 1952) from numbers not recovering from this exposure.

AMMONIA AND pH

In a first series of experiments, nymphs of each species were exposed to different ammonia concentrations at pH 6, 7 and 8. Free carbon dioxide concentrations and bicarbonate alkalinities were kept as far as possi-

ble to levels of the same order at the different pH values used. The results are given in tables 82 and 83, showing 1000-minute median lethal concentrations calculated as unionized ammonia fraction and expressed as mg/l N.

TABLE 82

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS AT DIFFERENT pH VALUES (20°C)

pH	Free carbon dioxide (mg/l)	Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
6.0	10	5	240	6.8 7.0 7.2 7.5 7.8 8.2	7.2 (7.0 to 7.5)
7.0	2	10	240	6.8 7.0 7.2 7.5 7.8 8.2	7.1 (6.9 to 7.3)
8.0	0.2	15	240	6.8 7.0 7.2 7.5 7.8 8.2	7.1 (7.0 to 7.3)

Tables 82 and 83 show that Baetis harrisoni nymphs were found to tolerate roughly twice the concentration of ammonia tolerated by Choroterpes bugandensis nymphs. For each species the median lethal concentrations of unionized ammonia were found to be independent of pH at the levels tes-

ted in spite of the fact that very much higher concentrations of ammonium ions were present at pH 6 than were needed at pH 8 for the same unionized ammonia concentration to be present. This seems to confirm that it is the unionized ammonia fraction and not the ammonium which is toxic to these animals. It also seems to confirm that while pH is critically important in determining the ammonia-ammonium proportions it does not in itself appear to affect the toxicity of ammonia.

TABLE 83

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT pH VALUES (20°C)

pH	Free carbon dioxide (mg/l)	Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentrations (mg/l as N), 95% confidence limits in brackets
6.0	10	5	272	3.4 3.5 3.6 3.8 4.0 4.2	3.7 (3.5 to 4.0)
7.0	2	10	261	3.4 3.5 3.6 3.8 4.0 4.2	3.5 (3.3 to 3.7)
8.0	0.2	15	263	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.4 to 3.8)

AMMONIA AND CARBON DIOXIDE

In a second series of experiments, nymphs of Baetis harrisoni and Choroterpes bugandensis were exposed to different ammonia concentrations in the presence of four very different concentrations of free carbon dioxide. Bicarbonate alkalinities and pH values were selected as far as possible to be of a similar order throughout.

The results shown in tables 84 and 85 reveal no evidence that mortality was in any way influenced by the concentration of free carbon dioxide present.

AMMONIA AND BICARBONATE

In a third similar series of experiments, nymphs of each species were exposed for 1000 minutes to ammonia concentrations at three widely differing bicarbonate concentrations. Once again, pH values and free carbon dioxide concentrations in these test solutions were selected to be at least of the same order.

The results of these experiments are shown in tables 86 and 87. From these results it seems that the ammonia tolerances of nymphs of both species were unaffected by bicarbonate alkalinity differences, at least over the fairly wide range tested. These results contrast with those of studies carried out with fish (Brown 1968), in which the toxicity of ammonia solutions was found to be influenced by the bicarbonate alkalinity.

TABLE 84

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS IN DIFFERENT FREE CARBON DIOXIDE CONCENTRATIONS (20°C)

Free carbon dioxide (mg/l)	pH	Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N) 95% confidence limits in brackets
0.1	7.0	5	240	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.0 to 7.5)
5	7.6	100	240	6.6 6.8 7.0 7.2 7.5 7.8	7.0 (6.8 to 7.2)
20	7.1	100	240	6.6 6.8 7.0 7.2 7.5 7.8	7.1 (7.0 to 7.3)
100	6.3	100	240	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.0 to 7.3)

TABLE 85

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT FREE CARBON DIOXIDE CONCENTRATIONS (20°C)

Free carbon dioxide (mg/l)	pH	Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N) 95% confidence limits in brackets
0.1	7.0	5	240	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.4 to 3.8)
5	7.6	100	240	3.4 3.5 3.6 3.8 4.0 4.2	3.5 (3.4 to 3.7)
20	7.1	100	240	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.4 to 3.8)
100	6.3	100	240	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.5 to 3.8)

TABLE 86

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS AT DIFFERENT BICARBONATE ALKALINITIES (pH 8.0)

Bicarbonate alkalinity (mg/l as $CaCO_3$)	Free carbon dioxide (mg/l)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
2	0.1	240	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.0 to 7.4)
20	0.4	240	6.6 6.8 7.0 7.2 7.5 7.8	7.3 (7.1 to 7.4)
200	4	240	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.0 to 7.5)

NYMPHS OF DIFFERENT SIZES

For each species, nymphs of widely differing sizes were exposed to a number of different ammonia concentrations and their mortality compared. At the end of each experiment the length of each individual, excluding the antennae and cerci, was measured under a microscope. From the results shown in tables 88 and 89 it seems that the different size groups of each species tested were similarly tolerant of ammonia.

TABLE 87

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT BICARBONATE ALKALINITIES (pH 8.0, 20°C)

Bicarbonate alkalinity (mg/l as $CaCO_3$)	Free carbon dioxide (mg/l)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
2	0.1	228	3.4 3.5 3.6 3.8 4.0 4.2	3.5 (3.3 to 3.8)
20	0.4	213	3.4 3.5 3.6 3.8 4.0 4.2	3.5 (3.3 to 3.8)
200	4	218	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.4 to 3.8)

TABLE 88

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS OF DIFFERENT SIZES (pH 8.0, 20°C)

Body length (mm)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
2.0 to 3.4	160	6.8 7.0 7.2 7.5	7.0 (6.9 to 7.3)
3.5 to 4.9	160	6.8 7.0 7.2 7.5	7.2 (6.8 to 7.5)

TABLE 88 (cont)

Body length (mm)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
5.0 to 6.5	160	6.8 7.0 7.2 7.5	7.2 (7.0 to 7.4)

TABLE 89

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS OF DIFFERENT SIZES (pH 8.0, 20°C)

Body length (mm)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
1.5 to 2.9	157	3.4 3.6 3.8 4.0	3.4 (3.2 to 3.7)
3.0 to 4.4	148	3.4 3.6 3.8 4.0	3.5 (3.2 to 3.8)
4.5 to 6.0	163	3.4 3.6 3.8 4.0	3.6 (3.4 to 3.9)

TIME MORTALITY

Time-mortality tests using mayfly nymphs are less satisfactory in some ways than are dosage-mortality tests. In order to count numbers dead and alive at intervals of time each individual must be prodded. This can only be done in an open container, where no effective control of water

flow conditions is possible. Moreover, it seems from results presented in previous sections that Baetis nymphs are sensitive to physical disturbances once they have started ecdysis.

Mortality of each species at time intervals in various ammonia concentrations in the lethal range was observed in this way. Median survival times (Bliss 1937) for these nymphs are shown in figures 53 and 54 as a function of unionized ammonia concentration. A small point to bear in mind in comparing time-mortality and dosage-mortality data might be mentioned. In the former, the numbers of animals actually living or dead are counted at each time interval. Dying animals are counted as alive. In the latter, numbers of animals eventually recovering and not recovering after a given exposure time are counted. In this case, dying animals are counted as dead.

From figures 53 and 54 it may be seen that even short exposures to high ammonia concentrations were lethal for Baetis and Choroterpes nymphs. Nymphs were also still found to be dying at the end of 1000 minutes' exposure and further mortality would apparently have occured if the experiment had been continued. This suggests that 1000-minute median lethal concentrations quoted earlier do not represent "incipient" lethal limits (Fry 1947) for prolonged exposure.

PREVIOUS EXPOSURE TO AMMONIA

In order to see what effects previous exposure to sub-lethal ammonia concentrations might have had on the ammonia tolerances of Baetis and Choroterpes nymphs, populations of each were kept in the laboratory for 48 hours

Figure 53

Median times of survival of Baetis harrisoni nymphs in ammonium chloride solutions at pH 8.0 and 20°C (concentrations given as unionized ammonia mg/l N).

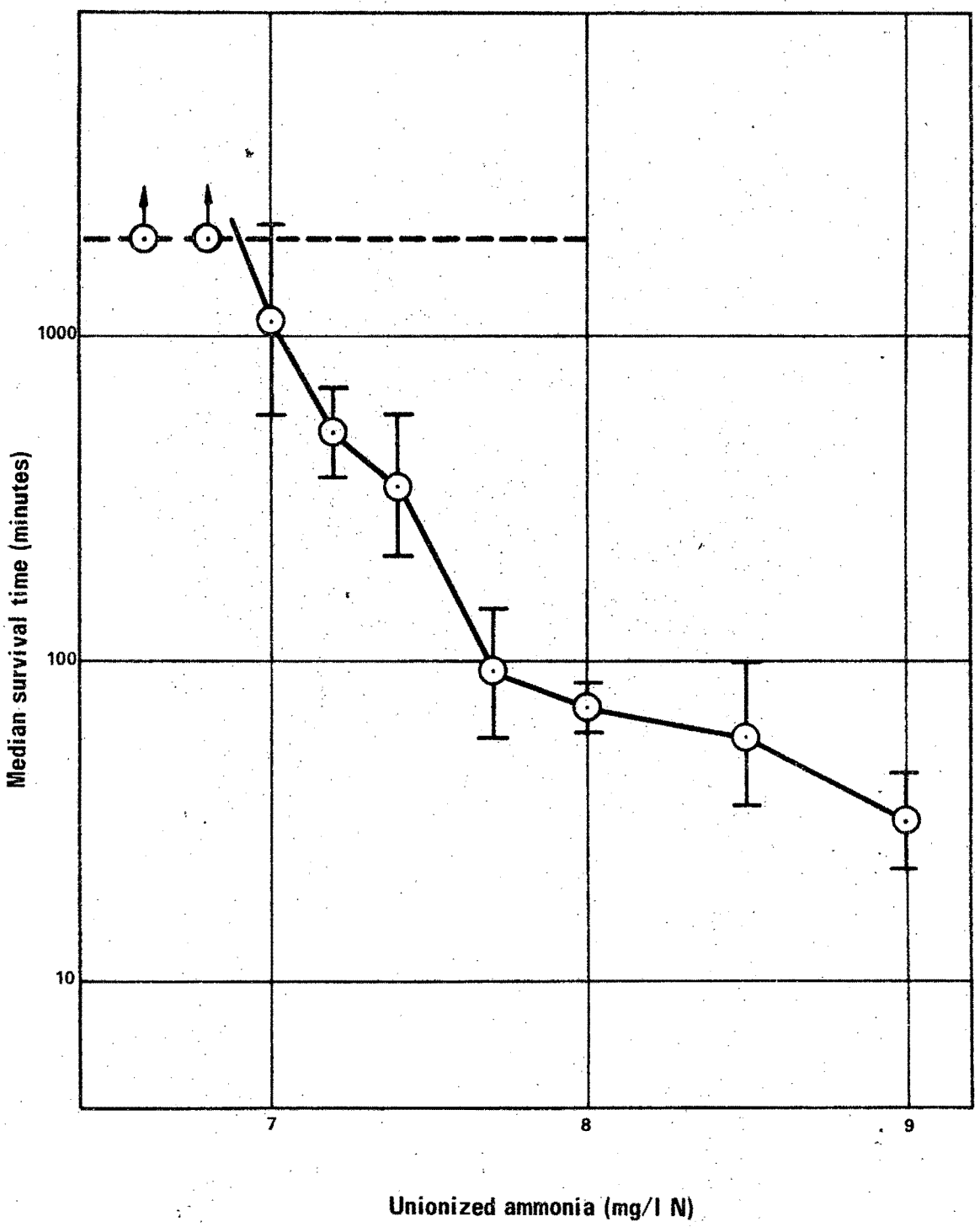
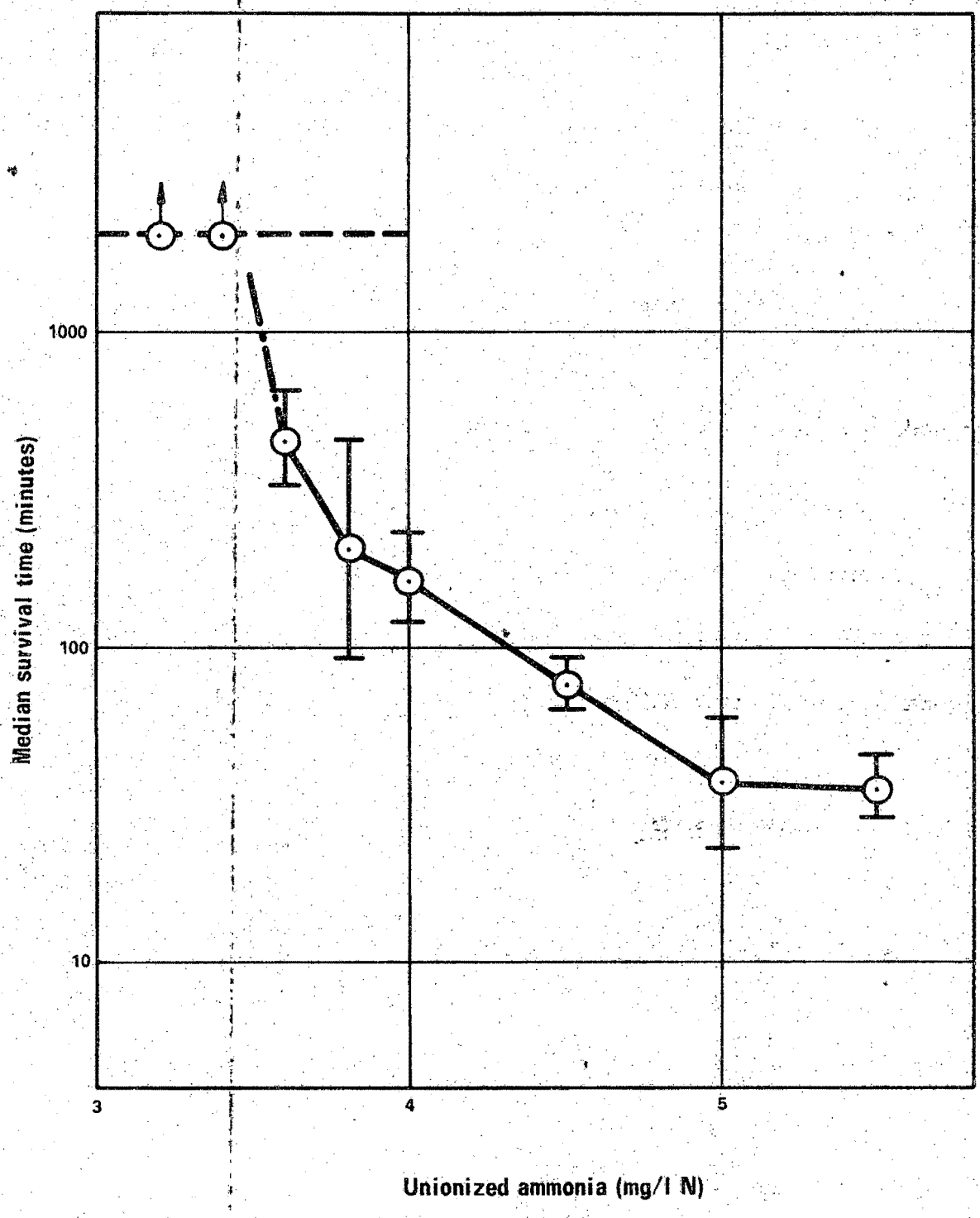

1000
100
10
Median survival time (minutes)
7
8
9
Unionized ammonia (mg/l N)

Figure 54

Median times of survival of Choroterpes bugandensis nymphs in ammonium chloride solutions at pH 8.0 and 20°C (concentrations given as unionized ammonia mg/l N).


1000
100
10
Median survival time (minutes)
3
4
5
Unionized ammonia (mg/l N)

in concentrations of ammonia of the order of 10 per cent of the 1000-minute median lethal concentration before being exposed for 1000 minutes to concentrations in the lethal range. The unionized ammonia concentrations in the dishes in which they were kept must have varied, since variations in pH of up to 0.5 of a pH unit occured. The nymphs were provided with an adequate food supply during this pre-test period. The results of these experiments, shown in tables 90 and 91, reveal that previous exposure to sub-lethal ammonia concentrations significantly decreased the ammonia tolerances of both species. Fish have also been found to be deleteriously affected by exposure to sub-lethal ammonia concentrations (Flis 1968).

FIELD OBSERVATIONS

Allanson (1961) has recorded occasional total ammonia-ammonium concentrations of up to 189 mg/l (as N) associated with pH values of the order of pH 7.5 in the polluted Jukskei River between Johannesburg and Pretoria. Although Baetis harrisoni was common elsewhere in this river system he did not find this species at the stations at which these very high ammonia concentrations occured. This is not surprising, since 189 mg/l total ammonia-ammonium would have represented unionized ammonia concentrations of around 2 to 10 mg/l (as N), depending on exact temperatures and pH values. The laboratory studies presented here have shown that these concentrations are in the lethal range for this species. Further downstream, where he recorded total ammonia-ammonium concentrations up to 59 mg/l quite commonly, Baetis harrisoni was present in every collection.

TABLE 90

1000- MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS PREVIOUSLY HELD FOR 48 HOURS IN CLEAN WATER AND IN A SUBLETHAL AMMONIA CONCENTRATION (pH 8.0, 20°C)

Unionized ammonia in holding tank(mg/l N)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets)
0.5 to 1	240	6.0 6.2 6.4 6.6 6.8 7.0	6.5 (6.3 to 6.8)
0	240	6.6 6.8 7.0 7.2 7.5 7.8	7.4 (7.2 to 7.6)

TABLE 91

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS PREVIOUSLY HELD FOR 48 HOURS IN CLEAN WATER AND IN A SUBLETHAL AMMONIA CONCENTRATION (pH 8.0, 20°C)

Unionized ammonia in holding tank (mg/l N)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l as N), 95% confidence limits in brackets
0.2 to 0.5	240	3.0 3.1 3.2 3.3 3.4 3.5	3.1 (3.0 to 3.3)
0	240	3.4 3.5 3.6 3.8 4.0 4.2	3.6 (3.5 to 3.7)

Visits were paid during the present study to sites on the Jukskei River where high ammonia concentrations were thought likely to occur. No exceptionally high concentrations of the order of those reported by Allanson were found, and pollution of the Jukskei River appeared to have decreased. Baetis harrisoni nymphs were collected at a site at which Allanson had recorded high ammonia concentrations and had found no Baetis harrisoni (his station 8).

Total ammonia-ammonium concentrations measured from time to time at this site varied from 10 to 61 mg/l (as N). However, higher values were rather rare and were all recorded during one two-week period. Samples were taken during one 24 hour period during this time of high ammonium concentrations. The temperatures, pH values and unionized ammonia concentrations found are shown in figure 55. The Baetis harrisoni population present appeared to be unaffected by the high ammonia concentrations present.

Total ammonia-ammonium concentrations measured in the Pienaars River, where Choroterpes bugandensis nymphs were found at all times of year, were generally much lower. The highest total ammonia-ammonium concentration recorded was 14 mg/l (as N), which correspond to an unionized ammonia concentration of 0.2 mg/l (as N). This was more than 10 times higher than the next highest concentration determined here. All of the values recorded therefore fell well within the range tolerated by this species.

During the same period while recordings shown in figure 55 were being made in the Jukskei River, a population of Choroterpes nymphs collected

in the Pienaars River was held overnight in the Jukskei River. For this purpose, a container was constructed consisting of a wire frame about 30 cm x 30 cm x 30 cm covered with coarse nylon gauze. The nymphs were transferred to this container and the container anchored in the flowing water for 1000 minutes. At the end of this period 26 of the 85 Choroterpes nymphs in the container were found to be dead. On a subsequent occasion a population, also collected in the Pienaars River, was held overnight in the same container at the place where they were collected. On that occasion none of 92 nymphs was observed to succumb.

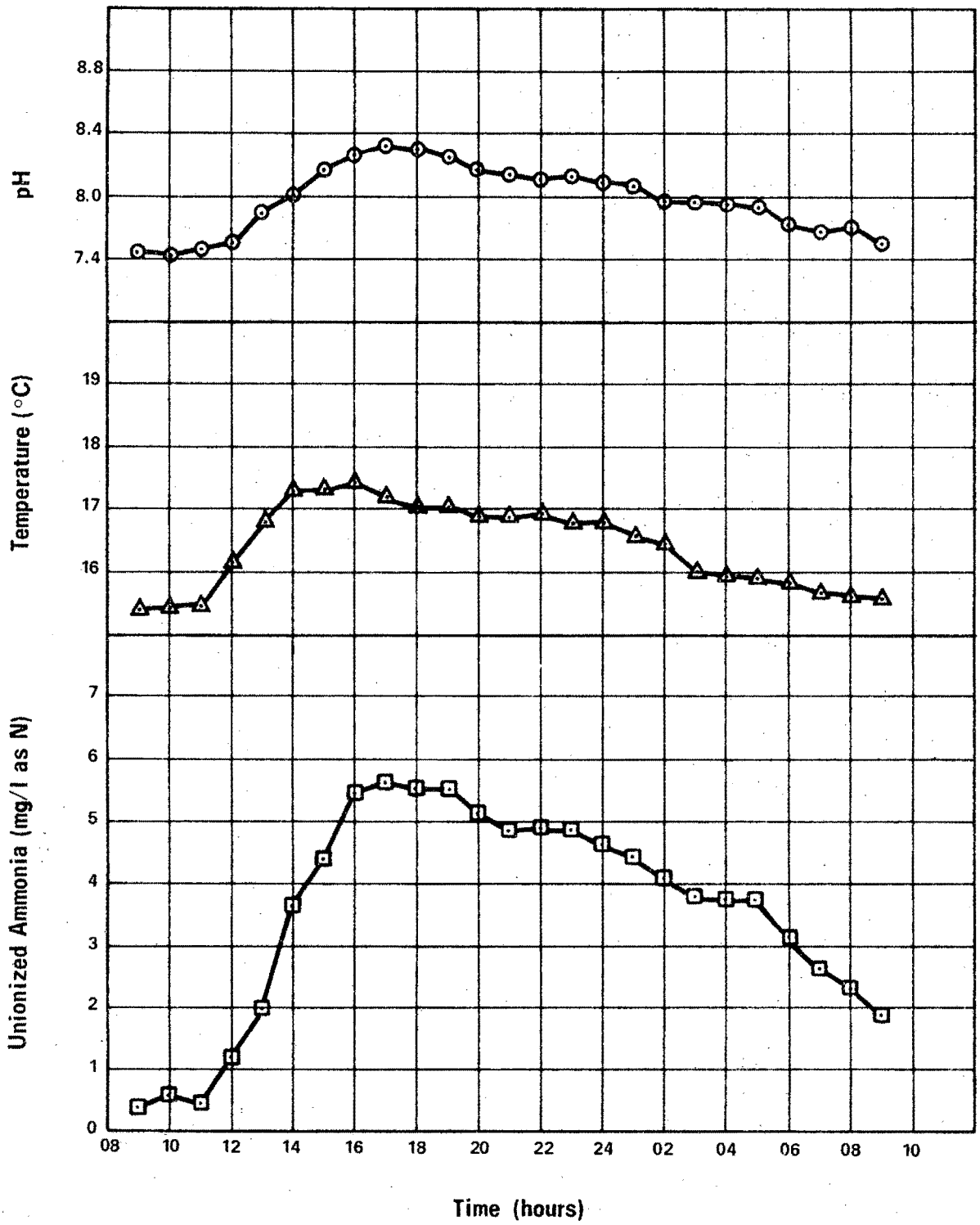
DISCUSSION

From the results presented here it seems that ammonia concentrations found to occur from time to time in the polluted Jukskei River exceeded the lethal limit for Choroterpes bugandensis and would have been sufficient to explain the absence of this species from the river. At the time of this study, occasional high ammonia concentrations in the Jukskei River were only barely within the range tolerated by the more tolerant Baetis harrisoni. At the time of Allanson's earlier survey it seems likely that Baetis harrisoni had been eliminated from places subjected to sporadic high ammonia concentrations. The return of this species to these situations seems, if this reasoning is correct, very striking evidence of recent improvement in the level of pollution of the river.

The lethal ammonia concentrations found here are of the same order as those reported by other authors for other mayflies and aquatic invertebrates. Stammer (1953) has reported the lethal ammonia concentrations

Figure 55

Fluctuations in pH, temperature and unionized ammonia concentration during 24 hours in the Jukskei River at a time when ammonia concentrations were unusually high.


pH
8.8
8.4
8.0
7.4
Temperature (°C)
19
18
17
16
Unionized Ammonia (mg/l as N)
7
6
5
4
3
2
1
0
08
10
12
14
16
18
20
22
24
02
04
06
08
10
Time (hours)

for nymphs of a mayfly, Ecdyonurus to be 3 mg/l unionized ammonia. He found lethal concentrations for nymphs of a stonefly, Perla, and larvae of a species of Chironomus tolerant of pollution to be 9 mg/l and 4 mg/l, respectively. In contrast, he found a leach and a number of oligochaetes and planarians to be less tolerant than any of these insects. Anderson (1944) found the lethal limit for a Daphnia species to be 8 mg/l unionized ammonia.

Carbon dioxide has been found to reduce the toxicity to fish of solutions of ammonium salts, primarily by reducing the pH of the water (Alabaster and Herbert 1954). No evidence was found in the results described here to suggest that free carbon dioxide at constant pH influenced the toxicity of ammonia to Baetis and Choroterpes nymphs. Tabata (1960), on the other hand, found the toxicity to fish of ammonia solutions to be reduced by the presence of carbon dioxide even at constant pH.

Lloyd and Herbert (1960) have shown that, for fish, the toxicity of ammonia was influenced not so much by the carbon dioxide concentration and the pH in the open water, but really by the pH of the water around the gills, the source of entry of ammonia into the fish. They found that respiratory carbon dioxide was often sufficient to reduce the pH of the water around the gills and consequently to reduce the toxicity of the solution. This occured especially in water of low carbon dioxide and bicarbonate concentrations because of the relatively low buffering capacity of these waters. Lloyd (1961a) and Brown (1968) have published data for computing the toxicity to trout of ammonia solutions at different levels of these factors.

In the present study neither the carbon dioxide concentration nor the bicarbonate alkalinity, which would have changed the buffering capacity of the

water, was found to influence the toxicity of ammonia-ammonium solutions to Baetis and Choroterpes nymphs. Respiratory carbon dioxide evidently did not reduce the pH of the water immediately surrounding the animals sufficiently to influence the toxicity of these solutions. Alternatively, if the pH of the water was influenced, this modified water must have been washed away by the current.

Because he found insects to be rather less susceptible to ammonia than were other invertebrates, including some found characteristically in polluted water, Stammer (1953) reasoned that their distribution in polluted streams would be determined by such other factors as the dissolved oxygen concentration. In the Jukskei River, at least, this has been found not to be the case. It was shown in an earlier section that Choroterpes buganden-sis nymphs would easily have been able to tolerate the low dissolved oxygen concentrations found at times in this river. They were in fact more tolerant of low oxygen than were nymphs of Baetis harrisoni. On the other hand, from the results presented here it seems that the lethal ammonia concentrations found to occur occasionally in the Jukskei River would be sufficient to explain the absence of Choroterpes. Comparison with past data (Allanson 1961) suggests that even Baetis harrisoni might at one time have been eliminated from the most heavily polluted stretches.

SUMMARY

1. Baetis nymphs tolerated higher ammonia concentrations (median lethal unionized ammonia concentration 7.0 to 7.2 mg/l N) than did Choroterpes nymphs (median lethal unionized ammonia concentration 3.5 to 3.7 mg/l N)

2. The toxicity of ammonia to nymphs of both species was proportional to the calculated concentration of unionized ammonia present and not to the ammonium ion concentration.

3. Free carbon dioxide concentration, pH and bicarbonate alkalinity in themselves did not affect the toxicity of any given concentration of unionized ammonia to nymphs of either species. All of them of course, and most especially pH, were responsible for determining how much unionized ammonia was present in the first place.

4. For both species, nymphs of different sizes did not differ significantly in ammonia tolerance.

5. Exposure to sublethal ammonia concentrations slightly decreased the subsequent ammonia tolerance of nymphs, the median lethal unionized ammonia concentration for Baetis being reduced to 6.5 mg/l N and that for Choroterpes being reduced to 3.1 mg/l N.

6. Concentrations of unionized ammonia in the polluted Jukskei River were found occasionally to exceed the lethal concentration for Choroterpes nymphs (up to 5.7 mg/l N).

7. These concentrations were close to the lethal concentration for Baetis, but were tolerated by nymphs in this part of the Jukskei River. Evidence suggests heavier past pollution had eliminated Baetis from some stretches, and that subsequent improvement had permitted Baetis to recolonize these stretches.

INFLUENCE OF TEMPERATURE AND DISSOLVED OXYGEN ON THE TOXICITY OF AMMONIA TO BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

INTRODUCTION

Several authors have shown the toxicity of poisons to fish and other aquatic animals to be influenced by temperature or to be increased in conditions of low dissolved oxygen concentration (Wuhrmann and Woker 1955). Ammonia was shown in the previous section to reach levels occasionally which would be lethal for mayfly nymphs in some polluted rivers. Temperature presumably influences the toxicity of ammonia first of all indirectly by shifting the equilibrium between the toxic unionized ammonia and the non-toxic or impermeable ammonium ions. This effect has been clearly demonstrated by Woker (1949). What is not known, however, is the extent to which temperature might influence the toxicity of a given concentration of unionized ammonia.

Dissolved oxygen concentration has been demonstrated to influence the toxicity of ammonia to fish (Wuhrmann and Woker 1953, Allen 1955, Downing and Merkens 1955, Merkens and Downing 1957). Lloyd (1961a) has shown that this effect is likely to have been caused by increased ventilation of fish at low oxygen levels. Increased ventilation presumably replaced more rapidly the water around the gill surfaces, the presumed site of entry of ammonia into fish. Lloyd suggests that water of higher pH and, consequently, higher unionized ammonia content replaced more rapidly the less toxic water containing respiratory carbon dioxide when the ventilation rate increased.

Results of experiments are reported here in which ammonia tolerances of nymphs of Baetis harrisoni and Choroterpes bugandensis at different temperatures and at different concentrations of dissolved oxygen are compared.

MATERIAL AND METHODS

Nymphs of Baetis harrisoni and Choroterpes bugandensis were collected in the Braamfontein Spruit and Pienaars River, held in the laboratory for a day and then exposed to different ammonia concentrations under strictly controlled conditions of temperature, pH, free carbon dioxide concentration and bicarbonate alkalinity, as has been described. Except where otherwise described, all experiments were carried out at a current speed of 10 cm/sec in tubes of 1.6 cm internal diameter. At this speed in these tubes the current was laminar. At the end of 1000 minutes' exposure the numbers of nymphs dying and recovering were counted and the median lethal ammonia concentration calculated from these counts by probit analysis (Finney 1952).

In each of the experiments described here nymphs were exposed to different ammonia concentrations at a number of different temperatures and dissolved oxygen levels. Each experiment required many more test tanks than were actually available and was therefore carried out in parts over several days. The combinations of temperature, oxygen and ammonia to be tested on each day were selected at random.

AMMONIA AND TEMPERATURE

In the first series of experiments, summer nymphs of each species were collected and were held in the laboratory for 24 hours at $10^{o}C$, $15^{o}C$, $20^{o}C$ and $25^{o}C$. Thereafter they were exposed for 1000 minutes at the same

temperatures to a number of ammonia concentrations in the lethal range and the mortality noted. The results are summarized in tables 92 and 93, respectively for Baetis harrisoni and Choroterpes bugandensis.

TABLE 92

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT TEMPERATURES (pH 8.0)

Temperature (^{o}C)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
10^{o}C	247	7.2 7.5 7.8 8.1 8.5 9.0	7.9 (7.7 to 8.3)
15^{o}C	252	7.0 7.2 7.5 7.8 8.1 8.5	7.5 (7.4 to 7.7)
20^{o}C	241	6.8 7.0 7.2 7.5 7.8 8.1	7.3 (7.1 to 7.4)
25^{o}C	142 attempting ecdysis 110 not attempting ecdysis	 6.4 6.6 6.8 7.0 7.2 7.5	6.8 (6.5 to 7.1) 7.0 (6.7 to 7.2)

TABLE 93

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATION FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT TEMPERATURES (pH 8.0)

Temperature (°C)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
10°C	240	3.4 3.5 3.6 3.7 3.8 4.0	3.7 (3.5 to 3.9)
15°C	240	3.4 3.5 3.6 3.7 3.8 4.0	3.7 (3.5 to 3.9)
20°C	240	3.4 3.5 3.6 3.7 3.8 4.0	3.5 (3.4 to 3.7)
25°C	240	3.4 3.5 3.6 3.7 3.8 4.0	3.5 (3.3 to 3.6)

Table 92 shows that Baetis harrisoni nymphs were significantly more tolerant of unionized ammonia at lower temperatures. This effect was quite separate from the influence of temperature on the ammonia-ammonium equilibrium.

At 25°C, at which temperature the nymphs were least tolerant of ammonia, more than half of the nymphs attempted ecdysis during the exposure period. This temperature was close to the upper lethal temperature for Baetis harrisoni, as has been reported in an earlier section. Nymphs in ecdysis have been shown in previous sections to be less tolerant of high temperatures and low dissolved oxygen concentrations than were nymphs not attempting ecdysis during the experiment. Within each group in the present experiment the nymphs attempting and not attempting ecdysis were distinguished. Median lethal ammonia concentrations calculated separately for nymphs attempting and not attempting ecdysis at 25°C are shown in table 92, but the difference between them was not found to be significant. At lower temperatures nymphs in and out of ecdysis appeared to be about equally tolerant of ammonia.

Table 93 shows that Choroterpes bugandensis nymphs at 10°C and 15°C were significantly more tolerant of ammonia at low temperatures than they were at 20°C and 25°C. However, the differences in median lethal concentrations at different temperatures were much less marked for Choroterpes than they were for Baetis.

AMMONIA AND DISSOLVED OXYGEN

In these experiments, nymphs were held in the laboratory for a day at 20°C in well aerated water before being exposed to different ammonia and dissolved oxygen concentrations. The lethal ammonia concentrations estimated from mortality at different combinations of these factors are shown in tables 94 and 95.

TABLE 94

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS AT DIFFERENT DISSOLVED OXYGEN CONCENTRATIONS

Dissolved oxygen (mg/l)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
7.7	267	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.1 to 7.4)
6.0	128 attempting ecdysis	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.1 to 7.4)
	174 not attempting ecdysis		7.2 (7.0 to 7.3)
4.5	105 attempting ecdysis	4.8 5.0 5.2 5.4 5.6 5.8	5.3 (5.1 to 5.5)
	189 not attempting ecdysis	6.6 6.8 7.0 7.2 7.5 7.8	7.0 (6.9 to 7.2)

In the experiment involving Baetis harrisoni, distinction was drawn between nymphs attempting and not attempting ecdysis during the course of the experiment. Lethal ammonia concentrations were computed both separately for each and jointly for the whole population. As shown in table 94, the ammonia tolerances of nymphs were unaffected by reduction in

TABLE 95

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT DISSOLVED OXYGEN CONCENTRATIONS

Dissolved oxygen (mg/l)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
7.7	240	3.4 3.5 3.6 3.7 3.8 4.0	3.7 (3.5 to 3.8)
3.0	240	2.8 2.9 3.0 3.1 3.2 3.3	2.9 (2.8 to 3.1)
1.0	240	2.4 2.5 2.6 2.7 2.8 2.9	2.7 (2.5 to 2.9)

dissolved oxygen concentration to 6 mg/l. At 4.5 mg/l dissolved oxygen, a concentration almost within the lethal range for this species, the median lethal ammonia concentration for nymphs which did not attempt ecdysis was not significantly different from that for nymphs in oxygen saturated water. Nymphs attempting ecdysis at this oxygen concentration, however, were found to be considerably less tolerant of ammonia. These results seem to suggest that the ammonia tolerances of Baetis nymphs were only reduced in dissolved oxygen concentrations almost low enough to have been lethal on their own.

Mortalities at these concentrations presumably resulted from the joint lethal action of ammonia and low oxygen.

In the case of Choroterpes bugandensis, nymphs did not appear to be less tolerant during ecdysis than at other times. As shown in table 95, in sharp contrast with what was found for Baetis, Choroterpes nymphs were found to be significantly less tolerant of ammonia at lower concentrations of dissolved oxygen.

Baetis and Choroterpes, as described in an earlier section, differed greatly in their dissolved oxygen requirements. The fact that the experiments to which tables 94 and 95 refer were carried out at completely different oxygen concentrations makes it a little difficult to compare the two sets of results. However, it is clear that the toxicity of ammonia to both Baetis nymphs in ecdysis and to Choroterpes nymphs was increased at low concentrations of dissolved oxygen.

AMMONIA AND WATER FLOW

Experiments were undertaken to compare the toxicities to Baetis harrisoni and Choroterpes bugandensis nymphs of ammonia solutions at different rates of water flow. These experiments resembled those just described in which nymphs were exposed to ammonia solutions at different concentrations of dissolved oxygen. The experiments were carried out at 20°C and in water containing 7.7 mg/l dissolved oxygen, approximately that of water in equilibrium with air at 20°C. Frequent measurements of oxygen concentration were made during the experiment and the concentration maintained by mixing a small amount of pure oxygen with the air being bubbled through the

water. As before, the animals were held in perspex experimental tubes through which a current of water was driven. Different current speeds were obtained using tubes of different internal diameter connected in series. The flow in each of the tubes was laminar ($Re < 2000$). Mortality was noted in each tube and lethal ammonia concentrations calculated for each current speed. The results obtained for Baetis and Choroterpes are summarized in tables 96 and 97.

TABLE 96

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS IN WATER FLOWING AT DIFFERENT RATES

Current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
7.0	laminar	1.6	262	6.6 6.8 7.0 7.2 7.5 7.8	7.3 (7.2 to 7.5)
12.5	laminar	1.2	257	6.6 6.8 7.0 7.2 7.5 7.8	7.2 (7.1 to 7.4)
22.2	laminar	0.9	272	6.6 6.8 7.0 7.2 7.5 7.8	7.0 (6.8 to 7.1)

TABLE 97

1000-MINUTE MEDIAN LETHAL UNIONIZED AMMONIA CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS IN WATER FLOWING AT DIFFERENT RATES

Current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Unionized ammonia test concentrations (mg/l N)	Lethal ammonia concentration (mg/l N), 95% confidence limits in brackets
7.0	laminar	1.6	231	3.4 3.5 3.6 3.7 3.8 4.0	3.6 (3.4 to 3.7)
12.5	laminar	1.2	238	3.4 3.5 3.6 3.7 3.8 4.0	3.6 (3.4 to 3.7)
22.2	laminar	0.9	236	3.0 3.1 3.2 3.3 3.4 3.5	3.3 (3.3 to 3.4)

Baetis harrisoni nymphs in and out of ecdysis in these experiments appeared to be equally tolerant of ammonia. The lethal ammonia concentrations were therefore calculated for both together. Both for Baetis and for Choroterpes the median lethal ammonia concentrations at the fastest current speed, 22.2 cm/sec, were slightly but significantly lower than those at slower current speeds. In previous sections it was seen that mortality was increased under certain circumstances at low rates of water flow as a result of

decreased oxygen availability. The fact that mortality was here increased at fast flow rates indicates that oxygen was not the limiting factor. Dissolved oxygen concentrations were well above lethal levels at all times. Moreover, no difference in sensitivity of Baetis nymphs in and out of ecdysis was noted, such as had been evident in all instances reported in previous sections where insufficient oxygen appeared to be available to nymphs.

DISCUSSION

The results reported here have shown that the toxicity of ammonia solutions to Baetis and Choroterpes nymphs was increased both at increased temperature and at decreased dissolved oxygen concentrations. This effect of temperature was quite separate from that on the dissociation coefficient of ammonia. Wuhrmann and Woker (1955) have listed several possible reasons for similar effects of temperature and dissolved oxygen on the toxicity of ammonia solutions to fish. Among the factors they list which might apply equally to mayfly nymphs are the possible effects of temperature and hypoxia on nymphal metabolic rates and hence on the metabolic energy available to counteract the effects of the ammonia. Hypoxia has been found to increase toxicity of ammonia to mice, also apparently as a result of its influence on metabolic rates (Warren and Schenker 1960).

In discussing how lowered dissolved oxygen concentrations might increase the toxicity to fish of ammonia and other poisons, Lloyd (1961 a) has suggested that the toxicity of these poisons was related to the rate of flow over the gills of water containing the poison. He reasons that in lowered oxygen the flow of water across the gills was increased and water

made less toxic by loss of poison into the animal and by reduction of the ammonia-ammonium ratio through the influence of respiratory carbon dioxide on the pH of the water was washed away. He has suggested that this phenomenon might explain other observed instances of increase in toxicity of solutions to fish. Increased toxicity of ammonia to Choroterpes nymphs in reduced dissolved oxygen concentrations could conceivably be explained in this way, since Choroterpes create water currents around themselves by means of their abdominal gills and the rate at which the gills beat was seen to increase in water of reduced dissolved oxygen content. This could not explain the increase observed in toxicity of ammonia to Baetis nymphs at low dissolved oxygen concentrations since the gills of these nymphs do not beat.

Lloyd's observations are also of interest in relation to the observation reported here that the toxicity of ammonia solutions to both Baetis and Choroterpes nymphs was increased at fast rates of water flow. The possibility seems to exist that this increased apparent toxicity might have resulted from more rapid replacement at faster current speeds of the possibly less toxic water surrounding the animals. Although the current speeds compared here are fairly fast, and stagnant conditions around the animals seem unlikely, no other explanation for the observed different toxicities suggests itself.

SUMMARY

1. The toxicity to Baetis and Choroterpes nymphs of unionized ammonia increased with increase in temperature (median lethal concentrations decreasing from 7.9 mg/l N at 10°C to 6.8 - 7.0 mg/l N at 25°C for Baetis and from 3.5 mg/l N at 10°C to 3.7 mg/l N at 25°C for Choroterpes).

2. The toxicity of unionized ammonia to Baetis nymphs was apparently unaffected by decrease in dissolved oxygen concentration (median lethal ammonia concentration 7.0 - 7.2 mg/l N in 4.5 - 8.0 mg/l dissolved oxygen), except in the case of nymphs attempting ecdysis in 4.5 mg/l dissolved oxygen concentration (median lethal ammonia concentration 5.3 mg/l N).

3. The toxicity of unionized ammonia to Choroterpes nymphs increased with decrease in dissolved oxygen (the median lethal concentration decreasing to 2.9 mg/l N in 3.0 mg/l dissolved oxygen and to 2.7 mg/l N in 1.0 mg/l dissolved oxygen).

4. Median lethal unionized ammonia for both Baetis and Choroterpes nymphs were lower in 22.2 cm/sec water current speed than they were in slower flowing water (7.0 mg/l N as opposed to 7.2 - 7.3 mg/l N for Baetis and 3.3 mg/l N as opposed to 3.6 mg/l N for Choroterpes).

TOXICITY OF COPPER, LEAD AND ZINC TO BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS NYMPHS

INTRODUCTION

Concentrations of the heavy metals copper, lead and zinc lethal to fish and other aquatic animals have been found quite commonly in rivers in different parts of the world. Reviews of published data (for instance Powers 1917, Ellis 1937, Doudoroff and Katz 1953, Skidmore 1964, Jones 1964) have revealed wide variations in concentrations found by different workers to be toxic to these animals. As these authors point out, the toxicity of cations may be expected to be influenced both by factors which determine their solubility and by factors which affect their toxicity and rate of uptake by animals. Solubility of all of them is determined by the pH and bicarbonate alkalinity. Other salts present in the water have in several instances been found quite drastically to affect toxicity, apparently through influence on the rate of uptake of heavy metals (for instance, Learner and Edwards 1963, Herbert and Wakeford 1964). Clearly, no study of the toxicity of these metals is very meaningful without reference to these factors.

Heavy metals in natural waters may originate either from natural geological sources or from industrial or mine pollution (Jones 1964). Very little is currently known of the possible occurrence of these metals in South African rivers. Copper salts are widely applied to waters at least in the Transvaal, apparently to control either algae or aquatic snails. Lead and zinc have been widely found in lethal concentrations elsewhere in the world.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected in the field, as has been described, and brought into the laboratory for study. After being held at 20°C in the laboratory for a day, random groups were transferred to perspex experimental tubes and exposed under controlled conditions to a number of different concentrations of the heavy metals concerned. All of these experiments were carried out at 20°C in well-aerated water. The animals were held in tubes of 1.6 cm internal diameter through which a laminar flow of water of 10 cm/sec was driven. pH values in all experiments were strictly controlled. At the end of 1000 minutes' exposure the numbers of nymphs dying and recovering were counted. Median lethal concentrations were calculated from these counts by probit analysis (Finney 1952).

COPPER

Nymphs of each species were exposed to different concentrations of copper sulphate, the copper salt most commonly used in the Transvaal to control algae and the one most commonly mentioned in the literature, at several different pH values. Each test solution was made up at pH 6, at which all the copper salt was easily dissolved. The pH value was then adjusted and the solution allowed to reach equilibrium for 24 hours before start of the experiment.

1000-minute median lethal copper concentrations for Baetis harrisoni and Choroterpes bugandensis nymphs at different pH values are shown in tables 98 and 99. A faint cloudy precipitate was seen in all of the more

TABLE 98

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS AT 20°C AND DIFFERENT pH VALUES

pH	Appearance of solutions	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
6.0	all clear	213	2.0 2.2 2.4 2.6 2.8 3.0	2.4 (2.2 to 2.7)
6.5	only dilute solutions clear	213	2.0 2.2 2.4 2.6 2.8 3.0	2.4 (2.2 to 2.6)
7.0	all cloudy	124	3.0 4.0 5.0 6.0	no mortality
7.5	all cloudy	117	3.0 4.0 5.0 6.0	no mortality

alkaline solutions. Most of the precipitate settled outside of the experimental tubes and was apparently not toxic. No mortality of either species occured in solutions of 100 mg/l copper at pH 10. Mortality of Baetis nymphs was observed only at pH 6 and pH 6.5, presumably because it was only in this acid water that enough copper could be dissolved. Choroterpes nymphs were rather less tolerant of dissolved copper. Mortality of

TABLE 99

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT 20°C AND DIFFERENT pH VALUES

pH	Appearance of solutions	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
6.0	all clear	240	0.4 0.5 0.6 0.7 0.8 0.9	0.7 (0.5 to 0.9)
6.5	only dilute solutions clear	240	0.6 0.7 0.8 0.9 1.0 1.1	0.9 (0.8 to 1.0)
7.0	all cloudy	120	1.0 2.0 3.0 4.0	no mortality
7.5	all cloudy	120	1.0 2.0 3.0 4.0	no mortality

this species also occurred only at pH 6 and pH 6.5 but not in more alkaline water. The median lethal concentration estimated for Choroterpes at pH 6 was slightly lower than that found at pH 6.5, ($p < 0.025$), suggesting perhaps that pH might have influenced the toxicity of copper as well as its solubility. However, this effect was not noted for Baetis.

LEAD

Nymphs of each species were exposed for 1000 minutes to different concentrations of lead nitrate. This was the salt most commonly used in published studies by other workers. These experiments were repeated at two different pH values. The concentrations used were found to be easily soluble throughout. The results of these experiments are summarized in tables 100 and 101.

TABLE 100

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS AT 20°C AND TWO pH VALUES

pH	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
6.5	220	13.5 14.0 14.5 15.0 15.5 16.0	14.6 (14.1 to 15.0)
7.5	227	13.5 14.0 14.5 15.0 15.5 16.0	15.1 (14.7 to 15.5)

Both species were found to be far more tolerant of zinc than they had been of copper. Once again, Baetis harrisoni nymphs were more tolerant than were those of Choroterpes bugandensis. Both species were found to be slightly less tolerant of lead at the lower pH value tested ($p < 0.025$ for Baetis, $p < 0.05$ for Choroterpes).

TABLE 101

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT 20°C AND TWO pH VALUES

pH	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
6.5	180	10.4 10.8 11.2 11.6 12.0 12.4	11.2 (10.6 to 11.9)
7.5	180	10.4 10.8 11.2 11.6 12.0 12.4	11.7 (11.2 to 12.2)

ZINC

Baetis harrisoni and Choroterpes bugandensis nymphs were also exposed to concentrations of zinc sulphate at two pH levels. Here again, the concentrations of zinc used were found to be easily dissolved in this water. The results of these experiments, shown in tables 102 and 103, show that both species were relatively tolerant of zinc and that pH did not apparently affect the toxicity of zinc to these species.

NYMPHS OF DIFFERENT SIZES

For each species, nymphs of different sizes were exposed for 1000 minutes to copper concentrations in the lethal range and the mortality noted. At the end of each experiment the length of each individual, excluding the

TABLE 102

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR BAETIS HARRISONI AT 20°C AND TWO pH VALUES

pH	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentrations (mg/l Zn), 95% confidence limits in brackets
6.5	231	12.5 13.0 13.5 14.0 14.5 15.0	13.8 (13.4 to 14.2)
7.5	240	12.5 13.0 13.5 14.0 14.5 15.0	13.6 (13.1 to 14.1)

TABLE 103

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS AT 20°C AND TWO pH LEVELS

pH	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
6.5	180	9.4 9.8 10.2 10.6 11.0 11.4	10.5 (9.9 to 11.1)
7.5	180	9.4 9.8 10.2 10.6 11.0 11.4	10.5 (10.1 to 10.9)

antennae and cerci, was measured under a microscope. Estimated lethal concentrations for each are shown in tables 104 and 105. As may be seen, nymphs of different sizes were similarly tolerant of copper. This experiment was carried out at 20°C and at pH 6.0.

TABLE 104

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS OF THREE DIFFERENT SIZES

Body length (mm)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
2.0 to 3.4	172	2.0 2.4 2.6 3.0	2.5 (2.3 to 2.8)
3.5 to 4.9	160	2.0 2.4 2.6 3.0	2.4 (2.2 to 2.7)
5.0 to 6.5	160	2.0 2.4 2.6 3.0	2.4 (2.2 to 2.6)

TIME MORTALITY

Nymphs of each species were placed in open gauze trays in each of a number of experimental tanks containing different concentrations of copper sulphate, lead nitrate and zinc sulphate solutions. These concentrations were all in the lethal range. At intervals of time the number of nymphs dead and alive were counted. Individuals had to be prodded in order to be sure whether they were alive or dead. Baetis nymphs attempting ecdysis

TABLE 105

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATION FOR CHOROTERPES BUGANDENSIS NYMPHS OF THREE DIFFERENT SIZES

Body length (mm)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
1.5 to 2.9	140	0.4 0.6 0.8 1.0	0.8 (0.8 to 0.9)
3.0 to 4.4	140	0.4 0.6 0.8 1.0	0.7 (0.6 to 0.8)
4.5 to 6.0	140	0.4 0.6 0.8 1.0	0.8 (0.6 to 1.0)

during these experiments were found to be sensitive to physical disturbance. A proportion was found to die even in the absence of dissolved heavy metals. For this reason, the survival times were calculated only for nymphs not attempting ecdysis. At each concentration tested, a median time of survival was estimated by probit analysis (Bliss 1937).

Median times of survival of Baetis and Choroterpes nymphs in different copper solutions are shown in figures 56 and 57. Comparable data for lead are shown in figures 58 and 59, and for zinc in figures 60 and 61. In each, median times of survival are seen to have increased with decrease in concentration of the metal concerned. Further exposure to the test solution after 1000 minutes would apparently have led to further mortality in most

instances, since the curves relating survival time and concentration show no sign of becoming asymptotic at long exposure times.

FEEDING DURING EXPOSURE

In an earlier section it was reported that availability of food during a 1000 minute test did not affect the tolerance by these nymphs of high temperature, provided that they had adequately been fed before the start of the experiment. Similar experiments were carried out here in order to check that results obtained in experiments were not influenced by the presence or absence of food at critical times. Nymphs of each species were held in the laboratory for 24 hours with algal food provided, as in all other experiments, and without food being present. Nymphs of each group were then exposed to different copper concentrations at pH 6 either in clean experimental tubes or in tubes containing algal food.

The results found are summarized in tables 106 and 107, respectively for Baetis and for Choroterpes nymphs. Surprisingly, the nymphs of both species starved for 24 hours before the experiment and provided with food during the experiment were found to be significantly less tolerant of copper than were nymphs starved both before and during the experiment. Baetis nymphs fed both before and during the experiment were slightly but significantly less tolerant than were those fed before but not during the experiment. This seems to indicate that the nymphs that were fed in copper sulphate solutions ingested copper with their food. The results also indicate that nymphs of both species starved before the experiment were less tolerant of copper than were nymphs fed before exposure. Median lethal concentra-

Figure 56

Median times of survival of Baetis harrisoni nymphs not attempting ecdysis in different concentrations of copper sulphate.

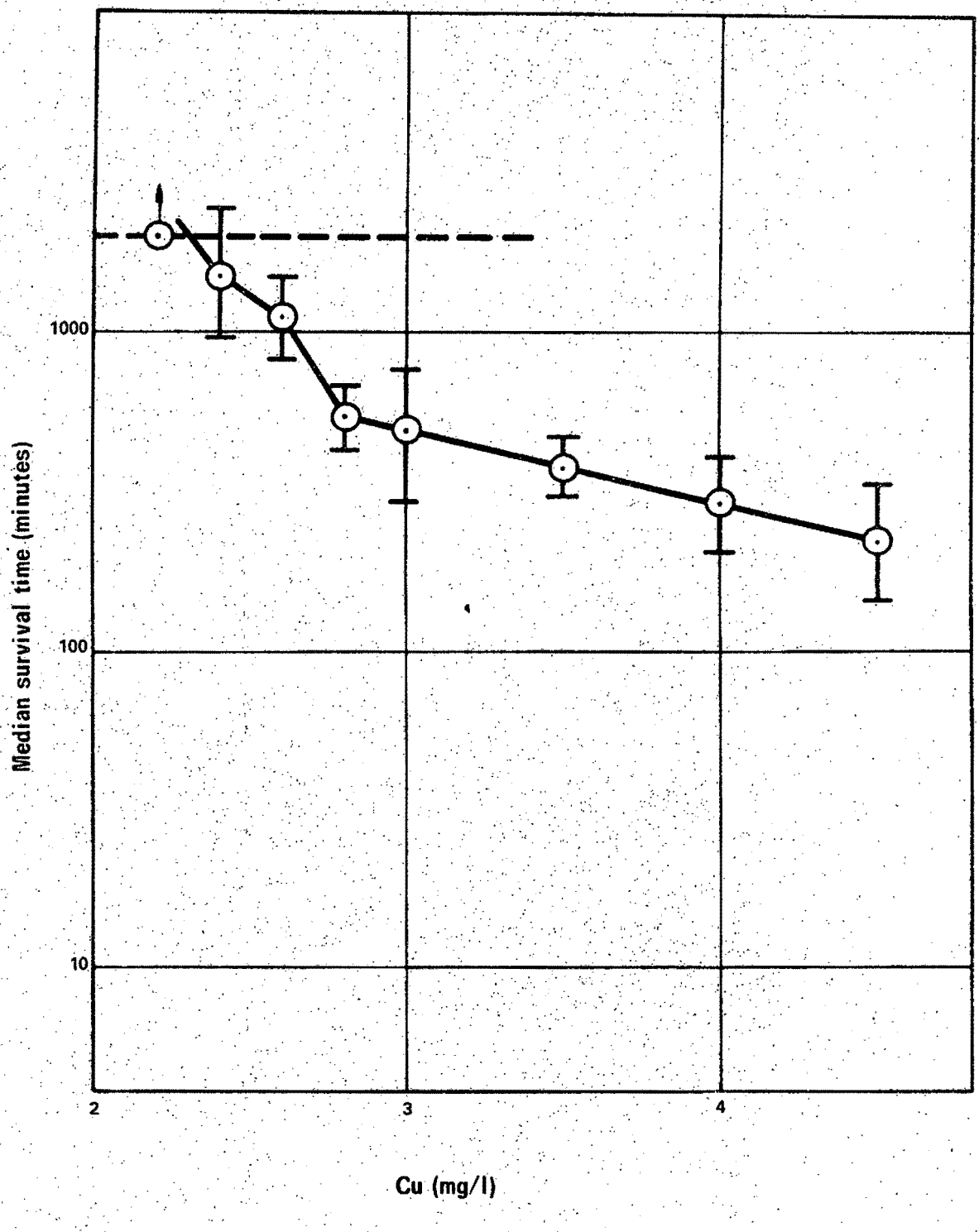

1000
100
10
Median survival time (minutes)
2
3
4
Cu (mg/l)

Figure 57

Median times of survival of Choroterpes bugandensis nymphs in different concentrations of copper sulphate.

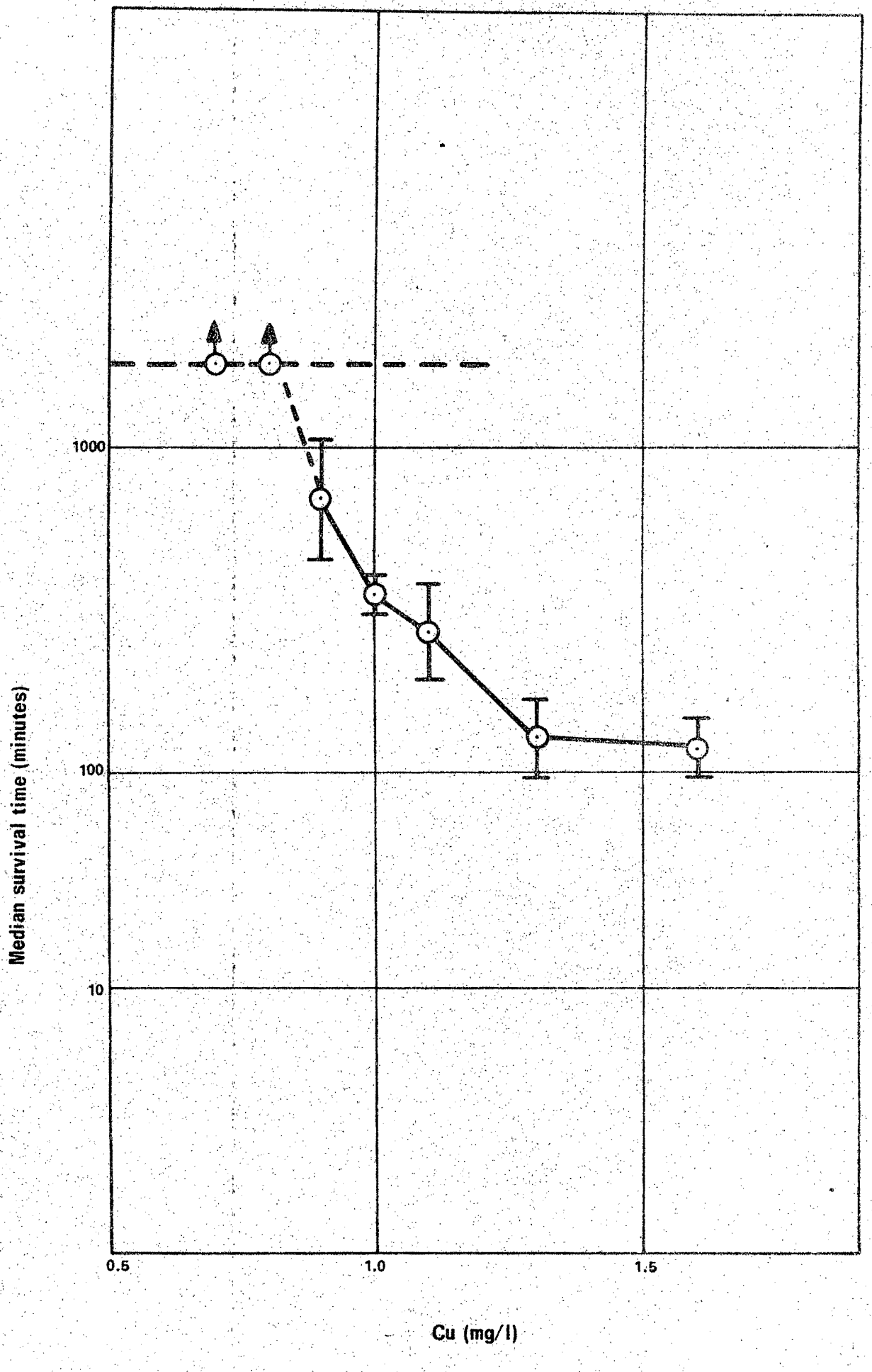

1000
100
10
Median survival time (minutes)
0.5
1.0
1.5
Cu (mg/l)

Figure 58

Median times of survival of Baetis harrisoni nymphs not attempting ecdysis in different concentrations of lead nitrate.

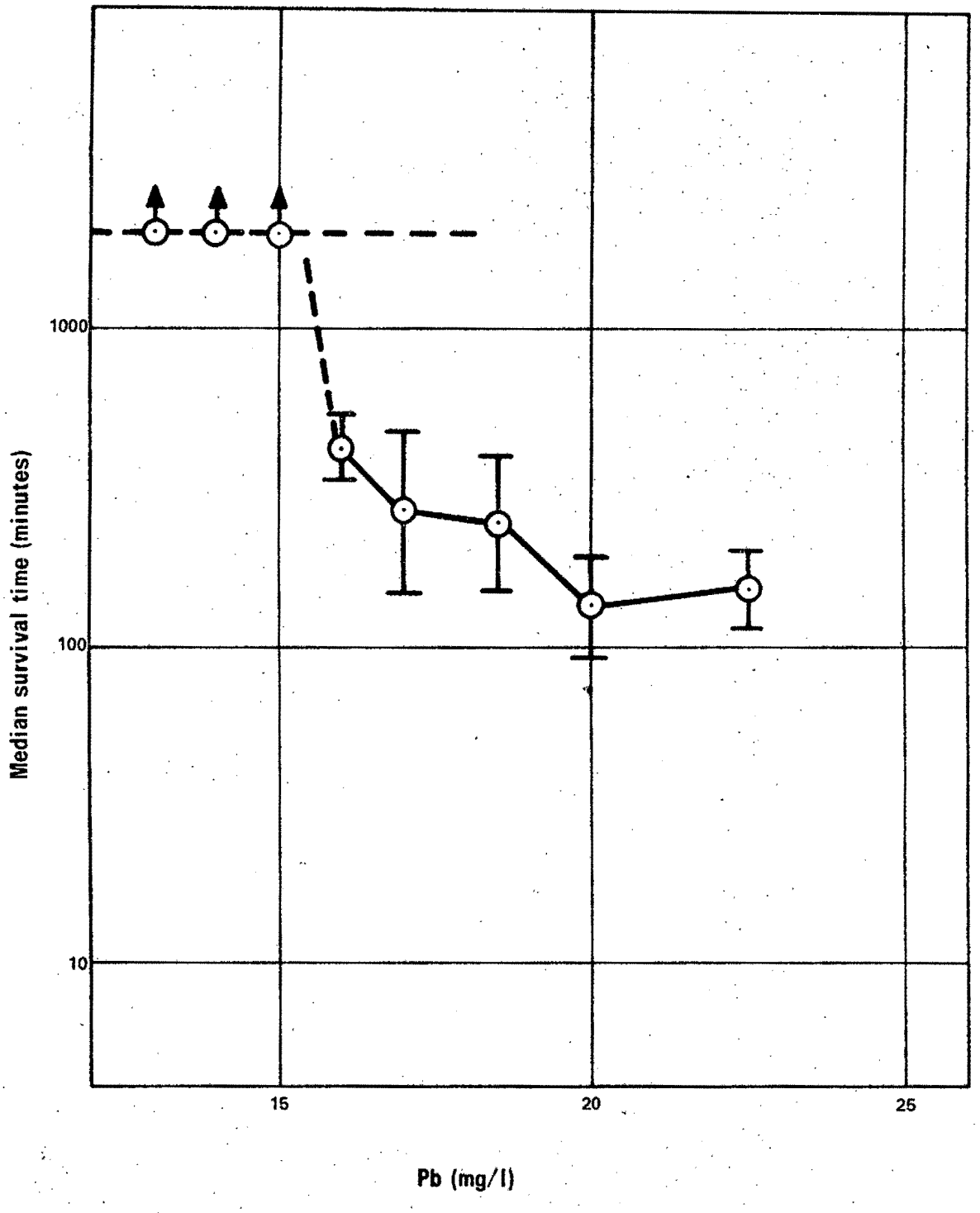

Median survival time (minutes)
1000
100
10
15
20
25
Pb (mg/l)

Figure 59

Median times of survival of Choroterpes bugandensis nymphs in different concentrations of lead nitrate.

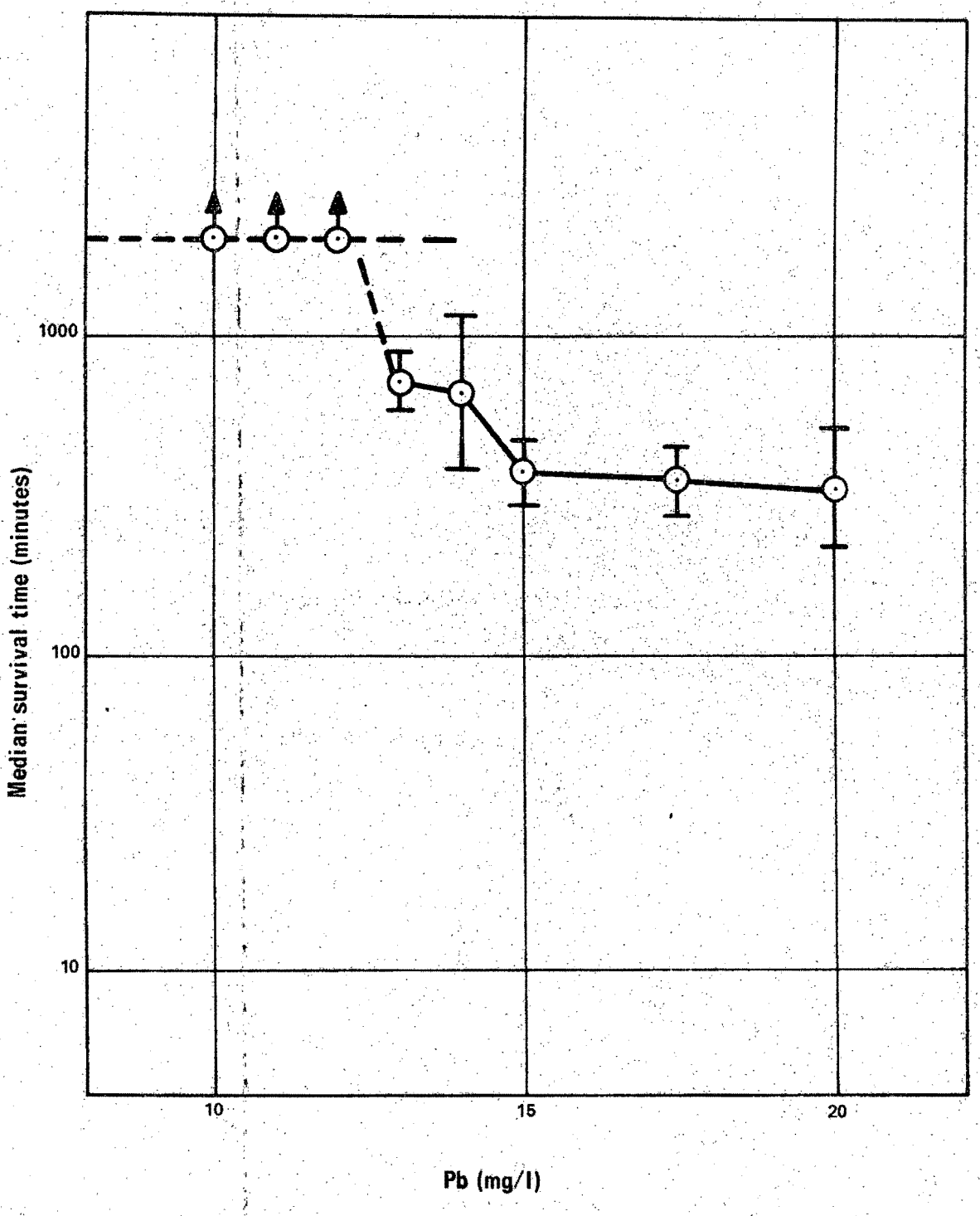

1000
100
10
Median survival time (minutes)
10
15
20
Pb (mg/l)

Figure 60

Median times of survival of Baetis harrisoni nymphs not attempting ecdysis in different concentrations of zinc sulphate.

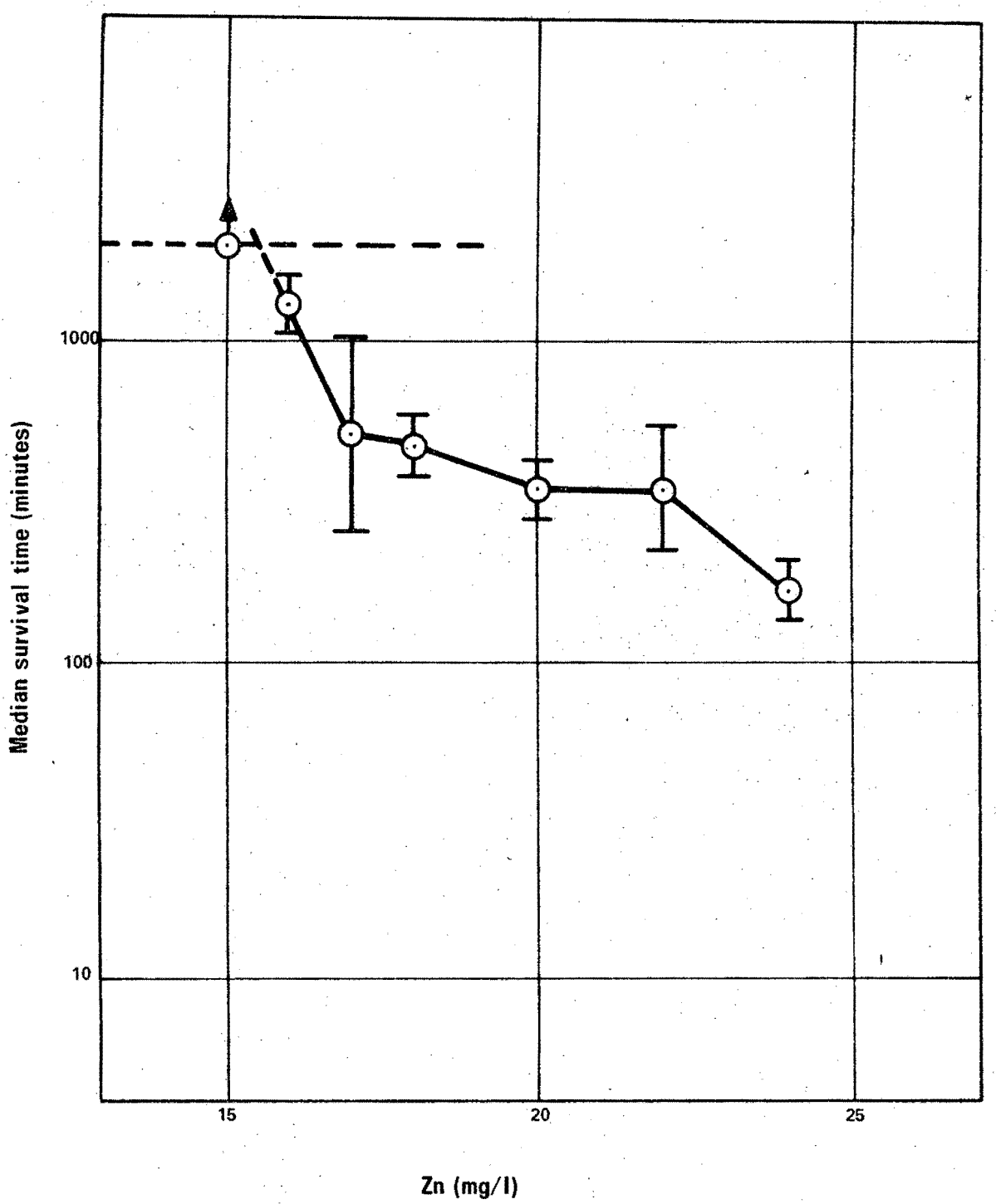
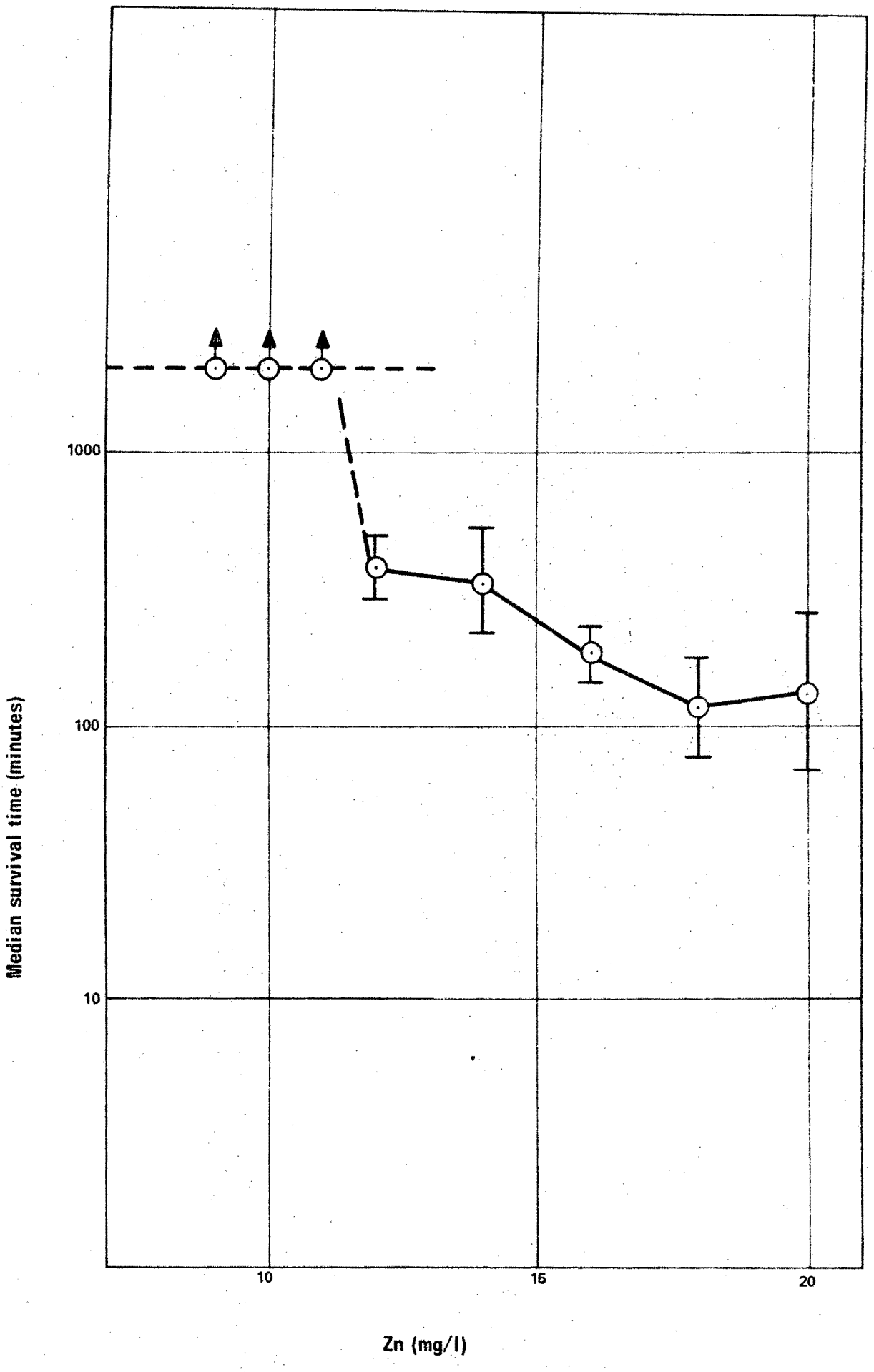

Median survival time (minutes)
1000
100
10
15
20
25
Zn (mg/l)

Figure 61

Median times of survival of Choroterpes bugandensis nymphs in different concentrations of zinc sulphate.


1000
100
10
10
15
20
Median survival time (minutes)
Zn (mg/l)

tions for nymphs fed before exposure were significantly higher than those for nymphs starved before exposure both when nymphs were fed and when they were not fed during the experiment.

TABLE 106

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS FED AND UNFED BEFORE AND DURING EXPERIMENTS

Before experiment	During experiment	pH	Appearance of solutions	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
fed	fed	6.0	clear	130	1.5 2.0 2.5 3.0	2.1 (1.7 to 2.4)
fed	unfed	6.0	clear	136	1.5 2.0 2.5 3.0	2.5 (2.2 to 2.7)
unfed	fed	6.0	clear	140	0.5 1.0 1.5 2.0	0.8 (0.6 to 1.0)
unfed	unfed	6.0	clear	138	1.5 2.0 2.5 3.0	1.9 (1.7 to 2.2)

FIELD OBSERVATIONS

Water samples were collected at various times during this study from places where either Baetis harrisoni or Choroterpes bugandensis were found or where it was thought concentrations of heavy metals might be present.

TABLE 107

1000-MINUTE MEDIAN LETHAL CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS FED AND UNFED BEFORE AND DURING EXPERIMENTS

Before experiment	During experiment	pH	Appearance of solutions	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
fed	fed	6.0	clear	240	0.2 0.4 0.6 0.8 1.0 1.2	0.7 (0.5 to 1.0)
fed	unfed	6.0	clear	240	0.2 0.4 0.6 0.8 1.0 1.2	0.9 (0.7 to 1.0)
unfed	fed	6.0	clear	240	0.2 0.4 0.6 0.8 1.0 1.2	0.2 (0.1 to 0.5)
unfed	unfed	6.0	clear	240	0.2 0.4 0.6 0.8 1.0 1.2	0.5 (0.3 to 1.0)

The determinations were very kindly carried out by Dr L.R.G. Butler of the National Physical Research Laboratory. In no instance was a concentration of any of these elements found to be within the lethal range as revealed by the laboratory experiments.

DISCUSSION

The results reported here relating to copper toxicity and solubility in water of different pH values suggest strongly that at pH 6.5 only just enough cupric ions could be dissolved in the water to kill Baetis nymphs. Samples of the test water used in these experiments were filtered and sent to Dr L.R.P. Butler of the National Physical Research Laboratory who kindly determined the concentrations of dissolved copper present. The highest concentrations of dissolved copper determined at pH 6.5 were of the order of 2.5 mg/l and the highest concentrations determined at pH 7.0 were of the order of 0.2 mg/l, even when much greater amounts of copper sulphate had been added to the water. The concentrations of dissolved lead and zinc determined at pH 6.5 corresponded to the amounts added, the highest being 16 mg/l lead and 15 mg/l zinc. The highest concentrations of dissolved lead and zinc determined at pH 7.5 were, respectively, 15.5 mg/l and 14 mg/l. They, too, were relatively insoluble in alkaline solutions.

Jenkins, Keight and Ewins (1964) have studied the solubility of these heavy metals in some detail. Their results indicate that solubility of copper and zinc, at least, ought to have been greater than was observed in the present study. This is surprising, since some trouble was taken preparing the solutions for these experiments. However, it is also known that the solubility of copper, lead and zinc is influenced by the hardness (i.e. the calcium and magnesium content) of the water (Water Pollution Research 1959, Lloyd 1960).

Results presented here have suggested that precipitated copper might have been toxic to mayfly nymphs if ingested. Howard, Halvorson and Walden (1964) have shown colloidal suspensions of copper salts to be toxic to fish. Copper can become bound to organic matter present in water in which form Sprague (1964a) has reported it to be harmless for fish, as has hexavalent copper (Pickering and Henderson 1966). In view of these possible complexities, predictions of the toxicity of given concentrations of copper under natural conditions should be applied with some caution.

The concentrations of copper, lead and zinc found here to be lethal for mayfly nymphs are roughly of the same order as those reported by other authors to be lethal for other freshwater invertebrates (for instance Jones 1941 a, b, Liepolt and Weber 1958, Learner and Edwards 1963, Fowler and Goodnight 1965). Clearly, copper, lead and zinc can be found in concentrations lethal to aquatic invertebrates in rivers in many parts of the world. With increasing industrial pollution the toxic effects of these metals may be expected increasingly to cause problems.

SUMMARY

1. Baetis nymphs were more tolerant of copper (median lethal concentration 2.4 mg/l Cu) than were Choroterpes nymphs (median lethal concentration 0.7 - 0.9 mg/l Cu).

2. Copper sulphate solutions were not lethal for either species in water more alkaline than pH 6.5. Apart from its influence on the solubility of copper salts, pH did not appear significantly to affect the toxicity of copper to Baetis and Choroterpes nymphs.

3. Both species were found to be tolerant of quite high concentrations of lead in solution (median lethal concentration 14.6 - 15.1 mg/l Pb for Baetis, 11.2 - 11.7 mg/l Pb for Choroterpes) and zinc (median lethal concentration 13.6 - 13.8 mg/l Zn for Baetis, 10.5 mg/l Zn for Choroterpes).

4. 1000-minute lethal concentrations of dissolved copper, lead and zinc were demonstrated to have been higher than the incipient lethal concentrations which would have caused 50 per cent mortality after prolonged exposure.

5. For each species, nymphs of different sizes were similarly tolerant of copper.

6. Nymphs of both species fed before being exposed to copper solutions were more tolerant of copper than were unfed nymphs.

7. Nymphs of both species fed during the experiments were less tolerant of copper than were animals starved during the experiment, possibly due to ingestion of copper with the food.

TOXICITY TO NYMPHS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS OF MIXTURES OF COPPER, LEAD AND ZINC IN SOLUTION, AND INFLUENCE OF CALCIUM AND BICARBONATE CONCENTRATIONS ON THE TOXICITY OF COPPER

INTRODUCTION

It has been seen in the previous section that copper dissolved in slightly acid water (pH 6.5 or less) could be lethal both for Baetis harrisoni and for Choroterpes bugandensis nymphs at levels which could conceivably occur in natural waters. Both species were found on the other hand to be relatively tolerant of zinc and lead in solution. Published information on interaction between these metals in their toxic action to mayflies is not available. However, information in available on their joint toxicity to fish (Doudoroff and Katz 1953, Jones 1964). Jones (1938 a, 1939 a, b) has reported antagonism (toxicity less than that predicted by addition) between the joint toxic effects of copper and lead on fish and certain invertebrates, while Doudoroff and Katz (1953) and Lloyd (1961 b) have found copper and zinc to be more toxic to fish in combination than would be predicted by addition (i.e. synergism). Some observations are reported here of the toxicity of mixtures of copper, lead and zinc solutions to Baetis and Choroterpes nymphs.

A great deal of published evidence is available to suggest that solutions of copper, lead and zinc might be less toxic to fish in water of high calcium or bicarbonate content (Lloyd 1960, Mount 1966, Pickering and Henderson 1966). It is not altogether clear from this information to what extent this might be due to the insolubility of carbonates of these metals and to what extent it might be due to antagonism, particularly involving calcium (Jones 1964). These factors are obviously of great importance in determining the

toxicity of metals to aquatic animals and observations are reported here of the toxicities of copper solutions to Baetis harrisoni and Choroterpes buganden-sis nymphs at different calcium and bicarbonate concentrations.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected in the Braamfontein Spruit and Pienaars River and brought into the laboratory. After being held in the laboratory for 24 hours in well-aerated water at selected temperatures they were exposed in experimental tubes, as has been described, for 1000 minutes under controlled conditions to different concentrations of the cations concerned. At the end of 1000 minutes' exposure the numbers of nymphs recovering and not recovering were counted. Lethal concentrations of combinations of the metals tested were then calculated by probit analysis (Finney 1952).

COMBINATIONS OF COPPER, LEAD AND ZINC

Nymphs of each species were held in the laboratory at 20°C, and exposed at the same temperature to different combinations of copper sulphate, lead nitrate and zinc sulphate. In order to facilitate comparison of results, all tests involving Baetis harrisoni were carried out in random batches as part of large factorial design experiment. The tests involving Choroterpes bugandensis were similarly combined in a single experiment taking several days to complete. All of these experiments were carried out at pH 6.5. Median lethal concentrations for Baetis nymphs in solutions made up of different proportions of copper, lead and zinc are shown in table 108. Equivalent

data for Choroterpes bugandensis are shown in table 109. Here the concentrations of each of the three metals is given as proportions of their individual median lethal concentrations estimated earlier and described in the preceeding section. This is a method widely used in estimating the toxicity to aquatic animals of mixtures of poisons (Lloyd and Jordan 1964).

TABLE 108

1000-MINUTE MEDIAN LETHAL MIXTURES OF COPPER, LEAD AND ZINC FOR BAETIS HARRISONI NYMPHS

Mixture proportions $\left(\frac{Cu}{2.4} : \frac{Pb}{15.0} : \frac{Zn}{13.7}\right)$	Number of nymphs	Mixture test concentrations $\left(\frac{Cu}{2.4} + \frac{Pb}{15.0} + \frac{Zn}{13.7}\right)$	Lethal mixture concentration $\left(\frac{Cu}{2.4} + \frac{Pb}{15.0} + \frac{Zn}{13.7}\right)$, 95% confidence limits in brackets
1 : 1 : 0	172	0.6 0.9 1.1 1.4	1.2 (1.0 to 1.4)
1 : 0 : 1	167	0.6 0.9 1.1 1.4	1.0 (0.8 to 1.2)
0 : 1 : 1	180	0.6 0.9 1.1 1.4	1.0 (0.7 to 1.2)
3 : 1 : 1	184	0.6 0.9 1.1 1.4	1.2 (0.9 to 1.4)
1 : 3 : 1	171	0.6 0.9 1.1 1.4	1.0 (0.9 to 1.2)
1 : 1 : 3	175	0.6 0.9 1.1 1.4	0.9 (0.7 to 1.2)

TABLE 109

1000-MINUTE MEDIAN LETHAL MIXTURES OF COPPER, LEAD AND ZINC FOR CHOROTERPES BUGANDENSIS NYMPHS

Mixture proportions $\left(\frac{Cu}{0.8} : \frac{Pb}{11.5} : \frac{Zn}{10.5}\right)$	Number of nymphs	Mixture test concentrations $\left(\frac{Cu}{0.8} + \frac{Pb}{11.5} + \frac{Zn}{10.5}\right)$	Lethal mixture concentrations $\left(\frac{Cu}{0.8} + \frac{Pb}{11.5} + \frac{Zn}{10.5}\right)$; 95% confidence limits in brackets
1 : 1 : 0	160	0.6 0.9 1.1 1.4	1.0 (0.9 to 1.2)
1 : 0 : 1	160	0.6 0.9 1.1 1.4	1.0 (0.8 to 1.2)
0 : 1 : 1	162	0.6 0.9 1.1 1.4	1.1 (0.9 to 1.3)
3 : 1 : 1	160	0.6 0.9 1.1 1.4	1.0 (0.8 to 1.3)
1 : 3 : 1	160	0.6 0.9 1.1 1.4	1.1 (0.9 to 1.4)
1 : 1 : 3	160	0.6 0.9 1.1 1.4	0.9 (0.7 to 1.2)

The individual three median lethal concentrations found earlier for Baetis harrisoni were 2.4 mg/l copper, 15.0 mg/l lead and 13.7 mg/l zinc. The test mixtures for these experiments were made up as proportions of the lethal concentrations, so that in each series the proportions:

$$\frac{Cu}{2.4} : \frac{Pb}{15.0} : \frac{Zn}{13.7}$$

were kept constant. The median lethal concentration of each mixture is expressed in tables 108 as:

$$\left(\frac{Cu}{2.4} + \frac{Pb}{15.0} + \frac{Zn}{13.7}\right)$$

where Cu, Pb and Zn represent the actual concentrations of these cations present as mg/l. In the case of Choroterpes the equivalent lethal concentration for the three metals taken singly, as reported in the previous section, were 0.8 mg/l copper, 11.5 mg/l lead and 10.5 mg/l zinc. The concentrations of the mixtures are expressed in table 109 as:

$$\left(\frac{Cu}{2.4} + \frac{Pb}{15.0} + \frac{Zn}{13.7}\right)$$

As may be seen from tables 108 and 109, the numerical values of $\left(\frac{Cu}{2.4} + \frac{Pb}{15.0} + \frac{Zn}{13.7}\right)$ for Baetis and of $\left(\frac{Cu}{0.8} + \frac{Pb}{11.5} + \frac{Zn}{10.5}\right)$ for Choroterpes were in all instances not significantly different from unity. There is nothing in these results to suggest that the joint toxic action of copper, lead and zinc might have been either antagonistic or synergistic. The tables also show that it was not possible to estimate the lethal concentrations of copper - lead - zinc mixtures with much accuracy. No explanation of this variability suggests itself, but similar variability in the estimates of lethal mixtures of metals has been found in experiments using fish (Brown 1968).

While these results suggest that, as a general rule of thumb, the toxicity of mixtures of copper, lead and zinc to Baetis and Choroterpes

nymphs might be predicted by addition of the proportions of the lethal concentrations present, they do not reject the possibility of antagonistic or synergistic interaction entirely. In order critically to have investigated the possibilities of antagonistic or synergistic interaction between copper, lead and zinc it would have been necessary to have tested many more mixtures of different proportions. It is quite possible that interaction might be found only in mixtures of certain proportions (Jones 1938a, Lloyd 1961b).

CALCIUM HARDNESS

Different groups of both Baetis harrisoni and Choroterpes bugandensis nymphs were exposed for 1000 minutes to different copper concentrations in the lethal range made up in water containing three different concentrations of calcium. The calcium concentrations were arrived at by adding calcium chloride to borehole water and subsequently adjusting the pH of each solution to 6.5. Mortality in each combination of calcium and copper was noted and the median lethal copper concentrations calculated for each calcium concentration are shown in tables 110 and 111 for Baetis and for Choroterpes respectively. As may be seen from these results, calcium had no apparent influence on the toxicity of copper to these animals.

BICARBONATE ALKALINITY

Similar experiments were carried out to compare the toxicity of copper solutions to Baetis harrisoni and Choroterpes bugandensis nymphs in water of two different bicarbonate alkalinities. Here the different solutions were made up by addition to borehole water of an amount of sodium bicarbonate calculated to be required at pH 6.5. The carbon dioxide content of the air to be

TABLE 110

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS IN DIFFERENT CALCIUM CONCENTRATIONS

Calcium concentration (mg/l Ca)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
2	141	2.0 2.4 2.6 3.0	2.6 (2.2 to 3.0)
20	137	2.0 2.4 2.6 3.0	2.6 (2.4 to 2.8)
200	140	2.0 2.4 2.6 3.0	2.4 (2.1 to 2.7)

TABLE 111

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS IN DIFFERENT CALCIUM CONCENTRATIONS

Calcium concentration (mg/l Ca)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
2	120	0.6 0.8 1.0 1.2	0.9 (0.7 to 1.1)
20	120	0.6 0.8 1.0 1.2	1.0 (0.9 to 1.1)
200	120	0.6 0.8 1.0 1.2	0.8 (0.7 to 0.9)

kept in equilibrium with the test solution and to maintain its bicarbonate alkalinity was also calculated. The pH of the test solution was then adjusted to pH 6.5 and a mixture of air and carbon dioxide bubbled through it. During the next 24 hours the bicarbonate alkalinity of each test solution was determined from time to time and adjusted where necessary by addition either of borehole water or of more sodium bicarbonate solution. When equilibrium had been reached, the copper sulphate was added. Thereafter the toxicity test was carried out as before.

TABLE 112

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR BAETIS HARRISONI NYMPHS IN WATER OF DIFFERENT BICARBONATE

Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
10	125	2.0 2.4 2.6 3.0	2.4 (2.1 to 2.7)
100	127	2.0 2.4 2.6 3.0	2.4 (2.2 to 2.6)

SUMMARY

1. Mixtures of copper sulphate, lead nitrate and zinc sulphate were toxic to Baetis and Choroterpes nymphs when:

TABLE 113

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR CHOROTERPES BUGANDENSIS NYMPHS IN WATER OF DIFFERENT BICARBONATE ALKALINITIES

Bicarbonate alkalinity (mg/l as $CaCO_3$)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
10	120	0.6 0.8 1.0 1.2	0.8 (0.6 to 1.0)
100	120	0.6 0.8 1.0 1.2	0.8 (0.7 to 0.9)

$$\left(\frac{\text{Cu in mixture}}{\text{toxic Cu on its own}}\right) + \left(\frac{\text{Pb in mixture}}{\text{toxic Pb on its own}}\right) + \left(\frac{\text{Zn in mixture}}{\text{toxic Zn in mixture}}\right) = 1$$

No evidence of interaction (antagonism or synergism) between toxic actions of copper, lead and zinc was found.

2. Neither calcium hardness nor bicarbonate alkalinity of the water was found to influence the toxicity of copper sulphate, lead nitrate and zinc sulphate to Baetis and Choroterpes nymphs.

INFLUENCE OF TEMPERATURE AND DISSOLVED OXYGEN ON THE TOXICITY OF COPPER, LEAD AND ZINC TO NYMPHS OF BAETIS HARRISONI AND CHOROTERPES BUGANDENSIS

INTRODUCTION

Because these have been found to occur in streams in different parts of the world as a result of industrial or mine pollution, considerable published data are available on the toxicity of the so-called heavy metals, copper, lead and zinc, to aquatic life. Reviews of this literature (for instance Doudoroff and Katz 1953, Jones 1964) reveal tremendous variability in concentrations found by different authors to be toxic. Concentrations of these cations toxic for a wide range of aquatic animals have been found quite commonly in some polluted rivers. At the same time, a number of such other factors as temperature, dissolved oxygen and other salts have been found to influence their toxicity very markedly (Lloyd 1960, 1965, Jones 1964, Pickering 1968).

It has been reported in a previous section that concentrations of copper lethal for nymphs of Baetis harrisoni and Choroterpes bugandensis, although not actually observed during the present study, might be expected to occur from time to time in acid streams either receiving industrial wastes or to which copper sulphate has been added for some reason. Most streams in the Transvaal are alkaline, however, and copper added to them would precipitate. Thus Allanson (1961) has determined up to 11 mg/l Cu in bottom sediments in the Jukskei River. Precipitated copper has been shown in a previous section not to be directly toxic to nymphs, but under certain circumstances to be taken up by nymphs with their food and to cause

mortality in this way.

Results are described in this section of experiments in which nymphs of both Baetis harrisoni and Choroterpes bugandensis were exposed to copper sulphate, lead nitrate and zinc sulphate solutions under different conditions of temperature, dissolved oxygen and water current speed. Both increased temperatures and decreased dissolved oxygen concentrations have been found by other authors to increase the toxicity of these metals to fish (Lloyd 1961b). Water current speeds are included here because water flow has been shown in a previous section to affect the availability of oxygen at least to Baetis nymphs. Moreover, Lloyd has produced evidence to show that the increased toxicity to fish of poisons at low oxygen concentrations could be ascribed to the increased flow of water over the gills at low oxygen concentrations.

MATERIAL AND METHODS

Baetis harrisoni and Choroterpes bugandensis nymphs were collected, as before, in the Braamfontein Spruit and Pienaars River. They were held for a day in the laboratory at 20°C before being exposed for 1000 minutes to the conditions being tested. The experiments described here were all carried out at pH 6.5. Temperatures, dissolved oxygen and pH values were all strictly controlled in all experiments, as has been described in earlier sections. From observed mortality at each combination of metal salt concentration and the other factors being studied, lethal copper, lead and zinc concentrations were calculated by probit analysis (Finney 1952).

TEMPERATURE

Median lethal dissolved copper concentrations for Baetis harrisoni

and Choroterpes bugandensis nymphs at 15°C, 20°C and 25°C are shown in tables 114 and 115. These experiments were carried out in summer in water containing 7.5 to 7.7 mg/l dissolved oxygen and in a laminar flow of 10 cm/sec in tubes of 1.6 cm internal diameter. The salt used was copper sulphate. Mortality was recorded after 1000 minutes and median lethal copper concentrations calculated by probit analysis (Finney 1952) for each temperature. Nymphs of both species were found to be significantly less tolerant of dissolved copper at 25°C than they were at 15°C and 20°C. Relative mortalities of nymphs in and out of ecdysis were compared in each case. In no cases were nymphs attempting ecdysis found to be less tolerant than other nymphs.

TABLE 114

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATION FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT TEMPERATURES

Temperature (°C)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
15	161	2.0 2.4 2.6 3.0	2.6 (2.3 to 2.8)
20	160	2.0 2.4 2.6 3.0	2.4 (2.2 to 2.7)
25	160	2.0 2.4 2.6 3.0	2.2 (2.0 to 2.3)

TABLE 115

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT TEMPERATURES

Temperature (^{o}C)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
15	160	0.6 0.8 1.0 1.2	1.0 (0.8 to 1.2)
20	160	0.6 0.8 1.0 1.2	0.9 (0.7 to 1.0)
25	160	0.4 0.6 0.8 1.0	0.6 (0.4 to 0.7)

Median lethal dissolved lead concentrations for Baetis harrisoni and Choroterpes bugandensis nymphs at 15^{o}C, 20^{o}C and 25^{o}C are shown in tables 116 and 117. These experiments were carried out in summer in well-oxygenated water flowing at 10 cm/sec. In the 1.6 cm diameter tubes used this flow was laminar. The salt used was lead nitrate. As may be seen, the toxicity of lead to both species was found to increase with increase in temperature.

Two further two experiments were carried out in which summer nymphs of each species were exposed to solutions of zinc sulphate at 15^{o}C, 20^{o}C and 25^{o}C. As before, the water was well-oxygenated at the current speed maintained at 10 cm/sec. The median lethal concentrations found are shown in

TABLE 116

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR SUMMER *EAETIS HARRISONI* NYMPHS AT DIFFERENT TEMPERATURES

Temperature (°C)	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
15	160	13.5 14.5 15.0 16.0	15.2 (14.7 to 15.9)
20	157	13.5 14.5 15.0 16.0	14.9 (14.3 to 15.5)
25	159	13.5 14.5 15.0 16.0	14.5 (13.9 to 15.1)

TABLE 117

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR SUMMER *CHOROTERPES BUGANDENSIS* NYMPHS AT DIFFERENT TEMPERATURES

Temperature (°C)	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
15	160	10.5 11.0 11.5 12.0	11.6 (11.1 to 12.1)
20	160	10.5 11.0 11.5 12.0	11.1 (10.7 to 11.5)
25	160	10.5 11.0 11.5 12.0	10.8 (10.3 to 11.3)

tables 118 and 119, respectively for Baetis harrisoni and Choroterpes bugandensis. As in the case of the other metals, the toxicity of zinc to nymphs of both species was found to increase with temperature, the median lethal concentration at 25°C being significantly lower than those at 15°C.

TABLE 118

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT TEMPERATURES

Temperature (°C)	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
15	160	12.5 13.5 14.0 15.0	14.5 (14.1 to 14.9)
20	160	12.5 13.5 14.0 15.0	13.9 (13.3 to 14.5)
25	160	12.5 13.5 14.0 15.0	13.6 (13.0 to 14.2)

DISSOLVED OXYGEN AND CURRENT SPEED

Nymphs of Baetis harrisoni and Choroterpes bugandensis, after 24 hours in the laboratory at 20°C in well-aerated water, were exposed for 1000 minutes to different concentrations of copper sulphate in the lethal range at different combinations of dissolved oxygen concentration and of water current speed. These experiments were carried out at 20°C. For each species these tests were carried out in random order over several days,

TABLE 119

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT TEMPERATURES

Temperature (^{o}C)	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
15	160	9.5 10.0 10.5 11.0	11.0 (10.5 to 11.6)
20	160	9.5 10.0 10.5 11.0	10.4 (9.8 to 11.0)
25	160	9.5 10.0 10.5 11.0	10.4 (10.0 to 10.8)

since the number of tanks required for the experiment exceeded the number available. As in the previous experiments, mortality of nymphs was noted and median lethal copper concentrations calculated in each case. The results obtained for Baetis and for Choroterpes respectively, are shown in tables 120 and 121. No suggestion was found to indicate that reduced oxygen levels or reduced current speeds had any effect at all on the toxicity of copper to Baetis or Choroterpes.

In two similar experiments, each also carried out piecemeal over several days, nymphs of Baetis harrisoni and Choroterpes bugandensis were exposed to different concentrations of lead nitrate at different combinations of dissolved oxygen and water current speed, all at 20^{o}C. Median lethal lead concentrations estimated from these experiments are shown in tables 122 and 123.

TABLE 120

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
8	4.0	laminar	2.6	156	2.0 2.4 2.6 3.0	2.5 (2.3 to 2.7)
	10.6	laminar	1.6	160	2.0 2.4 2.6 3.0	2.6 (2.3 to2.8)
5	8.6	laminar	1.6	159	2.0 2.4 2.6 3.0	2.4 (2.3 to 2.6)
	14.1	laminar	1.2	159	2.0 2.4 2.6 3.0	2.4 (2.2 to2.6)

As in the case of copper, neither lowered oxygen nor slow current speed appeared to affect the toxicity of lead to these species.

In two further experiments, nymphs of each of the two species were exposed to different concentrations of zinc sulphate at different combinations of dissolved oxygen and water current speed, all at 20°C. The median lethal zinc concentrations estimated from these results are shown in tables 124 and 125. As for copper and lead, the toxicity of zinc to Baetis and Choroterpes nymphs was unaffected either by decreased oxygen concentration or by slow water current speed.

TABLE 121

1000-MINUTE MEDIAN LETHAL COPPER CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Copper test concentrations (mg/l Cu)	Lethal copper concentration (mg/l Cu), 95% confidence limits in brackets
8	2.0	laminar	2.6	160	0.6 0.8 1.0 1.2	0.7 (0.5 to 0.9)
	9.4	laminar	1.2	160	0.6 0.8 1.0 1.2	0.9 (0.7 to 1.0)
3	2.0	laminar	2.6	160	0.6 0.8 1.0 1.2	0.8 (0.6 to 1.0)
	9.4	laminar	1.2	160	0.6 0.8 1.0 1.2	0.8 (0.6 to 1.0)

TABLE 122

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
8	4.0	laminar	2.6	160	13.5 14.5 15.0 16.0	14.8 (14.2to15.4)

TABLE 122 (cont)

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
8	10.6	laminar	1.6	160	13.5 14.5 15.0 16.0	15.1 (14.4to15.8)
5	8.0	laminar	1.6	160	13.5 14.5 15.0 16.0	15.0 (14.5to15.5)
	14.1	laminar	1.2	160	13.5 14.5 15.0 16.0	15.2 (14.6to15.8)

TABLE 123

1000-MINUTE MEDIAN LETHAL LEAD CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Lead test concentrations (mg/l Pb)	Lethal lead concentration (mg/l Pb), 95% confidence limits in brackets
8	2.0	laminar	2.6	157	10.0 10.5 11.0 11.5	11.0 (10.5to11.5)
	9.4	laminar	1.2	160	10.0 10.5 11.0 11.5	11.2 (10.6to11.8)
3	2.0	laminar	2.6	160	10.0 10.5 11.0 11.5	11.0 (10.4to11.6)
	9.4	laminar	1.2	160	10.0 10.5 11.0 11.5	11.1 (10.5to11.7)

TABLE 124

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR SUMMER BAETIS HARRISONI NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
8	4.0	laminar	2.6	160	12.5 13.5 14.0 15.0	14.0 (13.6to14.6)
	10.6	laminar	1.6	160	12.5 13.5 14.0 15.0	14.0 (13.5to14.5)
5	8.0	laminar	1.6	162	12.5 13.5 14.0 15.0	13.7 (13.1to14.3)
	14.1	laminar	1.2	161	12.5 13.5 14.0 15.0	14.0 (13.5to14.5)

TABLE 125

1000-MINUTE MEDIAN LETHAL ZINC CONCENTRATIONS FOR SUMMER CHOROTERPES BUGANDENSIS NYMPHS AT DIFFERENT COMBINATIONS OF DISSOLVED OXYGEN CONCENTRATION AND WATER CURRENT SPEED

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
8	2.0	laminar	2.6	160	9.5 10.0 10.5 11.0	10.6 (10.1to11.1)
	9.4	laminar	1.2	160	9.5 10.0 10.5 11.0	10.5 (10.0to11.0)
3	2.0	laminar	2.6	160	9.5 10.0 10.5 11.0	10.4 (9.9to10.9)

TABLE 125 (cont)

Dissolved oxygen (mg/l)	Water current speed (cm/sec)	Nature of flow	Tube diameter (cm)	Number of nymphs	Zinc test concentrations (mg/l Zn)	Lethal zinc concentration (mg/l Zn), 95% confidence limits in brackets
3	9.4	laminar	1.2	160	9.5 10.0 10.5 11.0	10.5 (10.1to10.9)

DISCUSSION

The observation made here that the toxicity of copper, lead and zinc salts to Baetis harrisoni and Choroterpes bugandensis nymphs was increased at higher temperatures is in general agreement with observations that have been made of the toxicity of these metals to fish (Brown 1968). Lloyd (1960), for instance, found survival times of trout in zinc sulphate solutions to be shorter at higher temperatures although he found the threshold concentrations to be similar at different temperatures.

Death of fish in solutions of copper, lead and zinc salts has been shown to be associated with respiratory stress, sometimes with excess production of mucus which appeared to coagulate and in various ways to interfere with gill function (Carpenter 1927, 1930, Ellis 1937, Jones 1938a, Westfall 1945, Schweiger 1957) and sometimes with damage to the gill tissue (Lloyd 1960). Jones (1947b) found the frequency of respiratory movements of fish in such solutions to double and their rates of oxygen uptake, after a short rise, to fall steadily to a fifth the original value, after which death followed rapidly. No evidence of similar effects of copper, lead or zinc salts on the respiration of Baetis or Choroterpes nymphs was noted in the present study. The

frequency of gill movements of Choroterpes nymphs was slightly, but not notably, increased in these solutions and slowed and stopped intermittently long before the animals died. The outward signs of distress and death were no different from those in other toxic solutions. The toxicity of copper, lead and zinc was not increased at low dissolved oxygen concentrations, as they might have been expected to have been if oxygen lack had been a reason for the death of the nymphs.

SUMMARY

1. The toxicity of dissolved copper to Baetis and to Choroterpes nymphs increased with increase in temperature (median lethal concentrations 2.6 mg/l at 15°C and 2.2 mg/l at 25°C for Baetis, 1.0 mg/l at 15°C and 0.6 mg/l at 25°C for Choroterpes).

2. The toxicity of dissolved lead to Baetis and to Choroterpes nymphs increased with increase in temperature (median lethal concentrations 15.2 mg/l at 15°C and 14.5 mg/l at 25°C for Baetis, 11.6 mg/l at 15°C and 10.8 mg/l at 25°C for Choroterpes).

3. The toxicity of dissolved zinc to Baetis and to Choroterpes nymphs increased with increase in temperature (median lethal concentrations 14.5 mg/l at 15°C and 13.6 mg/l at 25°C for Baetis, 11.0 mg/l at 15°C and 10.4 mg/l at 25°C for Choroterpes).

4. The toxicity of dissolved copper, lead and zinc to Baetis and to Choroterpes nymphs was not influenced by differences in either dissolved oxygen concentration or water current speed within the range tested (median lethal concentrations 2.4 - 2.6 mg/l Cu, 14.8 - 15.2

mg/l Pb and 13.1 - 13.6 mg/l Zn for Baetis, 0.7 - 0.9 mg/l Cu, 11.0 - 11.2 mg/l Pb and 10.4 - 10.6 mg/l Zn for Choroterpes).

GENERAL DISCUSSION

GENERAL REMARKS ON TOLERANCE LIMITS

As explained in the introductory section, laboratory experiments define the tolerance limits for a chosen exposure time which will kill 50 per cent of animals. Some individuals in a population will be killed by much less extreme conditions and some will survive conditions beyond the median limits. Limits which will either kill or be tolerated by 95%, 99% or all the animals in a large population cannot be estimated with precision. This has long been a problem in studies aimed at determining the concentrations of pollutants which will be "safe" to release into rivers, since fish have been found to die in rivers at concentrations of poisons considerably less than the threshold limits determined in the laboratory (Allan, Alabaster and Herbert (1954). For lack of a better criterion, most workers have recommended that 10 per cent of the threshold concentration for a poison, or for a combination of poisons, be considered a "safe" concentration which will not kill fish (Beak 1958, Jones 1964).

An important factor to be taken into account in interpreting the results of tolerance experiments in relation to the survival of animals in the field is the time they may be exposed to potentially lethal conditions. Occasional very short exposures to extreme conditions well outside the threshold limits might not be harmful, while long-term exposure to sub-lethal conditions could have deleterious effects not revealed by relatively short-term laboratory experiments.

Very little is known of the lethal effects on animals of exposure to fluctuating conditions in the lethal range. Fry (1947) found the survival time

of fish in fluctuating temperatures in the upper lethal range to be consistent with the relation:

$$\sum_{(i)} \frac{\text{time spent at temperature (i)}}{\text{median survival time at constant temperature (i)}} = 1$$

which is to say that the cumulative effect of the temperatures to which the animals were exposed was additive. Very detailed information on the temperature fluctuations is necessary to be able to predict the survival of animals in the field.

Very little is known, too, of the effects on animals of intermittent exposure to lethal conditions, although this evidently occurs commonly in the field. Tsukuda (1959) found repeated exposure to low temperatures not to affect the chill coma temperatures of individual fish. Ernst (1965), on the other hand, found the lethal effects of repeated chill coma on another fish species during successive nights to be additive when diurnal water temperatures rose a few degrees above the chill coma temperature. This additive effect disappeared if the fish were kept for a day or so at 20°C. Death during chill coma apparently resulted from renal failure. The fish were able to recover from its effects at 20°C but not at temperatures close to the chill coma temperature. Clearly, the mechanism of the lethal effect will determine whether or not animals will be killed by repeated short exposures to environmental extremes.

An attempt will be made here to define the tolerance limits for Baetis harrisoni and Choroterpes bugandensis nymphs in streams and to compare these limits with the information on the ecology of each species that was summarized in the introduction to this report. For most factors,

the 1000-minute median lethal limits determined in this study may be taken as reasonable approximations of the true limits as they might limit the distribution of the nymphs of each species in the field. They are applicable, for instance, to factors like temperature and dissolved oxygen which have been shown in relevant sections of the report normally in most South African rivers to fall within the range tolerated by both species but which from time to time for short periods reach lethal levels. They are applicable, too, to factors like pH which appear from the results described here to cause mortality only within the first 1000 minutes of exposure. They are probably not applicable to to copper, lead and zinc salts, or to salinity, which apparently continue to kill nymphs after the first 1000 minutes of exposure.

TOLERANCE LIMITS FOR BAETIS HARRISONI

Fast water flow

It has been shown here that Baetis harrisoni nymphs are dislodged by current speeds in excess of 0.5 - 0.6 m/sec actually impinging on the animals and estimated 0.1 cm from the surface of the rocks in the stream flow. It has also been shown that current speeds of this order might be expected to impinge on the animals when the current speed in the open flow is anything from 1.3 to 4.5 m/sec. These speeds have been measured during flooding even in normally sluggish streams. The observation of Harrison (1958a) and Allanson (1961) that nymphs were washed downstream in large numbers during floods has been confirmed.

It is interesting to note that Baetis harrisoni nymphs occur in large numbers in rivers where they may be washed away by floods perhaps several

times a year. As has been shown in another section, their positive rheotactic behaviour leads them to occupy sites in stony runs where they will be exposed to the full force of the current. During this study it was noted that recolonization of stony runs after floods took place at least partly through hatching of eggs deposited in the river before flooding began. It is not necessary to postulate, as Müller (1954) has done, that downstream drift of the nymphs is compensated for by upstream flight before oviposition of the subimagines and imagines.

Both Harrison and Elsworth (1958) and Oliff (1960a) found no Baetis harrisoni in the source and cliff waterfall zones of the rivers they studied but found large numbers in both the mountain torrent and the foothill stony run zones. Their absence from the topmost zones could conceivably be due to low temperatures found here. The results presented here suggest it is more likely to be due to the very fast rate of water flow (Harrison and Elsworth estimate speeds of the order of 3 m/sec) and the probable difficulty of recolonization in the face of such maintained flows.

Dissolved oxygen and slow water flow

Results presented here have shown Baetis harrisoni nymphs to be intolerant of low dissolved oxygen concentrations at slow rates of water flow, as has been found for other species of Baetis (Ambühl 1959). In completely stagnant water nymphs were found to die of oxygen lack even in water saturated with oxygen. Nymphs attempting ecdysis were considerably less tolerant of low dissolved oxygen concentrations than were nymphs not in ecdysis. For exposures of only a few hours' duration the lethal limit for the species will be determined principally by the tolerances of nymphs not

in ecdysis, as follows for summer nymphs:

Current speed	2.7 cm/sec	7-8 cm/sec	15-40 cm/sec
15°C	1.1 mg/l	0.7-0.9 mg/l	0.6-0.7 mg/l
20°C	1.6 mg/l	0.9-1.1 mg/l	0.6-0.8 mg/l
25°C	2.3 mg/l	0.9-1.4 mg/l	0.9-1.0 mg/l.

For longer exposures, the lethal limit for the species will be determined by the tolerances of nymphs in ecdysis, as follows for summer nymphs:

Current speed	2.7 cm/sec	7-8 cm/sec	15-40 cm/sec
15°C	6.3 mg/l	3.2-4.4 mg/l	1.4-2.3 mg/l
20°C	7.0 mg/l	3.6-4.4 mg/l	1.5-2.7 mg/l
25°C	> 8 mg/l	4.3-5.4 mg/l	1.9-2.9 mg/l.

Precise data are not available for winter nymphs but temperature tolerance data suggest that the dissolved oxygen requirements of winter nymphs at 15°C and 20°C might be similar to those of summer nymphs at these temperatures but that nymphs at 25°C might only survive at relatively fast rates of water flow and high dissolved oxygen concentrations.

As noted in the introduction, Baetis harrisoni nymphs are found almost exclusively in swift flowing water. Observations described here of their behaviour have shown that they tend to move into situations of faster flow. Determinations of their dissolved oxygen requirements at different water current speeds show not only that they are physiologically dependent upon water flow for their oxygen supply but also that they are best able to survive exposures to low dissolved oxygen levels in fast flowing water.

Observations made during this study have also shown that these nymphs are able to detect differences in the oxygen content of water flowing past them. They appear to be able to select positions where oxygen is best available to them.

It seems clear that the behaviour and dissolved oxygen requirements of Baetis harrisoni nymphs are extremely important in defining the nymphal habitat. These requirements will restrict the nymphs to positions on stones in current or, when nymphs are thrust into situations where no stones are available for them to sit on (Harrison 1958a), to situations on vegetation and other submerged objects where their dissolved oxygen and water flow requirements are met.

In spite of their sensitivity to dissolved oxygen lack, it is clear from the findings reported here that Baetis harrisoni nymphs can live in polluted streams in which low dissolved oxygen concentrations occur (Allanson 1961) if stones are available in swift flowing water.

The water flow and dissolved oxygen requirements of nymphs are sufficient to explain the apparent absence of Baetis harrisoni from the slower flowing lower reaches of many rivers (see introduction) and their comparative rarity in such rivers as the Sundays River (Forbes 1968) and the small coastal rivers of Natal (Brand et al 1967). They are also sufficient to explain the disappearance of nymphs from temporary streams when these streams dry up (Harrison 1958b, 1966a). However, they do not explain the apparent absence of this species from the lower Vaal River (Chutter 1967) and lower Orange River (Agnew 1965). As may be seen from table 1, habitat conditions on stones in current are found in most river systems in South Africa which fulfil the current and dissolved oxygen requirements of Baetis harrisoni.

High temperature

Upper lethal temperatures reported here for Baetis harrisoni nymphs were found to be lower than occasional very high temperatures that have been

recorded in certain South African streams in the past (see introduction) and in the Pienaars River during this study. Nymphs in ecdysis and last instar nymphs were found to be particularly sensitive to high temperatures. Very high water temperatures recorded here were associated with exceptionally hot spells and low stream flows and persisted for several days. Because the ecdysis rate was very high at these temperatures the upper lethal limits may be taken as those of nymphs in ecdysis, as follows for nymphs in oxygenated water flowing at 15 cm/sec:

Summer : 27.0 - 29.3°C

Winter : 25.4 - 25.8°C.

The precise lethal temperature for each population was evidently a function of the previous temperature history of the population. The highest lethal temperature shown was determined at a time when nymphs were seen surviving 28.2°C in the field. The upper lethal temperatures of winter nymphs increased with acclimation at increased temperature in the laboratory, but were not ever as high as those of summer nymphs. The upper lethal temperatures of last instar nymphs were about 2°C lower than those of smaller nymphs.

Harrison and Elsworth (1958) recorded a temperature of 32.5°C on one occasion in the Great Berg River at a station at which they collected Baetis harrisoni. Not even summer nymphs not in ecdysis tested during this study would have survived this temperature. Oliff (1960a) recorded a temperature of 28.9°C at a station in the Tugela River where Baetis harrisoni occurred. At least some mortality of nymphs attempting ecdysis

tested during this study would have been expected at this temperature. Whether or not Baetis harrisoni nymphs were exposed to these actual temperatures, it is clear from this information that temperatures in the lethal range for this species must occur occasionally in a great many streams in South Africa.

Upper lethal temperatures of Baetis harrisoni nymphs were found to be decreased at low water flow rates and low dissolved oxygen concentrations, as follows for winter nymphs in ecdysis:

Current speed	2.7 cm/sec	7-8 cm/sec	15-40 cm/sec
D.O. 8 mg/l	24.0°C	25.5-26.2°C	25.9-26.8°C
D.O. 6mg/l	20.9°C	24.9-25.4°C	24.6-25.8°C
D.O. 4mg/l	< 17°C	23.3-23.9°C	24.3-24.5°C.

Although upper lethal temperatures for summer nymphs might be expected to be uniformly higher under comparable conditions, it seems highly likely that lethal combinations of high temperature, slow water flow and low dissolved oxygen concentration might be expected from time to time in small sluggish streams in most parts of southern Africa. Occasional periods of low flow and high temperatures might be survived by diapause eggs (M.V. Eksteen unpublished data) but the absence of Baetis harrisoni from the sluggish Pienaars River and from certain other Transvaal streams (J.D. Agnew unpublished data) might be explained in terms of the temperature, dissolved oxygen and water flow requirements of the nymphs.

Low temperature

Summer Baetis harrisoni nymphs were found to enter chill coma at 5.7°C and winter nymphs to enter chill coma at 3.4°C. They took more than a day to die in chill coma. As has been described in the introduction, even

lower temperatures than these have been recorded during winter in South African streams. Oliff (1960a), for instance, recorded a temperature of -1.1°C at a station in the Tugela River system where this species occurred. Baetis harrisoni has been found in the upper Caledon River, where low winter temperatures might be expected. It is not known whether or not the species occurs in the headwaters of the Orange River in Lesotho, where low temperatures occur throughout the year. Conceivably, nymphs from these areas might be more tolerant of low temperatures than were those tested here.

Total dissolved solids

The 1000-minute median lethal total dissolved solids concentration for Baetis harrisoni nymphs, using borehole water to which sodium chloride had been added, was found to be 12.2 g/l. This is greatly in excess of any concentration in which this species has been found in the field. Forbes (1968) has found nymphs of another baetid mayfly, Cloeon crassi, in water of high dissolved solids content in the upper estuary of the Sundays River. He found this species able to hyporegulate, for short periods at least, in water of up to 20 g/l dissolved solids content (both diluted sea water and river water with sodium chloride added). However, mortality took place after long exposures to much more dilute solutions. He observed median survival times of 24-35 hours in water of 10.4 g/l salt content and 216-259 hours in water of 7 g/l salt content. His results suggest that Cloeon crassi might have been less tolerant of salt solutions than was Baetis harrisoni,

although the latter is nowhere found in saline water. His results also suggest that 1000-minute median lethal salt concentrations determined in the laboratory are much higher than the actual salt concentrations that would limit the distribution of mayfly nymphs in nature.

pH

The lethal low pH values determined here for Baetis harrisoni, pH 4.1 -4.3 at 20°C, and the lowest pH value in which nymphs have been collected in the field (pH 4.3, Harrison and Elsworth 1958) are practically identical. Although the pH values in acid streams in the Cape Province, Natal and the Transvaal remain fairly constant, there seems every reason to believe that 1000-minute tolerance limits determined in the laboratory correspond quite closely to actual long-term tolerance limits for nymphs in the field. Certainly there seems no doubt that the distribution of Baetis harrisoni nymphs in acid streams, whether soft, poorly buffered and peat-stained as in the Cape Province or hard, well-buffered and not peat-stained as found elsewhere in areas of acid mine drainage, will be restricted by the lethal pH value.

Lethal pH values were found to be higher at higher temperatures than they were at lower temperatures (from pH 3.9 at 10°C to pH 4.7 - 4.9 at 25°C). The lethal value was also increased slightly both at low dissolved oxygen concentration (pH 4.3 - 4.5 in 4 mg/l dissolved oxygen as opposed to pH 4.1 - 4.3 in 6 and 8 mg/l dissolved oxygen) and in very slow flowing water (pH 4.4 - 4.7 at about 2.7 cm/sec flow as opposed to pH 4.1 - 4.3 at all other flows tested).

In the experiments described here, soft, poorly buffered borehole water was used to which hydrochloric acid was added to achieve the pH values desired. This water differed from that typically found in Cape acid streams principally in having no peaty organic matter dissolved in the water. It differed from the water of streams receiving acid mine drainage principally in having a low calcium and magnesium content, in having a negligible sulphate concentration, in being low in total dissolved solids and in being poorly buffered. The possible effects of these differences in water chemistry on the pH tolerances of Baetis nymphs were not tested. Possibly, nymphs might have been more tolerant of low pH values in well buffered water, since respiratory carbon dioxide might further reduce the pH of unbuffered water in the immediate vicinity of the animals. Chemical factors which would probably be correlated with the buffering capacity of the water include the hardness and the total dissolved solids content of the water. Even without this information, however, it is clear that the results reported here are sufficient to explain the distribution of Baetis harrisoni nymphs in acid streams.

High pH values may accur in polluted rivers either as a result of industrial pollution or diurnally as a result of algal activity (Liebmann 1951). The tolerance limit appears to be adequately defined for Baetis harrisoni nymphs by the 1000-minute median lethal value (pH 10.7-10.8). Time-mortality tests have also shown that nymphs will survive exposure for 3-4 hours to pH 11.0-11.2. These values are a good deal higher than any in which the species has been found.

Ammonia

Wuhrmann and Woker (1948) and others have shown the "unionized" or "free" ammonia component of ammonia-ammonium solutions to be far more toxic to aquatic animals than the ionic ammonium component. For any given total ammonia-ammonium concentration the concentration of unionized ammonia is determined by the temperature and the pH of the solution and may be calculated. In one polluted river studied here it was found that high ammonia concentrations recorded in the past (Allanson 1961) were sufficient to explain the past absence of Baetis harrisoni from certain stretches. During this study ammonia concentrations in the same river, although still relatively high, were found not to exceed the lethal concentration and Baetis harrisoni were found to have returned to the stretches from which they had previously been absent.

Quite apart from its effect on the ammonia-ammonium equilibrium, water temperature was found to influence the toxicity to Baetis harrisoni of unionized ammonia:

Temperature	10°C	15°C	20°C	25°C
Lethal unionized ammonia	7.9mg/l N	7.5mg/l N	7.3mg/l N	7.0mg/l N.

Temperature was the only factor found significantly to influence the toxicity of unionized ammonia to Baetis harrisoni nymphs, or at least to those not in ecdysis.

The unionized ammonia concentration rises several fold during each day in polluted streams in which there is appreciable algal activity, as a result of diurnal rise in pH. Exposure to lethal unionized ammonia concentrations

is therefore normally intermittent. The 1000-minute lethal limit is probably the best measure available to define the actual tolerance limits for nymphs in the field.

Heavy metal salts

Copper, lead and zinc salts are found in streams at high concentrations normally only as a result of pollution. Only very high concentrations of lead and zinc salts were found to be toxic to Baetis harrisoni nymphs. Copper salts were much more toxic to nymphs and were reasoned to be far more likely to be found in toxic concentrations. However, no instances are known where copper might have eliminated Baetis harrisoni from a section of stream.

Time-mortality experiments show that tolerance limits for nymphs in the field for long exposures to copper and other metallic salts will be lower than the 1000-minute lethal concentrations determined in the laboratory. For long-term exposure, it might be best to assume that only concentrations less than 10 per cent of the 1000-minute median lethal concentration, i.e. about 0.25 mg/l Cu, will be tolerated.

For relatively short-term exposures, the tolerance limits may be taken to be represented by the following 1000-minute median lethal concentrations determined in the laboratory:

Temperature	15°C	20°C	25°C
Lethal concentration	2.6 mg/l	2.4 mg/l	2.2 mg/l.

These concentrations could only become dissolved in acid water (pH 6.5 or less). Copper salts precipitating at higher pH values have also been found

to be toxic to nymphs, presumably because they were taken up with the food.

TOLERANCE LIMITS FOR CHOROTERPES BUGANDENSIS

Fast water flow

Choroterpes bugandensis nymphs were dislodged by slower current speeds (0.3 - 0.4 m/sec actually impinging on the animals and estimated 0.1 cm from the substratum) than were Baetis nymphs. However, Choroterpes nymphs were found in the field to be less liable to be washed away during comparably strong floods than were Baetis nymphs. They escaped the full force of the flood largely by living on the undersurfaces of stones. When exposed to fast water currents in the field they were better able than were Baetis nymphs to escape into crevices and small depressions on the rock surface where they were protected from the full force of the current. Very large numbers were only washed away by floods sufficiently strong to roll the stones they occupied. As in the case of Baetis, the egg patches stayed behind and hatched after the flood had passed.

Dissolved oxygen and slow current speed

As has been discussed in the relevant sections of this report, Choroterpes bugandensis nymphs were able to tolerate far lower dissolved oxygen concentrations than were Baetis nymphs. They were also only very little dependent upon water flow for their oxygen supply as shown by their median dissolved oxygen requirements:

Current speed	< 0.2 cm/sec	2.6 cm/sec	12 cm/sec
15°C	0.53 mg/l	0.47 mg/l	0.44-0.48 mg/l
20°C	0.60 mg/l	0.53 mg/l	0.55 mg/l
25°C	0.72 mg/l	0.69 mg/l	0.70-0.71 mg/l.

As seen from these results, the dissolved oxygen requirements of Choroter-

pes nymphs were influenced far more by temperature than they were by water current speed.

The results of the experiments described here suggest that 1000-minute median lethal dissolved oxygen concentrations would fairly adequately define the tolerance limits for nymphs in the field. During times of general deoxygenation in the Pienaars River, considerable differences in dissolved oxygen concentration were found from place to place within the stream bed. These variations would have favoured the nymphs since, like Baetis nymphs, they were able to detect differences in oxygen content in the water with which they came into contact and, presumably, to orientate themselves accordingly. Fluctuations in dissolved oxygen content were also found to occur during the day and night at these times. In general, higher temperatures and higher dissolved oxygen concentrations were found during the day and lower temperatures and dissolved oxygen concentrations were found during the night.

Low dissolved oxygen concentrations which would have been lethal for Choroterpes nymphs were not found in rivers during this study. However, as has been discussed, such low concentrations might be expected to occur from time to time in the water between and beneath rocks in stream beds where Choroterpes nymphs live. Tolerance of low dissolved oxygen concentration has been suggested to be a necessary adaptation to life in these situations.

Although Choroterpes nymphs were found to be tolerant of low dissolved oxygen concentrations it has been suggested in a section of this report that they might not be tolerant enough to live in sluggish polluted streams. While they would tolerate the levels of dissolved oxygen found in the surface water the accumulation of organic matter in the spaces between and beneath stones

might lead to intolerable deoxygenation of the water in the situations where Choroterpes nymphs would be found.

High temperature

Choroterpes nymphs were also found in this study to be far more tolerant of high temperatures than were Baetis harrisoni nymphs, the median lethal temperatures determined falling within the following range:

Winter 35.4 - 35.8°C

Summer 35.9 - 36.2°C.

The differences in lethal temperature of nymphs collected at different times of year were attributed to physiological compensation to the temperatures at which the nymphs had been living. Lethal temperatures were changed to some extent by acclimation to different temperatures in the laboratory.

Choroterpes nymphs were found on one occasion in the field to survive temperatures of up to 32.5°C. The 1000-minute median upper lethal temperatures estimated from experiments described here seem to represent fairly adequately the actual limits of tolerance for nymphs in nature.

Low temperatures

Summer Choroterpes bugandensis nymphs were found to enter chill coma in the laboratory at 6.5°C and winter nymphs to enter chill coma at 5.5°C. Death of nymphs in chill coma took some 36 hours. As described in the introduction, even lower temperatures than these occur quite commonly in winter in streams in many parts of South Africa. Oliff (1960a), for instance, has recorded a temperature of -1.1°C in a stream in which Choroterpes elegans nymphs were found.

Total dissolved solids

The 1000-minute median lethal total dissolved solids concentration determined for Choroterpes bugandensis nymphs in borehole water to which sodium chloride had been added was 10.6 g/l. This is very greatly in excess of any salt concentration in which this species has been found. As discussed for Baetis harrisoni, the 1000-minute lethal concentrations determined here were probably rather higher than the actual tolerance limits for nymphs in the field.

pH

The lowest pH value of water in which Choroterpes bugandensis have been collected was pH 6.0 (see introduction). 1000-minute median lethal low pH values determined in the laboratory were a little lower than this:

Temperature	10°C	15°C	20°C	25°C
Lethal pH	5.0	5.1	5.2	5.3 .

It seems clear from these results that these nymphs would be prevented from colonizing the acid streams of the southern and south-western Cape Province and the Natal and Transvaal streams receiving acid mine drainage by the lethal action of the acid water alone.

The 1000-minute high lethal pH values determined in this study (pH 10.2 - 10.3) are higher than any in which Choroterpes bugandensis nymphs have been collected. As in the case of Baetis harrisoni these appear adequate to define the tolerance limits for nymphs in the field. For short exposures, time-mortality experiments have shown that pH 10.4 could be tolerated for 3-4 hours.

Ammonia

As in the case of Baetis the toxicity of ammonia-ammonium solutions to Choroterpes nymphs at different pH values was consistent with the view that the toxicity of these solutions was proportional to the unionized ammonia fraction. Apart from its influence on the ammonia-ammonium equilibrium, temperature also influenced the toxicity of unionized ammonia to nymphs:

Temperature	10 - 15°C	20 - 25°C
Lethal ammonia	3.5 mg/l N	3.7 mg/l N.

The toxicity of unionized ammonia to Choroterpes nymphs was also influenced by the dissolved oxygen content of the water:

Dissolved oxygen	7.7 mg/l	3.0 mg/l	1.0 mg/l
Lethal ammonia	3.7 mg/l N	2.9 mg/l N	2.7 mg/l N.

Unionized ammonia concentrations were determined in the Jukskei River which alone could have accounted for the absence of Choroterpes bugandensis nymphs from these stretches. It is suggested that ammonia might be the most important component of sewage which affects aquatic animals directly.

Heavy metal salts

As was found for Baetis, copper salts were found to be toxic to Choroterpes nymphs at concentrations which could conceivably occur in South African streams, while lead and zinc salts were found to be toxic only at concentrations which might only be found in streams receiving very heavy industrial pollution.

As for Baetis the actual tolerance limits of these salts for Choroterpes nymphs are evidently lower than the 1000-minute median lethal concentrations determined in the laboratory. If the limit in practice is taken to be about 10 per cent of the 1000-minute lethal concentration it must follow that nymphs could live in the field in water containing up to about 0.1 mg/l copper.

For relatively short-term exposures, the following 1000-minute median lethal concentrations apply:

Temperature	15°C	20°C	25°C
Lethal concentration	1.0 mg/l	0.9 mg/l	0.6 mg/l.

TOLERANCES AND POLLUTION

It has long been known that the entry of sewage and other pollutants into rivers causes the invertebrate fauna characteristic of unpolluted water immediately to be replaced by a community of animals characteristic of polluted water. Downstream from the source of pollution, self-purification is seen progressively to occur and the fauna gradually recovers. The effects of pollution appears to be due to the introduction of organic matter and the resultant increase in micro-organisms feeding on this organic matter. Sensitive animal species are eliminated by the anaerobic conditions produced. Self-purification takes place as the organic matter is used up and its deleterious side-effects disappear. These phenomena and the animal communities characteristic of different classes of polluted water were described by Kolkwitz and Marsson (1909). From the animals present in rivers it then became possible to assess the degree of pollution using Kolkwitz and Marsson's "Saprobic System". This system has subsequently been expanded and revised

(Helfer 1931, Liebmann 1951) and is still widely used today (Bick 1965).

Kolkwitz and Marsson and later workers were deeply impressed with the differences they observed in dissolved oxygen content between polluted and unpolluted waters. In the sluggish water courses they investigated the deleterious effects of pollution must have been due almost entirely to oxygen lack. The importance of oxygen was highlighted, for instance, by Fehlmann (1917) who found nymphs of a mayfly, Hexagenia, in a fairly heavily polluted stream wherever the dissolved oxygen content was raised. In sluggish waters Hexagenia had only been found in unpolluted (oligotrophic) water. Thienemann (1954) has reviewed many years' work all confirming the great importance of dissolved oxygen concentrations in the ecology of larval Chironomidae.

The first workers to demonstrate that lethal factors other than low dissolved oxygen were present in polluted waters were Steinmann and Surbeck (1918, 1922). I have not seen this work in original, but much of it has been described by Harnisch (1951) and Bläsing (1953). Steinmann and Surbeck found several substances, especially ammonia, to kill freshwater organisms, especially fish. This work appears not to have been well received in all circles (Thienemann 1920). Because nothing was yet known of the role of pH in determining the toxicity of these factors, the results of subsequent investigations were contradictory, and the influence of toxic substances on aquatic animals in polluted waters discounted by many workers.

All of the systems currently in use for the assessment of pollution from the fauna present are based on empirical information (Bick 1963, Beak 1965). This applies equally to methods developed in South Africa

(Harrison 1958b, Allanson 1961, Chutter 1967 and in press, Pretorius 1969).

The methods developed in Europe have mostly been based on observations made of the effects of pollution on the biota of sluggish streams. Because dissolved oxygen concentrations become drastically reduced in such streams these methods have been based largely on the presumed oxygen requirements of different forms. The fact that these methods might not be applicable in swifter flowing waters was vividly demonstrated by Zimmermann (1961). Zimmermann allowed treated domestic sewage to flow down two long open channels inclined at different angles and observed the changes in both chemical composition and invertebrate fauna down the length of these channels. He found that self-purification in the faster flowing channel proceeded more rapidly than that in the slower flowing channel. He also found that communities present at the downstream end of the slower flowing channel in relatively clean water were present in the faster flowing channel much further upstream in relatively polluted water. From these observations Zimmermann concluded that criteria would have to be developed for flowing waters which would have to be different from those used for stagnant and sluggish water. Working on a polluted but relatively swift flowing Transvaal river, Allanson (1961) found that he could not distinguish the zones of pollution of the Saprobic System in spite of the relatively heavy pollution present. This confirmed that low dissolved oxygen was not nearly as important a factor in these swift streams as it was in sluggish channels.

The results of the present study have revealed that a mayfly of unpolluted streams, Choroterpes bugandensis, was far more tolerant of low oxygen levels than was a species common in polluted streams, Baetis harri-

soni. Furthermore, examination of their respective habitats has shown that the oxygen tolerances of these species might reflect the relative extremes to which they might be exposed in the field. Thus ability of Choroterpes bugandensis to tolerate low oxygen has been shown to be an adaptation to life beneath stones in sluggish streams and would not necessarily equip it to survive polluted conditions. Because it lived on top of stones Baetis harrisoni, on the other hand, was able to tolerate the lowest dissolved oxygen concentrations brought about by pollution in the Jukskei River. Since swift streams have high re-aeration rates dissolved oxygen levels might perhaps not fall any lower than this.

The present study has shown that Choroterpes and, to a lesser extent, Baetis nymphs are eliminated from polluted streams by the toxic action of various factors. The circumstances under which various factors can become lethal have been described in relation to the habitat of each species and the conditions to which each is likely to be exposed.

The ammonia concentrations found lethal for these nymphs are very similar to those found by Stammer (1953) and Wuhrmann and Woker (1958) to be lethal for mayfly nymphs. Stammer found other invertebrates of polluted waters to be far less tolerant of ammonia than were these insects. He has concluded that ammonia was the factor most likely to be lethal for Annelida, Turbellaria and Protozoa even in stagnant water, but that oxygen would be likely to be the limiting factor for mayflies and other insects under almost any circumstances. In the present study it was seen that ammonia concentrations in the Jukskei River were sufficient to explain the absence of Choroterpes from this river. Concentrations had evidently been so high in

the past that even Baetis had been eliminated from certain stretches.

Attention has been given in this study only to the acute toxicities of different poisons to Baetis and Choroterpes nymphs. Nothing is known of possible deleterious effects on the nymphs of long-term exposure to sub-lethal concentrations of poisons. However, Pallii and Markobatova (1963) showed that the growth of dragonfly nymphs was slowed in the presence of non-lethal concentrations of various substances.

Observations reported upon here have shown that nymphs of both Baetis harrisoni and Choroterpes bugandensis react to water of different temperature or to solutions of some poisons by apparently orthokinetic movements. It has been reasoned that these movements might under certain circumstances result in the nymphs moving to more favourable situations. This might only offer them limited protection against pollution. It seems likely that animals living in the free-flowing water will usually be exposed to whatever water is brought downstream.

In order to see to what extent different species were affected by the sudden onset of lethal conditions, Wuhrmann and Woker (1958) conducted an experiment in which they introduced 10.5 mg/l chlorine gas into a small unpolluted trickle and observed its effects on the numbers of invertebrate species downstream. This concentration of chlorine was lethal and they found all of the animals that live in the free water to be killed off immediately. These include Gammarus and all Ephemeroptera except burrowing Ephemera. The burrowing mayflies and worms and the encased Trichoptera and Chironomidae were apparently unaffected, although they too would have been killed if they had been exposed to the water containing chlorine.

Wuhrmann and Woker have concluded from this that the behaviour of invertebrates is of great importance in determining whether or not they will be killed by pollution. Those that live in inaccessible places are likely to be passed by, while those that live in the free flowing water will be exposed to whatever is carried down by the river. This is an illustration of one of the conclusions to be drawn from the present study, namely that the effects of pollution on invertebrates will depend on the situations they occupy in the stream.

The results of this study offer no easy method for the assessment of pollution. Neither Baetis nor Choroterpes is particularly suited for use as a test organism to assay the toxicity of polluted waters. Their presence or absence is also not immediately indicative of a particular water quality class. What has been provided is a definition of the habitat of each and of their requirements in terms of some important physical and chemical variables, several of which are related to water pollution. The presence or absence of either species in biotopes that fulfil the physical characteristics required implies, at least, that hydrochemical factors at this site have or have not kept within the tolerance limits. Ecological interpretation of the factors that might influence the relative abundance of these and the other invertebrates present is of course still necessary in order to assess pollution in streams, but the results of the present study will in many instances assist this interpretation.

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