An investigation into the temperature optima of two populations of the blue-green algae nostoc commune (vaucher)

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AN INVESTIGATION INTO THE TEMPERATURE OPTIMA OF TWO POPULATIONS OF THE BLUE-GREEN ALGAE NOSTOC COMMUNE (VAUCHER).

by

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1 Introduction

Blue-green algae occur in a very wide range of habitats throughout the world. The surface layers of agricultural and desert soils, streams, lakes, hot springs, and most marine habitats all support populations of these organisms.

One aspect of these organisms which is important in determining their widespread occurrence is their frequent tendency to be favoured by high temperatures. Hot springs provide the most striking example of the success of blue-green algae at high temperatures and in recent years these have been the subject of a great deal of research (Castenholz 1973).

As a result of these studies the growth optima of the characteristic blue-green algae inhabiting these springs are now very clearly determined. They have been shown to be adapted genuinely to their environment: the temperatures at which they occur being generally quite close to their experimentally determined growth optima (Brock 1967, Peary and Castenholz 1964). Mastigocladus laminosus has been found by Castenholz (1973) to have two major physiological strains which have been found in every major thermal area of the world. The first is a high temperature form which grows above a temperature of 58°C up to 64°C, and the second, a low temperature strain which has an upper temperature limit of 57-58°C. Peary and Castenholz (1964) showed that a Synechococcus lividus population distributed along a temperature gradient from 75°C to 53°C in a thermal spring consisted of at least four distinct strains, differing in their temperature optima and tolerance ranges.

However, these detailed studies on the growth optima of different strains of blue-green algae in hot springs have not been complemented by similar studies on characteristic species in other habitats.

The temperature optima of a large number of blue-greens have been determined (e.g. Bunning and Herdtle 1946, Kratz and Myers 1955, Allen 1956, Fay and Fogg 1961), but these were mainly obtained from laboratory cultures and little attempt was made to relate these to the temperature of the habitat from which the species originally came. Studies on more than one population of an algal species do not appear to have been undertaken.

The present study was carried out to obtain information on the temperature optima of different populations of one particular species to determine whether they varied and if they were related to the temperature of the habitat from which they were taken.

The species chosen for study was Nostoc commune Vaucher which 'dominates the vegetation of shallow pools on exposed limestone from the Arctic to the tropics', (Whitton and Sinclair, in press.). The global occurrence of this alga is well illustrated by records in the literature : it has been reported in Egypt (Taha and El Refai 1963), France (Hérisset 1952), Aldabra Atoll (Whitton 1971), the U.S.A. (Shields 1957), and the Antarctic (Holm-Hansen 1964, Fogg and Stewart 1968).

The two populations selected for study were taken from Aldabra Atoll in the Indian ocean, and Tarn Moor in Cumberland, England. The sites were chosen because of their contrasting

climates, particularly with respect to temperature. The climate of Aldabra Atoll is tropical and alternately wet and dry, with an average temperature of 32°C in the dry season and 22°C in the wet. That of Tarn Moor is continually wet, with an average temperature of 9°C.

The short time available for the study ruled out the use of time-consuming and demanding techniques for measuring the effect of temperature on the alga. A technique which could be quickly mastered and easily carried out was required. Consequently the acetylene reduction technique was utilised as it was a rapid, simple method, and required only one major piece of equipment - a gas chromatograph, whose operation could be mastered in a short time. Furthermore because of its rapidity large numbers of replicates could be used when necessary.

This procedure involves using the nitrogenase catalysed reduction of acetylene to ethylene together with gas chromatographic analyses and is based on the inhibition of nitrogen fixation by acetylene (Schöllhorn and Burris 1966) and the reduction of acetylene to ethylene (Dilworth 1966).

The technique was first utilised with blue-green algae by Stewart et al (1967, 1968) who advocated its use as a simple and inexpensive rapid-scan method for detecting potential nitrogen-fixing organisms in nature. The technique is now widely used (Hardy et al 1973).

Because the reduction of acetylene is an indirect index of nitrogen fixation, critical observations on acetylene reduction

should normally be verified by demonstrating fixation of $^{15}N_2$ under the same conditions (Stewart 1973). However, this was not possible because of the short-term nature of the study. Also, unequivocal proof that the algae is responsible for the acetylene reduction demands the use of pure cultures.

In this study, assays for acetylene reduction were carried out at varying temperatures and the rates of reduction obtained were used as an index to assess the effect of temperature on the alga under investigation.

Material taken from the field rather than laboratory cultures was used in investigations because it is not possible to ascertain that cultures are representative of the natural material. The temperature optimum of a culture could also be influenced by the favourable conditions in which it is grown. Thus to try and procure optima which were as indicative as possible of the true values the field material was always used.

As the colonies of N.Commune from Aldabra Atoll were in a dried state, it was first necessary to determine that they could be re-wetted satisfactorily and then be able to reduce acetylene. Hence, part of the study was taken up with investigations to determine if this could be achieved and if so, from the data obtained, to devise standard conditions for the pre-treatment of the material before it was used in any experiments on temperature.

2 Information on Algal Sites

2.1 Aldabra Atoll

The information given below is intended as background information only and further details can be obtained from the sources quoted in the text.

Aldabra Atoll (latitude 9° 24'S, longitude 46° 20'E) is situated 420 km northwest of Madagascar and 640 km from the East African mainland in the southwest Indian ocean. It is formed of coral reefs elevated 5 to 10 m above present sea-level (Stoddart et al 1971).

The atoll is elongated east-west, with a maximum length of 34 km and a maximum width of 14.5 km. Its total area, bounded by the edge of the peripheral intertidal reef flat, is 365 km^2, and of this land occupies 155 km^2. The land rim consists of four main islands, varying in size: South Island (110 km^2), Middle Island (26.4 km^2), Polymnie (1.8 km^2), and West Island (9.3 km^2).

West Island and Polymnie are separated by Main Channel, 600 m wide, and Middle and South Islands are separated by the narrower East Channel.

A map showing the main features of the atoll and further details of the topography is given in Stoddart et al (1971).

The surface of the atoll consists of two basic types of rock termed Champignon and platin (Fryer, 1911, Stoddart and Wright 1967). Champignon is used for deeply pitted and irregular solution-fretted reef rock, and platin for smooth-surfaced pavement-like cemented limestones.

The climate is a wet and dry tropical climate with a 5 or 6 month wet season in most years. Summer maximum temperatures average about 29°C, and winter minimum temperatures 19°C (Farrow 1971). However, these are air temperatures and ground temperatures are probably a few degrees higher. Temperatures of freshwater pools show a diurnal variation, with temperatures reaching 30°C in the middle of the day, and dropping to the lower twenties at night (Donaldson and Whitton in press). The mean annual rainfall of 670 mm is lower than atlas predictions and places Aldabra in the most arid sector of the Western Indian ocean.

The flora and vegetation differs markedly from the sand cays of sea-level atolls, and because of its size, elevation, and proximity to continental land the terrestrial biota is larger than is usual on coral atolls.

Both terrestrial and freshwater algae are found on the atoll of which the blue-green algae are the far most abundant group.

The different types of algal habitat have been classified by Whitton (1971) according to the following five factors:

1) relation to water level; 2) depth of soil overlying rock; 3) size of habitat especially pools; 4) effect of surrounding vegetation; 5) effect of tortoises.

Further details of algal habitats are given by Whitton and Donaldson (2 papers in press).

Nostoc commune is commonly found on bare platin surfaces generally above the water-level, especially in small depressions

which are frequently filled with masses of irregular colonies. These colonies imbibe water very rapidly after a storm and maybe re-moistened and dried out many times especially during the wet season (Whitton 1971, A. Donaldson, pers.comm.). Depressions deep enough to have a permanent covering of water during the wet period have a much more varied flora.

The masses of N.commune colonies usually overlay a thin layer of humus which is thought to come from the alga itself. In some places a few angiosperms germinate and grow rapidly directly in the algal mounds.

Nostoc sphaericum colonies occur in small, shallow pools, and are generally wet for a longer period of time than the N.commune colonies.

The colonies of the Nostoc sp are also found on the bare platin in a similar situation to these of N.commune.

2.2 Tarn Moor

Further information on this area can be obtained from Holdgate (1955).

Tarn Moor is an area of undulating moorland lying to the west of Sunbiggin tarn, in Cumberland, England (map reference NZ 8502, latitude 54° 28'N, longitude 2° 30'W). It forms an uneven plateau about 250 m above O.D. with ridges to the north and south rising to somewhat over 300 m.

The rock beneath most of the moor is a Carboniferous limestone locally interrupted by bands of sandstone, and covered by drift. The drainage of the district is complex and there are

many springs and swallow holes.

The climate is wet, the annual rainfall averaging 1300 mm. The mean annual temperature is around 9°C, with an average summer temperature of about 14°C and winter of 3°C. This data has been derived from information given in the Climatological Atlas (1952) and thus can only be considered approximate.

Calluna vulgaris is dominant over most of the area with Nardus stricta as the most frequent associate in the drier parts and Sphagnum spp , Molinia caerulea, and Trichophorum caespitosum on the damper slopes.

The numerous hollows of the plateau contain deep peat, and support mires of many kinds, ranging from acid bogs to alkaline fens. Around them on the lower parts of the sloping valley sides, there are belts dominated by small Carex spp which separate the valley mires from the Calluna moor of the ridges. These 'wet-flush' areas, receive drainage from the slopes above, seepage from the deeper layers of the drift where these are exposed, and locally from springs.

Nostoc commune is found on the edges of these flushes near the surrounding vegetation, occurring in the surface water but often out of it. The colonies are gelatinous, pale-greeny brown, and irregular in shape. The size of colonies varies from a few mm in diameter to over 25 mm.

3 Materials and Apparatus

3.1 Algae

Aldabra Atoll samples were collected by A. Donaldson during the period April to June 1973. The colonies of each species were air-dried in the shade and then placed in polythene bags which were sealed to make them as air-tight as possible. Since collection the samples have been stored in the dark at room temperature.

Nostoc commune colonies were taken from an area of platin near the research station on West Island. Colonies of Nostoc sphaericum were collected from the edge of a small, shallow pool in the Bassin Flamant area of South Island. Those of the Nostoc sp. were collected from an area of platin in the Bassin Cabris area of West Island.

Nostoc commune from Tarn Moor was collected in August 1974 from wet flush areas, the colonies being found near the edges, partly mixed with the surrounding vegetation. Some of the colonies were situated in a slight flow of water while others were in relatively dry areas. The colonies were carried back to the laboratory in a polythene bag and kept at a constant temperature of 20°C under slightly moist conditions.

3.2 Medium

Medium was preferred to distilled or tap water for re-moistening dried algal colonies, as it would ensure that they were all in a standard environment.

The medium used is based originally on the medium of Allen

and Arnon (1955) and was designed for the growth of blue-green algae under nitrogen fixing conditions. As the medium was only being used for re-moistening and not for culture experiments it was diluted 1:4 with distilled water.

The medium was free of combined nitrogen and contained the following:

K_2HPO_4,	250 mgl^{-1}
$CaCl_2.2H_2O$,	66.2 mgl^{-1}
$MgSO_4.7H_2O$,	200 mgl^{-1}
NaCl	230 mgl^{-1}
Iron (As Fe-EDTA)	4 mgl^{-1}

and 1 ml of microelement stock, containing

Mn, Mo, Zn, Cu, Bo, Co, W, Ni and Cr.

The pH was between 7.2-7.5. The microelement stock was modified from the original of Allen and Arnon (1955) in that the salts containing the micronutrients were changed to eliminate nitrogen. Phosphate was added separately after the other components to avoid precipitation.

A modified version of the formula for Fe-EDTA was used in making up this salt. The formula was as follows:

12.7 g. Na.EDTA were mixed with 9.7 g. $FeCl_3 6H_2O$ and made up to 1 litre with distilled water. This gave 2 g. Fe per litre. Thus 2 ml of this solution would give 4 mg. Fe per litre.

3.3 Gases

All experimental gases, i.e. acetylene, ethylene, and the gas mixture of argon, oxygen and carbon dioxide, and the gases used in gas chromatography, nitrogen, hydrogen, and compressed

air, were all obtained commercially from the British Oxygen Company.

3.4 'Cross Gradient' Apparatus

This apparatus, which will be called the 'Cross Gradient' by the author, is based, but with several modifications on the original of Edwards and Van Baalen (1970) which was used for the culture of benthic marine algae under varying regimes of temperature and light intensity.

The Cross Gradient consists of a 25 mm thick aluminium plate 820 mm. square set in polystyrene for insulation, supported by a steel frame, and enclosed in a steel cabinet. One side of the cabinet can be let down for access to the plate. One side of the plate is heated and the other cooled by pumping water from two thermostatically controlled water baths through borings in each side of the plate from the front to the back. The flow of heat through the aluminium plate produces a linear temperature gradient perpendicular to the direction of the water flow. The magnitude of the gradient may be controlled by adjusting the temperatures of the two baths.

Light is provided by 20 plain white fluorescent tubes parallel to the aluminium plate. (Because of practical difficulties, warm white fluorescent tubes which were used for light in other experiments could not be provided). The fluorescent tubes are mounted on a board and suspended above the plate by adjustable clamps fitted to the frame; the tubes may be raised or lowered to adjust the light intensity in the apparatus. Pairs of tubes can

be switched on or off independently of the others to give the required light intensity which is measured with a Corning-Eel photoelectric photometer.

The aluminium plate is divided into squares by means of a grid composed of thin lengths of wire stretched across it so that the position of an experimental flask etc. can be accurately noted and measurements of the temperature and light intensity at its position determined.

The temperature of the plate at any point is determined by placing a small mercury thermometer in an experimental flask at that point and reading the temperature after thirty minutes.

3.5 Apparatus used for incubating algae at constant temperatures

For constant temperatures of 25°C and 32°C, growth rooms kept permanently at these temperatures were used. Continuous light was provided by warm-white fluorescent tubes and measured with a Corning-Eel photoelectric photometer. Positions in the growth rooms where the light intensity was at 3,000 ± 300 lux were found and used in all experiments requiring these temperatures and light conditions.

A constant temperature of 20°C was provided by a small water tank 1,000 mm x 800 mm, filled with distilled water and illuminated from beneath by warm-white fluorescent tubes set to give continuous light of 3,000 ± 200 lux. A Camlab coding unit kept the temperature constant.

For temperatures of 39°C and 43°C a larger water tank, 1,000 x 1,000 mm similar to the one described above was made use of.

4 Methods

4.1 Chlorophyll a extraction and measurement

Choice of method

The generally accepted method of quantifying rates of acetylene reduction obtained in experimental investigations is to measure the total nitrogen or protein in the sample(s) used and express the amounts of acetylene reduced (or ethylene produced) as a function of this value. However, in this study the measurement of total nitrogen was not considered very feasible. The standard method of Kjeldahl digestion and then measurement of nitrogen by an automatic Nitrogen analyser can take up a considerable amount of time largely because of the preparation involved and is a process which cannot easily be speeded up. Thus as a large number of replicates were planned in many experiments it was thought that the measurement of nitrogen would be too time-consuming.

Another method which could be completed relatively quickly was thus required. Measurement of photosynthetic pigments especially chlorophyll *a* was then considered as a well-tried, quick, and simple method which was available (Talling and Driver 1963, Talling 1969). Chlorophyll *a* is usually estimated because this is normally the most abundant and important pigment in living material and most information has been acquired on it.

Tests were carried out on the algal species under investigation and these confirmed that it contained chlorophyll *a* in amounts which could easily be estimated on a spectrophotometer and showed that the extraction of the pigment from a sample could be

completed in a matter of minutes. The method thus appeared to be satisfactory for use in the study.

It was finally decided to use this method because of the condition of the material under study. The colonies of Nostoc commune from Aldabra Atoll had been collected in the field and air-dried (see Section 3.1). They had quantities of soil and other debris adhering to their outer surfaces which were difficult to remove even when the colonies were re-moistened. Furthermore many colonies appeared to contain appreciable amounts of dead material (A. Donaldson pers. comm.). Thus if these colonies were measured for total nitrogen, nitrogen would have been contributed by dead as well as live material and most probably by debris. As only live material could be expected to reduce acetylene the nitrogen value would thus be unrepresentative of that actually involved in reduction.

However, chlorophyll a is only present in live material, and hence would only represent material responsible for acetylene reduction. This would make chlorophyll a more suitable for quantifying acetylene reduction than total nitrogen.

A short experiment was carried out in which total nitrogen was measured in algal samples of similar size. Tremendous variation occurred even though the samples had been carefully selected. This provided support for the view that in this case nitrogen was not suitable for quantification purposes, and thus it was definitely decided to use chlorophyll a by which to quantify acetylene reduction.

Extraction of chlorophyll a

This technique is based on an outline given in Talling (1969)

but with some modifications.

Chlorophyll *a* was extracted by placing the algal sample in 100% methanol heated to boiling by means of a hot plate. The heating of the methanol greatly accelerates the extraction. For 1 ml algal samples a volume of 10 ml of methanol was sufficient for the extraction but for the larger samples used in re-moistening experiments 25 ml were used. A time of 10-15 minutes was allowed for each extraction to ensure that all the chlorophyll in the sample was extracted. When this had occurred the alga took on a bleached appearance. The 150 ml conical flask used as the extraction vessel was fitted with a reflux condenser to prevent the methanol boiling off and leaving the sample dry.

When the extraction was complete the methanol was filtered to remove algal and other debris. A sinta glass filter with a Whatman 21 mm GF/C glass fibre filter paper, supported in a millipore funnel which was connected to a small vacuum pump - a source of reduced pressure, was used for this purpose. The filtrate was collected in a 10 ml or 25 ml Pyrex volumetric flask according to the sample size and if necessary the volume was made up with fresh methanol passed through the filter. (Despite the use of the reflux condenser it was not possible to stop the loss of methanol entirely). This procedure was completed as quickly as possible to reduce degradation of chlorophyll while in the solvent to a minimum. As a further precaution against this, the volumetric flasks containing the filtrate were covered with a layer of aluminium foil. The sample was then taken to the spectrophotometer in an adjacent room to measure the amount of chlorophyll *a* contained in it.

Spectrophotometric estimation of chlorophyll a

A 10 mm spectrophotometer cell was filled with solvent and the optical density of the chlorophyll a in the solvent measured over a wavelength range of 430-750 nm on a Perkin-Elmer 402 Ultra-violet visible spectrophotometer. The optical density at 665 nm was then read off from the spectrophotometer trace.

The concentration of chlorophyll a in the solvent was obtained by using the following relationship proposed by Talling and Driver (1963) and Talling (1969).

$$\text{Chl.}a = 13.9\ D665$$

where: D665 = optical density at 665 nm measured with a path length (cell thickness) of 10 mm

Chl.a = concentration of chlorophyll a in $\mu g ml^{-1}$ of solvent.

13.9 = a constant.

From this relationship the concentration of chlorophyll a in 1 ml of methanol could be obtained and hence the concentration in the total amount of solvent used.

4.2 Acetylene reduction technique

For carrying out acetylene reduction assays two types of incubating chambers were used: 7 ml serum bottles fitted with screw tops and rubber liners and 250 ml conical flasks fitted with No. 57 Suba-seal closures. To prevent possible contamination by ethylene from earlier assays (Waughman 1971) the liners and closures were aired for at least two weeks after completion of an experiment before they were used again. If found to be badly

holed from syringes then they were not re-used because of the possibility of leakage occurring and constant checks to detect this condition were made.

The 250 ml conical flasks were used in assays involving re-moistening of algal colonies and then determination of acetylene reduction over a long period of time. They were chosen for the following reasons. Firstly they were convenient to handle when setting up these assays and then when carrying them out. Secondly, Waughman (1972) and Sprent (1969) both found when using root nodules that inhibition of acetylene reduction occurred in a small volume of gas hence a large volume of gas was used as a precaution against that occurring in these assays. Finally, with a large gas phase the depletion of acetylene due to its reduction and its removal in gas samples taken for analysis would not be too significant.

The sample size when using 7 ml serum bottles was 1 ml of alga in 1 ml of medium, leaving a gas phase of 5 ml. For assays in 250 ml conical flasks the usual sample size was 1 or 3 colonies plus the required amount of medium to re-moisten them. In some assays more than 3 colonies were used and details are given in the appropriate part of the text. The gas phase volume thus varied according to the sample size.

In preliminary experiments the air in the incubating chambers was removed by flushing with a gas mixture of 79.97% Argon, 20.00% oxygen, and 0.03% Carbon dioxide, the gas being introduced through the rubber seals with a hypodermic needle and venting it through a second needle. However, it was found that

the rates of acetylene reduction in flushed and unflushed flasks were of the same order and as Stewart et al (1971) and Sprent (1970) had also found that no advantage was gained from air removal, this procedure was dispensed with in actual experiments. Hence a considerable amount of time was saved in setting up assays.

For assays in serum bottles, 1 ml of acetylene which gave a concentration of 16% (by volume) was injected into the gas phase to start the assay. When using 250 ml flasks the amount of acetylene added was also calculated to give a concentration of 16%. After injection the pressure was allowed to equilibrate with the atmosphere through the hypodermic needle.

For practical reasons the acetylene was conveyed to the place of the experiment in a football bladder fitted with a 'T' junction and suba-seals and samples taken when required.

When determining the source of acetylene reduction immediately after dried colonies of alga were re-moistened the acetylene was added to the flask before the medium, and the assay started on the addition of the medium.

Constant temperatures and light conditions were provided by means of the apparatus described in Sections 3.4 and 3.5. An equilibration period of thirty minutes was allowed in experiments with varying temperatures, and then the assays started with the injection of acetylene.

In time-course experiments 1 ml samples of gas were taken at varying intervals of time for analysis by gas chromatography. After four gas samplings an equivalent amount of air was added back to the flasks to restore the gas pressure inside. For

time-courses in a serum bottle, 1 ml of air was added back to the bottle immediately after the gas sample had been taken. Over the 1 hour incubation only three samples were taken as it was thought a larger number would have disturbed the environment in the bottles to a state where erroneous results would have occurred.

In all assays acetylene was used as an internal calibration to check for depletion of the acetylene by its reduction to ethylene or by removal in gas samples, and to check for faulty technique and readings when using the gas chromatogram.

Ethylene was estimated by withdrawing a 1 ml sample of gas from the incubating chamber with a syringe and injecting directly into a Varian Aerograph model 1200 gas chromatographic apparatus fitted with a 180 cm x 3.2 mm steel column containing Poropak R. The column was run at a temperature of 100°C using nitrogen at a flow rate of 24 ml/minute as the carrier gas.

The peaks for ethylene values obtained on the gas chromatogram were converted into volumes of ethylene (nannomoles) by relating them to a standard peak obtained from a known volume of ethylene.

The standards were obtained using the following procedure. The volume of a 1 litre volumetric flask containing a few glass beads was accurately determined by filling it with water. The flask and beads were dried and the flask fitted with a suba-seal closure. 1 ml of ethylene was injected into the gas phase and allowed to mix with the air for a period of one hour. The glass

beads facilitate the mixing of the gases when the flask is shaken. Four to six 1 ml samples of gas were then taken from the flask, injected into the gas chromatograph and the resulting ethylene peaks read off. The mean of the values obtained was calculated and taken as the standard peak.

The volume of the ethylene in a 1 ml sample was easily calculated knowing the volume of the volumetric flask. Thus all peaks for ethylene could then be converted into a volume of ethylene per 1 ml. The chromatograph was calibrated in this way each time it was used.

Once the concentration of ethylene in the 1 ml sample was known the total concentration in the incubating chamber used for the assay was then calculated.

Ethylene production was expressed as nannomoles of ethylene produced per microgram of chlorophyll a. - nm C_2H_4 µgChl.a^{-1}.

The ethylene contamination of the acetylene was determined in each experiment. For time-course assays in 250 ml conical flasks a 1 ml sample of gas was taken immediately after the start of the assay and analysed for ethylene. With assays in 7 ml serum bottles, controls with no alga in, were always included and then 1 ml gas samples from these analysed for ethylene. The ethylene contamination was allowed for in the final calculations of the results.

Dark controls in time-course assays were supplied by painting 250 ml flasks black with aluminium paint and then covering them with aluminium foil.

4.3 Measurement of moisture uptake by dry algae

A dry algal colony was removed from the storage bag and immediately weighed on a Mettler balance.

The colony was placed in the bottom of a small, plastic, petri-dish in a constant temperature room at 32°C. A few ml of medium sufficient to cover the colony to a depth of 10 mm were then added. The colony was removed at varying time intervals, excess moisture quickly removed and weighed on a balance adjacent to the room. This procedure was completed as quickly as possible and the colony returned to its petri-dish.

The removal of excess moisture before weighing posed a constant problem. This was blotted up with fine tissue paper but this had to be done very carefully or water could easily be extracted from the colony itself. Every precaution was taken to prevent the latter possibility happening but it was difficult to stop it entirely.

At the end of the experiment the colony was oven-dried at 105°C for 12 hours and the dry weight of the colony obtained.

The percentage moisture content of the colony was obtained by using the following formula (Showman and Rudolph 1971):

$$\% \text{ moisture content} = \frac{Wt - Wd}{Ws - Wd} \times 100$$

where: Wt = weight (g.) at time t.
Wd = dry weight of the colony.
Ws = final weight of the colony.

5 Results

5.1 Experiments on moisture uptake and acetylene reduction by Aldabra material

Because the samples of Nostoc commune were in a dried condition they had to be re-moistened for use in experimental studies. Before using them in any experiments on temperature it was essential to have all colonies fully re-moistened and in a similar condition with respect to acetylene reduction rates. These experiments were therefore carried out to provide information on moisture uptake and rates of acetylene reduction after re-moistening, so that a standard set of conditions for pre-treatment of colonies could be made up.

As a precaution against Nostoc commune proving unsatisfactory, two other species of Nostoc from Aldabra, Nostoc sphaericum and an undetermined Nostoc sp. were also tested in a similar manner. The results of experiments on these species are hence included in this section.

All the experiments were carried out at a constant temperature of 32°C, as this had been found to be the optimum for several Aldabrian species (Whitton Pers. Comm.) and a growth room at this temperature was readily available.

Moisture uptake experiments

When dry, colonies of all species were a browny-black colour and were hard and rather brittle, but within two or three minutes of medium being added they became green in colour, especially those of N.commune, soft, pliable, and had increased in size. The

Figure 1: Rate of Moisture Uptake by N.commune

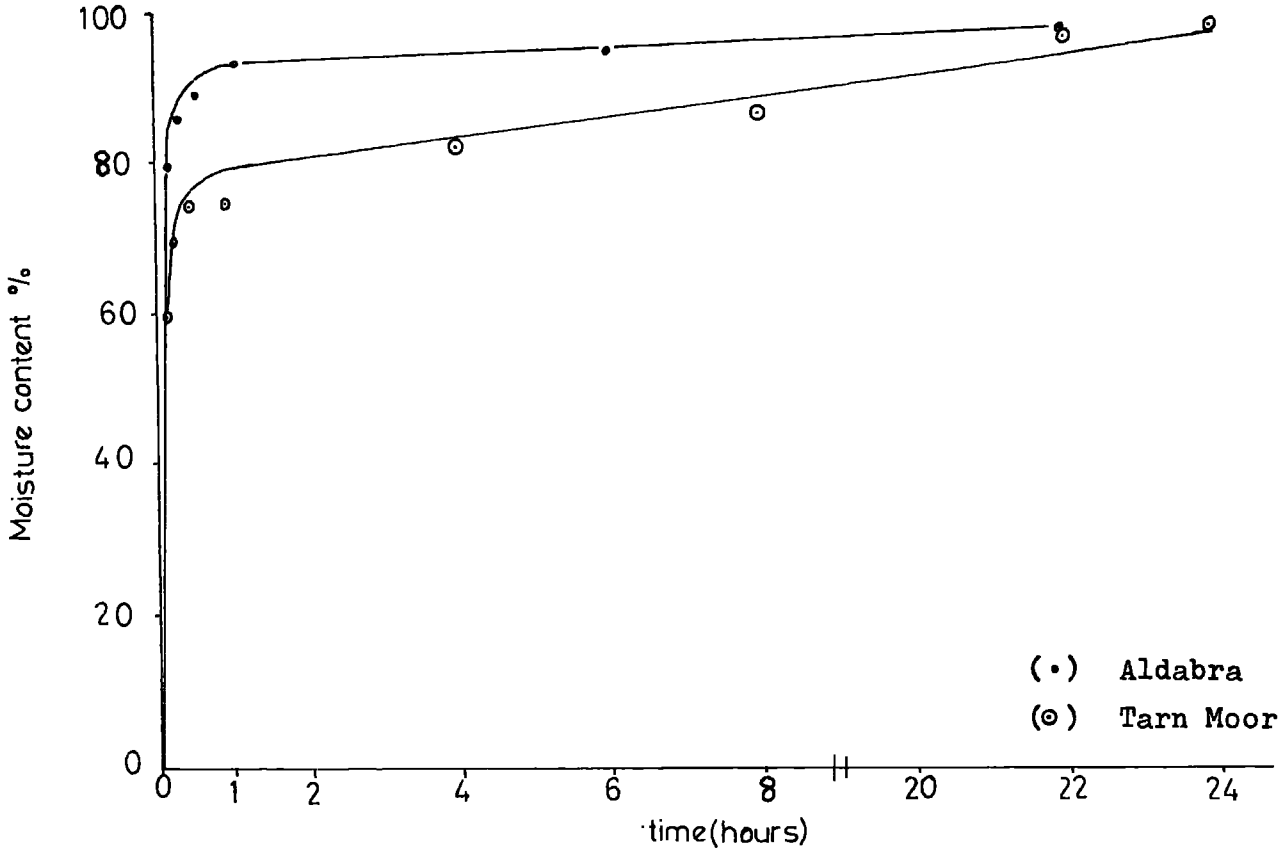
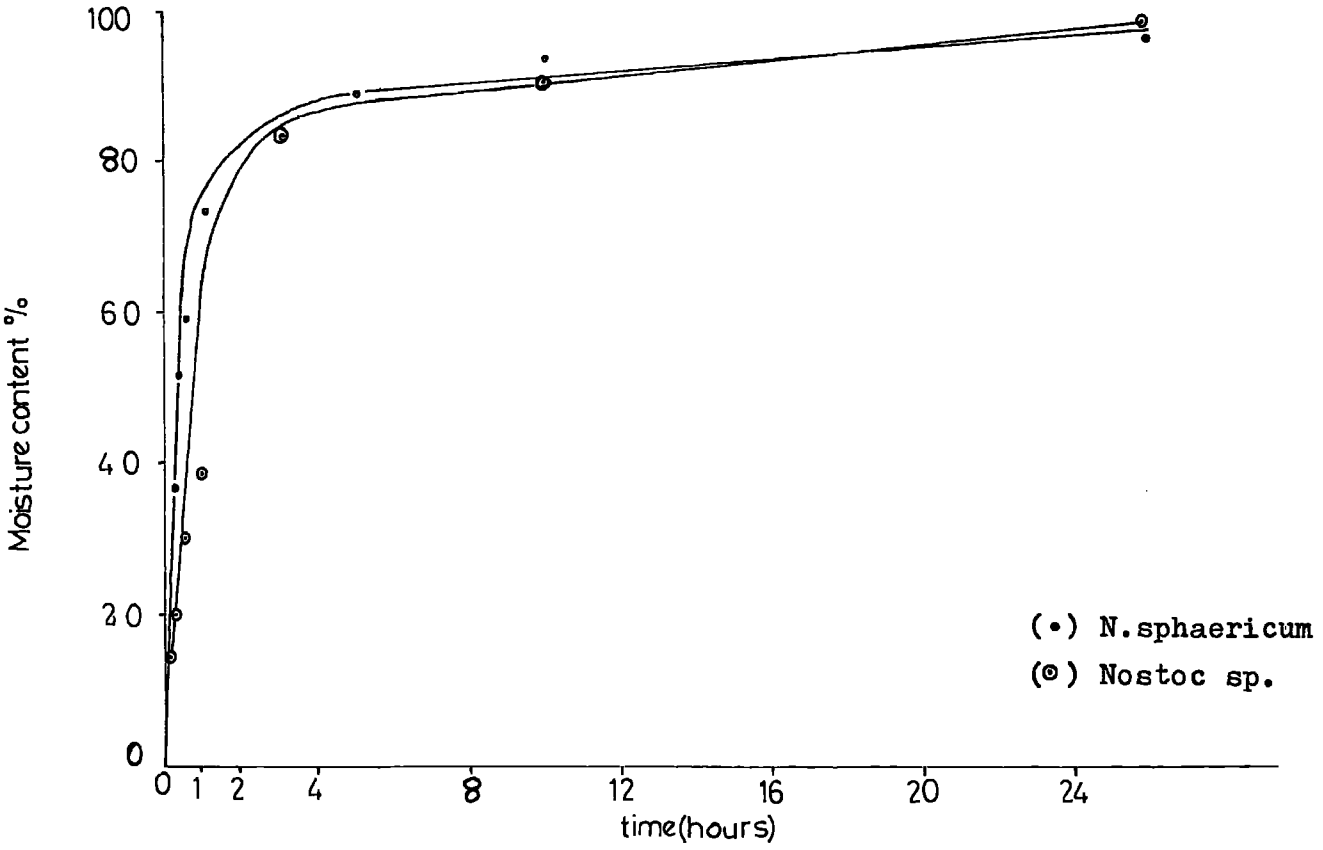


Figure 2: Rate of Moisture Uptake by N.sphaericum and Nostoc sp.



All points are the mean of 3 replicates.

rate of uptake of moisture was measured for each species (Section 4.3).

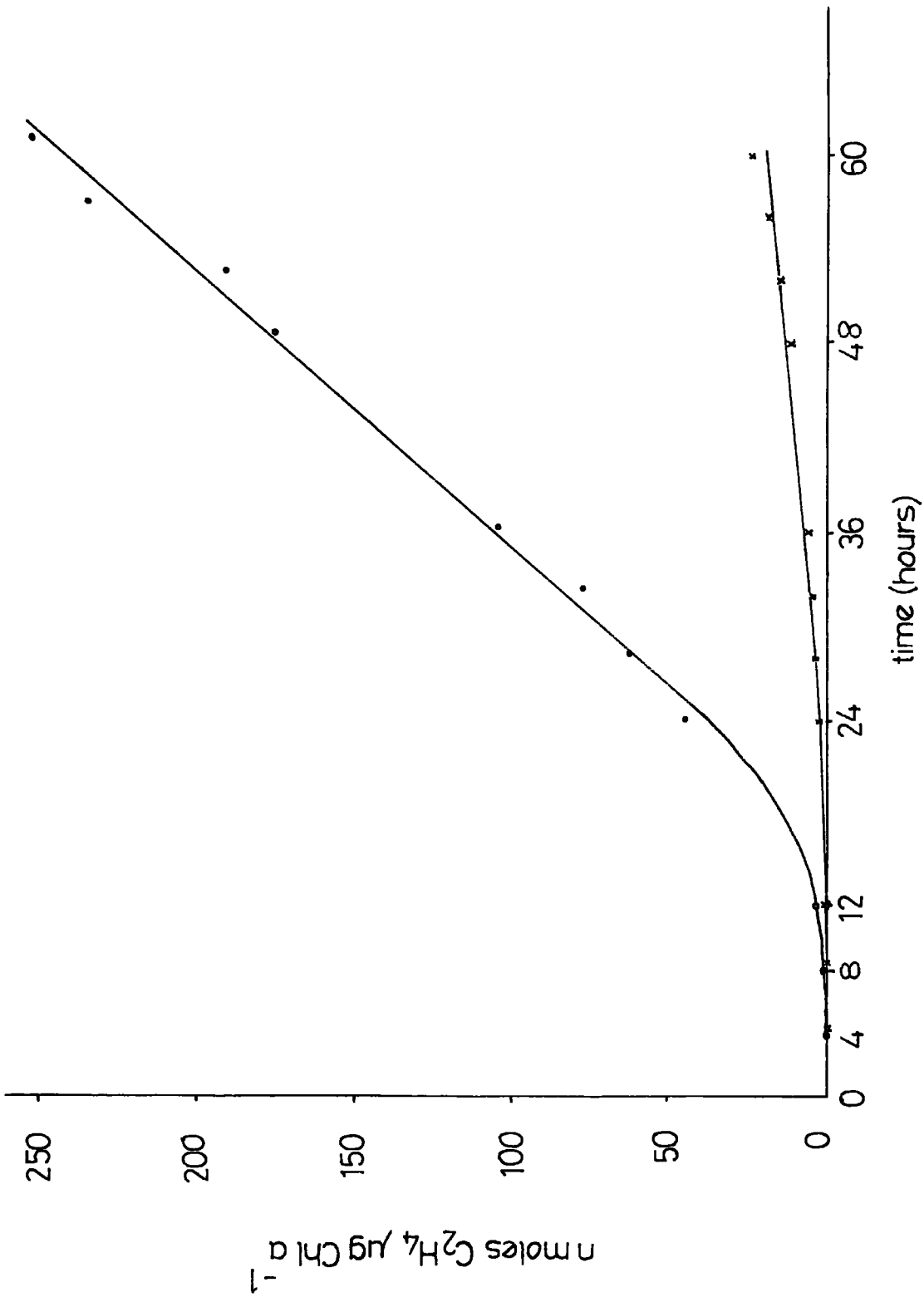
Within five minutes of the addition of medium, colonies of N.commune had reached a moisture content of 80% (Fig. 1), but after this period of rapid uptake of moisture the rate began to slow down and level off and maximum water content was reached after about 22 hours. All colonies tested re-moistened satisfactorily.

As an interesting comparison some colonies of Tarn Moor N.commune were air-dried and their capacity for moisture uptake investigated. The curve for moisture uptake (Fig. 1) is similar to that of the Aldabra colonies but the rate of uptake is somewhat slower. 80% moisture content was only achieved after one hour but maximum water content was reached about the same time as with the Aldabra colonies.

The corresponding results for the other two Aldabra species are shown in Figure 2. The curves for moisture uptake are similar to these for N.commune but the rates of uptake are much slower. The colonies of these two species are small in comparison to these of N.commune and the slower rates could be due to having a smaller surface area for moisture uptake and the fact that they are more compact, particularly those of the Nostoc sp.

A number of N.sphaericum colonies tested did not become spherical or even round but assumed a disc-like shape. This was also found in later experiments. Whether or not this was due to

FIGURE 3: TIME COURSE OF ACETYLENE REDUCTION BY ALDABRA N. COMMUNE AFTER REMOISTENING. (•) LIGHT and (×) DARK. ONE SAMPLE OF 3 COLONIES. TEMPERATURE 32°C



the effects of drying or they were disc-like in nature is an interesting question.

Acetylene reduction by re-moistened colonies

In these experiments the algae were assayed for acetylene reduction immediately after they had been re-moistened.

The following standard amounts of medium were added to a colony to re-moisten it; 6 ml/colony of N.commune, 1-5 ml/colony of N.sphaericum, and 2 ml/colony of Nostoc sp. These amounts were set from knowledge gained in the moisture uptake experiments and in small experiments on individual colonies. They were calculated to fully re-moisten the colony but to leave a small amount in excess to allow for above-average size colonies.

A preliminary investigation on N.commune showed that on re-moistening reduction of acetylene commenced after a period of 4-6 hours and then the rate of reduction gradually increased but it had not reached a constant level when the experiment had to be terminated after 20 hours.

The experiment was then repeated and continued until a constant rate of reduction had been achieved. A sample size of three colonies was chosen to ensure a reasonable rate of reduction.

Acetylene reduction commenced between 4 to 8 hours after re-moistening (Fig. 3) and the production of ethylene increased steadily until a constant rate was attained after a period of 24 hours. Reduction continued at this rate until 60 hours when the experiment was terminated.

Reduction in the dark was extremely low (Figure 3) and most probably represents that due to heterotrophic bacteria.

This was a good indication that the greater part of acetylene reduction in the light was due to N.commune.

The findings of the experiment were substantiated using only one colony. The time-course of ethylene production (Fig. 4) was very similar to that for the previous experiment (Fig. 3). The curve is the mean of two replicates, a third giving much lower results. It was thought that this might be because of natural variation between colonies but on checking the experimental flask it was suspected of being contaminated and the result was discarded.

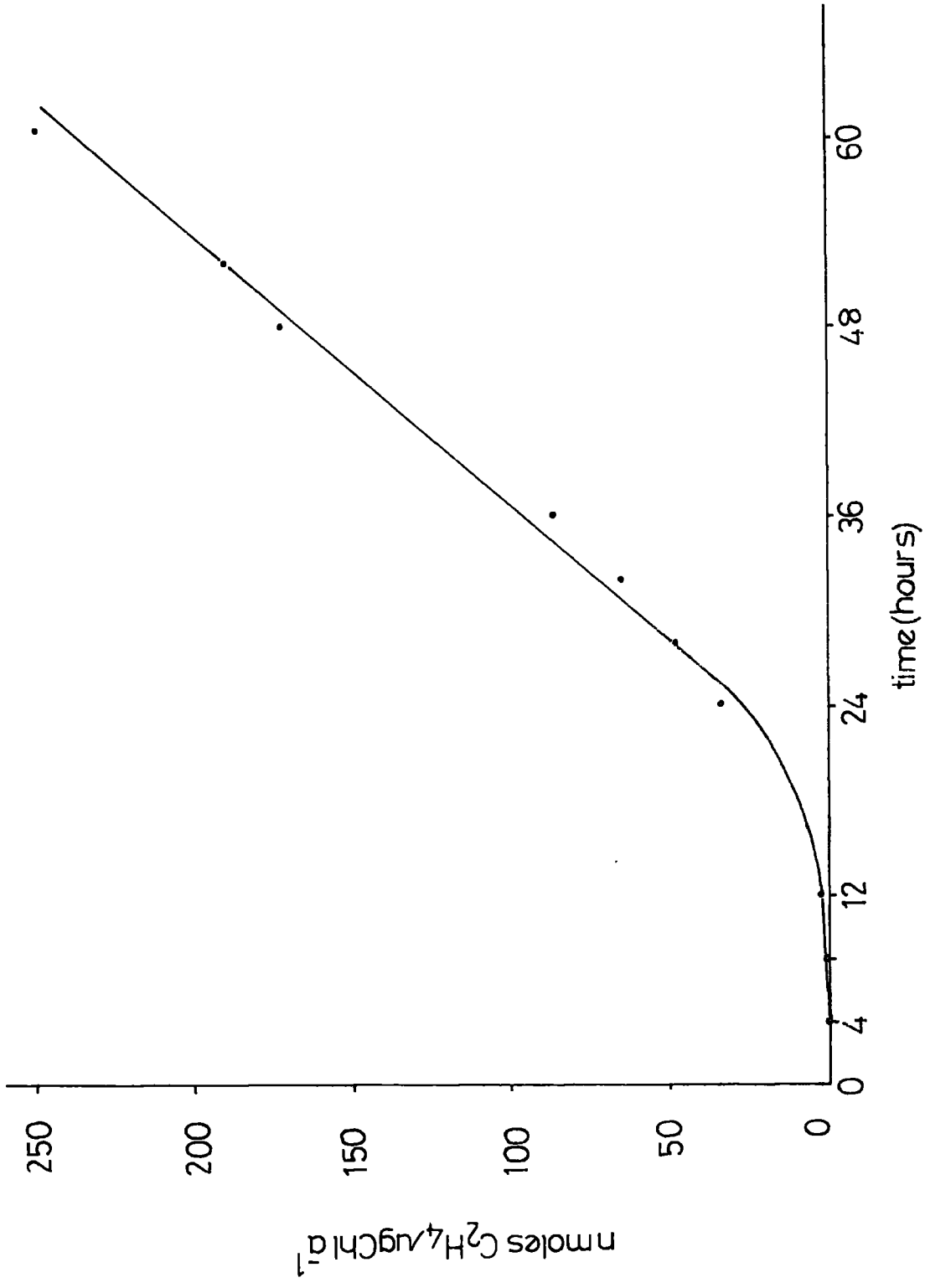
A further check was then made on the time-course of ethylene production and an attempt to gain a more accurate picture of the time-course immediately after acetylene reduction commenced, was made.

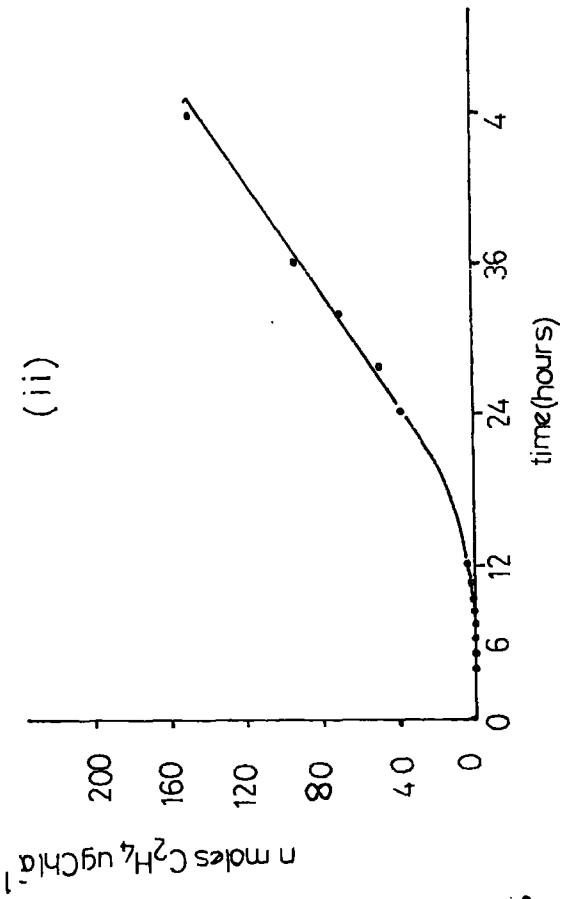
Figure 5(i) shows the time course of ethylene production over the first 12 hours of the experiment. Reduction is indicated as starting after 4 hours but only one replicate had started at that time, the others did so between 4 and 5 hours. The progressive increase in ethylene production during the first 20 hours of reduction is clearly demonstrated. The inset of the complete time course of acetylene reduction (Fig. 5(ii)) shows that it is similar to earlier results.

Thus it appeared that the course of acetylene reduction followed by a colony after re-moistening was fairly standard, with a linear rate being achieved after about 24 hours.

The time course of acetylene reduction by N.sphaericum colonies (Fig. 6) was similar to that obtained with N.commune,

Figure 4: Time Course of Acetylene Reduction by Aldabra N.commune after remoistening. Sample Size 1 colony. Each point is the mean of 2 replicates. Temperature 32°C.





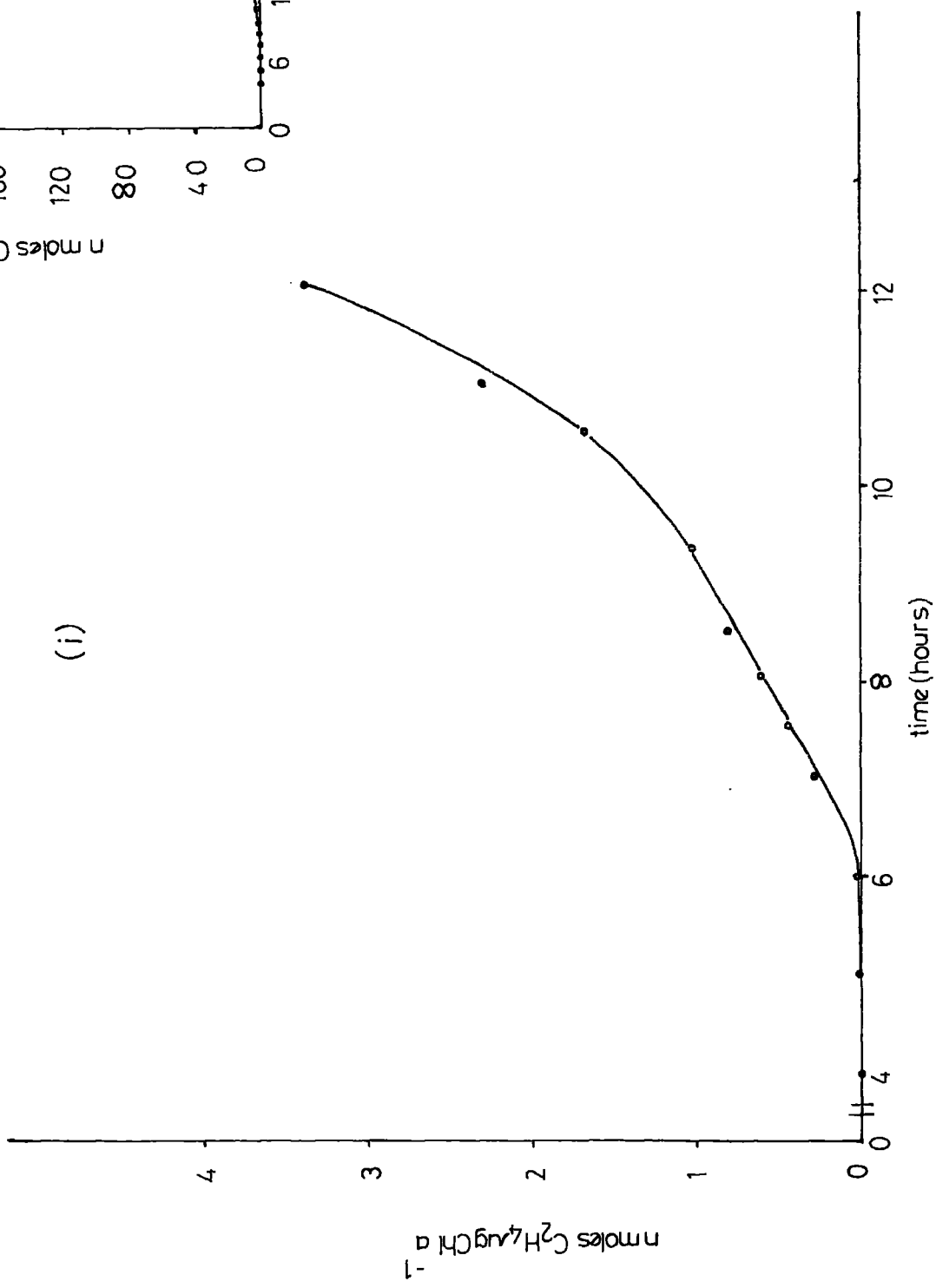


Figure 5: Time Course of Acetylene Reduction by Aldabra N.commune.

i) Over the first eight hours of reduction

ii) Full time course

Sample size 1 colony. Each point is the mean of 3 replicates. Temperature 32°C.

Figure 6: Time Course of Acetylene Reduction by N.sphaericum in the light (•) and dark (x) after remoistening. One sample 10 colonies. Temperature 32°C.

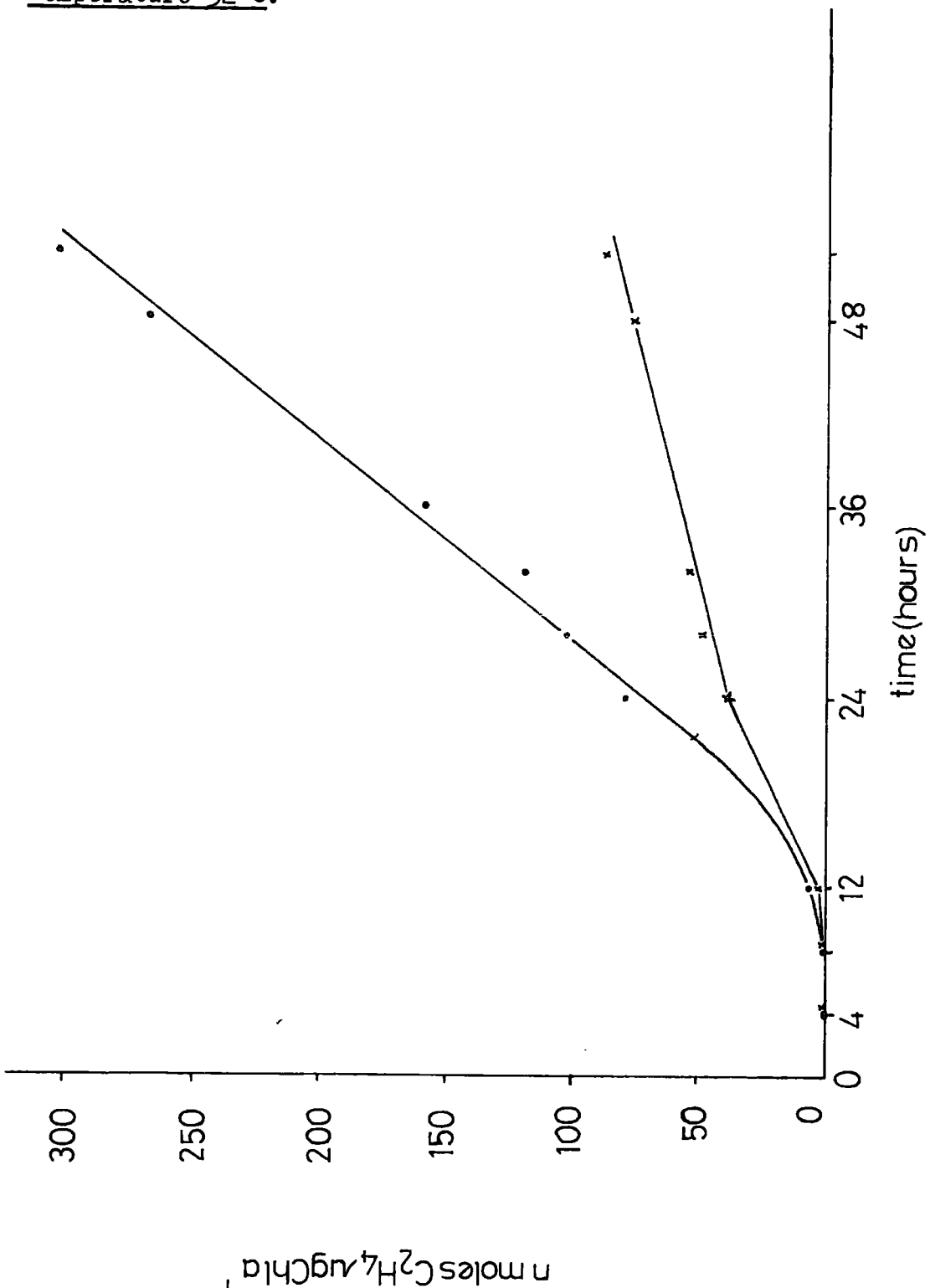
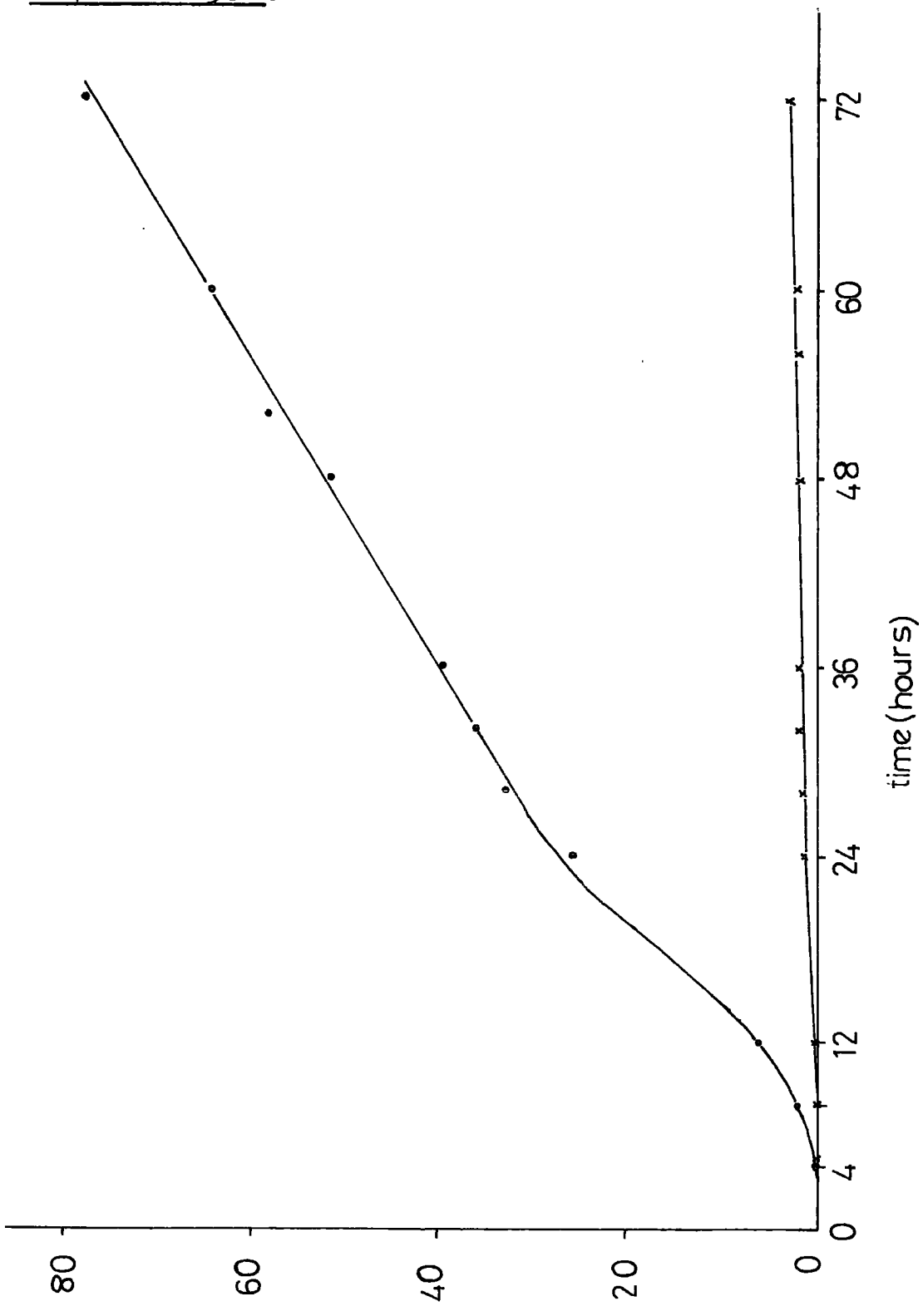


Figure 7: Time Course of Acetylene Reduction by Nostoc sp. in the light (•) and dark (x) after remoistening. 1 Sample 5 colonies. Temperature 32°C.



although a linear rate of reduction appeared to be reached a little earlier, i.e. after about 20 hours. Reduction in the dark was considerably higher than with N.commune.

Similar results were also obtained with colonies of the Nostoc sp. (Fig. 7). In this case reduction in the dark was again extremely low.

Acetylene reduction by colonies re-moistened under standard conditions for a standard period of time

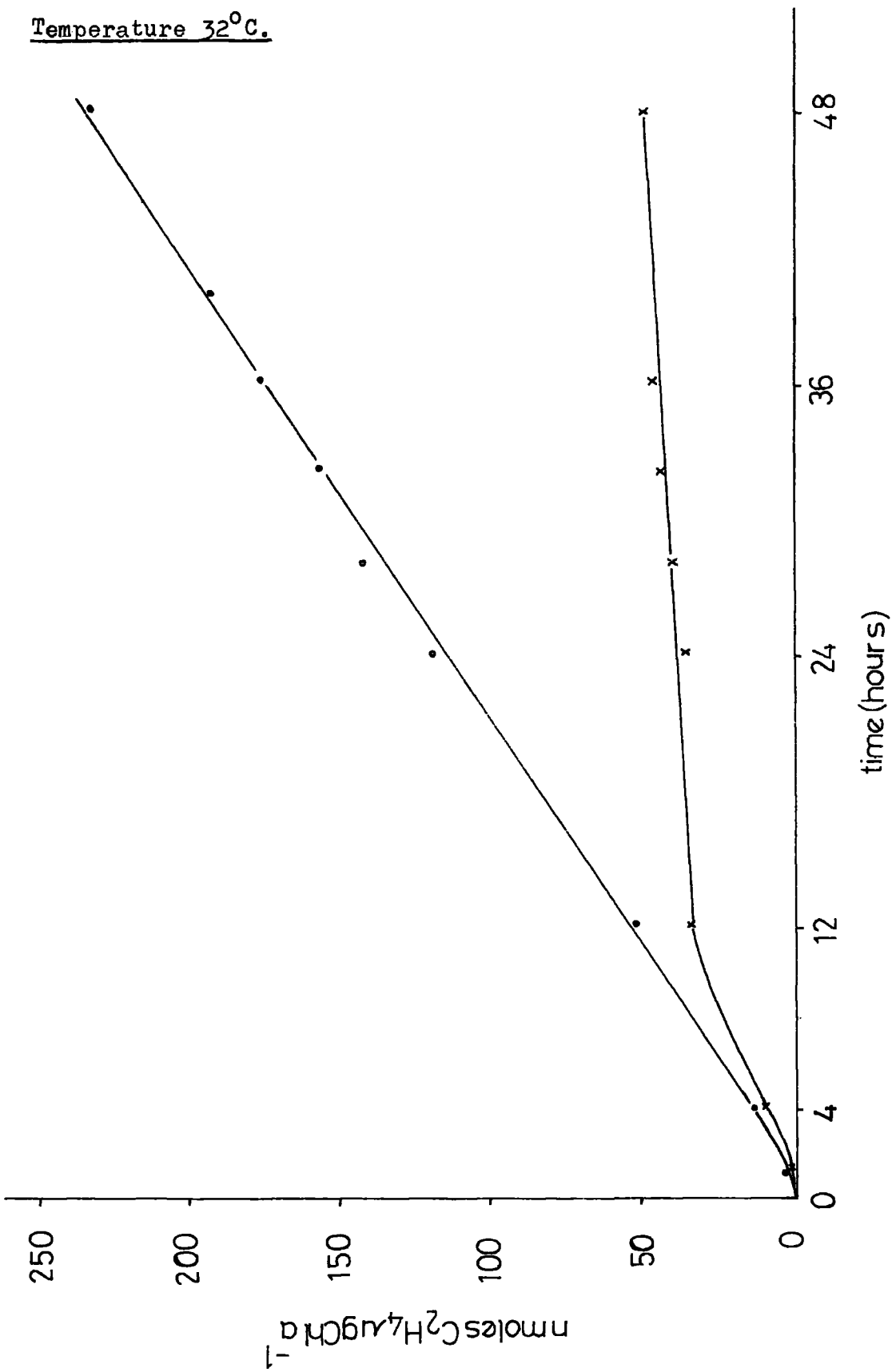
On the basis of information gained from the above experiments the following procedure was suggested as the standard pre-treatment for colonies of N.commune before using them in any experiments on the effect of temperature.

The required number of dried colonies were to be placed in a glass trough and re-moistened with the appropriate amount of medium. The trough, covered with a glass plate to prevent evaporation of moisture was then to be placed at a constant temperature of 32°C and light intensity of 3000 ± 300 lux for a period of 48 hours.

The period of 48 hours was chosen to ensure that all colonies were fully re-moistened, and in a state, which if in an experiment, they would be reducing acetylene at a constant rate.

To check that these conditions were appropriate, dry colonies of N.commune were treated in the above manner and then assayed for acetylene reduction. The time course of ethylene production was linear (Fig. 8) showing that the colonies were in fact reducing acetylene at a constant rate.

Figure 8: Time Course of Acetylene Reduction by Aldabra N.commune in the light (•) and dark (×) after pre-treatment in the standard manner. Sample size 1 colony. Each point is the mean of 3 replicates. Temperature 32°C.



Colonies in the dark control reduced acetylene at a rate similar to these in the light for 12 hours but then the rate dropped very sharply and remained at a very low level for the rest of the experimental period. At first it was thought that this was an indication of heterotrophic acetylene reduction by the alga. However, Paul et al (1971) found that algal crusts would reduce acetylene for some time after being placed in the dark, and this appeared to be a similar situation.

As a result of this experiment the conditions outlined above were adopted as the standard pre-treatment for colonies of Aldabra N.commune.

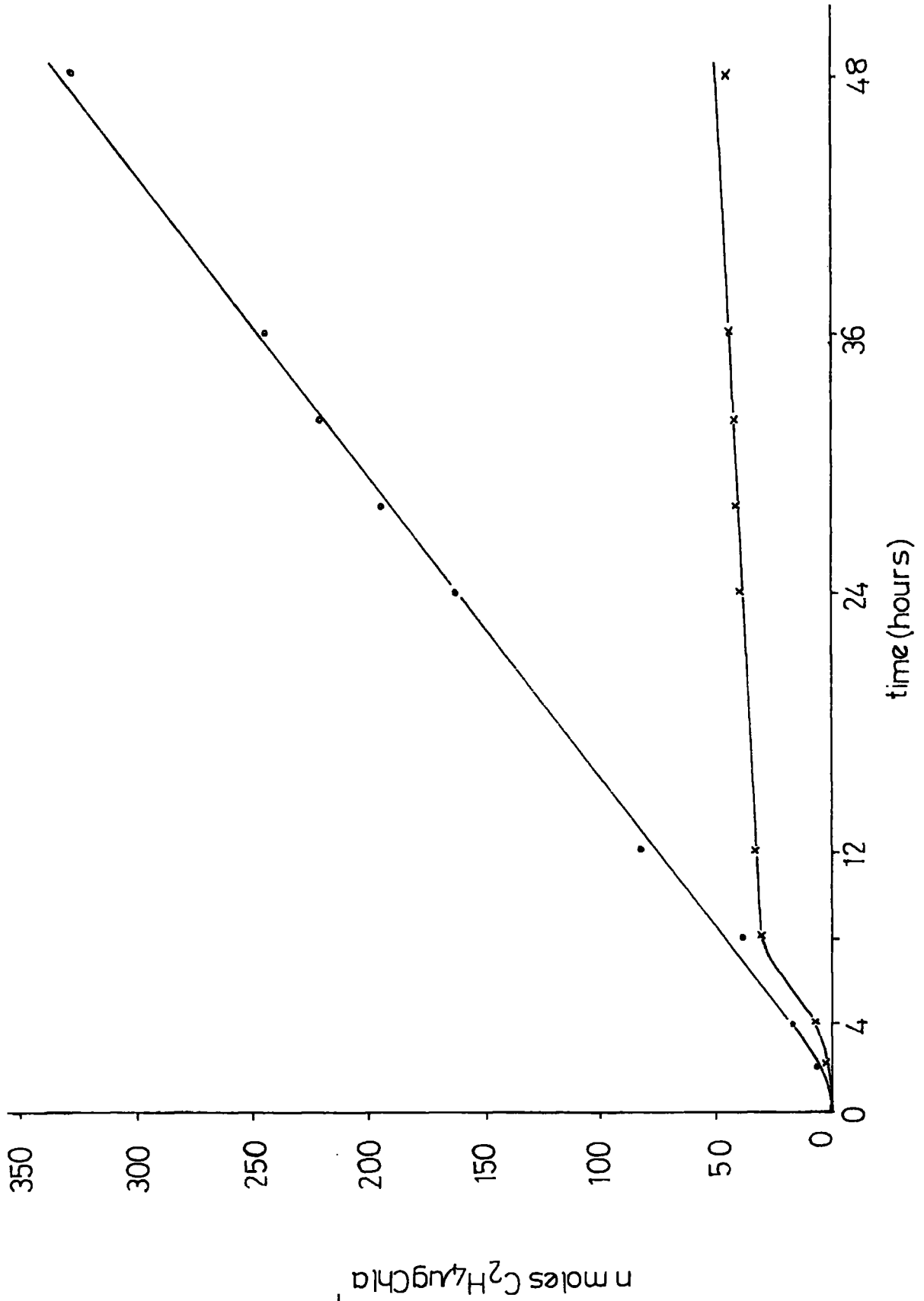
5.2 Acetylene reduction by Tarn Moor N.commune

The colonies were tested for the ability to reduce acetylene using the same procedure as with Aldabran material, only no re-moistening was necessary.

A sample size of three colonies was used. The time course of ethylene production is shown in Figure 9. After a slight lag in the first two hours the production of ethylene was linear and continued to be so until the experiment was terminated after 48 hours.

Colonies in the dark control reduced acetylene at a rate similar to those in the light for a period of 8 hours, after which the rate diminished to a very low level and remained at this level for the rest of the experimental period. This was a very similar situation to that found with Aldabra N.commune in the dark (see Fig. 8).

Figure 9: Time Course of Acetylene Reduction by Tarn Moor N.commune in the light (•) and dark (x). Sample size 3 colonies. Each point is the mean of 3 replicates. Temperature 20°C.



5.3 Acetylene reduction by algae under assay conditions used for experiments on the effect of temperature

Experiments to investigate the effects of temperature were carried out in 7 ml serum bottles. The standard incubation period was one hour to minimise the chances of the alga adapting to the temperature and to enable a direct comparison of rates of acetylene reduction.

For a direct comparison of rates to be made the rate of reduction over the incubation period should be linear. Thus the time course of ethylene production over one hour in the serum bottles was determined for the two N.commune populations and also for the other two Aldabra species.

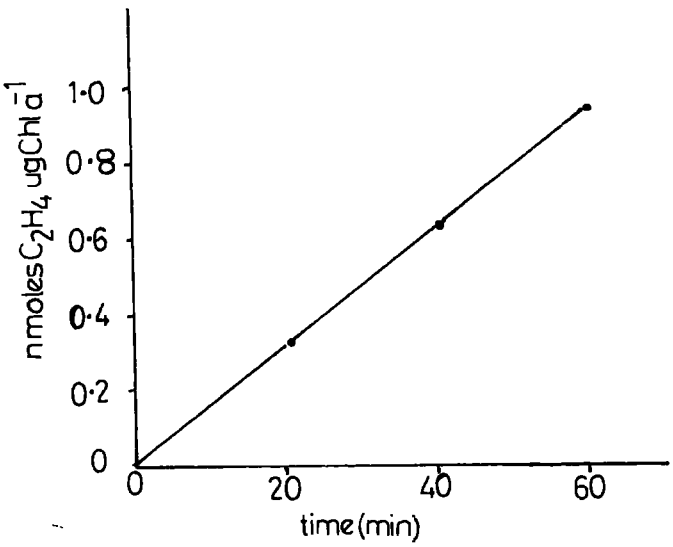
The time-course of ethylene production was linear for all the four algae (Fig. 10).

From these experiments mean rates of acetylene reduction were obtained for the four algae tested. These are given in Table 1. Considerable variation in rates was found between the four replicates of each species which is not apparent in the data given. This was most marked in Aldabra N.commune.

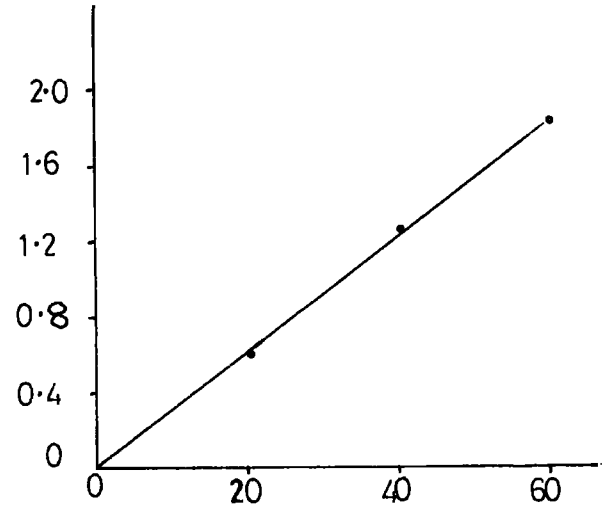
The mean rates differed quite markedly and were compared statistically.

The mean rate for Aldabra N.commune was significantly different from that of N.sphaericum at the 0.05 level, and from the rate for the Nostoc sp. at the 0.02 level. The mean rate of N.sphaericum was significantly different from that of the Nostoc sp. at the 0.001 level.

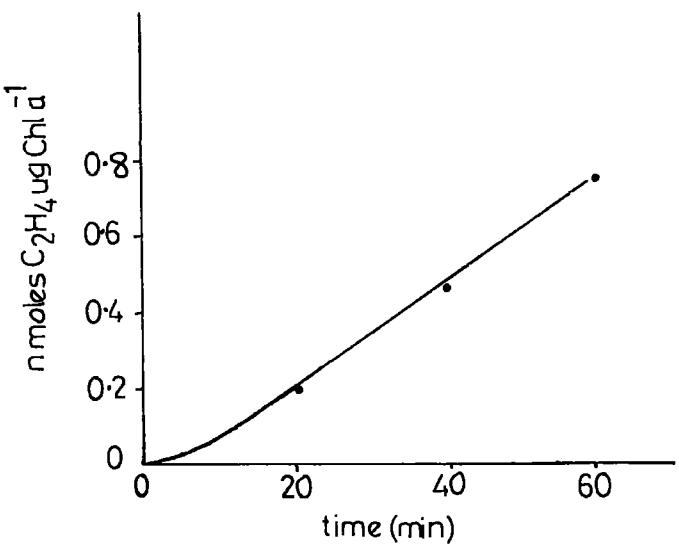
Figure 10: Time course of acetylene reduction over one hour by each algal species.



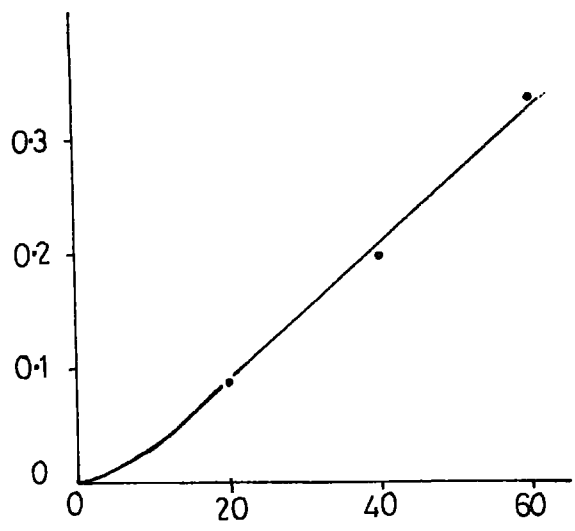
i) Tarn Moor N.commune.
Temperature 20°C.



ii) Aldabra N.commune.
Temperature 32°C.



iii) N.sphaericum.
Temperature 32°C.



iv) Nostoc sp.
Temperature 32°C.

Each point is the mean of 4 replicates.

TABLE 1

RATE OF ETHYLENE PRODUCTION BY EACH OF THE ALGAL SPECIES INVESTIGATED

Species	Rate and S.D. n moles C_2H_4 $\mu gChla^{-1}h^{-1}$
Tarn Moor Nostoc commune	0.940 ±0.240
Aldabran Nostoc commune	1.8309 ±0.2849
Nostoc sphaericum	0.7636 ±0.1224
Nostoc sp.	0.3429 ±0.0608

TABLE 2

MEAN RATES AND STANDARD DEVIATION OF C_2H_4 PRODUCTION BY ALDABRAN NOSTOC COMMUNE AT VARIOUS TEST TEMPERATURES AFTER PRE-INCUBATION AT THESE TEMPERATURES

Pre-incubation temperature °C	Test temperature °C		
	20	25	32
20	0.9001 ±0.6627	0.9152 ±0.6029	1.1594 ±0.6720
25	0.8161 ±0.4648	0.6730 ±0.6047	1.7086 ±0.9044
32	0.3764 ±0.1819	0.9010 ±0.4263	1.1580 ±0.7302

All rates in n moles C_2H_4 $\mu gChla^{-1}h^{-1}$

The mean rates of the two N.commune populations were not significantly different.

5.4 The effect of temperature on the rate of acetylene reduction by Aldabra N.commune

These experiments were a preliminary to determining the temperature optimum of this alga and were designed to provide information on the effect of various temperatures on the alga and thus give some indication of where the optimum was.

The three temperatures used, 20°C, 25°C, and 32°C covered a range of temperatures the alga would probably have experienced regular ly in its natural environment.

Colonies were re-moistened in the standard way (see Section 5.1) at a constant temperature of 32°C. They were then assayed for acetylene reduction at temperatures of 20°C, 25°C, and 32°C. Eight replicates were placed at each temperature and the mean rate of acetylene reduction subsequently determined.

As it was possible that the colonies could have adapted to the temperature at which they were pre-incubated, the experiment was repeated with the alga pre-incubated first at 25°C, and then at 20°C.

The mean rates of acetylene reduction for each test temperature, and each experiment are shown in Table 2.

There was considerable variation in rates between replicates at each temperature in all three experiments. The standard deviations give some indication of the magnitude of the variation.

In all three experiments acetylene reduction was highest at 32°C, which indicated that the temperature optimum of the alga would most probably lie in the thirties. Of the three values for acetylene reduction at 32°C, two were in remarkably close agreement, but the third was considerably higher. However, a comparison of the rates statistically using the 't' test revealed that they were not significantly different.

With the other two temperatures the situation was not quite as clear. Acetylene reduction at 25°C was greater than at 20°C except when the alga was pre-incubated at 25°C, then the reduction at 20° was greater than that at 25°C. The difference between the latter rates did not prove to be significant and probably occurred because of the variability of the material with respect to acetylene reduction. As with the rates for 32°C, those at 25°C and 20°C were not significantly different.

That the rates of acetylene reduction at each test temperature were not significantly different seemed to indicate that the pre-incubation temperature was not affecting the acetylene reducing activity of the alga to any great degree.

When the experiments were considered separately and acetylene reduction values for each temperature compared, some inconsistencies in the results became apparent.

1. Pre-incubation temperature 20°C

No statistically significant differences were found between any of the acetylene reduction values.

2. Pre-incubation temperature 25°C

In this experiment acetylene reduction at 32°C was

significantly different from that at 25°C at the 0.02 level. It was also significantly greater than acetylene reduction at 20°C ($p = 0.02$). The difference between acetylene reduction at 25°C and 20°C was not significant.

3. Pre-incubation temperature 32°C

In contrast to the previous experiment the difference between acetylene reduction at 32°C and 25°C was not significant, although the difference between the values at 32°C and 20°C was ($p = 0.02$). In this case acetylene reduction at 25°C was significantly different from that at 20°C at the 0.05 level.

It is clear from these results that the differences in acetylene reduction between temperatures were not consistent. However, it is evident that acetylene reduction is greater at the higher temperatures. The inconsistency between the results was most probably due to the great variability of the material with respect to acetylene reduction. The variation between material will be discussed later.

Similar experiments were not carried out on Tarn Moor N.commune as this was not available at the time.

5.5 Temperature optima experiments

For determination of the temperature optima of the alga under investigation the cross gradient apparatus (Section 3.4) was utilised. The apparatus was calibrated to give a temperature gradient from 50°C to 21°C and the fluorescent lamps were adjusted to give a continuous light intensity of 3000 ± 300 lux over the area of the aluminium plate used in experiments (hence a cross gradient of temperature and light was not used).

The results were expressed graphically by plotting acetylene reduction against temperature. It was hoped that this would show the temperature optimum relatively clearly. However, in all experiments the material showed considerable variation with respect to acetylene reduction and relatively sharp optima were not obtained. No statistical method of clarifying the results was available and hence it was only possible to determine the range of temperature in which the optimum appeared to lie.

Experiments on Aldabra N.commune

As the pre-incubation temperature could influence the temperature optimum of the alga, it was pre-incubated at several temperatures, in order that the results could be compared and an accurate assessment of the optimum made.

The range of temperatures used in an experiment varied according to the pre-incubation temperature of the alga and details of the range are given where appropriate.

Experiment 1:

The alga was pre-incubated at 32°C and then assayed for acetylene reduction at 22-46°C. To ensure the full range of temperatures was covered 30 replicates were used.

The results are shown in Figure 11. The optimum appeared to be at 35°C but could quite easily have been at 31°C or 33°C. Above 39°C acetylene reduction diminished very sharply and at 46°C was very low. The situation was similar in the lower twenties.

Experiment 2:

The alga was again incubated at 32°C in an attempt to clarify the previous results. On the evidence of these results the range of temperature was decreased to 26-42°C. This allowed more replicates to be assayed in the thirties, the range at which the optimum appeared to lie.

The highest rates of acetylene reduction were now found at 37°C and 39°C (Fig. 12).

On amalgamating the results of the two experiments it was clear that the optimum was somewhere in the 30-39°C range (Fig.13). There was a slight tendency for the higher rates of acetylene reduction to be found in the higher rather than the lower thirties.

Experiment 3:

The alga was pre-incubated at 25°C and assayed at the temperature range used in Experiment 1.

The highest rates of acetylene reduction were in the 26-31°C range rather than in the thirties (Fig. 14). Acetylene reduction again decreased sharply above 40°C.

Experiment 4:

As the results from the above experiment differed from these obtained when the alga was pre-incubated at 32°C, it was decided to repeat the experiment to verify them.

Pre-incubation was at 25°C, with a temperature range as for experiment 2. Acetylene reduction was highest at 28°C, 39°C, and 41°C (Fig. 15). Generally the higher rates were in the 30-39°C in contrast to the earlier results.

On combining the results of the experiments (Fig. 16) the situation was not clarified any further. The optimum could have been anywhere in the 26-41°C region.

Experiment 5:

The alga was pre-incubated at a high temperature - 39°C. Temperature range as in Experiment 2.

The results from this experiment were much clearer than before. The optimum was between 36°C and 40°C, the rates at these temperatures with one exception being considerably higher than those at the other temperatures (Fig. 17).

Experiment 6:

The alga was pre-incubated at 43°C, the temperature range for assaying was 30-46°C.

All values for acetylene reduction were very low (Fig.18) probably reflecting the fact that the incubation temperature of 43°C was near the edge of the alga's tolerance limits. Activity was slightly higher in the 35-41°C range.

Summary of Results

When all the results are taken together it is relatively clear that acetylene reduction is greatest in the 30-39°C region. The temperature optimum thus appears to lie somewhere in this range. It is not possible to be more accurate although there is a tendency for activity to be highest in the higher rather than the lower thirties.

For the purpose of further discussion the above conclusions will thus be used.

Figure 11: Plot of acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 32°C.

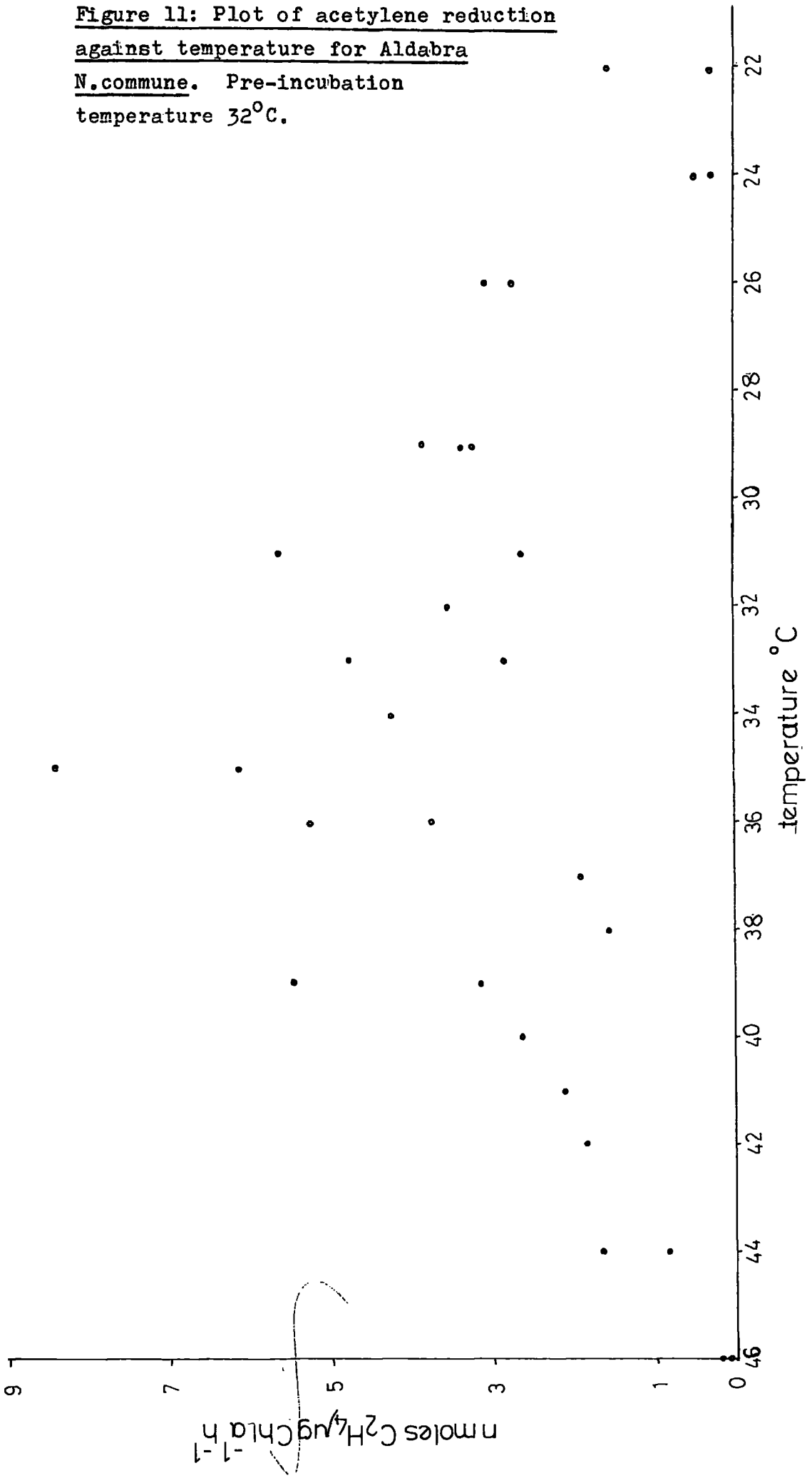


Figure 12: Plot of acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 32°C.

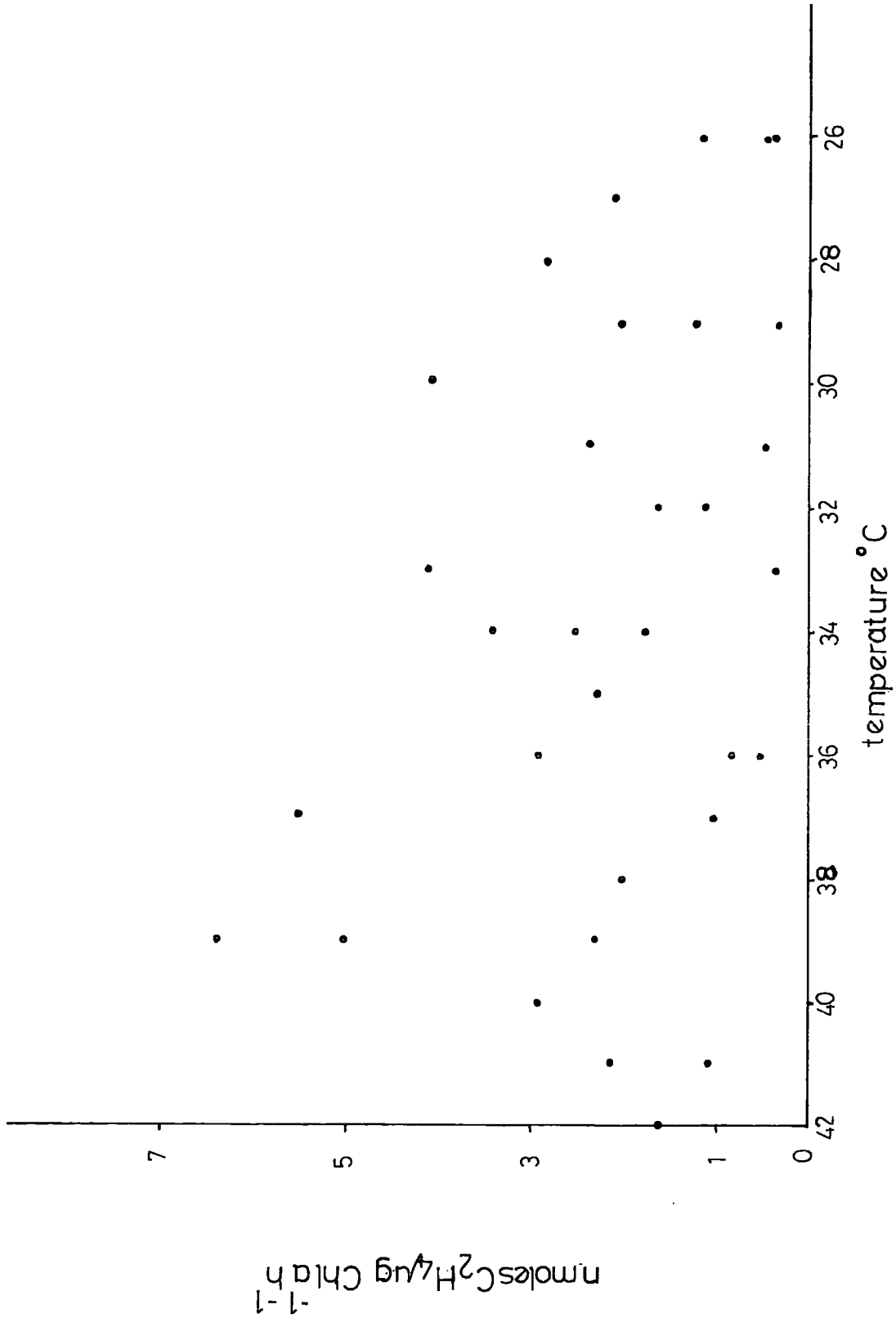


Figure 13: Combined plot of data shown in Figs. 11 and 12

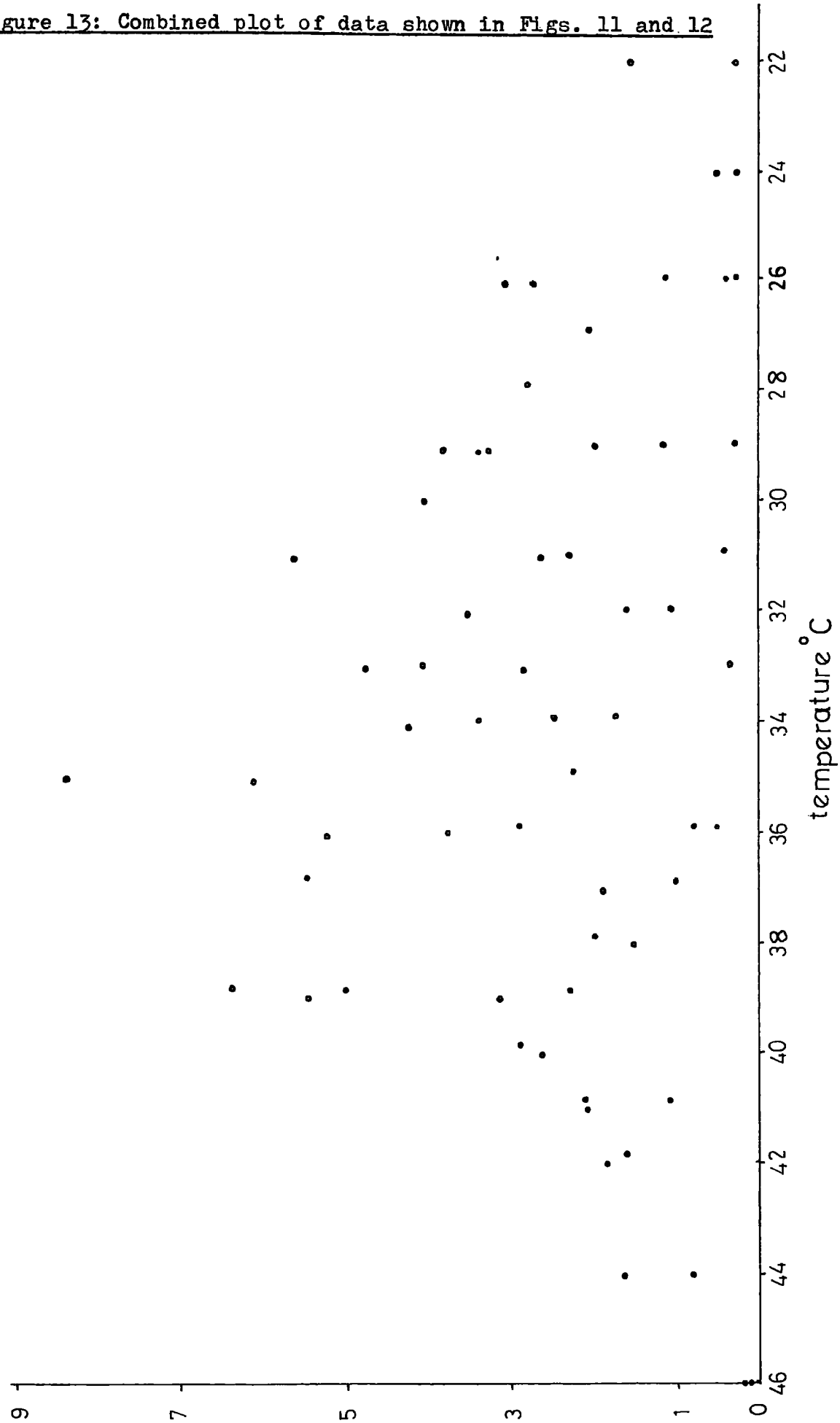


Figure 14: Plot of acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 25°C.

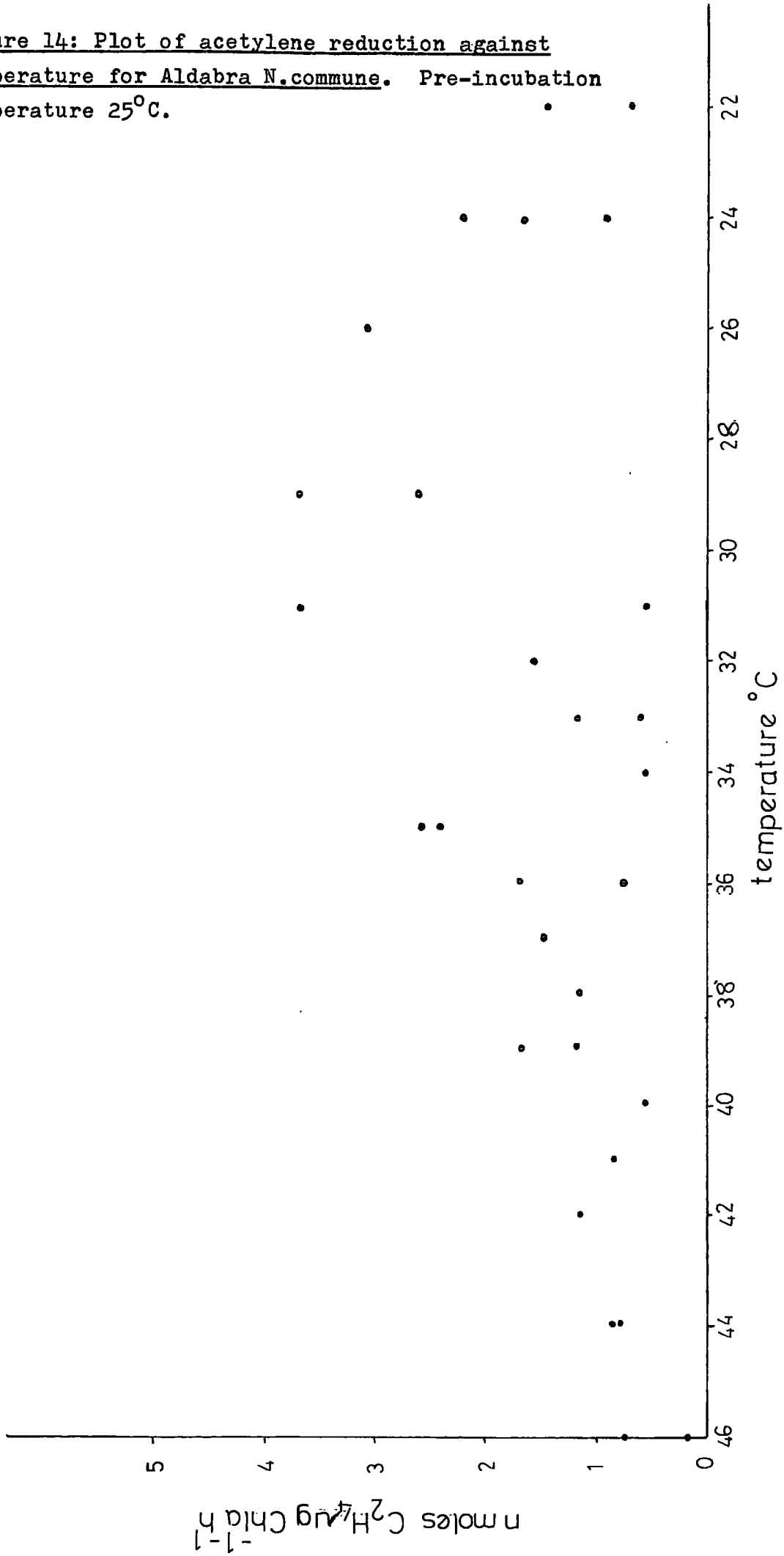


Figure 15: Plot of acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 25°C.

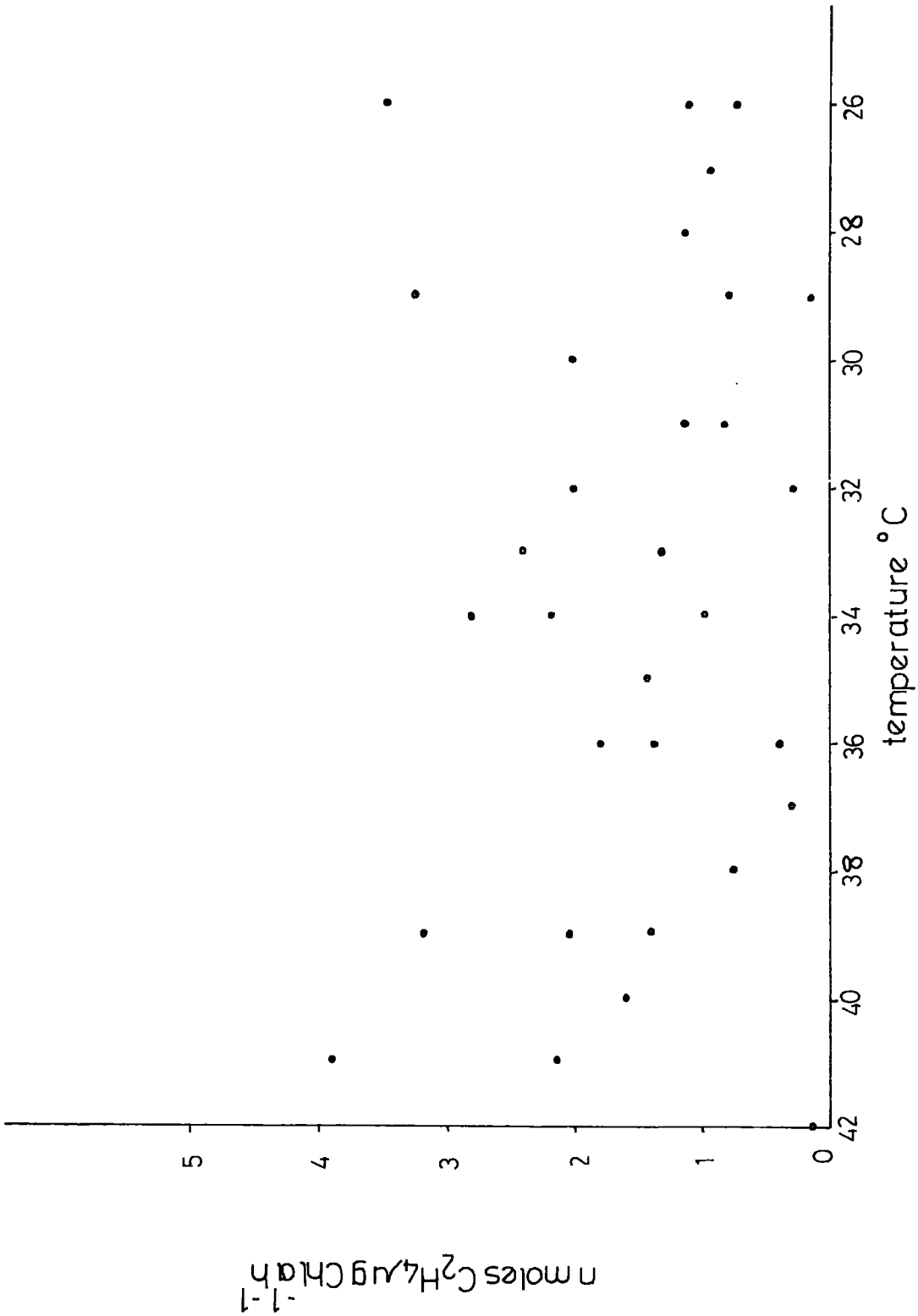


Figure 16: Combined plot of data shown in Fig. 14 and 15.

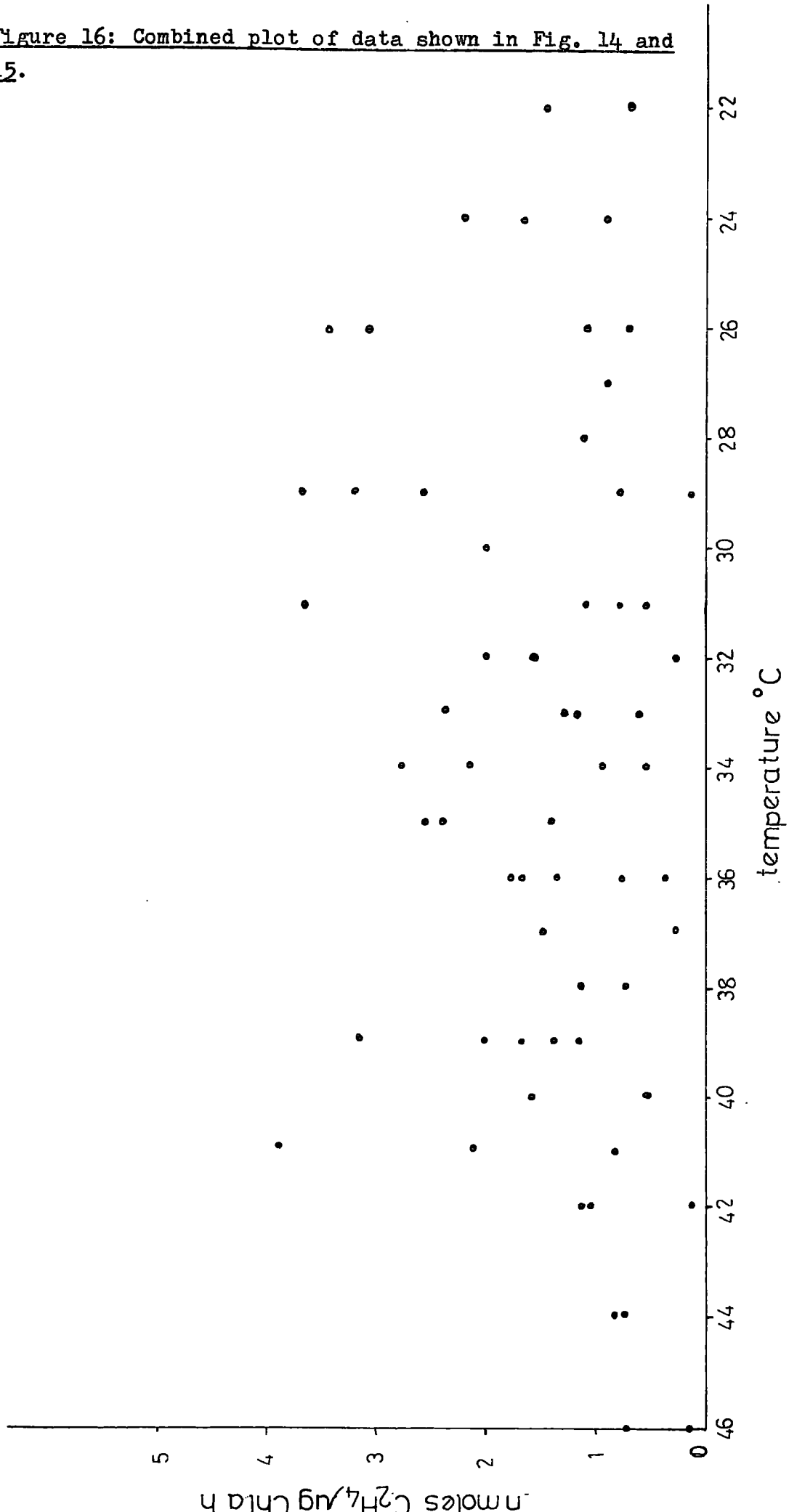


Figure 17: Plot of acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 39°C.

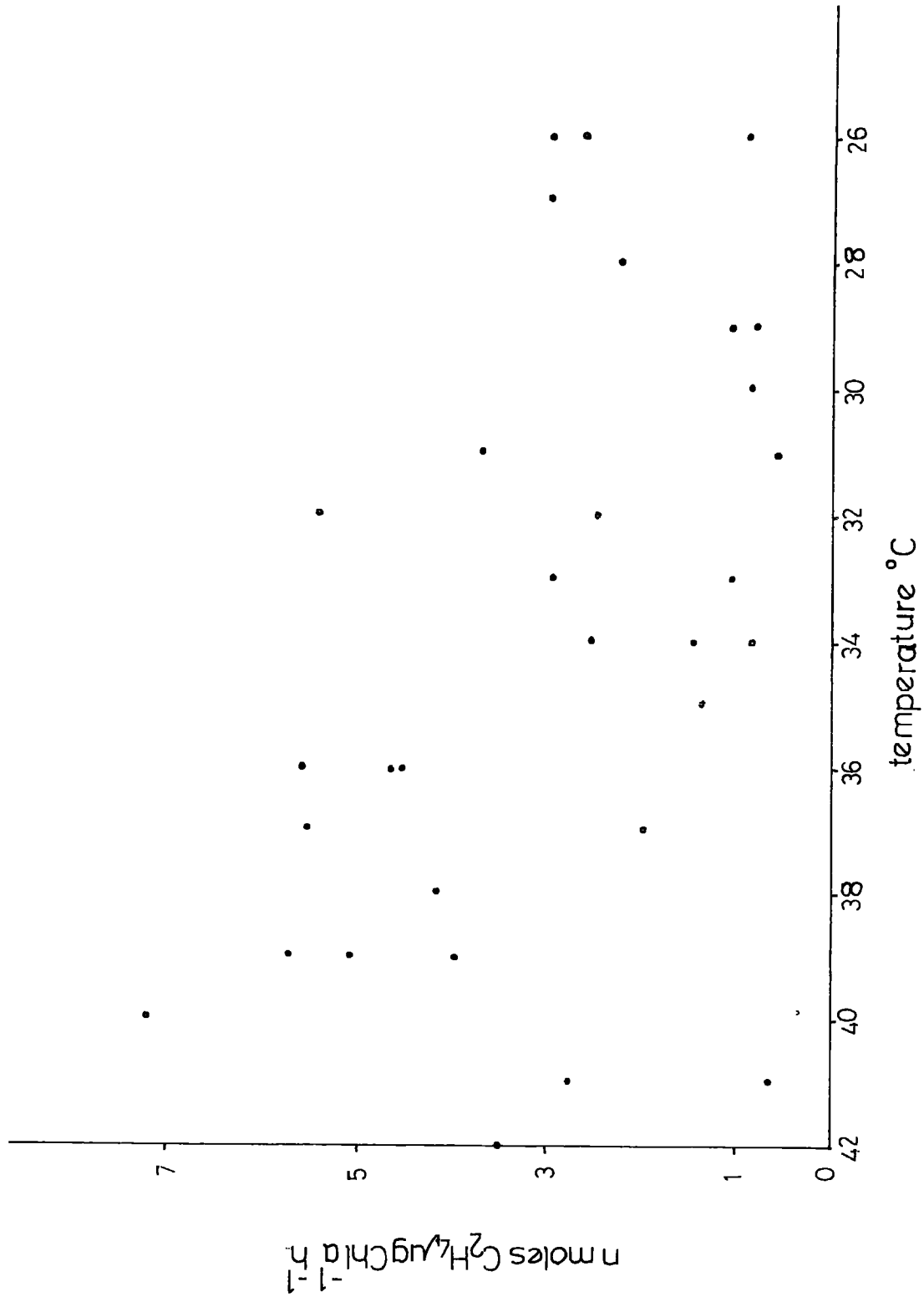
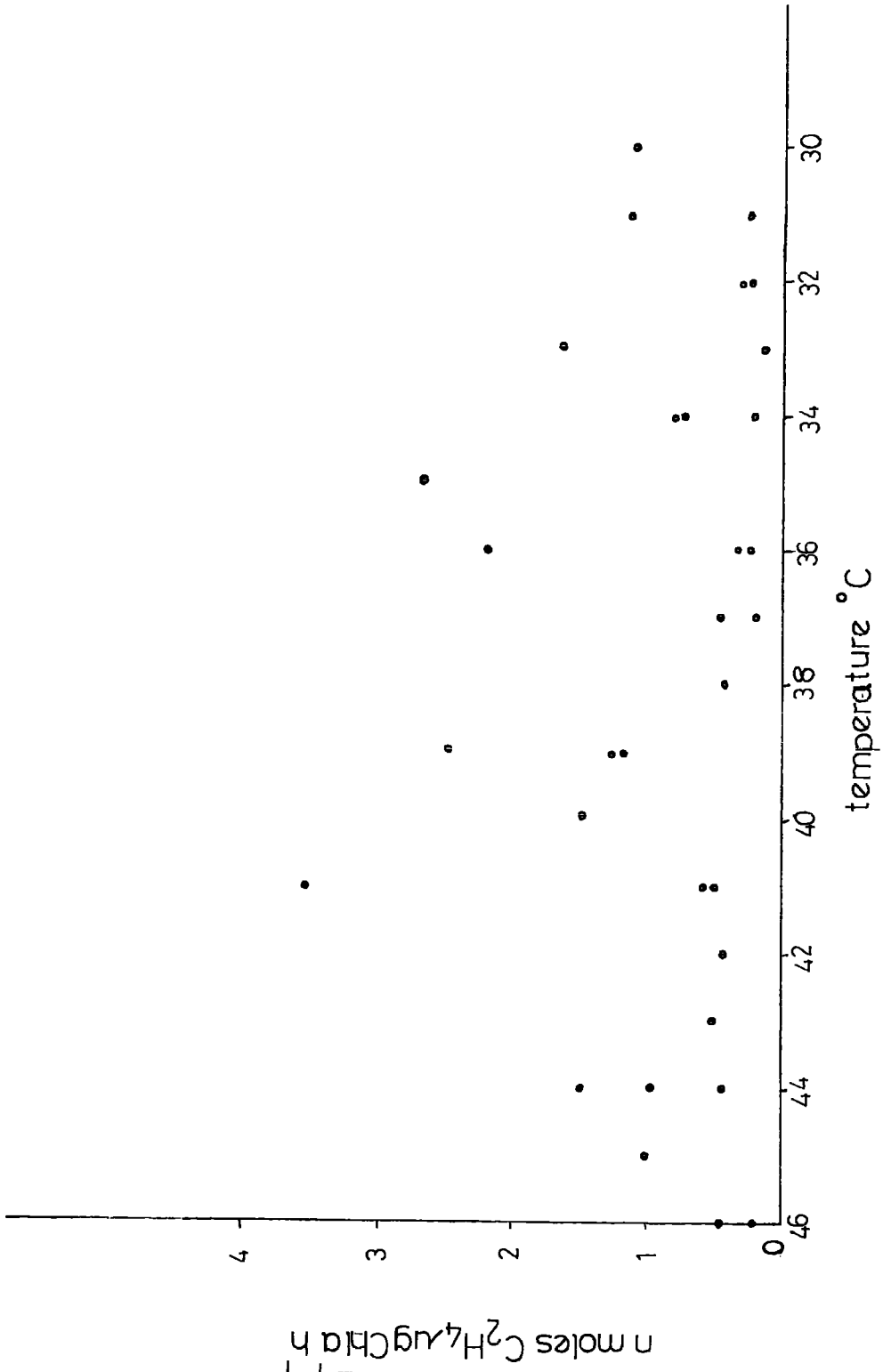


Figure 18: Plot for acetylene reduction against temperature for Aldabra N.commune. Pre-incubation temperature 43°C.



Experiments on Tarn Moor N.commune

The procedure was as before, with the temperature range for all experiments being 20-38°C. The cross gradient apparatus could not be calibrated to encompass lower temperatures which the alga would normally experience as it was being used in conjunction with other workers experiments. However, preliminary investigations had indicated that the temperature optimum was above 20°C so the lack of lower temperatures was not considered to be too critical.

The alga was pre-incubated in the same way as the Aldabra material (see Section 5.1), differing only in the respect that no re-moistening was required, and consequently only a small amount of medium to ensure a moist environment was added.

Experiment 1:

Alga were pre-incubated at a temperature of 20°C.

Acetylene reduction values were in the same range for the majority of the temperatures, being highest at 29, 30 and 35°C, (Fig. 19).

Experiment 2:

Pre-incubation temperature was increased to 25°C. Reduction rates were considerably higher than those in the previous experiment. Acetylene reduction was greatest in the 24-29°C range, rates at the other temperatures being of much the same order (Fig. 20). Rates at 36-38°C were very low.

Experiment 3:

Pre-incubation temperature was 32°C. Acetylene reduction activity was high in the 29-33°C range, with one exceptionally high

value of 32°C (Fig. 21).

Summary of Results

Acetylene reduction activity was generally highest in the 26-32°C range in all experiments. The temperature optimum hence appears to be somewhere in that range. For the purpose of further discussion it will be taken that the optimum is in the range stated above.

Experiments on N.sphaericum and Nostoc sp.

Only one experiment was carried out on each species, and in both cases the pre-incubation temperature was 32°C and the temperature range 22-46°C.

Nostoc sphaericum data was extremely variable and it is only possible to say that the temperature optimum lies in the 31-41°C range (Fig. 22).

The Nostoc sp. showed the least variability of all the algae and the temperature optimum is probably between 34° and 37°C (Fig. 23).

Figure 19: Plot of acetylene reduction against temperature for Tarn Moor N.commune. Pre-incubation temperature 20°C.

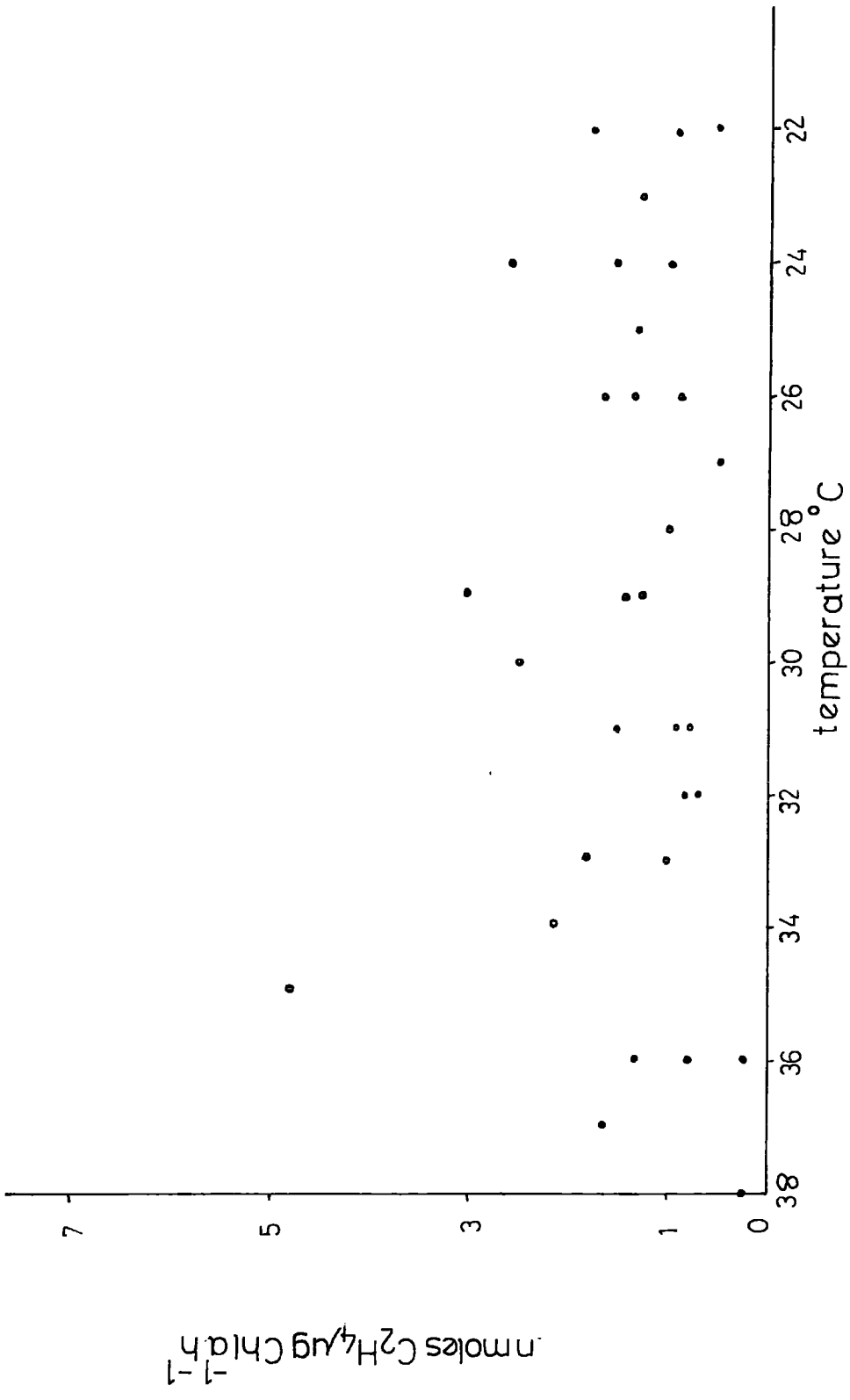


Figure 20: Plot of acetylene reduction against temperature for Tarn Moor N.commune. Pre-incubation temperature 25°C.

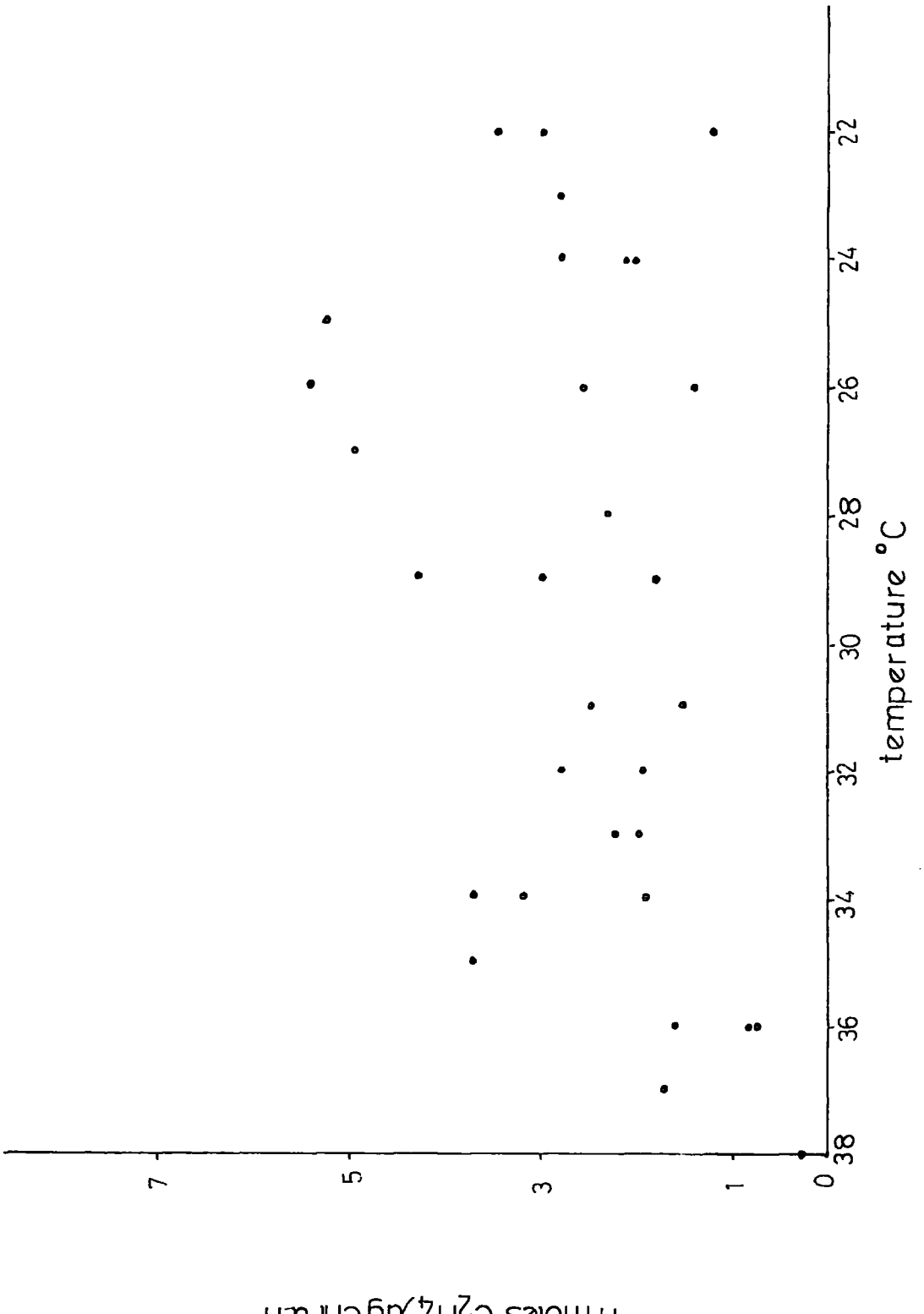


Figure 21: Plot of acetylene reduction against temperature for Tarn Moor N.commune. Pre-incubation temperature 32°C.

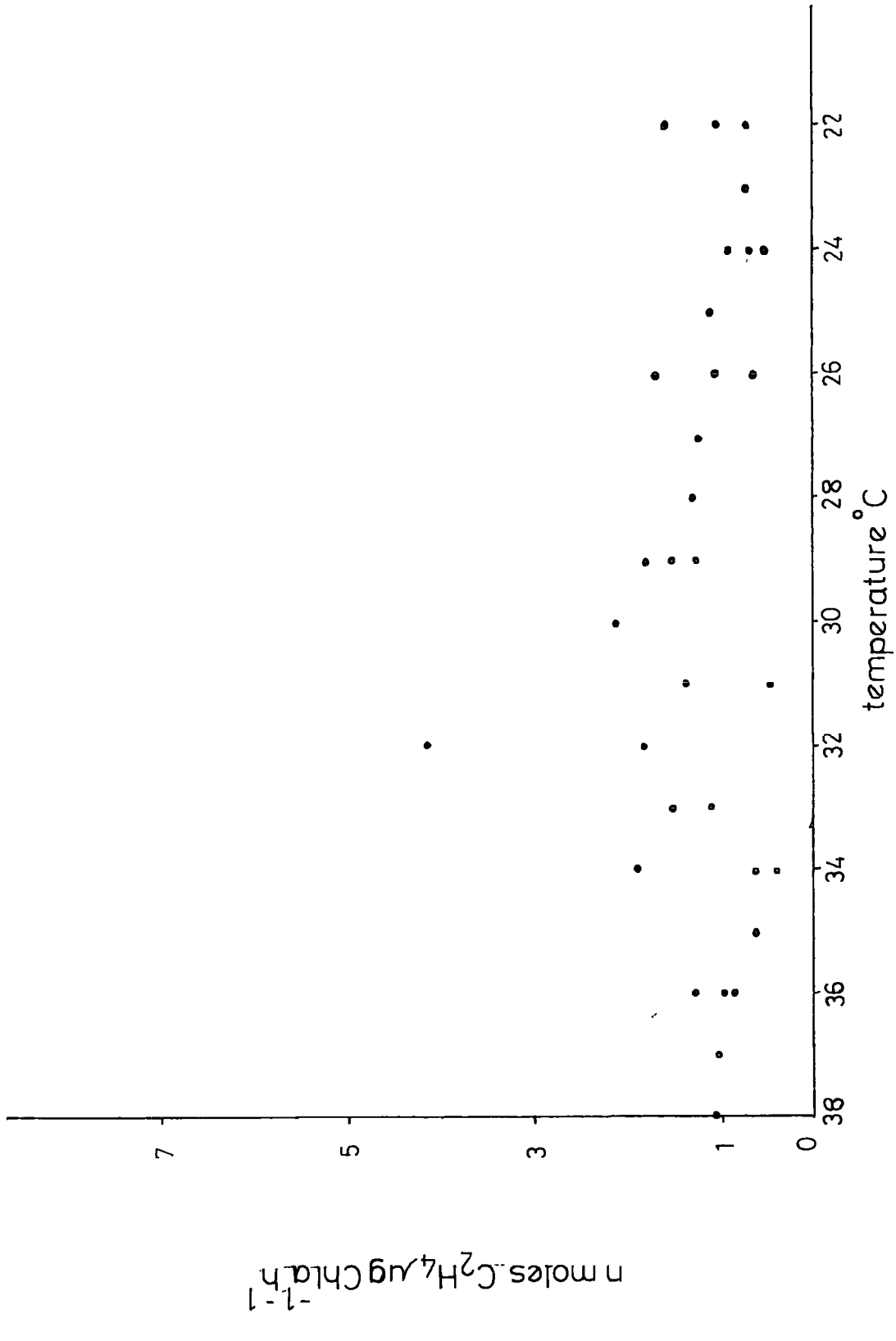


Figure 22: Plot of acetylene reduction against temperature for N.sphaericum. Pre-incubation temperature 32°C.

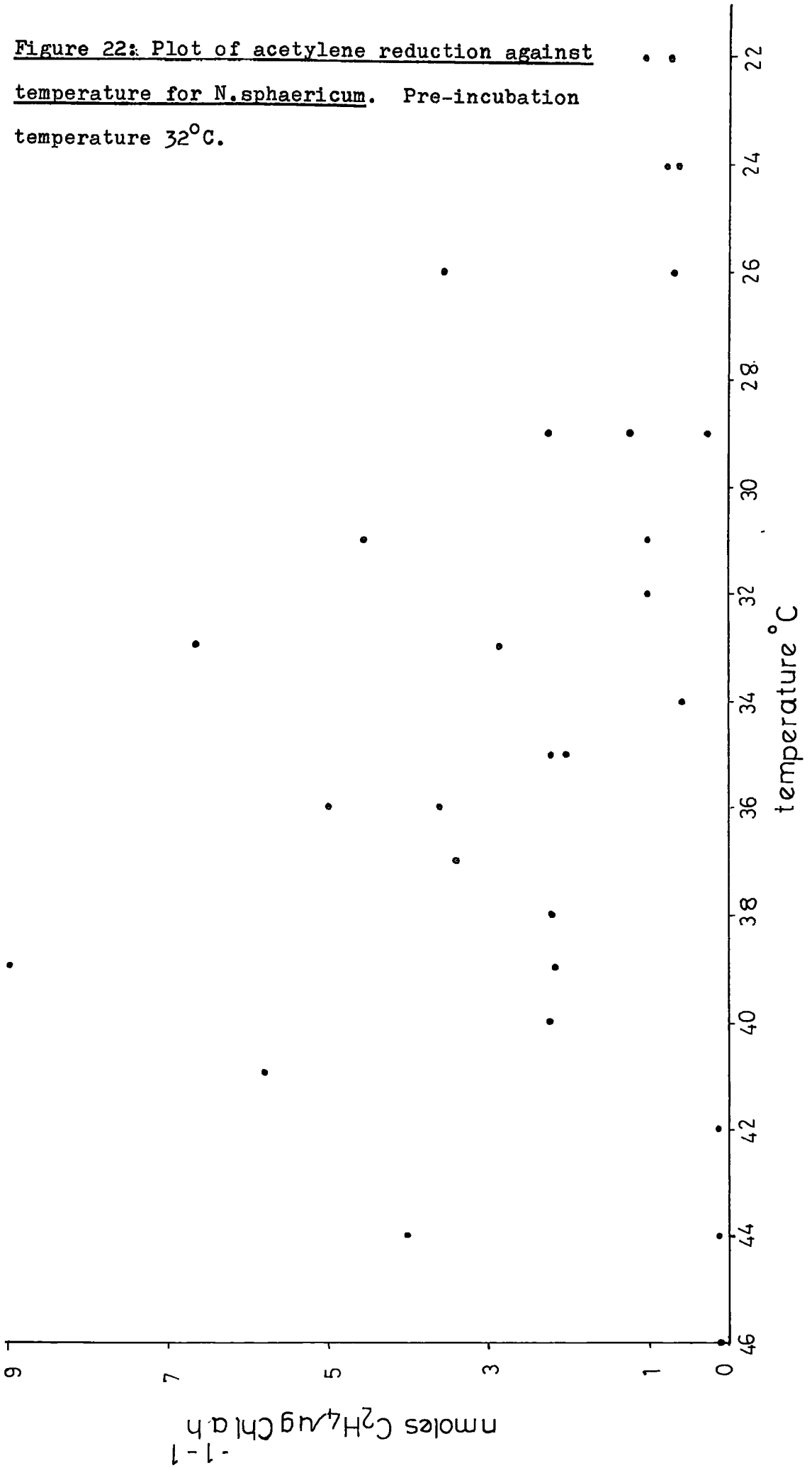
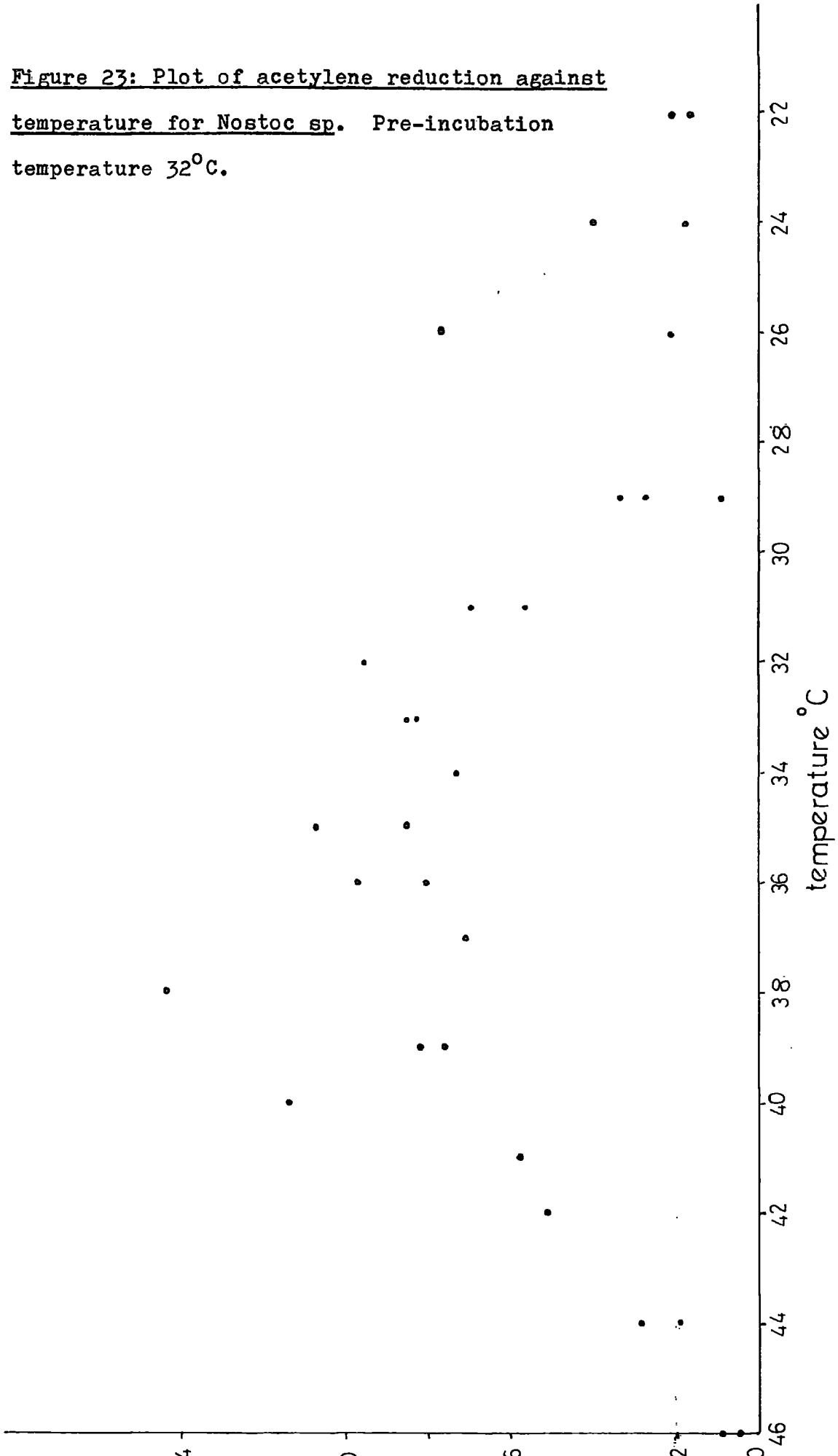


Figure 23: Plot of acetylene reduction against temperature for Nostoc sp. Pre-incubation temperature 32°C.



6 Discussion

6.1 Revival of Aldabran algae

The successful recovery of the Aldabran algae after they had spent over twelve months in the dried state, illustrates their ability to withstand being in this condition. On Aldabra, these algae, particularly N.commune, are frequently in a dried condition, even during the wet season (Whitton 1971). Thus it is essential for their survival that they can retain their vitality during these periods and recover quickly when conditions once more become favourable. Whitton (1971) observed that colonies of N.commune imbibed water very rapidly after a storm, and this has been confirmed experimentally in this study. Nostoc sphaericum and the Nostoc sp also imbibed moisture quickly although not as fast as N.commune. Hence these algae appear to be well adapted to the conditions they experience in their natural environment.

Retention of vitality throughout long dry periods is a characteristic feature of many terrestrial algae, particularly in those which form algal crusts, (Shield and Durrell 1964, Mayland et al 1966). Durrell and Shields (1961) showed that blue-green algae in soil crusts absorbed moisture very rapidly, after a period of drought. These results were similar to those obtained for the Aldabra species in this study.

The length of time that an alga can survive in the dried state will presumably vary with the species and strain. However, Lipman (1941), successfully grew N.commune from an herbarium sheet, in culture, after it had spent 87 years as a dried specimen. This

evidence indicates that this, and possibly other species may be available to survive for even lengthier periods.

Drying down algae should thus be a perfectly feasible and convenient method of preserving them for further use or until they could be properly investigated. They would take up very little storage space and need hardly any maintenance. It would also allow samples collected at different times to be compared.

Before it could be used, experiments would have to be carried out to determine, that an alga could be re-moistened, and to investigate possible effects of drying on the alga. Even if it were not possible to do this with every algal species, it would still be a very useful method.

6.2 Temperature optima

Although the variability of the material prevented accurate determinations to be made it is clear from the results obtained that the two Nostoc commune populations have very different temperature optima. The optimum for Aldabra N.commune lies somewhere in the 30-39°C region, most probably in the higher rather than the lower thirties; the Tarn Moor N.commune has its optimum in the 26-32°C range.

The Aldabra population thus appears to be well adapted to the temperatures it encounters in its natural habitat. During the wet season - the growing period - the average air temperature on Aldabra is about 29°C and one would expect the alga to be several degrees higher than the air temperature due to insolation. On very hot days the algal temperatures would probably be in the high

thirties and might possibly reach the forties. Thus with an optimum in the thirties the colonies of N.commune can take full advantage of these temperatures.

However, the Tarn Moor N.commune has an optimum temperature which is several degrees higher than even the highest temperature it would ever experience in its habitat. The average annual air temperature is only about 9°C, and in summer it is only about 14°C. Temperatures above 20°C do not occur very often. Even allowing for insolation it is unlikely that algal temperatures would reach the 26-32°C range, and if so very rarely. Hence it does not appear to be genuinely adapted to its environment.

This latter result is in agreement with the findings of Kratz and Myers (1955), Fogg (1956), and Allen and Stanier (1968), that blue-green algae from temperate habitats often show temperature optima some degrees higher than the temperatures they would usually encounter in nature. Allen and Stanier found that of twenty-nine pure strains of blue-green algae collected at temperatures never in excess of 28°C, every strain could grow at 35°C, eleven at 40°C, and two at 45°C. They then made use of this fact to select for blue-green algae from a mixed collection of algae, by incubating the samples at 35°C.

Castenholz (1967) holds the view that probably a majority of the blue-green algae have tendencies in the direction of thermophily. He suggests (Castenholz 1973) that this general 'thermal tendency' of the blue-green algae may be a characteristic retained by these organisms since their evolution in the warmer Precambrian era.

Similar results for N.commune were obtained by Taha and El Refai (1963). They found that a local strain of this alga in Egypt had a temperature optimum for growth of about 35°C. Above 35°C, the rate of growth decreased sharply and the alga would not grow at 45°C. They did not comment on the relation of this to the temperatures the alga experienced in its natural habitat, but it seems highly probable that they would be in the high thirties.

Despite its apparent liking for high temperatures the adaptability of N.commune to almost any temperature is illustrated by its occurrence in the Antarctic (Holm-Hansen 1964, Fogg and Stewart 1968, Horne 1972). Fogg and Stewart (1968) found that appreciable nitrogen fixation occurred at temperatures in the vicinity of 0°C but thought that the bulk of fixation probably took place when temperatures of the micro-environment reached 10°C or more. It would be interesting to determine the temperature of these populations of N.commune in the laboratory.

The optima of the N.sphaericum and Nostoc sp also appeared to be in the thirties, the latter species giving fairly clear results. They thus appear to be well adapted to the temperatures of their environment. These results are similar to those reported for other Nostoc sp. (Allison et al 1937, Kratz and Myers 1955).

It is not clear in most cases to what extent the high temperature optima of blue-green algae in the laboratory is due to the favourable laboratory environment. The temperature optimum of an alga may be dramatically or subtly influenced by light

intensity, day length, nutrient concentration and availability, CO_2 concentration, pH, constancy of the several factors, and preconditioning (Castenholz 1969). It thus follows that the only way to ascertain the real optimum of a species is to determine this for natural material in its natural environment. If this study had continued this would have been one of the top priorities.

I used medium to re-moisten the Aldabra material, so it is fairly certain that they were not lacking nutrition. However, on Aldabra it could be quite possible for a nutrient(s) especially a micronutrient to be lacking and this could effect growth and the temperature optimum. Taha and El Refai (1962) have indicated that calcium, molybdenum, and cobalt, are required for the best growth of N.commune.

It is also certain that the conditions under which the experimental algae were pre-incubated bore no resemblance to those they would encounter in nature. Several of the above mentioned factors particularly light intensity would have been very different from those in the natural state. Hence pre-incubation could well have influenced the temperature optima of the algae.

It is virtually impossible, anyway to duplicate the conditions an alga would experience in its natural habitat in the laboratory. The only way to obtain approximate conditions would be to vary the environment of the alga, until the experimentally determined temperature optimum in the laboratory was similar to that determined from natural material in the field. However, this would be very time-consuming and in most cases uneconomical to carry out.

Finally, two other points must be mentioned. The first is that these results are with respect to the effect of temperature on the nitrogenase complex of the alga as indicated by its acetylene reducing ability. Therefore measurement of another physiological parameter might give rather different results, although it seems unlikely that it would differ markedly. Secondly, the results are for the particular set of environmental conditions and material used in this study and again a different set of conditions and material may produce its own particular results.

6.3 Variability of the material

The magnitude of the variation of the material investigated was one of the main features to emerge from the study. It must be stressed that this is in respect of acetylene reduction and other physiological parameters may not have shown a similar variation. It would be interesting to know whether or not this would be the case.

The sample of Aldabra N.commune was only small and hence it was not possible to be selective in anyway when using the alga in experiments. Colonies differed greatly in size and shape, and presumably in age, and could have been affected by drying down, e.g. they could have been photo-oxidised, or by their storage in the dry state at low temperatures. Any of these factors could have contributed in producing variation between the material. Alternatively, as the sample size was small it may well have been just natural variability.

Because of the relatively dry spell in the earlier part of 1974 the population of N.commune on Tarn Moor was very low.

Consequently when samples were collected it was not possible to be selective because there just wasn't enough Nostoc. Thus I could not select for a particular size or shape of colony from the material, to use in experiments, in order to reduce the variation.

Laboratory cultures of the alga with uniform age and size material would probably have given less variability but these were not utilised for two reasons. Firstly, it was not possible to obtain cultures in the short time available for the study. Secondly it is virtually impossible to ascertain that cultures are representative of the natural material or that the laboratory conditions bear any resemblance to the alga's natural habitat.

The Nostoc sp showed the least variability of all the material. This was probably because the colonies of this alga were much smaller than the N.commune ones and much more uniform in size and shape. Consequently it was relatively easy to have replicates which were fairly uniform in experiments.

The cross gradient apparatus was utilised to determine the temperature optima for several reasons. It had been found when using laboratory cultures in the apparatus, that relatively sharp temperature optima could be obtained (B.A. Whitton pers. comm.). Secondly it could be calibrated to give a convenient range of temperatures which could be maintained very easily. Finally, it was thought that a large number of replicates covering a wide temperature range would give an accurate picture of the effect of temperature on the alga, and enable the temperature optimum to be

determined very accurately.

However, on reflection it may have been better in view of the variability of the material to have selected a number of temperatures and placed several replicates at each one. This would have reduced variability by allowing a mean value to be obtained, and have allowed a statistical comparison of the results to be made. But this was done to an extent in the experiment to investigate the effect of temperature on Aldabra N.commune and the results produced were not very satisfactory.

6.4 Acetylene reduction by the algae

Unequivocal proof that the algal species investigated were reducing the acetylene requires the use of pure cultures. However, the low acetylene reduction in dark controls indicated that the alga was responsible for the majority of the reduction.

If acetylene reducing ability is taken as an indication of nitrogen fixation (Stewart 1968) then the results provide further evidence of the nitrogen fixing ability of N.commune and N.sphaericum. The former has been shown to fix nitrogen by Hérisset (1952) and Fogg and Stewart (1968), whilst Pankow and Martens (1964) showed this in the latter. For conclusive evidence that the algal species used were nitrogen fixers, similar results should be obtained using the uptake of N_2^{15}, (Stewart 1973).

The linearity of the time course of acetylene reduction, especially over a long period of time, by both N.commune populations suggests that they would be suitable for further research in this direction. They might be particularly useful in long-term experiments for investigating the effect of changing environmental

conditions on the rate of acetylene reduction.

A notable feature to emerge from the re-moistening experiments on the Aldabra species was the lag of about four hours or more before acetylene reduction commenced. This was probably due to the metabolic processes of the algae being gradually re-activated, particularly the nitrogenase complex which is responsible for the acetylene reduction. Whether this actually occurs in the field or whether it is just an artefact because of the length of time the colonies had been dried is an interesting question. The fact that it occurred in all three species tends to rule out the latter view. Investigation of this phenomenom could be a topic for future research. To clarify the situation similar experiments on material in the field are needed, and these could be very rewarding.

The effect of temperature on acetylene reduction as shown by the temperature experiments on Aldabra N.commune, and the temperature optima experiments is similar to that found by other workers, (Hardy et al 1968, Dart and Day 1971, Gibson 1971, Wheeler 1971). In all cases acetylene reduction activity increased with temperature until it peaked about the optimum, and above that it decreased sharply and at relatively high temperatures practically stopped. However, all these workers were using legumes or non-legumes and although I have searched the literature I did not come across any reports on the effect of temperature on acetylene reduction in algae. Hardy et al (1973) in an extensive review does not quote any papers either.

6.5 Further study

The immediate aim of any further study would be to investigate the variation shown by the material and suggest ways of reducing it. For instance if material was to be dried it would be best to have a standard procedure for doing this as well as one for re-moistening it. Or variability might be greatly reduced by using one particular size of colony. If the variability could be reduced to some acceptable level results would then become much easier to interpret.

To verify and clarify the situation regarding the temperature optima, further experimentation is needed. Experiments could be carried out on cultures as well as natural material. For truly realistic values to be obtained natural material in its natural habitat must be used.

As only two populations of N.commune were investigated in this study, other populations both in this country and in other parts of the world could be studied as well. From this information it would be possible to ascertain the amount of variation in the temperature optima of N.commune populations, throughout the world.

Finally nitrogen fixation by N.commune could be investigated using the acetylene reduction technique. The Aldabra population appears to be very important in the nitrogen economy of Aldabra Atoll and estimations of the contribution of the alga to the nitrogen budget of the atoll could probably be obtained. (Whitton 1971).

Summary

An investigation was carried out to determine the temperature optima of different populations of the blue-green algae Nostoc commune. The two populations investigated were from Aldabra Atoll in the Indian ocean, and Tarn Moor, Cumberland, England. To measure the effect of temperature on the alga, the acetylene reduction technique was utilised.

Dried material of Aldabra N.commune was re-moistened successfully and shown to reduce acetylene at a linear rate. Similar results were obtained for dried material of Nostoc sphaericum and a Nostoc sp also from Aldabra Atoll.

A standard method of re-moistening dried algal material was devised and used in the study.

Nostoc commune from Tarn Moor was shown to reduce acetylene at a linear rate.

Each algal species reduced acetylene at a linear rate under the assay conditions utilised in experiments to measure the effect of temperature on the algae. Rates of acetylene reduction differed between the species.

A preliminary investigation showed that acetylene reduction by Aldabra N.commune was highest at a temperature of 32°C, indicating that the temperature optimum would probably be within a few degrees of this temperature.

The two N.commune populations exhibited very different temperature optima. The optimum of Aldabra N.commune was somewhere in the 30-39°C range indicating that the alga was adapted to the

temperatures it encountered in nature. N. commune from Tarn Moor had its optimum in the 26-32°C range, which was several degrees higher than the temperatures it experienced in its natural habitat. This finding was similar to earlier ones in which blue-green algae from temperate habitats were found to have temperature optima several degrees higher than the temperatures they encountered in nature.

N. sphaericum and the Nostoc sp. were found to have temperature optima in the 30-39°C range. This indicated that they were adapted to the temperatures of their natural habitat.

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