Some aspects of the effect of temperature on the respiratory and cardiac activities of the Cichlid Teleost *Tilapia mossambica*

by

V. JOSMAN

Thesis presented to Rhodes University
for the degree of Master of Science

Department of Zoology and Entomology
Rhodes University
Grahamstown
South Africa

November, 1971.

CONTENTS

(contd. p. ii

CONTENTS (contd. from p. i)

ABBREVIATIONS USED

%	percentage
°/oo	parts per thousand
/	per or or
<	less than
>	greater than
+ve	positive
-ve	negative
°C	degrees Centigrade
cm	centimetre
contd.	continued
etc.	et cetera
Fig.	Figure
ff.	following pages
gm	gram
h	hours
i.e.	that is
l	litre
log.	logarithm
m	metre
mm	millimetre
mV	millivolt
min.	minute
MS 222	tricaine methane sulphonate
p.	page
pp.	pages
ppm	parts per million
pers. comm.	personal communication
Hg	mercury

P.T.O.	Please turn over
P	probability
s	second
S.E.	standard error of the mean
thou.	thousandths of an inch
v	coefficient of variation
vs	against

I. Introduction.

The importance of the cichlid teleost Tilapia mossambica as a protein source, coupled with its remarkable adaptability, has resulted in its introduction into many water systems throughout the tropical, sub-tropical and even temperate regions of the world. However, its successful exploitation of these waters is dependent very largely upon the value of minimum temperatures and their duration. For e.g. Long et al (1961) has drawn attention to the tremendous mortalities of T. mossambica that occur in shallow water bodies during the precipitous temperature decreases that accompany the winter monsoons in Vietnam and other eastern countries, even at temperatures as high as 14 or 16 °C. Coche (1967) does not recommend stocking with T. mossambica where temperatures are not above 14 °C all the time. Allanson et al (1962) conclude, after an experimental study, that low temperatures (13 °C or lower) in South African highveld dams in winter are certainly an important factor in the extensive mortalities of T. mossambica that have been reported from these dams. Jubb (1961) also reports that this species is often killed during a severe winter in Rhodesia.

Tilapia is a thermophilic genus, and T. mossambica is one of the more thermophilic members of the 7 spp. of Tilapia studied by Fukusho (1968). Laboratory experiments by Badenhuizen (1967) and Donnelly (1969a) indicate that preferred temperatures for juveniles of T. mossambica are 27.0 - 33.5 °C and 34.3 - 36.5 °C respectively, and Fukusho (1968) shows that similar sized T. mossambica have a maximum cruising performance at 32 °C. Donnelly (1969b) has shown that under natural conditions, young T. mossambica of under 17 - 19 cm total length have a diurnal rhythm of migration, moving into warm, shallow waters (so-called "nurseries") at 10.00 - 12.00 h, remaining there until 20.00 - 22.00 h when these waters cool down. The fish then go into deep waters to rest.

It is possible that an excessive laboratory stay at uniform temperature may tend to abolish or alter these diurnal temperature responses.

Optimal temperatures for T. mossambica are normally considered as ± 25 to 30 °C and Jubb (1961) has indicated (his Fig. 1) that the optimum range for this species is ± 21.1 to 26.7 °C (70 - 80 °F). Long et al (1961) has suggested 20 - 36 °C as the temperature range for this fish, and that above and below this range lethal temperatures are approached; 20 - 16 °C and below are considered to be lower sub-lethal temperatures. Likewise Jubb (1961) indicates that optimum conditions for this species fall off below 18.3 °C (65 °F) as metabolism is greatly retarded, and Coche (1967) reports that T. mossambica will not breed below 15 °C. At temperatures below 16 °C this fish becomes very lethargic, will not feed and thus does not grow, shoes little sign of gut peristalsis and has such insignificant ventilatory movements that they are hardly discernible. Some fish held in fresh water at 14 °C show degenerative changes in kidney microstructure, seen also in all fish after 4 days at 11 °C (Allanson, 1966, and Allanson & Cross, 1970) and possibly associated with the decreased urine production that has been reported under these conditions (Minshull, 1967). Bok (1968) and Allanson et al (1971) report significant decreases in plasma osmolarity and in plasma sodium and chloride concentrations in fish held in fresh water at 11 °C (only small decreases were noted in similar fish held in 5% sea water). This feature was also recorded in fresh water after transfer to 15 °C by the same authors, but to a smaller extent, and few experimental animals were utilised. Solomon & Allanson (1968) report significantly lowered total serum protein concentrations in fish held in fresh water at 11 °C, but not in fish held in 5% sea water at the same temperature.

The present work on some aspects of the effect of temperature on respiratory and cardiac activities contributes to investigations into the effects of sub-lethal and lethal low temperatures on T. mossambica carried out in the Department of Zoology and Entomology at Rhodes University, some of which has been referred to above.

It is well known that both heart and respiratory ventilatory rates vary with the environmental temperature in poikilotherms such that these rates usually increase with increasing temperature over the range of temperature at which the animal in question can survive (e.g. Hasan & Qasim, 1960; Kropp, 1947; Labat, 1966; Long et al, 1961, and Tsukuda 1961). The pacemaker fibres of the heart in fish and other vertebrates are very temperature sensitive and it is these fibres which are largely responsible for the rate with which the heart beats. Other cardiac functions are also affected by temperature, the various time intervals of the electrocardiograph (ECG) being lengthened by low temperatures, and in carp and other teleosts, Labat (1966) has shown that low temperatures (10 - 0 ^{o}C) have a particularly marked effect on the duration of the Q-T interval and T hump of the ECG. Both of these periods are increased.

During acclimation to non-lethal low temperatures, poikilotherms show changes in metabolic rate. The pattern of change is quite variable in different animals, and even for different organs, tissues and cell homogenates (Prosser, 1964, Fig. 5, p. 17). Usually the acclimatory changes have a clear physiological significance, e.g. an increase in metabolism at low temperatures permits increased activity, growth etc. 1.)

1.) The use of the term acclimation in this report will follow that described by Bullock (1955), and will thus be used for those regulatory modifications in response to a changed environment that occur during the life of an individual, usually within days or weeks.

in contrast to non-acclimated animals at this temperature which may be virtually immobile. Other acclimation patterns are not so obviously significant and, in some animals, physiological functions may not show an acclimatory change at all, even at temperatures at which the animal is known to survive quite readily.

The ventilatory rate reflects the over-all metabolism of the organism, and such a relationship between opercular movement rate and oxygen consumption has been recorded for fish (Sumner & Wells, 1935) and Tsukuda (1961) reports that the temperature - respiratory frequency curves reflect the temperature - oxygen consumption relations at least within a moderate temperature range. Metabolic changes during thermal acclimation may therefore be reflected in ventilatory rate changes, as found by Sumner & Wells (1935), Meuwis & Heuts (1957), and Freeman (1950). The opercular rate has also been utilised as an indicator of thermal acclimation by Precht et al (1966) and Peak et al (1967).

Heart rate is also reported to be directly related to metabolic rate in both poikilotherms and homoiotherms (Prosser & Brown, 1961, p. 274). This relationship has been illustrated in specific studies, for e.g. by Owen (1969) for the teal (Aves), by Morhardt & Morhardt (1970) for a variety of rodents, and by Spitzer et al (1969) in a study in which oxygen consumption, heart and ventilation rates were measured in bluegill sunfish under varying conditions of water oxygenation. The graphical data of this study on the effects of hypoxia indicate that a linear relationship exists between oxygen consumption and heart rate of these fish at 13°C and 25°C, and a nearly linear relationship at 30°C. This is illustrated in Fig 1a below by re-plotting the data given by Spitzer et al (1969).

Figure 1a.

The oxygen consumption of the bluegill sunfish Lepomis macrochirus (from Spitzer et al, 1969, Figs. 2 & 4) as plotted against the changes of ventilation (V) and heart rate (HR) during changes in the oxygen tension of the water as recorded at 13°, 25° and 30°C. The numbers next to plots indicate the oxygen tensions in mm Hg at the extremes of the oxygen tension range, and the arrow indicates the direction of increasing oxygen tension. Graphs drawn in by eye to indicate the trends present.

● 13°C(HR) ◈ 25°C(HR) ○ 30°C(HR)

⊙ 13°C(V) ▲ 25°C(V) ✱ 30°C(V)

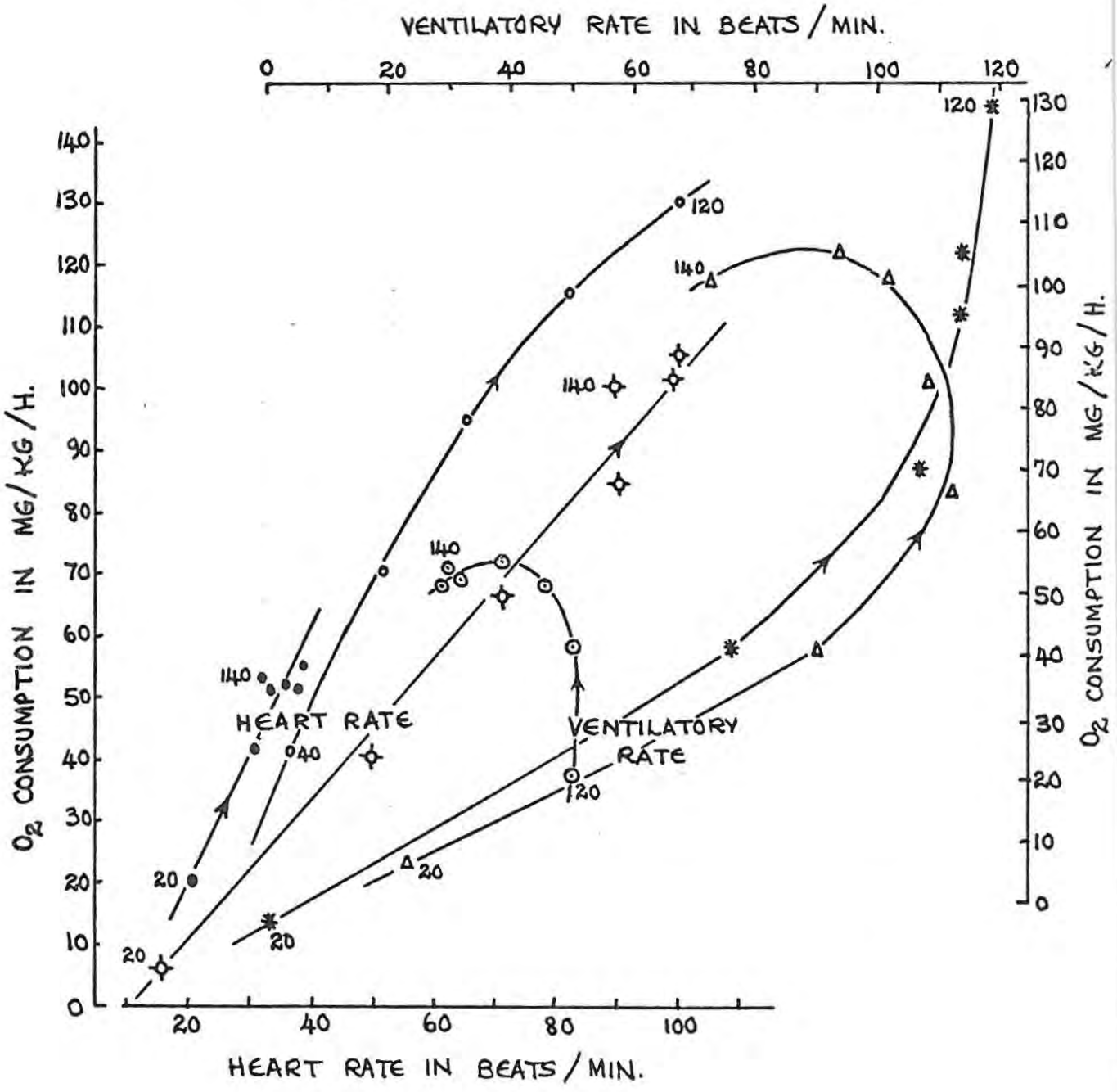


Fig. 1a also indicates that ventilation rate of bluegills is not linearly related to oxygen consumption under the progesssively hypoxic experimental conditions except at 30°C.

The ventilatory and heart rates of fish are not necessarily indicators of over-all respiratory or cardiac function respectively, as stressed by Tsukuda (1961, p. 36), but they may be indicators of whether acclimation to temperature change is occurring or not because of their relationship to the rate of metabolism. It is also important to remember that the process of acclimation itself requires energy and thus depends on metabolism.

Acclimation of heart-rate to temperature is well known in poikilotherms and in fish, (e.g. Jankowsky, 1968) although Prosser (1962) p. 12 reports that goldfish heart shows no metabolic acclimation to cold. However, few other aspects of heart function during acclimation have been studied. Labat (1966) presents rate-temperature information for heart rate in barbel and in intact and bi-vagotomised carp acclimated to different temperatures (his Figs. 48 to 52); he also gives the variations in heart rate in a number of intact and bivagotomised carp during a large portion of a seasonal cycle (his Fig. 56) which, on analysis, indicate that winter fish have heart rates which are raised relative to spring fish at the same low temperatures predominantly because of a lack of vagal inhibition. On the other hand Randall & Smith (1967) note that in Tinca, on average, heart rate is lower in winter, but these authors do not give the conditions under which these measurements were recorded, e.g. temperature. Hart (1957) reports seasonal changes, in relation to heart rate, in blood pressure, ventricular weight and possibly stroke volume in 3 spp. of fresh water fish, such that blood pressure is higher, stroke volume lower and ventricular weight greater in winter, and Tsukuda (1961) shows heart rate adaptation in 18° as compared to

23° and 28° C acclimated fish (Oryzias and Lebistes).

As already mentioned, T. mossambica shows decreased plasma osmolarity and lowered sodium and chloride concentrations in fresh water at temperatures of 15° to 11° C (Bok, 1968, and Allanson et al, 1971). These plasma electrolyte changes have been associated with kidney disorganisation in this fish under these same conditions by Allanson (1966) and Allanson & Cross (1970), a feature these authors consider may, in turn, be associated with cardiac malfunction. On the other hand it must be pointed out that Prosser et al (1970) consider lowered salt concentrations in fresh water fish in the cold an energy-saving feature, seen for e.g. in the cold-acclimated goldfish, and also reported by Houston & Madden (1968) for cold-acclimated carp.

The relationship between plasma constituents and their concentrations, temperature acclimation and the normality of the general physiological function of the organism is not a simple one, as pointed out by Meincke (1970), p. 289. Each species must, therefore, be studied individually and from as many angles as possible in order to gain some insight into the problem, as has been done by Houston and co-workers (e.g. Houston et al, 1970). Body electrolyte regulation is often considered important from the point of view of membrane-dependent phenomena (nervous and muscular activities), and, as suggested by Houston & Madden (1968) and other authors, also for the regulation of intermediary metabolism, particularly with regard to the enzymes involved. Obviously metabolism is, in turn associated with the energy-consuming processes involved in electrolyte regulation, so these may form a feedback system as is also pointed out by Houston et al (1970).

T. mossambica may be particularly sensitive to plasma ionic changes, as suggested by the fact that the cold-induced osmotic

breakdown mentioned above has been implicated in secondary chill coma (involving obvious central nervous breakdown, opercular movement cessation etc.) which occurs below about 11 °C in fresh water animals during slow temperature decreases (Allanson et al, 1971; Bok, 1968 and Solomon & Allanson, 1968) Also coma and death occur at a lower temperature in water of higher total dissolved solids (TDS = 130 ppm) as compared to borehold water (TDS = 19 ppm) Allanson et al, 1962, and as pointed out by Jubb (1967b), at its most southerly limit of distribution in South Africa, T. mossambica is more common in salt water estuaries than it is in fresh water, and this fish can tolerate lower temperatures in saline water than it can in fresh water. In addition water-electrolyte balance regulation of fish is influenced by the mass transport activities of the cardio-vascular and respiratory systems (Houston & Madden 1968), as these activities affect gill water and ion exchanges, oxygen uptake and distribution and, indirectly, metabolism (Houston et al, 1968). These are important concepts to be borne in mind in relation to the present work on T. mossambica.

Thus, to summarise these introductory remarks relevant to the present investigations, ventilatory and cardiac functions are important because of their close relation to metabolism and as indicators of metabolic acclimation, and also because of a possible relationship to kidney function and osmotic regulation in this cold-sensitive fish.

After some preliminary investigations which included the basic morphology of the heart, the characteristics of the electrocardiographs (ECGs) and of the electrical recordings of the ventilatory activity of the opercular/buccal musculature, and the possible long-term effects on ventilatory functions of handling and the anaesthetic used, my investigations have been concerned primarily with changes in some

respiratory and cardiac functions with temperature changes (particularly decreases) under different acclimation régimes both as the temperature changed and for a few days thereafter (i.e. short-term changes, Parvatheswararao, 1968), the time at which recordings were made being recorded so that any tendency towards a diurnal rhythm could be detected. The variables studied included the durations of both the cardiac and ventilatory respiratory cycles and the inversely related heart and ventilatory rates. The times taken for the myocardium to transmit potentials and to contract and relax again were estimated by measuring the distances between specific, clear deflections of the ECG, and the T-hump duration was observed. The height of the QRS complex was determined in some cases as well as whether a cardio-respiratory correlation exists or not. Thus it was hoped to ascertain whether or not acclimation or other changes of any of these physiological functions occurred during the experimental periods, particularly when the temperature was lowered to values in the lower sub-lethal range for this fish. Some introductory comments pertinent to the actual temperature changes, times used etc. are given prior to the experimental results (pp. 60 to 62).

II. Materials and methods.

(a) Obtaining and holding of fish.

Tilapia mossambica was caught in North End Lake, Port Elizabeth. This lake, formerly called Salt Lake, is a lake of salinity 1 to 3°/oo (Jubb, 1967a) and is always warm as its water is circulated through the adjacent cooling system of a large power station. T. mossambica was introduced into the lake in 1961 and has flourished; this is again an indication of the thermophilic nature of this fish.

Fish were transported as rapidly as possible to Grahamstown in open buckets which allowed splash, and were transferred either to 44-litre glass holding tanks, or to larger concrete tanks or enamel baths. During the winter season the tanks or baths were heated so as to maintain a temperature of 21 to 22°C as these fish are susceptible to fungal attack (Saprolegnia) if water temperatures should remain below 20°C for any length of time (room temperature is usually about 18°C). Fish were fed daily on a mixture of yellow/white maize meal and fish meal, and tanks were cleaned daily. The water was aerated and, in the case of the 44-litre holding tanks, was supplied with running water. All water supplied was tap water of total dissolved solids (TDS) of approximately 322 ppm, and a pH of 8.4. Under the holding conditions fish maintained their weight well, and in the large concrete baths they grew and sometimes bred. An artificial light-dark régime was not used, only natural light from the windows.

(b) Heart dissection.

The morphology of the heart was ascertained purely in support of the physiological work, and the resulting drawings thus do not represent

an anatomical study, and are therefore diagrammatic. Some fish that had succumbed were dissected fresh or after preservation in formalin. As the fish were usually small (11 - 14 cm standard length), a binocular microscope was used for the dissections. The operculum was removed on one side and the heart exposed and examined from this side. Water flow from a rubber tube with a finely drawn glass nozzle was used to determine the presence of and effectiveness of valves.

(c) Implantation of electrodes.

Fish 11 - 15 cm standard length (distance between the snout and the base of the caudal fin), weight 35 - 90 gm, were selected at random from the holding tanks, and were rapidly anaesthetised by transferring them to a solution of Sandoz MS 222 (150 - 170 mg/l) freshly made up in tap water at approximately the same temperature as the holding tank. Fish took 2 to 5 min to become fully anaesthetised (complete cessation of opercular and all other movements and lying ventral side up). Fish were gently dried, weighed on a rough balance to the nearest 0.1 gm, and their standard length was measured to the nearest 0.1 cm.

Leads and electrodes were prepared previously in the following way: A plastic-insulated copper wire lead was soldered to a 5 cm length of fine shellac-insulated copper wire 0.0254 - 0.0324 cm (10 - 12 thou) in diameter. The cut end of this fine wire and the region of soldering were later insulated with nail varnish or an araldite glue. A tiny region approximately 1 mm in length, and half way along the fine wire, scraped all around to remove the insulating shellac, was then 'silvered' with solder. This small region formed the electrode. Electrodes were inserted into the fish in the region of the heart using the "bipolar" method of Labat (1966) in order that the fish be symmetrically harnessed without mechanical stress on the electrodes, as the experimental period would be a long one. However, a "unipolar" method (only the positive

(+ve) electrode implanted) would have been satisfactory from an electrophysiological point of view, as used for e.g. by Serfaty & Reynaud (1956); alternatively only one, the +ve electrode, need be near the heart, the other being implanted in the back or tail (e.g. Remorov, 1964).

A sharp, gently curved size 12 syringe needle was inserted through the ventral body wall of the anaesthetised fish near the midline in the gular region, about 4 mm anterior to the posterior edge of the operculum. The needle point was pushed dorsally at first, then more posteriorly and laterally so that the point again penetrated the body wall just posterior to the operculum and in front of the pectoral fin (drawing 1, Fig. 1b).

The free end of the fine copper wire was then inserted into the bore of the syringe needle from the point end (drawing 2, Fig. 1b) and when it came out the other end, the syringe needle was withdrawn (drawing 3, Fig. 1b).

The silvered electrode region was then positioned approximately 5 to 7 mm inside the ventral skin surface (i.e. in the region of the heart), and was held in this position after insertion through a small cylinder of firm plastic by bending the wire back over the cylinder and then round a few times (drawing 4, Fig. 1b).

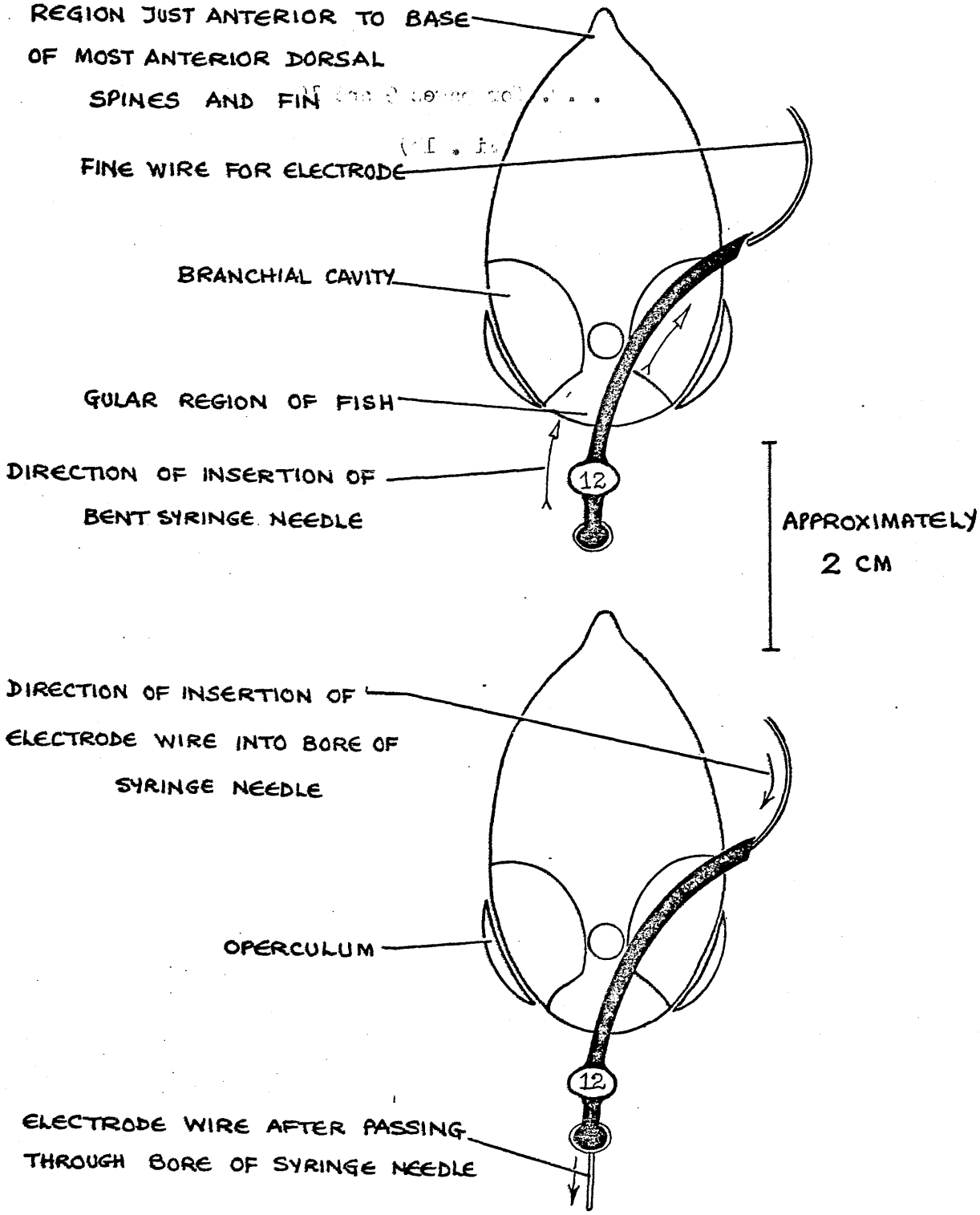
A second electrode was similarly implanted on the other side of the fish, one being used as a +ve, the other as a negative (-ve) electrode. The leads from the 2 electrodes were then passed through holes in a perspex holder before being firmly tied, together with a piece of foam plastic to prevent slipping, with strong cotton thread (drawing 4, Fig. 1b). Thus the fish was harnessed just posterior to the opercula, and on recovery from the anaesthetic could move and ventilate quite freely. The operation took under 5 minutes, and recovery of the fish took 5 - 15 minutes. A few fish did not recover.

P.T.O. for pages 9 and 10

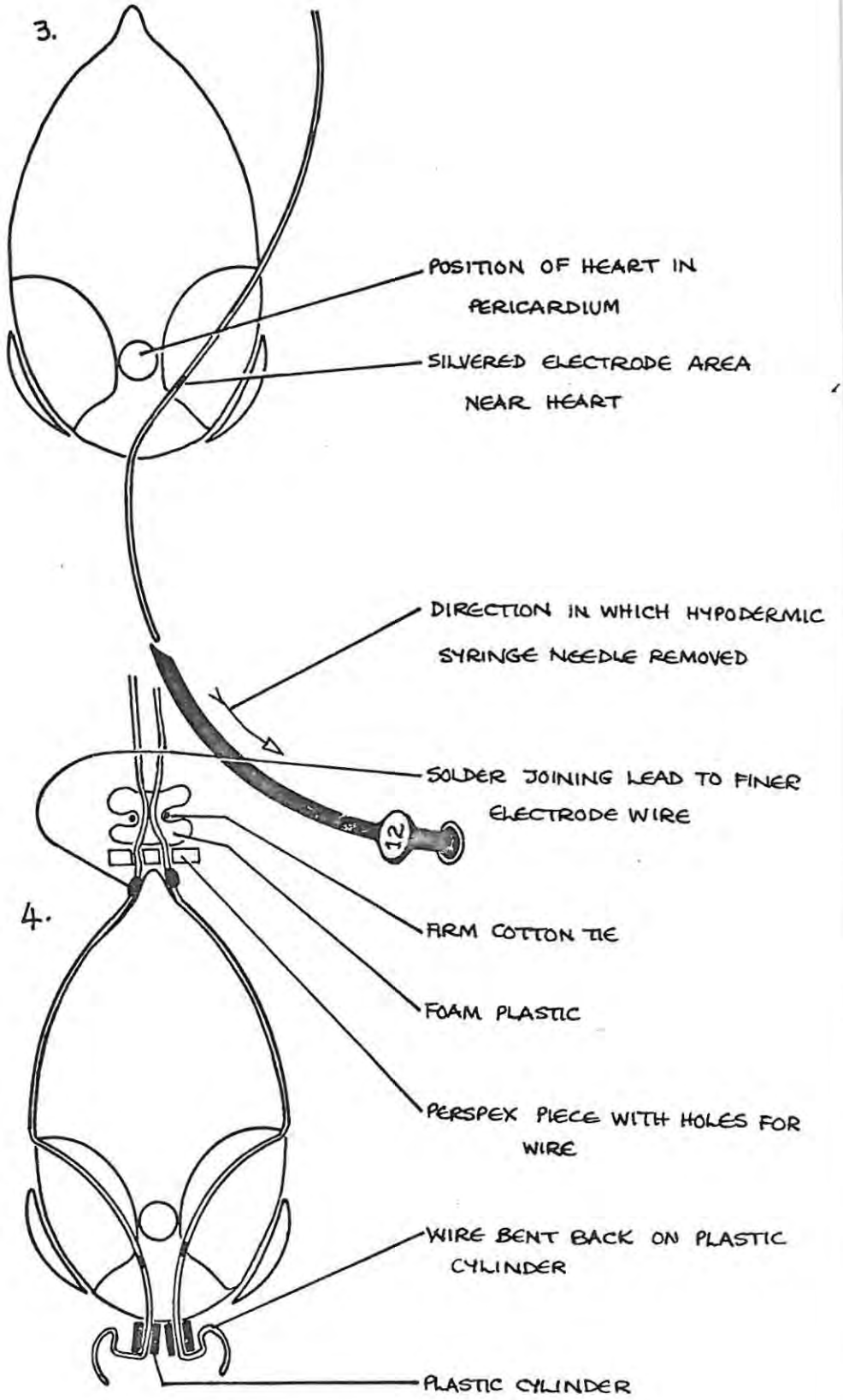
(Fig. 1b)

Figure 1b

Diagrams illustrating successive stages during the insertion of electrodes into the gular or heart region of T. mossambica. The fish is shown in T.S. through the heart region as seen from the front of the fish. See opposite for explanations of each of the stages shown.



1. Hypodermic syringe needle inserted from ventral side of fish and electrode wire about to be pushed into bore of the needle.
2. Electrode wire pushed through needle.
3. Syringe needle removed leaving electrode wire through fish.
4. Positive and negative electrodes in position and made firm so that fish "harnessed" by the electrode wires.



The fish was then placed in a labelled experimental chamber of transparent plastic, and the leads passed through a hole in the opaque lid where they were firmly tied with strong cotton together with a large piece of foam plastic so that the lead ends did not slip down into the fish chamber (Fig. 2). This allowed free turning of the electrode leads as the fish moved.

(d) Holding of fish post-anaesthesia.

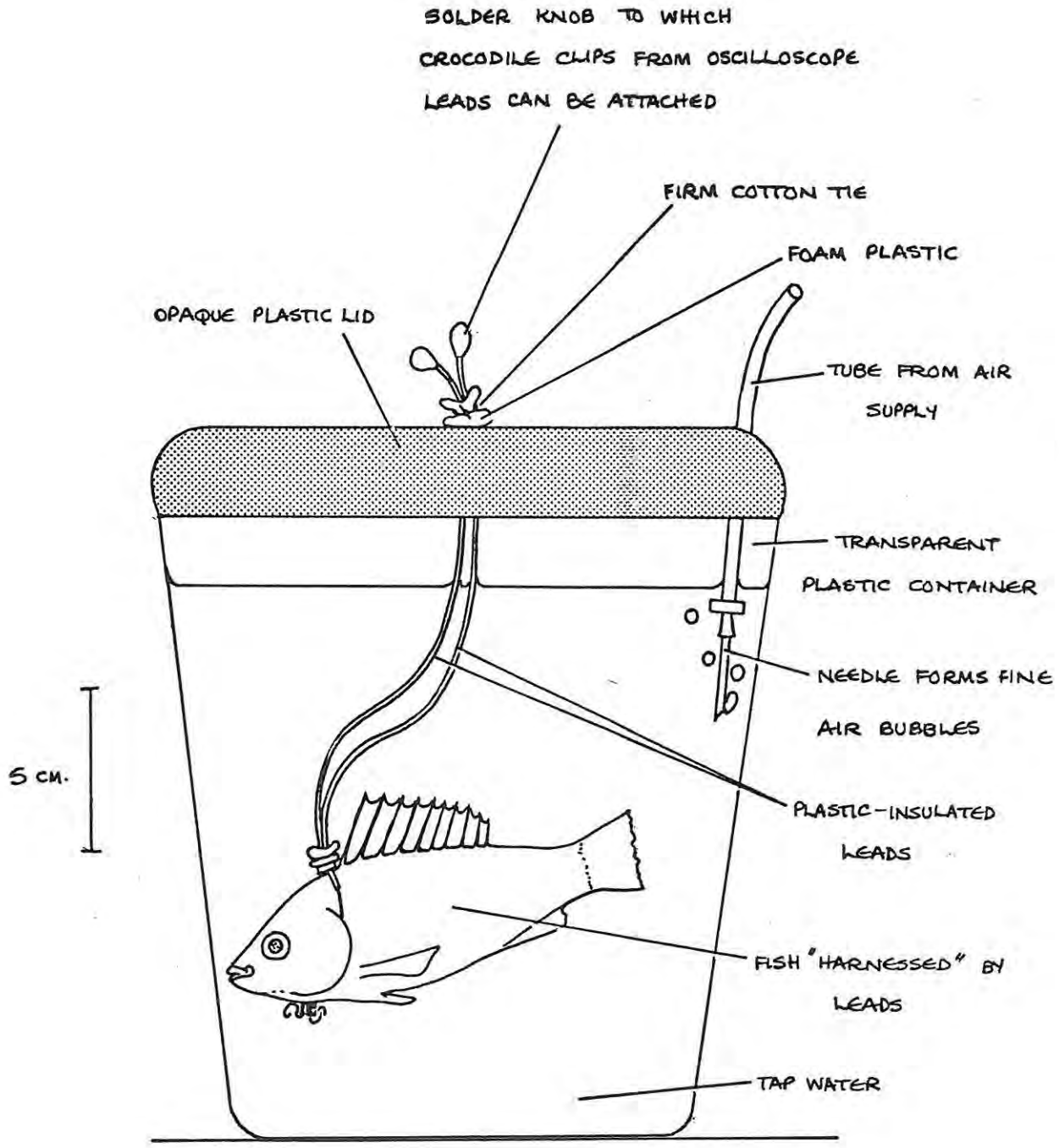
The plastic fish chambers were placed in a plastic baby bath which served as a constant temperature water bath by placing a Bühler of other heater-stirrer in the water at one end. Space was left between adjacent fish chambers to allow free water circulation and the water was found to be of equal temperature throughout the bath. The apparatus was kept in a constant temperature room with a temperature $1 - 2\ ^{o}C$ lower than the required water bath temperature. Each chamber was supplied with a bubbler which maintained good oxygen levels (oxygen content checked using the Winkler technique). Water was gently siphoned off from the fish chambers daily and replaced with water at the same temperature. During long experiments fish were fed daily if the experimental temperature was $25\ ^{o}C$ or above, and, as little food was normally taken, uneaten food was removed after 1 h. Fish held at lower temperatures were not fed. The constant temperature room had a light-dark régime of 12 h each, lights being operated automatically at about 6 a.m. and 6 p.m. by means of a Venner time switch Type B3.

Temperatures were changed as follows:-

Increasing the temperature: The setting of the Bühler or other heater-

Figure 2.

Diagram of fish with electrodes implanted as seen swimming in the experimental chamber.



stirrer was altered as desired, and the temperature in the fish chambers changed fairly rapidly. Temperatures were checked with an alcohol-in-glass thermometer to within 0.1°C. These thermometers were later calibrated against an accurate mercury-in-glass thermometer. Room temperatures were also altered so that they were 1 to 2°C below the desired water temperature.

Decreasing temperature: If the rate of temperature decrease required was slow, the heater-stirrer and room temperatures were altered as for Increasing temperature above. However, if rapid temperature decreases were required (e.g. a 7 or 8°C decrease in 2 hours), the alterations in air and consequently water temperature were speeded up by the judicious addition of ice cubes and iced water at 10 to 15 min intervals both to the water bath and to the fish chambers, after removal of a little of the warmer water by syphoning in each case.

(e) Recording of electrocardiographs and other electrical phenomena.

A Cossor oscilloscope camera was adapted for use on a Telequipment Laboratory Oscilloscope with two type C amplifiers. These amplifiers can, theoretically, display a potential difference of as little as 0.1 mV on the screen as a 1 cm deflection. This apparatus was found to be suitable for the recording of ECGs by recording ECGs from Xenopus laevis and comparing these with ECGs of this animal reported in the literature (see section VI (a)).

The Cossor camera has an electrically driven motor which can move 35 mm film across the back of the camera at a rate that can be varied between 0.127 and 63.5 cm/s (0.05 to 25 inches/s) according to the manufacturers. This allows continual recording when the oscilloscope beam is arrested as a vertically moving 'spot'. Film speeds commonly used

were 0.634 and 1.270 cm/s. These speeds were checked and calibrated by running paper film through the camera and marking it with the aid of a stop watch. The above speeds were found to average 0.678 and 1.434 cm/s respectively. These checked figures were those used in all relevant calculations.

The early siting experiments were done using a blue-sensitive paper film (Kodak Linagraph RP.30), but as this film was old and tended to develop gray rather than white and also tended to tear at the sprockets, negative film was subsequently used. Both Kodak Tri-X Pan and Ilford HP3 film (both approximately ASA 400) were found to be sensitive enough and suitable in that tearing was negligible.

A fish chamber (with air bubbler removed) was taken from the water bath, dried to reduce water evaporation on the outside, and placed on a 'floating earth' (a polystyrene box covered with thick aluminium foil) which was in turn placed above the oscilloscope resting on the metal framework of the oscilloscope trolley. After the fish had settled down (if any excitement was noticed), a recording was taken. Interference from surrounding electrical machinery was reduced still further by surrounding the fish chamber with aluminium foil-coated boxes which were in direct contact with the 'floating earth'. The earth of each of the Telequipment type C amplifiers was also electrically connected to the 'floating earth' (all other earths e.g. taps, leads to copper plates buried in the soil with rough salt, etc. were all found to be unsuitable).

For each recording the following information was noted:

Temperature of the water in the fish chamber before and after a recording (this changed very little, maximum change 0.2 °C).

The rate of the opercular/gular movements if visible (in some later experiments only).

Air temperature (at the beginning of a series of recordings).

Film speed.

Fish name (a letter of the alphabet or a Roman numeral).

Y-axis amplification and which amplifier (upper/lower) was used.

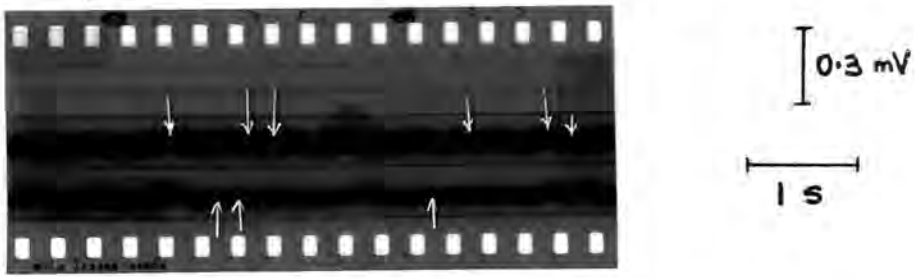
Date and time.

The negative film was marked by scratching before and/or after the recording was made for later identification. Film was cut into ± 2.3 m lengths for tank development using ribbons, and the pieces were later re-joined with sellotape. A strip of film approximately 1 m long was exposed for each fish or concurrent pair of fish (if both oscilloscope beams were used). An example of developed film showing an ECG recording is given in Fig. 3.

Figure 3.

Developed negative film showing oscillograph traces.

Arrows indicate deflections of an ECG.



(f) The measurement of rates and intervals.

Negative film was placed on a glass sheet 0.4 X 15 X 100 cm supported over a white surface in bright light. For each fish at each recording the opercular/buccal cycles in 8 cm were counted wherever possible. From these the respiratory rate and the duration of the related respiratory cycle were calculated. The QRS complex height was measured ± 6 times and, if possible, the QRS complex duration for the upper and lower experimental temperatures. From 8 to 40 of each of the

cardiac intervals visible (R-R, R-T and P-R; see Fig. 9 for details) were measured and averaged. The R-R interval is a measure of the time period between two consecutive heart beats, the R-T interval is a measure of the time period for which the ventricle remains contracted, and the P-R interval is a measure of the time taken for the excitatory impulse to travel from the atrium to the ventricle. It was not possible to measure the duration of the T-hump accurately as it was not at all clear as to where this deflection began or ended; however over-all changes in T-hump duration were noted.

The distances on the film for the respiratory cycle lengths, cardiac intervals etc. were then converted to time in seconds using the conversion factors given in (e) above. Respiratory rate measurements as calculated from the film usually compared fairly well with those observed visually although some difficulties were encountered because of inconsistencies between the trace and the visual observations, and because of some strange deflections of an unknown nature. These problems are discussed in section VI (b).

(g) Post-mortem dissections to locate electrode positions.

Fish were anaesthetised at the end of an experimental series and allowed to die. The positions of the silvered electrode areas of both the +ve and -ve electrode were recorded, particularly in relation to the heart and the pericardium. The presence of tissue damage caused by the electrodes or the leads, or of fungus infection, were also noted.

III. Results.

(a) The morphology of the heart of T. mossambica.

In general, the heart of T. mossambica is an advanced teleost heart, with conus very reduced and a well developed bulbus (Goodrich, 1958). The arrangement of the heart chambers is typical with sinus venosus and atrium dorsal to the ventricle and bulbus (see Fig. 4). The fish used (standard length approximately 11 to 14 cm) were usually those used for the physiological experiments, and the hearts of these fish were approximately 1 to 1.5 cm long and were therefore rather tricky to investigate. Only one large specimen with a heart of 2.5 cm long was available for dissection.

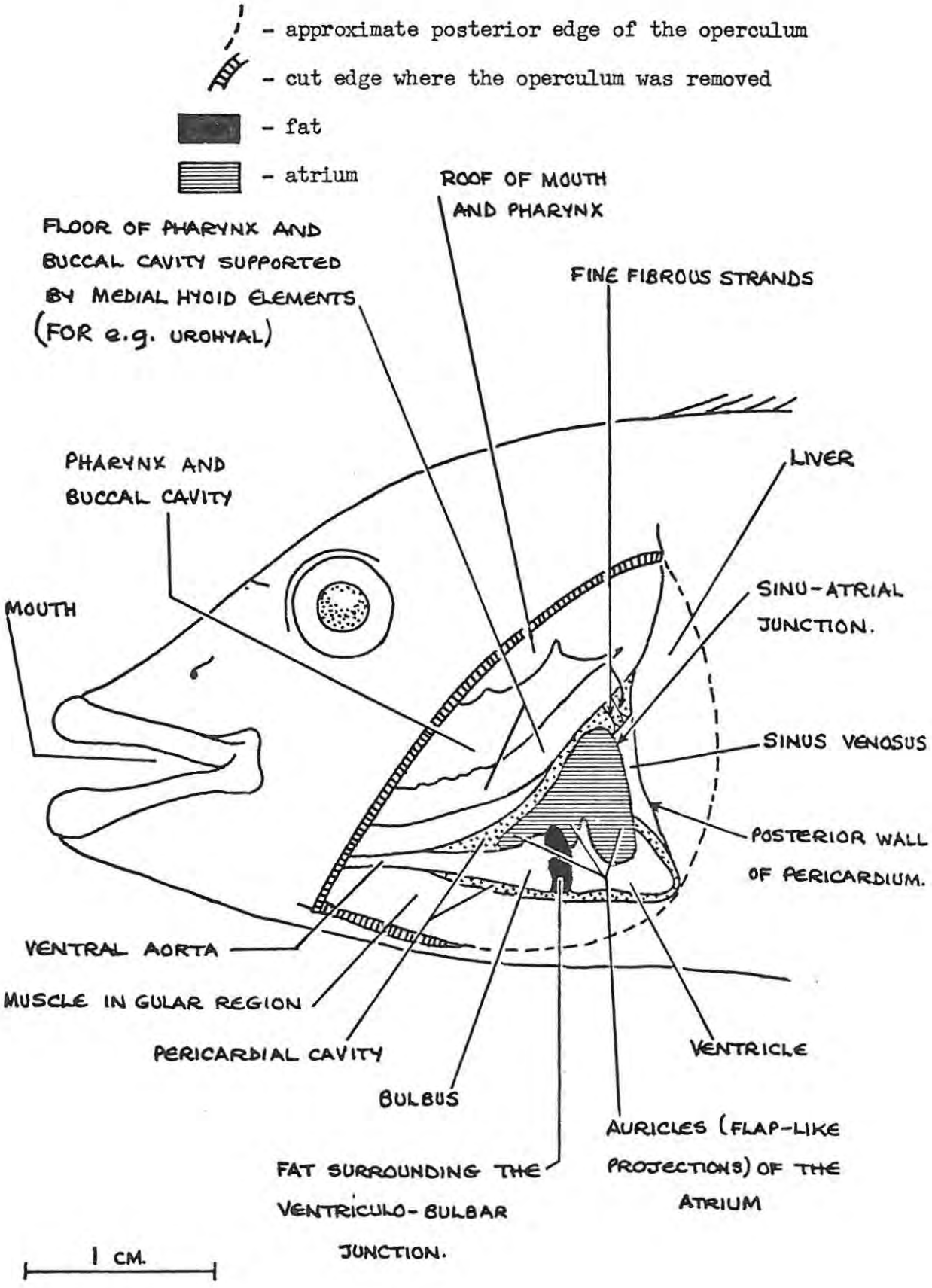
PERICARDIUM

The heart fits tightly into the pericardium which is a bag of varying thickness and composition. Posteriorly the pericardium is pigmented, strong and fibrous, and together with a muscular septum separates the liver and other organs in the coelom from the heart and the pericardial cavity. Elsewhere the pericardium is tenuous, transparent and thin and is closely applied to the muscles of the gular region ventrally and to the inner borders of the posterior pairs of gills. No pericardio-peritoneal canals were seen.

Please consult Figs. 4, 5, 6 and 7 which illustrate the following description. Fig. 4 shows the heart in situ as seen from the left had side. Fig. 5 is a ventral view of the heart showing atrial asymmetry (found fairly commonly), and the sinu-atrial valves. Fig. 6 is an enlarged drawing of the heart, seen from the left hand side, but removed from the fish. In Fig. 7 the sinus venosus, atrium, ventricle, ducts of Cuvier and bulbus have been slit open in order

Figure 4.

Diagram of the branchial chamber and pharynx area of Tilapia mossambica (animal's left hand side) with the operculum, gills, and part of the pericardium removed to show the heart.



to illustrate the morphology of the valves and other internal structural features; the heart is again viewed from the left hand side.

SINUS VENOSUS

The sinus venosus is a very thin-walled chamber containing very little (if any) muscle. Together with the two ducts of Cuvier the chamber is T-shaped with the base of the T resting against the fibrous posterior wall of the pericardium and receiving the hepatic veins from the liver through holes in the pericardial wall. Some small veins also enter the ducts of Cuvier through the posterior pericardial wall. The sinus venosus, in some cases, is slightly displaced to one or other side of the midline. It is often anchored to the dorsal wall of the pericardium by fine fibrous strands (Fig. 4).

SINU-ATRIAL JUNCTION

In smaller fish no valves could be seen between the sinus venosus and atrium; however one larger specimen was available and clearly indicated two thin semi-circular valves at this aperture which approach each other to close the aperture. The opening and closing of these valves was clearly seen using fine jets of water from the sinus venosus and atrial sides respectively. These features are illustrated in Figure 5. The larger flap is placed on the dorsal side.

ATRIUM

The atrium is a large, thin-walled chamber of variable shape and dimensions. There is a more or less central chamber

contd. on p. 22

Figure 5.

Diagram of the heart of Tilapia mossambica removed from the fish and seen from the ventral side. Sinus venosus and bulbus have been cut open to show internal structural features.

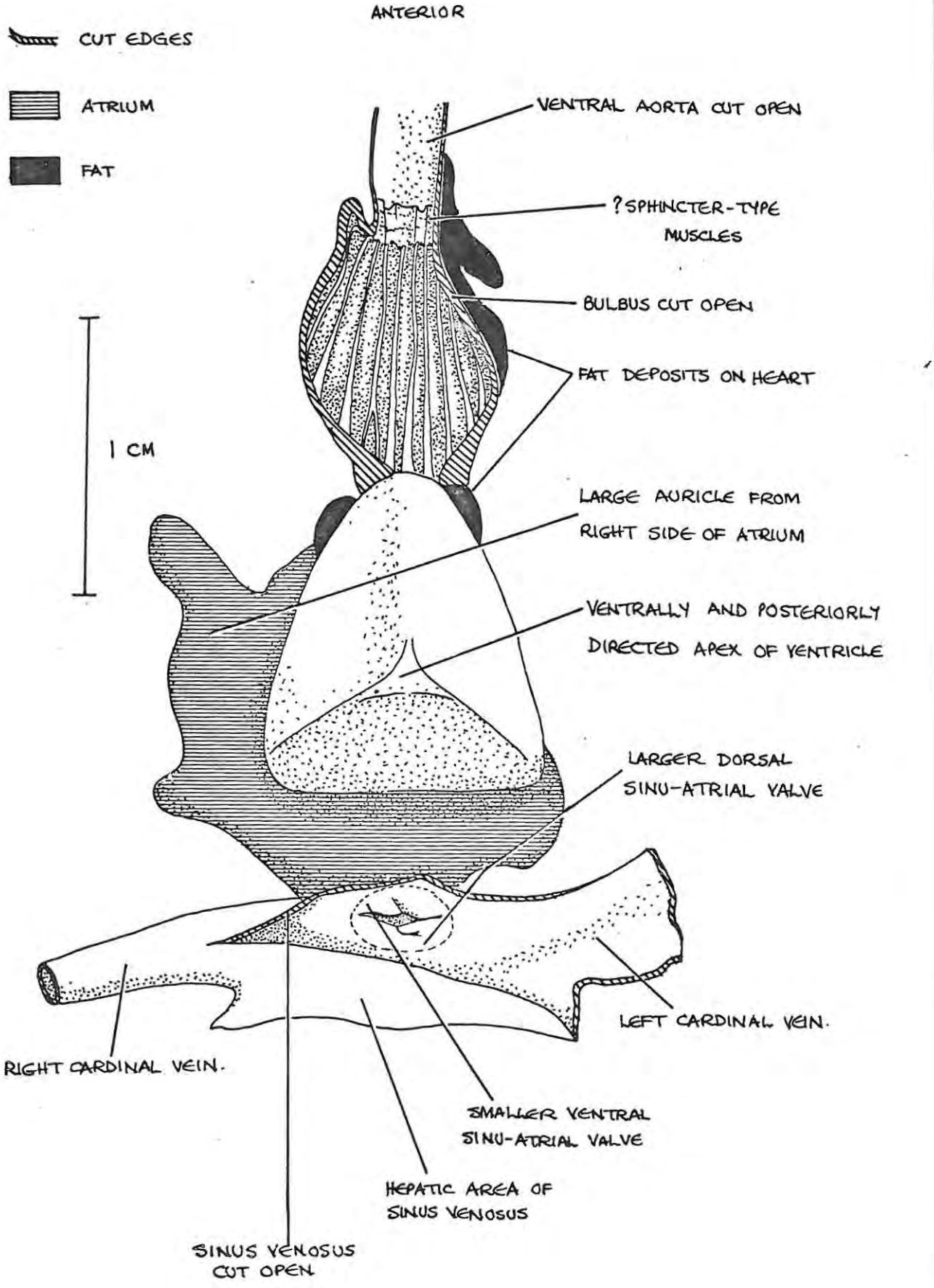
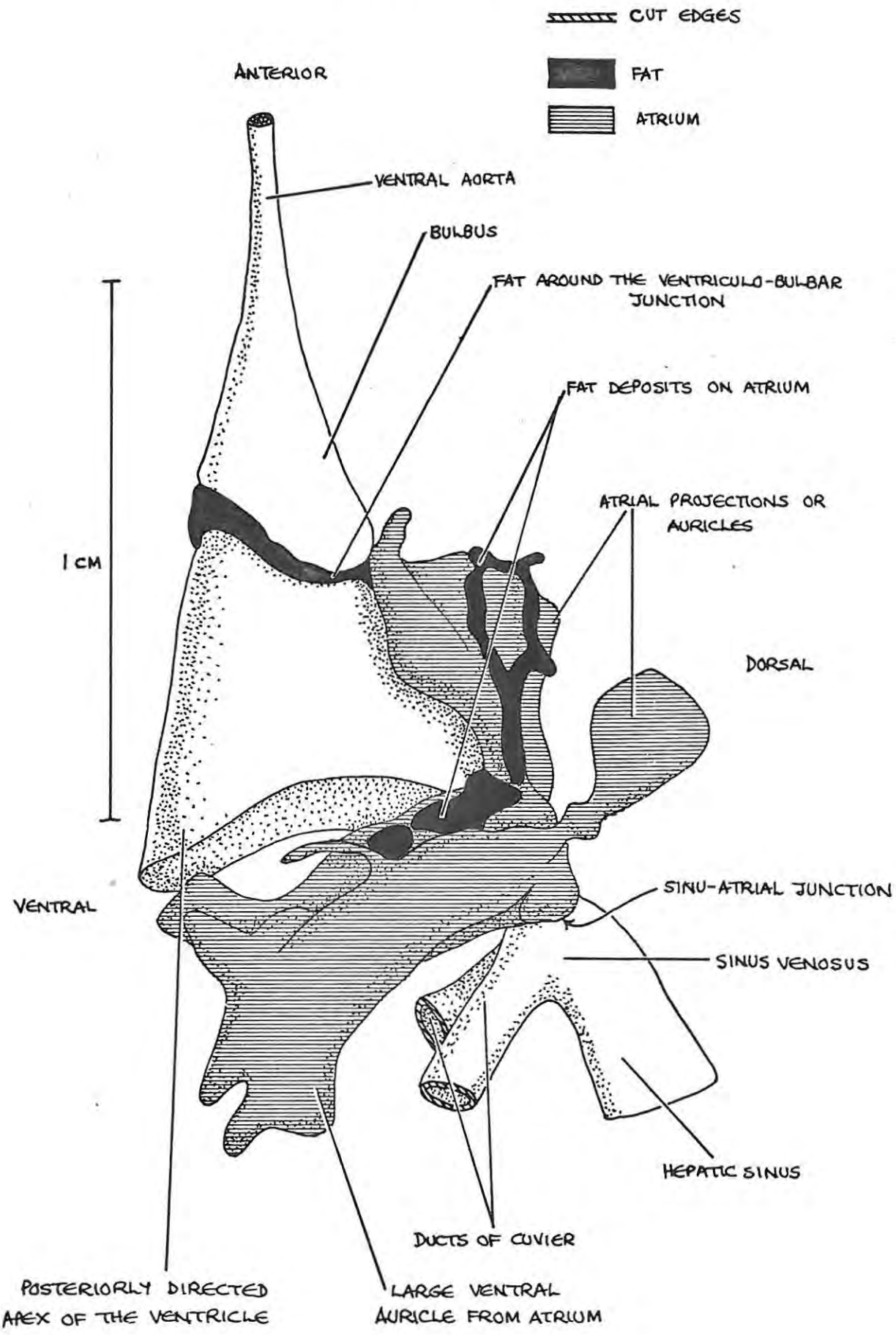


Figure 6.

Diagram of the isolated heart of Tilapia mossambica as seen from the left hand side.



from which there are a number of anterior, dorsal and latero-ventral extensions or lobes, the 'auricles'. The atrium of some fish is thus not bilaterally symmetrical. The various 'auricles' of the atrium seem to fill up the space available within the pericardial cavity dorsal and lateral to the ventricle. The lumen of the 'auricles' is crossed by strands of muscle, the musculi pectinati (Fig. 7). These strands prevent excessive dilation of the atrium and are connected to the muscle within the outer wall of the atrium.

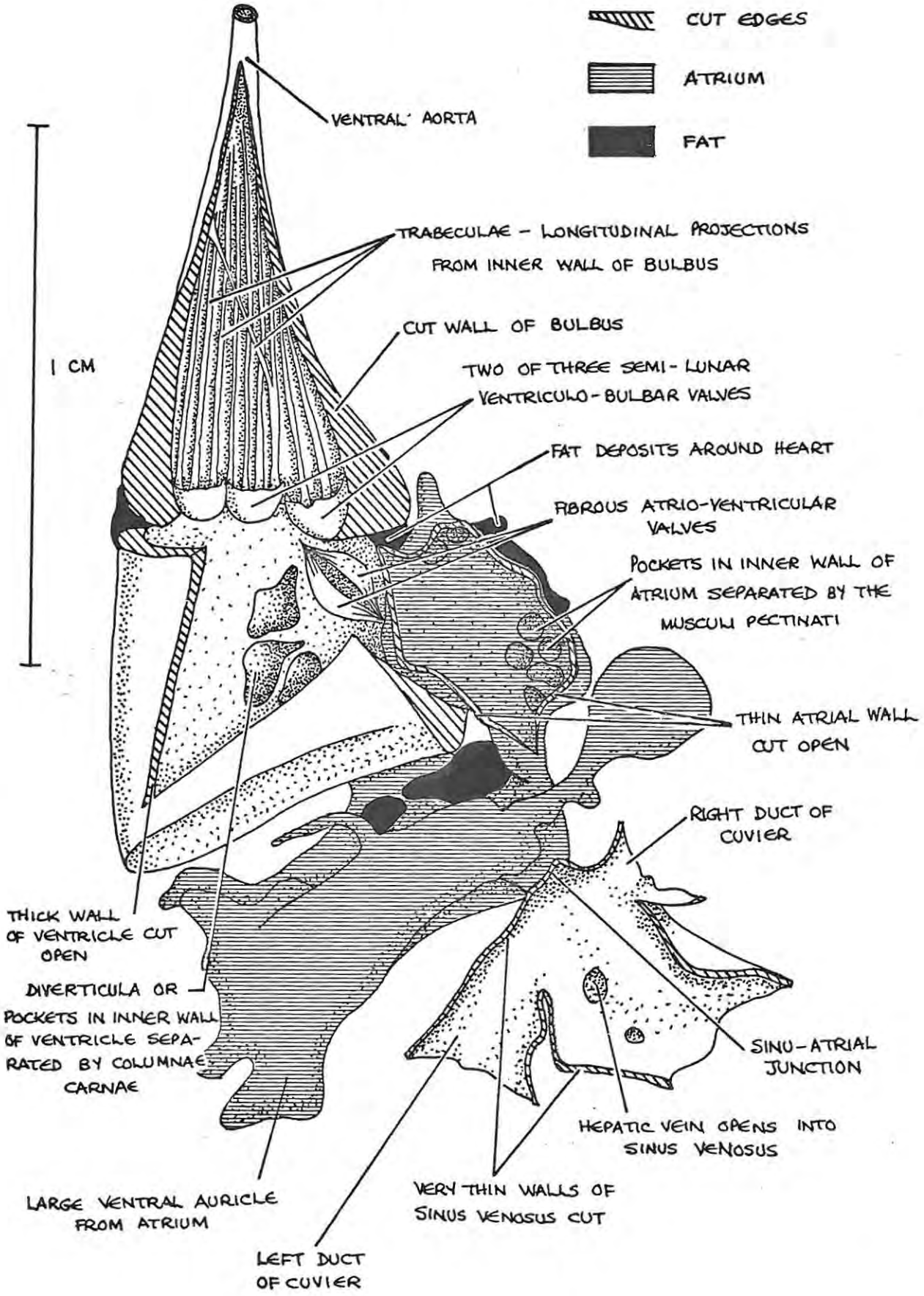
ATRIO-VENTRICULAR APERTURE

The atrio-ventricular aperture is on the ventral floor of the central atrial chamber and is a circular opening protected by a pair of pocket-like membranous flaps, the atrio-ventricular valves. The two valves are situated roughly on the right and the left, but the slit-like opening between them is usually at an angle to the longitudinal axis of the heart (Fig. 7). One of the valves may be larger than the other. These valves prevent back-flow from the ventricle to the atrium during the ventricular contraction. Water directed at the valves from a fine tube inserted into the ventricle causes the valves to fill out and their inner edges to come together so closing the aperture. There are no chordae tendinae attached to these valves. The valves are, however, strengthened with fibrous material at the ends of the elongate aperture between them. This fibrous strengthening may prevent tearing of the membranous valves and may also prevent the valves from being forced up into the atrium.

Figure 7.

Diagram of the heart of Tilapia mossambica opened to show some internal features as seen from the left hand side.

(Compare to Figure 6 where heart unopened)



It is interesting to note that the atrio-ventricular and the ventriculo-bulbar apertures are close together, both being at the anterior end of the ventricle. The same arrangement is seen in other teleosts, e.g. Salmo salar (Goodrich,(1958)), and in higher vertebrates. It is caused by the extensive folding of one heart chamber on another as the heart evolves from a straight tube to a complexly bent S-shaped structure.

VENTRICLE

The ventricle is ventral to the atrium and posterior to the bulbus. The ventricular wall is very muscular and thick, and by far the most powerful pumping chamber of the heart (Fig. 7). The ventricle is, however, a relatively small chamber when compared to the atrium, and of roughly pyramidal shape with the apex pointing caudally. The ventricular lumen consists of a small central chamber with numerous smaller pockets, the walls of which are formed by the columnae carnae, muscular strands similar to the musculi pectinati of the atrium. These strands, crossing the lumen of the ventricle except in the region of the anterior central chamber, are part of and continuous with the muscles of the middle region of the ventricular wall. The columnae carnae (the trabeculae of Grassé, (1954)) prevent excessive dilation of the ventricle.

VENTRICULO-BULBAR APERTURE.

The ventriculo-bulbar aperture, at the anterior end of the ventricle, is guarded by two or three pocket-like membranous valves (much deeper than the atrio-ventricular valves) which are, according

to Goodrich (1958), equivalent to the anterior valves of the conus. The region of the valves thus corresponds to the conus of Elasmobranch fish and is termed the bulbus cordis. These ventriculobulbar valves meet in the middle of the aperture when they are filled with blood under any pressure from the bulbus, and thus the aperture is effectively closed and backflow is prevented when the ventricle relaxes at the end of systole. One or two of the valves are situated ventrally, and one dorsally. There are often fat deposits in this region on the outside of the heart (Figures 4, 5, 6 and 7) and these may also be found more anteriorly on the bulbus, and more dorsally on the atrium.

BULBUS

The bulbus is the most anterior chamber of the heart, although it is considered by many as an enlargement of the ventral aorta, i.e. a differentiation of the arterial trunk, (Grassé, 1954). The bulbus continues into the ventral aorta without much definitive boundary, although in some fish it appears as though there may be sphincter-type muscles here (see Fig. 5).

The bulbus is a cone-shaped chamber with the narrow apex directed anteriorly. The ventricle is continuous with the broad end of the cone at the ventriculo-bulbar aperture as discussed above. The wall of the bulbus is variable in thickness (see Fig. 7) being thickened posteriorly but narrowing at the apex of the cone anteriorly as the bulbus merges with the relatively thin-walled ventral aorta. In the thickened regions, numerous longitudinal projections, the trabeculae (Singh, 1960) project into the lumen of the bulbus and even continue into the cup-like opening of the three ventriculo-bulbar valves.

(b) The electrocardiograph of T. mossambica and the effect of electrode poisitioning.

A number of ECGs were recorded from within the pericardium using dissected, anaesthetised fish. The electrodes were placed at different points in relation to the heart exposed in air, and Fig. 8 gives some examples of these recordings where the positive electrode alone is near the heart and has been placed next to the atrium, ventricle or bulbus. Insufficient recordings were made in order to pick up any consistent differences between traces recorded when the positive electrode was near different regions of the heart. The ECG recorded from the exposed heart can be compared fairly well to the theoretical scheme suggested by Labat (1966) (see Fig. 9) in some cases (Fig. 10B), but not in others (Fig. 10A), depending on the position of the positive electrode relative to the ventricle in any particular case.

In the two traces in Fig. 10 (A and B), the forms of the QRS complex and directions of the P and T deflections change such that the two traces are nearly mirror images of each other. This is to be expected in this particular case because the positive electrode is situated in opposite positions in relation to the heart - in the one case on the left of the ventricle, in the other case on the right. Thus if the dominant or main direction of depolarisation in the ventricular musculature is towards the left, the main deflection is a positive one if the positive electrode is on this side (Fig. 10B), and a negative one if the electrode is on the other side (Fig. 10A). This interpretation is based upon the fact that if a depolarisation front approaches the positive electrode of a pair, it causes a positive deflection.

contd. on p. 30.

Figure 8.

ECGs recorded from the exposed heart of a Tilapia mossambica where the positive electrode has been placed at different points in relation to the heart.

A - Positive electrode next to the atrium

B - Positive electrode next to the ventricle

C - Positive electrode next to the bulbus

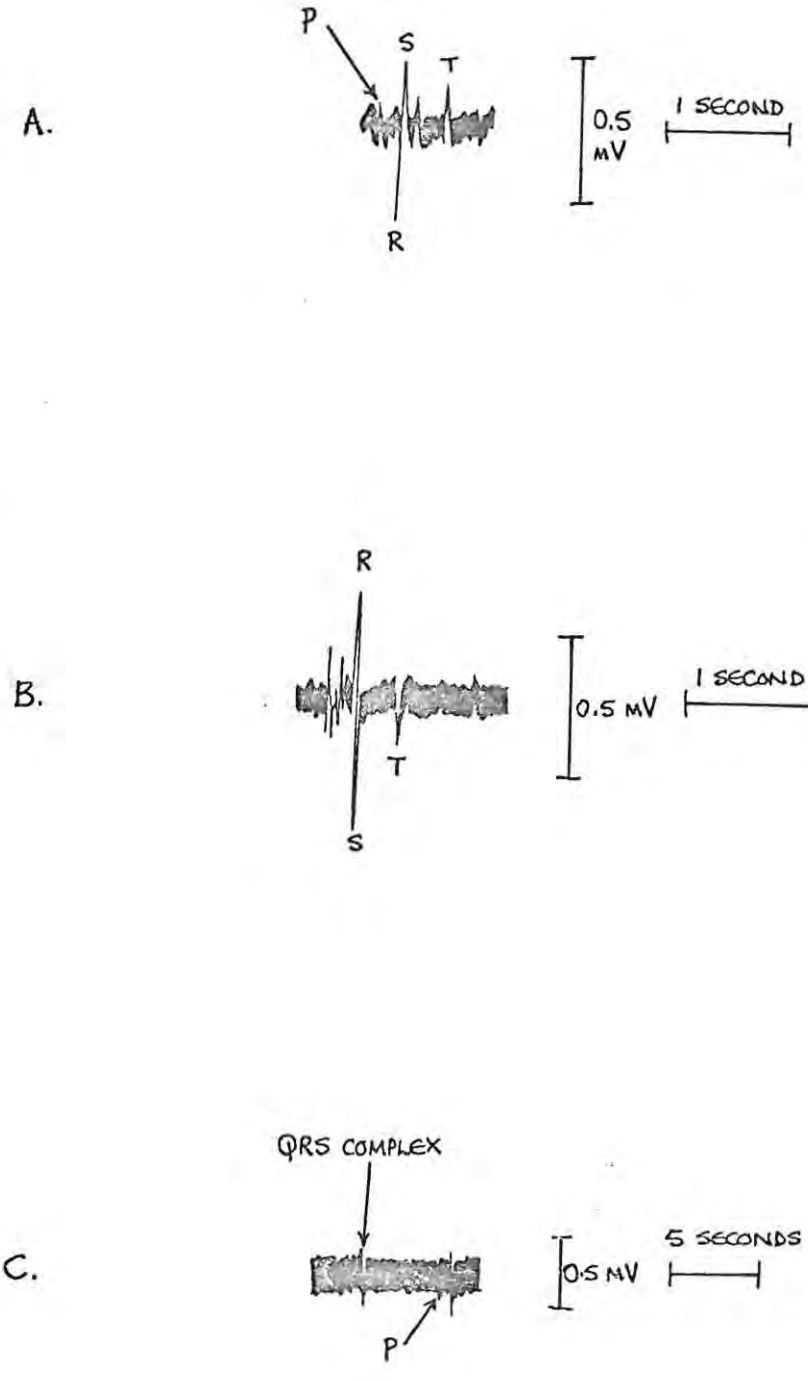
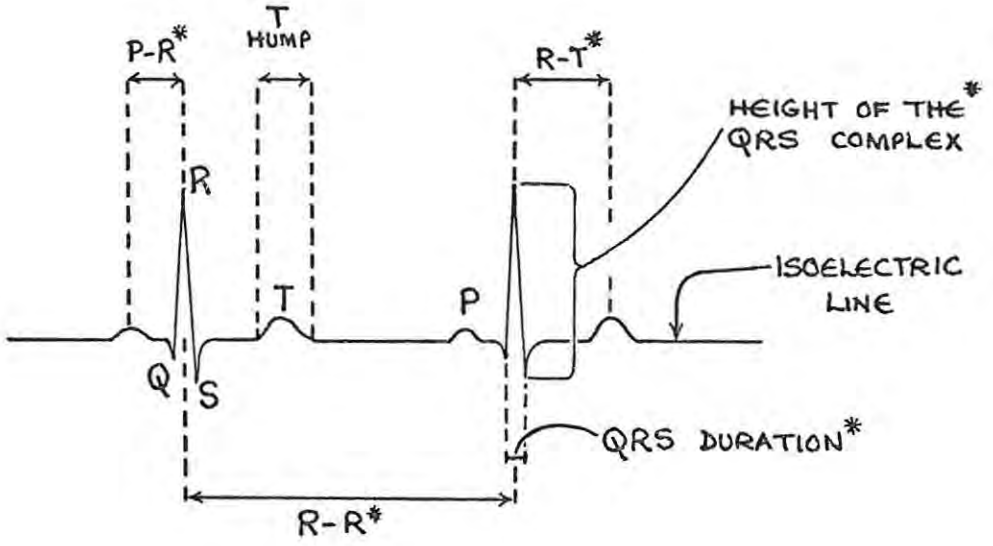


Figure 9 .

Theoretical teleost ECG taken from Labat (1966) showing characteristic deflections and also the cardiac intervals as measured* in the present experiments.



The P deflection corresponds to the depolarisation and contraction of the atrium;

The QRS complex corresponds to the depolarisation and contraction of the ventricle;

The T deflection corresponds to the repolarisation and relaxation of the ventricle, with the T hump a measure of the time these take;

The R-R interval is thus a measure of the time period between consecutive heart beats;

The P-R interval is a measure of the period between the onset of the atrial and the onset of the ventricular contraction, and thus of the speed of conduction between these two chambers;

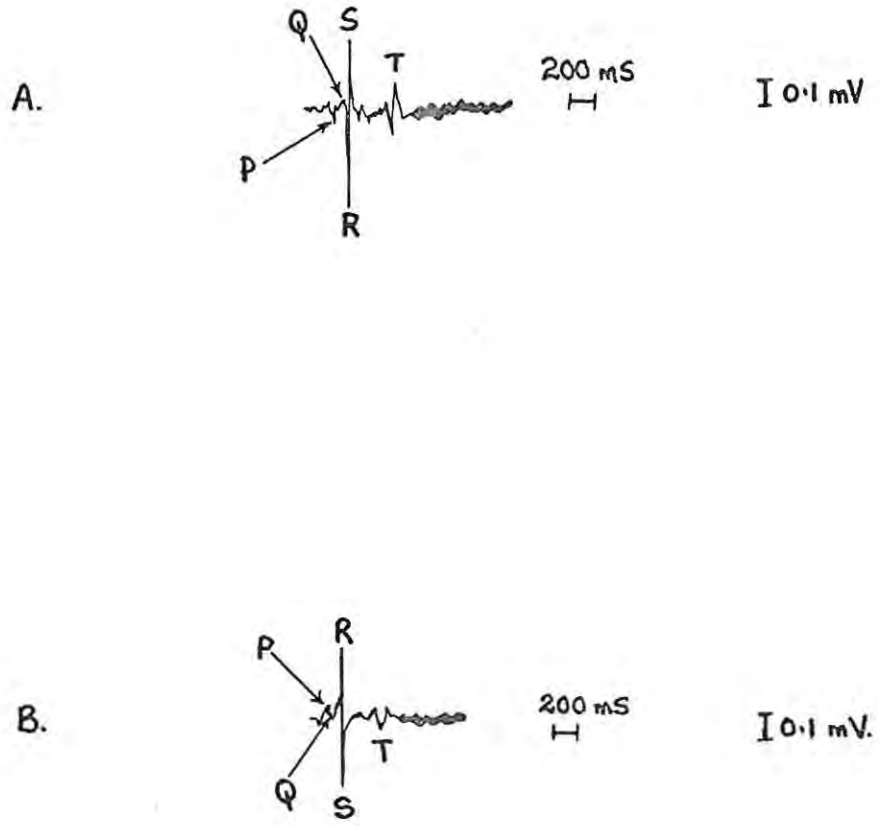
The Q-T interval is a measure of the time for which the ventricle remains contracted (R-T measured in this investigation).

Figure 10.

ECGs recorded from the dissected and exposed heart of an anaesthetised T. mossambica showing the characteristic deflections of the ECG and their associated names as used in this study

A - positive electrode on right of ventricle

B - positive electrode on left of ventricle



ECGs recorded in this way from the exposed heart were very clear and prominent when compared to recordings using implanted electrodes. The QRS complex recorded from exposed hearts was often from 0.3 and 0.7 mV in height as compared to 0.01 to 0.17 mV where electrodes were implanted. These differences may be due to electrical potential dissipation in body fluids, to the insulating properties of body tissues (particularly the pericardium, Oets (1950)) and to the greater distance between the myocardium and the receiving electrodes, all in the case of the implanted electrodes.

As Fig. 9 indicates, Labat (1966) suggests that normal teleost ECGs, recorded from electrodes in the gular region, show 3 positive (+ve) deflections, the P, R and T deflections. He reports deviations from this pattern only where stress or other abnormal conditions are present. However other published work and my results from similarly placed electrodes indicate that P and T are not always +ve deflections, even in seemingly normal fish; likewise the most prominent deflection of the QRS complex is not always +ve (see Table 1, p. 37).

The cardiac intervals chosen must be easily and reliably measured. Fig. 9 indicates how I have measured the intervals used in this investigation, and it is clear that I have measured the R-T interval instead of the more conventionally used Q-T interval. The reasons for this use of R instead of Q are:- (i) Frequently only one deflection represents the QRS complex, or one deflection of the QRS is much clearer than the others and is regularly present in any one trace. (ii) The whole of the QRS is of very short duration when compared to the cardiac cycle duration (0.012 to 0.022 : 1) or even to the R-T interval duration (0.056 to 0.068 : 1); thus measuring the shorter R-T instead of the Q-T introduces, at the most, a $\pm$ 3.4% error. Thus

except where the pattern shown can be easily and directly equated to the normal pattern as illustrated in Fig. 9 (small, negative Q and S and larger, positive R deflection), I have considered the QRS complex as a unit with a dominant deflection as a reference or measuring point that is, during any one recording, fairly consistent and either positive or negative. I have, for the purpose of this study, called this deflection the R deflection, although it may in reality correspond to an S or even a Q deflection. In the well displayed traces obtained in human electrocardiography, the Q (if large) or q (if small) deflection is always negative (-ve), the R or r is always positive (+ve) and the S or s is always -ve; a qR deflection recorded from a particular lead (small q, large R) would be distinguished clearly from an rS deflection from the same lead (small r, large S). However, in this report, the former qR would be referred to simply as 'R deflection +ve', and the latter as 'R deflection -ve'. The QRS pattern seen in Fig. 10A is also termed 'R deflection -ve' but, because of the electrode positioning next to the exposed heart in this case as has been discussed above, the R is probably correctly named although it is -ve.

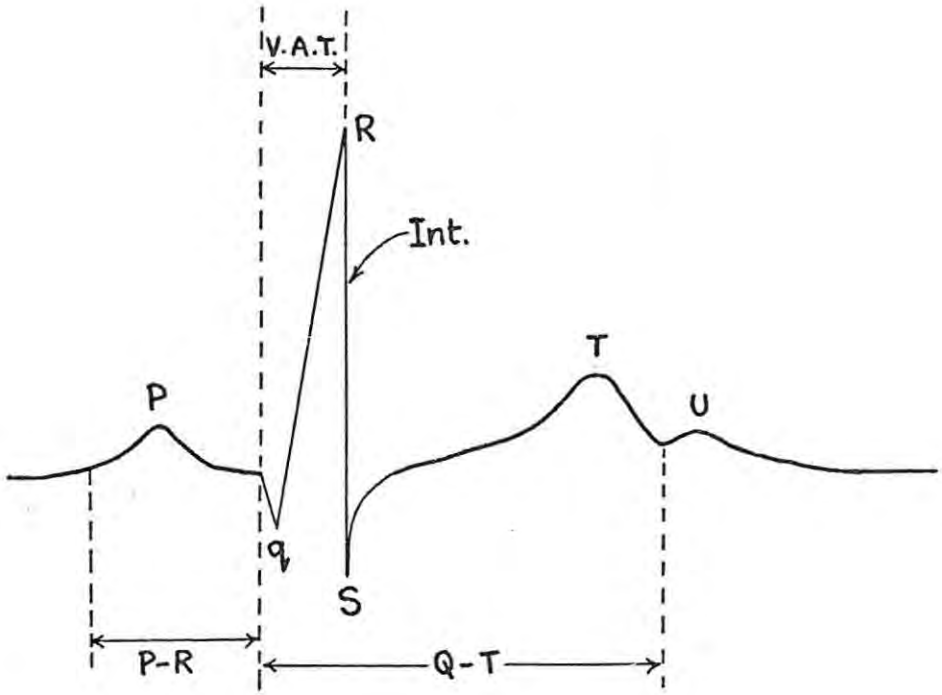
Returning again to the measurement of the R-T interval. I have used the interval between the R deflection and the peak of the T deflection (see Fig. 9). I am unsure what practise is followed by other workers on fish electrocardiography in the measurement of the Q-T (R-T), as they do not explain exactly how their measurements have been made. The practise in human electrocardiography is to measure from the beginning of the QRS complex (see above) to the end of the T hump, as is described by Schamroth (1966) (see Fig. 11). But Schamroth (1966) and Peters & Mullen (1966) find that it is difficult to measure the Q-T interval accurately because of the gradual slopes of the T limbs, thus Q-T measurements may be variable and are usually conservative. My

Figure 11.

Diagram illustrating the various names and measurements used on ECG traces in human electrocardiography (Schamroth, 1966).

V.A.T. - the ventricular activation time.

Int. - the intrinsicoid deflection.



method of measuring the R-T is thus one way of overcoming this difficulty, and as the QRS complex and T hump were often of fairly short duration (particularly the former, as already mentioned), the R-T interval used here would be fairly closely comparable to a Q-T interval as measured in human electrocardiography. The R-T values measured were also found to be very constant during any one recording, and three examples of this interval recorded from one of the fish from the last experiment (see section (g) (fish 0) are shown in Fig. 12. In this Figure a photograph is given of an example in each case, together with the mean value of the R-T interval, the 1.96 X S.E. value as well as the co-efficient of variation (v). The relatively small 1.96 X S.E. and v values indicate the consistency of the R-T measurements.

Similarly, my method of measuring the P-R interval differs from that used in human electrocardiography (Fig. 11), where this interval is measured from the beginning of the P deflection to the point where the trace leaves the iso-electric line at the very beginning of the Q deflection. The amount of film available as well as developing time considerations did not allow further expansion of the time scale (a faster film speed) such that the P-R and other intervals could be measured as in human electrocardiography. However, as with the present R-T interval measurements, the P-R interval measurements were very consistent. I notice that Boyer (1966), working on a variety of reptiles, had similar difficulties and also decided to use only the various peaks of the deflections in his ECGs in order to obtain consistent interval measurements.

Another feature of interest in the ECGs recorded from Tilapia mossambica during these experiments was that, in a few of the fish,

the P deflection sometimes varied in sign in the same fish at different times. Also the shape of the QRS complex tended to alter slightly such that the more prominent or R deflection therefore changed in sign. An example serves to illustrate this tendency. In fish A of the last experiment (section (g)), at 14.1°C, the QRS complex changes within a few hours as illustrated by the consecutive recordings given in Fig. 13. The more prominent -ve deflection, taken as R, in the top photograph (Fig. 13A) at zero time (0 h) becomes reduced and a +ve deflection becomes the dominant hence the R deflection after only 3.5 h (B). The last photograph (C) at 13.5 h also shows a +ve R deflection.

The position of the implanted electrodes in these fish is very difficult to correlate with any specific features of the ECG, in distinct contrast to the excellent correlations normally obtained in human electrocardiography. However, a few general comments can be made with reference to the six fish used in the last experiment (section (g)). The general features of the ECG for each of the six animals is given in Table 1 together with the position of the +ve and -ve electrodes in each case. Previous recordings with the +ve electrode on the animal's left hand side (in contrast to all recordings in these experiments where the +ve electrode was always on the right hand side) do not indicate any essential differences in the ECGs.

Where one or both of the electrodes were located within the pericardium, and where the fish survived (e.g. fish T, Table 1), the recordings commonly indicate clear and fairly consistent ECGs. The more usual result of pericardium penetration was, however, death after some hours or even days, as was found in four other fish. Post mortem examinations sometimes revealed heart damage, but usually no damage could be seen. More commonly, however, the electrodes were located in muscle, or in the branchial cavity (Fish I, A, R, V and O).

contd. on p. 38

Figure 13

Changes in sign of the R deflection in fish A during a period of 13.5 h at 14.1°C.

A - Time zero (0 h) B - 3.5 h C - 13.5 h

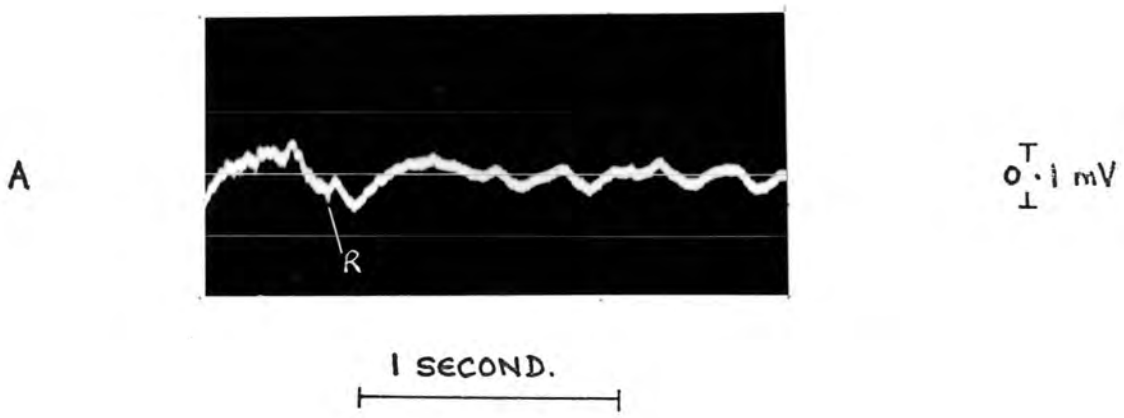


Table 1.

Electrode positioning, ECG characteristics and ECG variability in the six fish of the last experiment (see section (g)). In all cases the positive electrode was on the right side of the fish.

Fish name	Most common directions of the ECG deflections			Positions of the implanted electrodes	
	P	R	T	+ve electrode	-ve electrode
I	-ve constant	+ve constant	+ve constant	in gular muscle ventral to the heart.	in branchial cavity in connective t. outside the pericardium.
A	-ve constant	+ve variable	+ve constant	in branchial cavity.	in branchial cavity in connective t. outside the pericardium.
R	+ve constant	+ve constant	+ve constant	in gular muscle; may be 'second' electrode area in the branchial cavity where insulation damaged.	in the branchial cavity
T	-ve constant	-ve varies at 25.8°C	+ve constant	inside the pericardium next to the ventriculobulbar junction	in the branchial cavity.
V	-ve constant	+ve constant	+ve constant	in gular muscle; wire penetrates pericardium elsewhere.	in the gular muscle.
O	-ve or +ve variable	-ve or +ve variable	-ve or+ve variable	in muscles behind the bone of the pectoral girdle.	inside thick connective tissue next to the pericardium.

In the 3 fish where the +ve electrode was in the ventral gular musculature (Fish I, R and V), the ECG pattern of deflections was very consistent. There was slight variability where the +ve electrode was within the pericardium itself and was thus next to the heart (Fish T, see above), and moderate to tremendous variability where the +ve electrode was in the branchial cavity (Fish A) or behind the bones of the pectoral girdle (Fish O). The most consistent results were thus obtained where the +ve electrode was within muscle proximal to the heart.

If a depolarisation front approaches the +ve electrode, this causes a +ve deflection; if it moves away, a -ve deflection results (Oets, 1950, Fig. 4, Schamroth, 1966, Fig. 101). A repolarisation front causes a -ve deflection on approach, a +ve one on moving away from the +ve electrode. These deflections are seen as long as the polarisation front does not, at the same time, move relative to the 2 electrodes in the same way. In these investigations the 2 electrodes were never immediately next to each other, and the -ve electrode was never on a line joining the +ve electrode and the heart.

The fish with the +ve electrodes in the gular musculature (Fish I, R and V) show +ve R and T deflections, although the sign of the P deflection varies (Table 1). The T deflection may be very prominent (p. 119 and Fig. 20C, p. 53, the superimposed trace) and may even be mistaken for a respiratory deflection. The +ve R and T deflections suggest that if the +ve electrode bears a constant relation to the ventricular musculature, the predominent direction of depolarisation of the ventricular musculature is towards the ventral side of the animal, and that of repolarisation is towards the dorsal side. These interpretations are illustrated in Fig. 14. Where the +ve electrode is to the right of the heart (Fish A and T), or behind the heart (Fish O), the R deflection sign is variable, although the T is always +ve except in the latter fish. If an electrode is placed at approximately a right angle to

P.T.O. for pages 39 and 40.

(Fig. 14)

Legend for Figure 14

(opposite)

Above: Diagram showing a view of the right side of the head of T. mossambica with the branchial cavity and pericardium opened. The three main positions of a +ve electrode near the heart have also been indicated.

Below: Drawings A, B and C to show the heart as in the upper drawing but enlarged. In each case one positive electrode position is indicated together with the possible ECGs recorded from this electrode.

A - +ve electrode ventral to the heart in the gular musculature

B - +ve electrode in the branchial cavity or in the pericardium lateral to the heart

C - +ve electrode behind part of the pectoral girdle postero-lateral to the heart

KEY

atrium

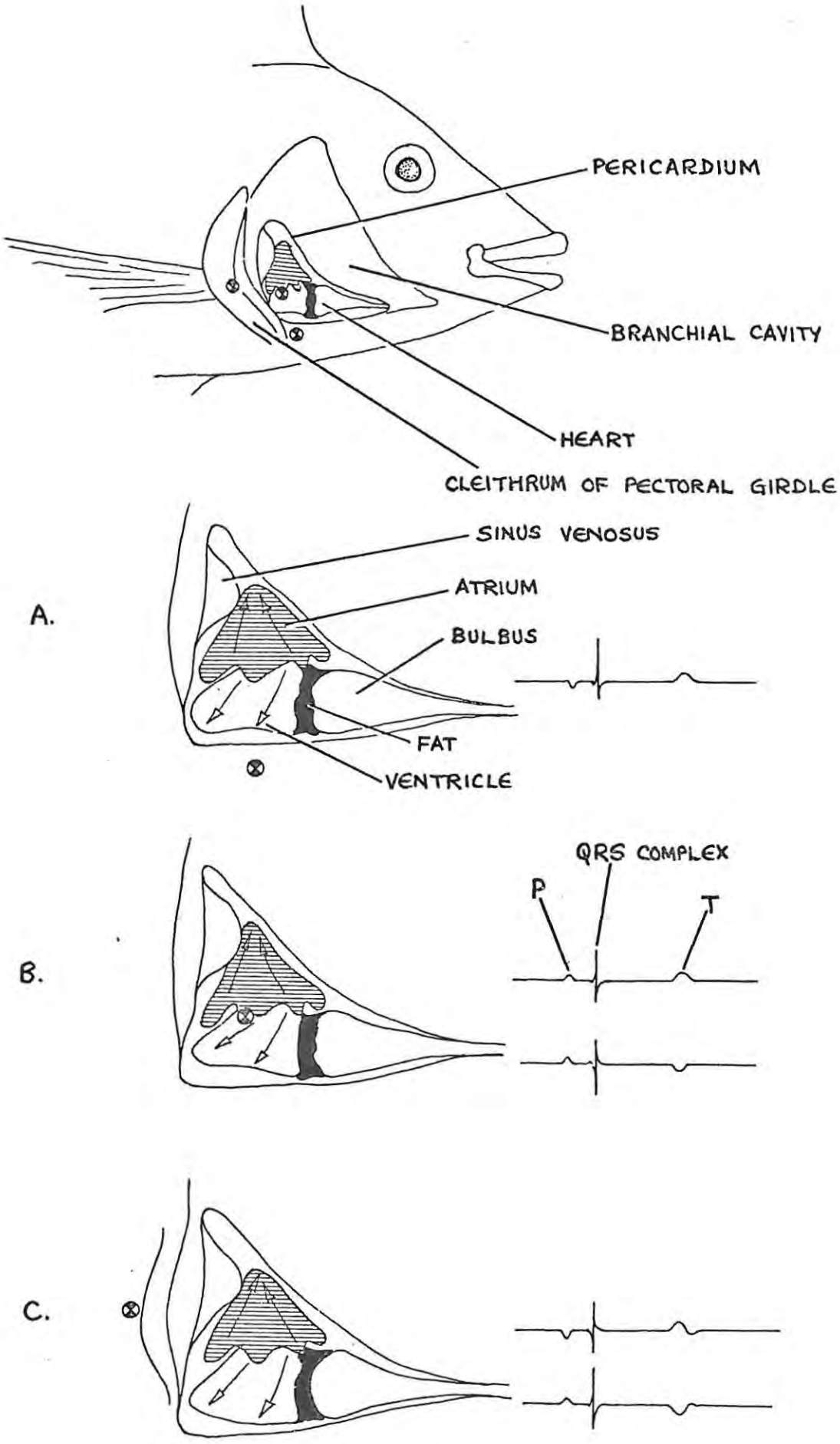
fat

-ve electrode

direction of depolarisation of the myocardium

direction of repolarisation of the myocardium

Figure 14.


PERICARDIUM
BRANCHIAL CAVITY
HEART
CLEITHRUM OF PECTORAL GIRDLE
A.
SINUS VENOSUS
ATRIUM
BULBUS
FAT
VENTRICLE
B.
QRS COMPLEX
P
T
C.

a depolarisation front, the deflection recorded may be +ve, -ve or absent, but is usually small and equiphasic (Schamroth, 1966). This range is seen in the results obtained for the R deflection in these three fish (Fig. 14 B and C).

The P deflection is normally negative; this may indicate that the depolarisation in the atrial lobes is normally in a dorsal direction and probably depends on the position of these lobes in any one fish, as described earlier (section III(a), Figs. 6 and 7). As these lobes or 'auricles' are often fairly long, and as their ends may come to lie ventrally next to the ventricle, contraction of the myocardium comprising their walls in a dorsal direction may expel blood out of the 'auricle' towards the main atrial lumen.

The ECGs of the 6 fish varied also in the magnitude of the deflections, i.e. in the voltages recorded. The mean height of the QRS complex has been plotted for each fish against fish weight in Fig. 15. The slope of the regression curve which has been fitted for these points ($y = -0.0067 + 0.000944x$) does not differ significantly from a horizontal line ($P<0.10$); the regression curve is drawn in on Fig. 15. These results suggest that there is no consistent change in QRS total voltage with fish weight. Heart weight probably varies in proportion to body weight, and the cumulative electrical changes produced by the ventricular myocardium may be expected to increase with an increase in the number of muscle fibres depolarising. However, this would only be noticeable if recordings were made from electrodes in comparable electrical contact with the ventricle in all fish. The variable electrode placement relative to the ventricle in these experiments would certainly mask any such trend.

Fig. 15 also shows that the three fish with the more consis-

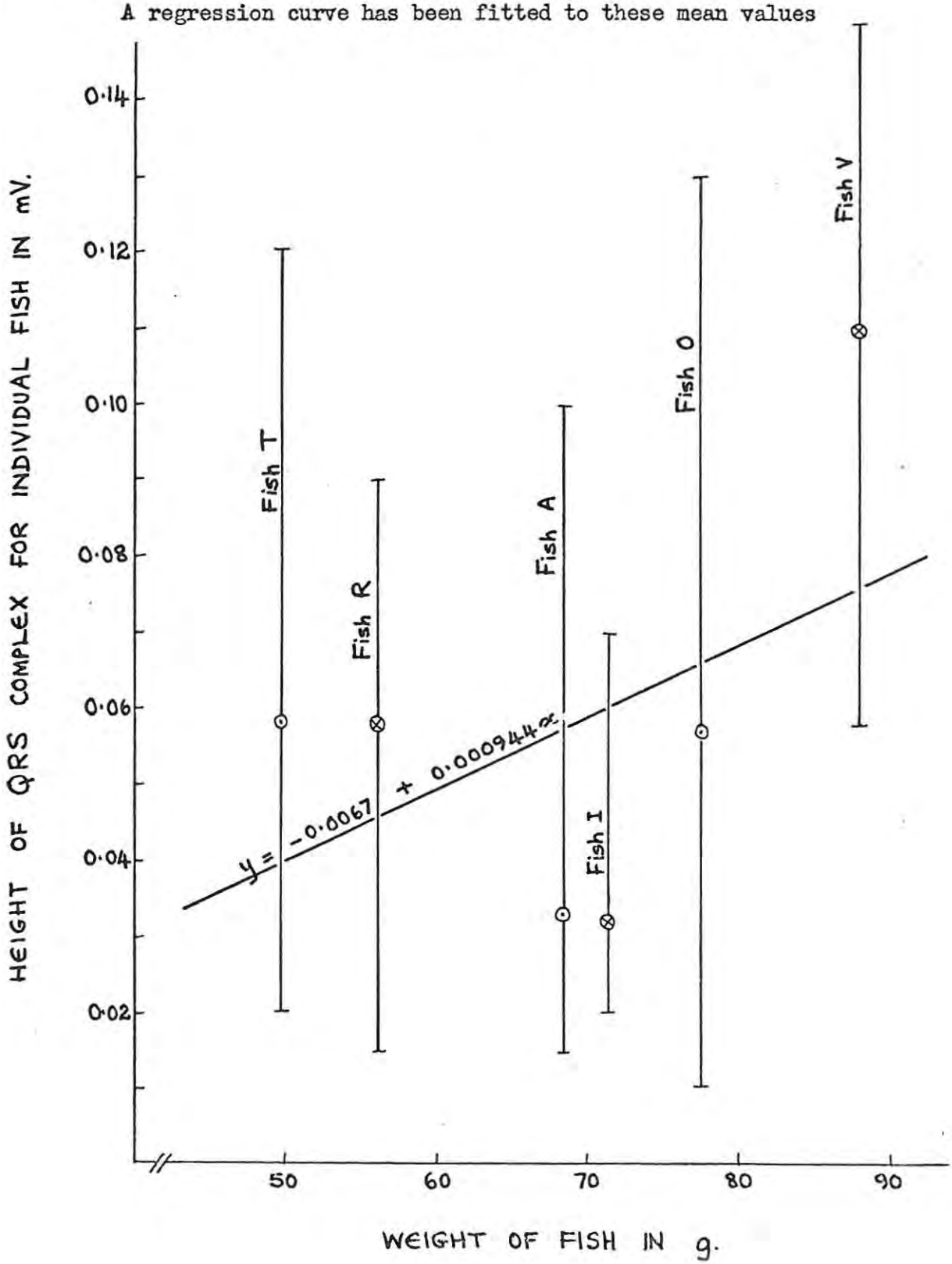
Figure 15

Mean magnitude of the QRS complex in the six fish of the last experiment (section (g)) and their total variation, shown plotted against the weight of the fish.

⊙ - mean QRS of fish with electrodes not situated in gular muscle;

⊗ - mean QRS of fish with electrodes situated in gular muscle;

A regression curve has been fitted to these mean values



tent ECG patterns (fish I, R and V) do not have a more prominent QRS than the other three fish (with the possible exception of Fish V). The mean range of the QRS values does, however, seem a little smaller for these three fish (mean range 0.072 mV) when compared to the other three fish (mean range 0.102 mV) as can be seen in Table 2. When the range in QRS values is taken as a % of the mean value for the individual fish, this difference between the two groups of fish becomes even more striking and the means (for the three fish in each group) of these percentages together with the respective 1.96 X S.E. values indicate that there is a significant difference between the two groups of fish at the 95% level (Table 2). Thus this again indicates that when the positive electrodes are embedded in muscle near the heart, the ECG has greater constancy. One reason for this could be that where the +ve electrode is in a fluid-filled cavity, electrical potential may be partially dissipated by short-circuiting. The extent to which this occurs may depend on slight position shifts of the electrode, or on the amount of pericardial fluid (Guyton, 1966, p. 216) or on the volume of respiratory water passing through the branchial cavity per opercular beat, etc. Where the +ve electrode is in muscle, however, the transfer of electrical potential differences occurs via the extracellular tissue fluids (Schamroth, 1966), and its efficacy is probably dependent on such factors as its volume, closeness of inter-cellular adhesions, and its ionic composition. As these factors are probably fairly constant, the conducted electrical changes are thus also fairly constant.

Table 2.

Amplitudes of the QRS complex for the six fish of the last experiment (section (g)), the ranges of the values measured in absolute terms and as %s of the respective means.

	Name of fish	Average magnitude of QRS in mV	Total range of the QRS complex in mV	Range of QRS as a % of the mean	Mean magnitude of the QRS for 3 fish in mV	Mean range of the QRS complex for 3 fish in mV	Mean range of QRS for 3 fish as a % of the mean with 1.96 X S.E.
(i)	I	0.032	0.020 → 0.070	156%	0.067	0.072	123 ± 33.4%
	R	0.058	0.015 → 0.090	129%			
	V	0.110	0.058 → 0.150	84%			
(ii)	A	0.033	0.015 → 0.100	258%	0.049	0.102	213 ± 40.2%
	T	0.058	0.020 → 0.120	172%			
	O	0.057	0.010 → 0.130	210%			

(i) - the three fish with the +ve electrode situated in the gular muscles

(ii) - the three fish with the +ve electrode not situated in the gular muscles

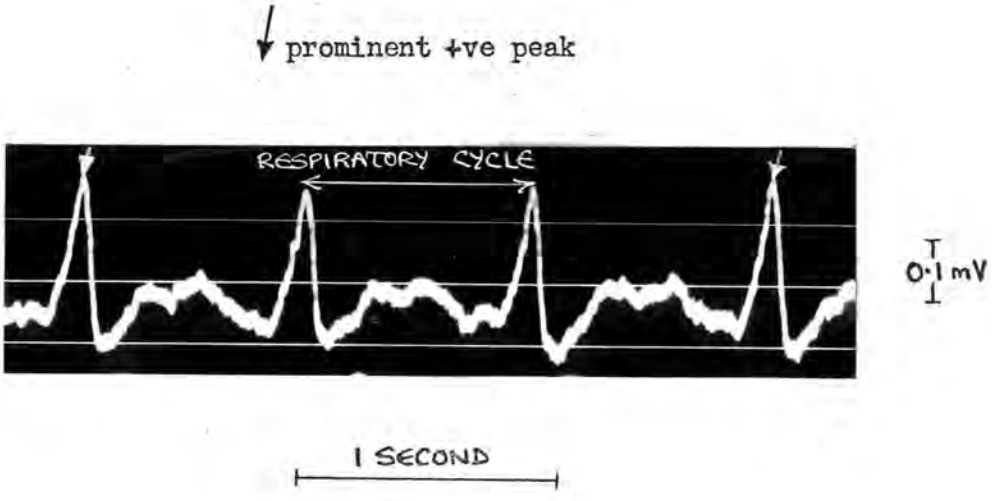
(c) Oscillographic recordings of respiratory activity in T. mossambica.

As reported by many authors (e.g. Labat, 1966), the electrical activity of the respiratory muscles of fish is frequently picked up by electrodes in the region of the heart. My results also often indicated these potentials. Proof that these are probably caused by opercular, buccal and other respiratory movements is discussed briefly in section VI (b).

Unlike the ECG, the electrical changes produced by the successive contractions and relaxations of the various muscles responsible for the respiratory movements are not seen as discrete deflections from an iso-electric base line; they result rather in a continually changing oscillograph. The trace usually indicates one or more rather prominent deflection as can be seen in Fig. 16. These deflections probably indicate large depolarisations in one or more muscles at certain stages of the respiratory cycle in directions approximately towards or away from the +ve electrode. Correlations between the traces and mouth opening or closing, or the contraction of the individual respiratory muscles, were not investigated.

Figure 16.

Typical oscilloscope recording of the electrical changes resulting from the respiratory movements of T. mossambica.

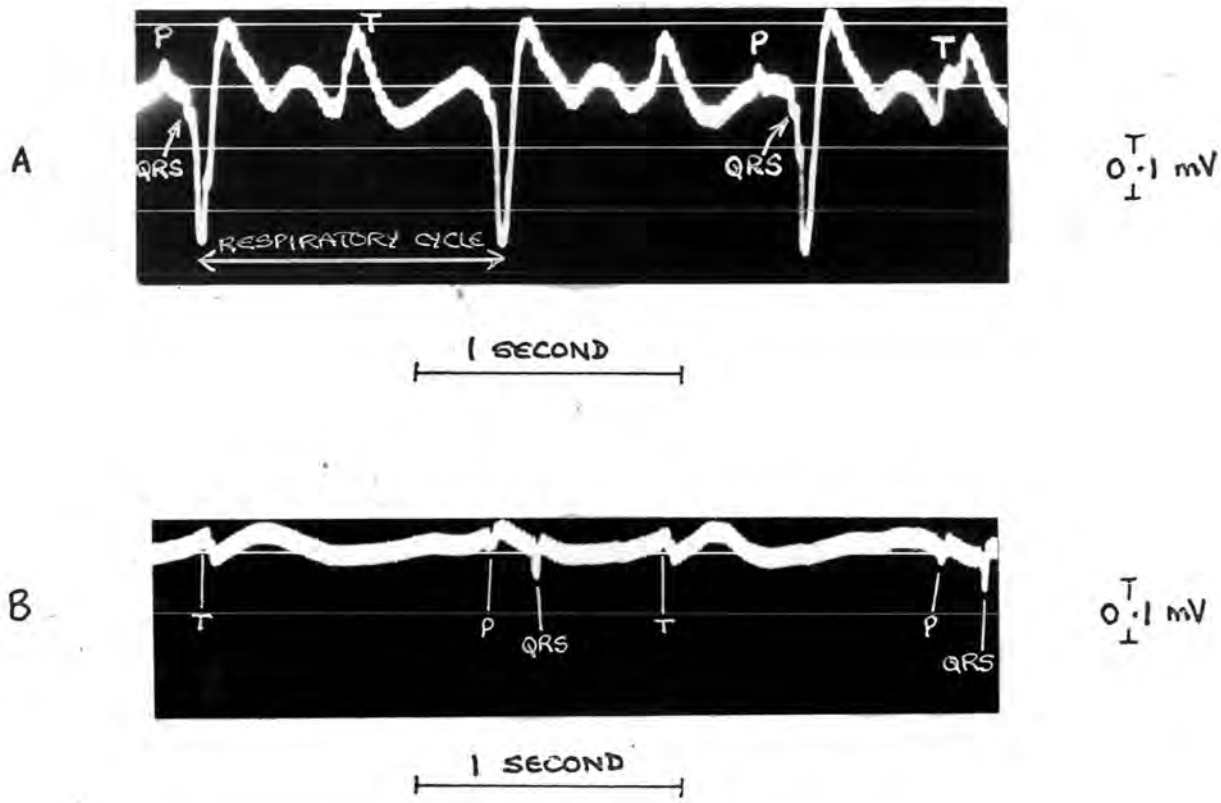


In many of the recordings, the ECG is seen superimposed upon the electrical record of the respiratory activity as seen in Fig. 17.

Figure 17.

ECGs superimposed upon recordings of electrical respiratory activity in two fish of the last experiment (section (g)) at 21.1°C

A - Fish R B - Fish T



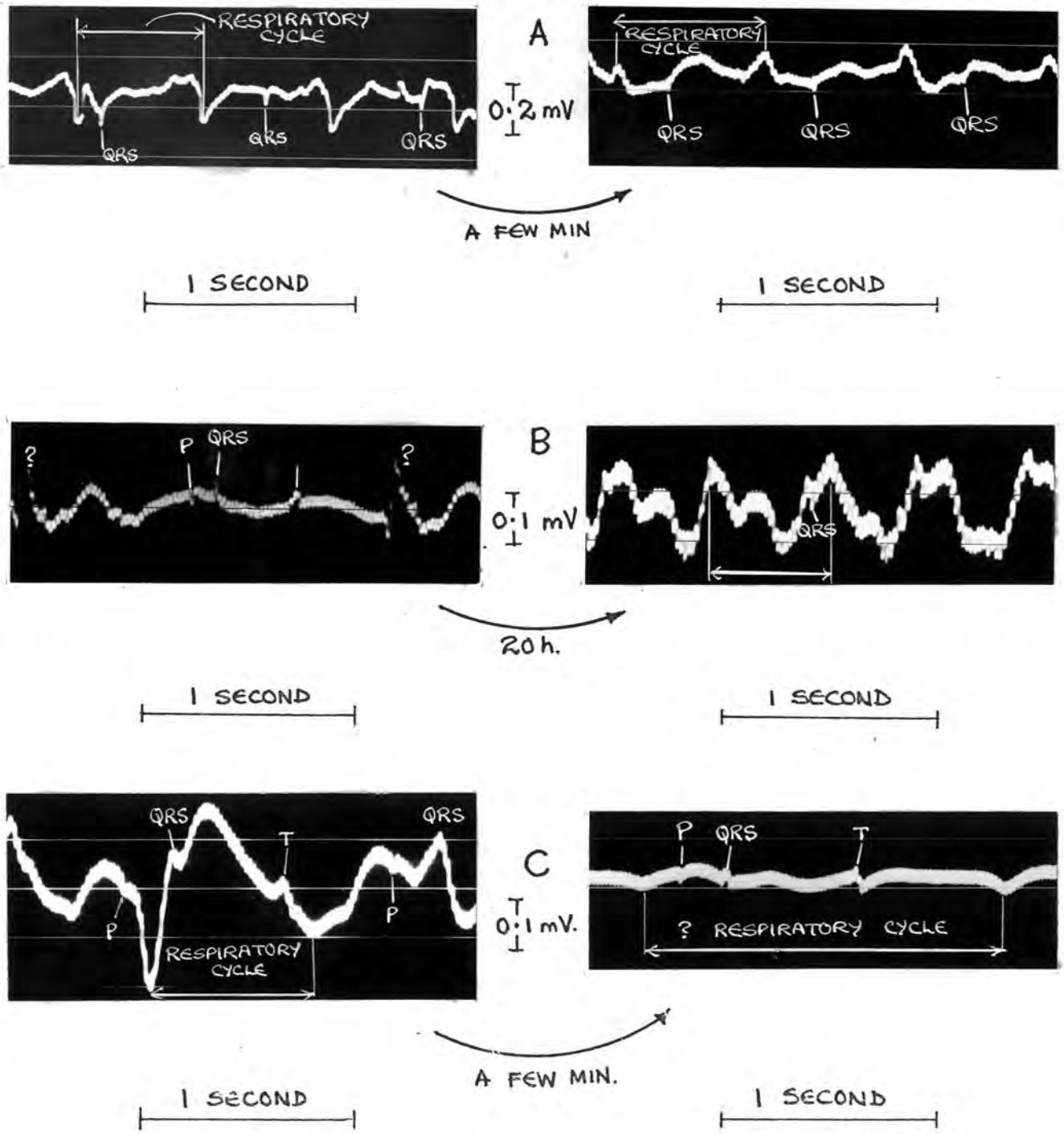
In Fig. 17 A, the respiratory activity is very prominent; this makes it difficult, in many cases, to distinguish the ECG. In Fig. 17 B, however, the electrical potentials caused by respiratory movements are barely visible, whereas the ECG is prominent. In any one fish, the prominence of the respiratory activity and of the ECG can be very variable, even at the same temperature, on the same day, or within

minutes, as can be seen in Fig. 18 for Fish 0 and I of the last experiments (section (g)).

Figure 18.

Variability in the prominence and appearance of respiratory activity and ECGs in Fish 0 and I of the last experiment (section (g)) when measured at the same temperature within a short time*.

A - Fish 0 at 21.8°C; * a few minutes B - Fish I at 25.8°C; * 20 h C - Fish I at 17.8°C. * a few minutes



In the example given in Fig. 18C, although no extraordinary motility or other signs of excitement were noted in the fish during the recording of the earlier trace (left hand side), the fish may well have been excited in view of the noticeable increases in both heart and respiratory rate as compared to a few minutes later on (right hand trace) when the fish had probably settled down. In Fig. 18A, the recordings were made about 1 h after the electrodes had been implanted under anaesthesia, so there may well be rapid changes in the respiratory pattern at this stage as discussed in section (d) below. A possible explanation of the variability in the example illustrated in Fig. 18B cannot be offered and, in fact, I am very unsure as to whether the indicated respiratory cycles are valid or not. This problem is discussed later in section VI (b).

Correlations between the presence of electrical respiratory activity on the traces and the position of the electrodes are difficult using the information available. This information using the six fish of the last experiment (section (g)) is summarised in Table 3. From this Table it can be seen that the three fish (Fish I, R and V) with +ve electrodes in the gular musculature always gave traces of electrical respiratory activity, except at 14.1°C as discussed below. This correlates with the regular ECG recordings in these same three fish. In the three fish (Fish A, T and O) where +ve electrodes were not in the gular muscles, the electrical respiratory activity was not always seen on the traces, although it was present in most cases.

Table 3 also indicates that there is a variability in the electrical record of respiratory activity with changes in temperature. The most outstanding feature of this is that at the lowest experimental temperature used (14.1°C), the recordings of the respiratory activity

Table 3.

Visibility of electrical record of respiratory activity on oscilloscope traces at different experimental temperatures.

	Fish Name	Visibility of electrical record of respiratory activity at following temperatures:			
		25.8°C	21.1°C	17.8°C	14.1°C
(i)	I	visible.	visible and prominent.	visible.	seldom visible.
(i)	R	visible.	visible, often prominent	visible, often prominent	often not seen
(i)	V	normally visible	normally visible	normally visible	normally visible
(ii)	A	visible	visible and prominent	visible	sometimes not seen
(ii)	T	usually not seen	visible and prominent	usually not seen	usually not seen
(ii)	O	normally very clear	clear and very prominent	clear, often prominent	clear, but may be absent

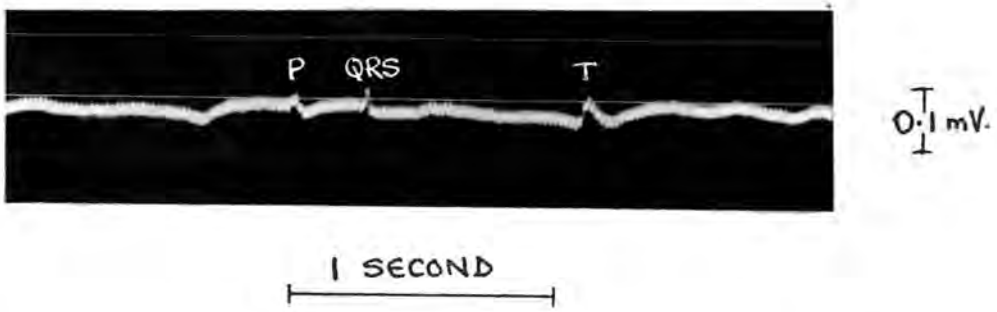
(i) The 3 fish with the positive electrode situated in the gular muscle

(ii) The 3 fish with the positive electrode not situated in the gular muscle

are difficult to discern, or otherwise no record of respiratory activity could be seen at all as is illustrated in Fig. 19 for Fish R, a fish which normally gives consistent electrical respiratory activity.

Figure 19.

Oscillographic recording from Fish R at low temperature (14.1°C) showing the absence of any electrical respiratory activity.



This frequent absence of recorded electrical respiratory activity at 14.1°C may be related to the increase in the opercular/buccal respiratory cycle duration at this temperature (discussed later in section (g)), and also may be because of a pronounced decrease in respiratory muscle activity. This would explain the increasing difficulty in visually observing and hence counting the opercular/buccal respiratory movements in fish at this temperature. Thus both the visual and oscillographic methods employed were sometimes unable to record opercular/buccal respiratory activity. I wonder if the more generally used mechano-electrical transducers (Shelton & Randall, 1962) would have been more sensitive to these very slight movements; I doubt it.

Another marked effect was that when the temperature was raised from 17.8 to 21.1°C (see section (g)), the electrical recordings of the respiratory muscle activity suddenly became very prominent in some of

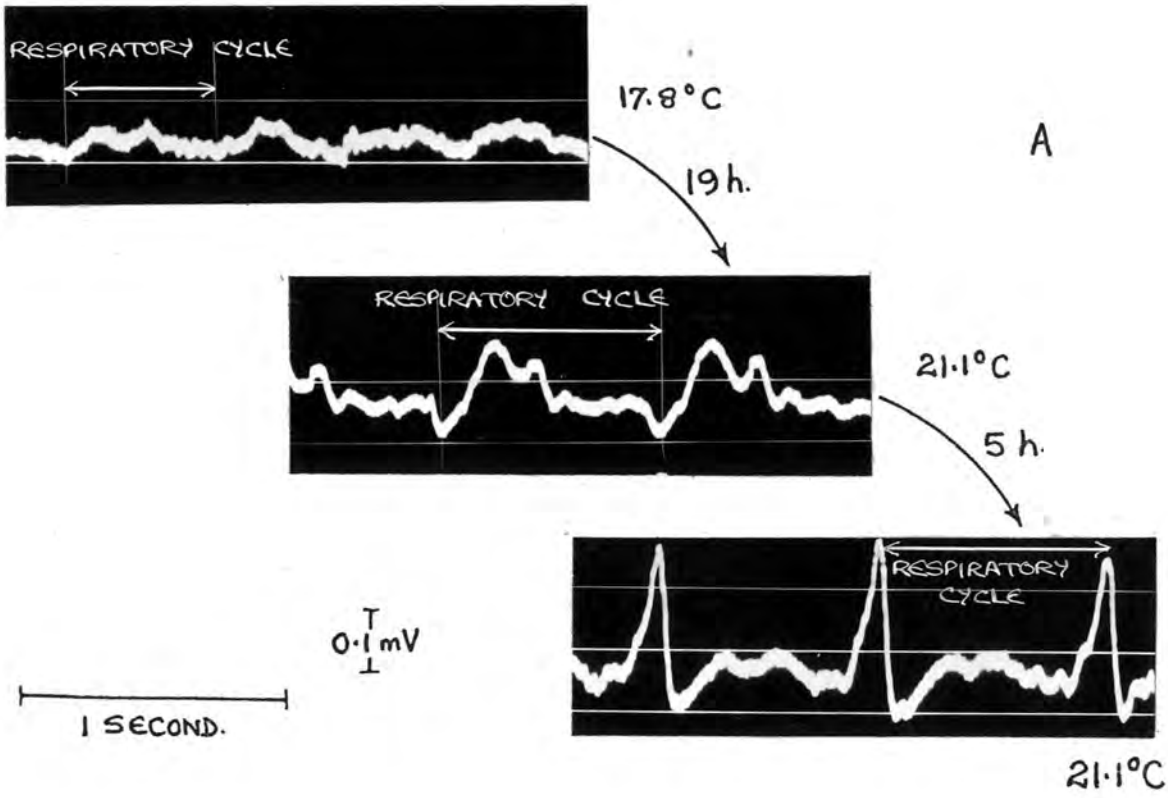
the fish. In these cases the traces were usually more prominent than traces obtained at a similar temperature during a temperature decrease from 25.8°C (see section (g)). Some examples can be seen in Fig. 20 where traces recorded before and after the temperature increase are given for three fish as well as traces recorded previously at approximately 21°C during a temperature decrease from 25.8°C. Fish A (Fig. 20A) is a typical example of the changes that occur, as are Fish T and I (Fig. 20D), whereas Fish R and O are more exceptional (Figs. 20B and 20C) in that the electrical respiratory activity is frequently also prominent at 17.8°C (Table 3).

It would have been interesting to ascertain if there is any correlation between the experimental temperature and the magnitude of the electrical respiratory traces. It is also possible that these values may change with time at a new experimental temperature and thus indicate some sort of acclimation. There was not time to investigate these aspects more fully here.

Thus although the methods used for recording the opercular/buccal respiratory activity were not those more generally used, they have provided a fair amount of information about the opercular/buccal respiratory cycle length and hence about the related respiratory rate. These results have also indicated that the opercular/buccal respiratory muscle activity as recorded electrically may also indicate alterations with changes in the experimental temperatures. The latter aspects will be discussed more fully later in section (g). And finally they allowed an investigation of a possible cardio-respiratory correlation, i.e. whether the heart tends to beat at any particular stage of the respiratory cycle or not. The findings of this investigation are also reported in section (g).

P.T.O. for pages 52 and 53

(Fig. 20)


RESPIRATORY CYCLE
17·8°C
19 h.
A
RESPIRATORY CYCLE
21·1°C
5 h.
RESPIRATORY CYCLE
0·1 mV
1 SECOND.
21·1°C

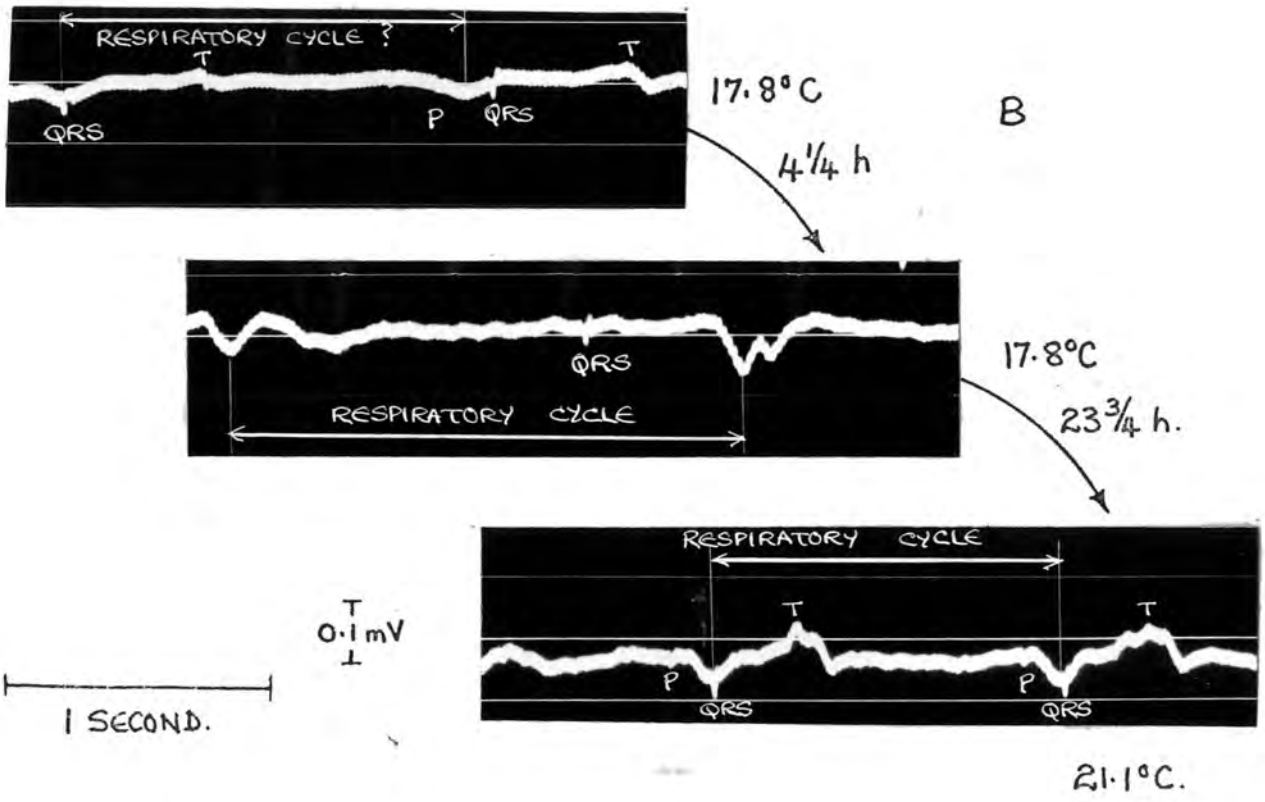
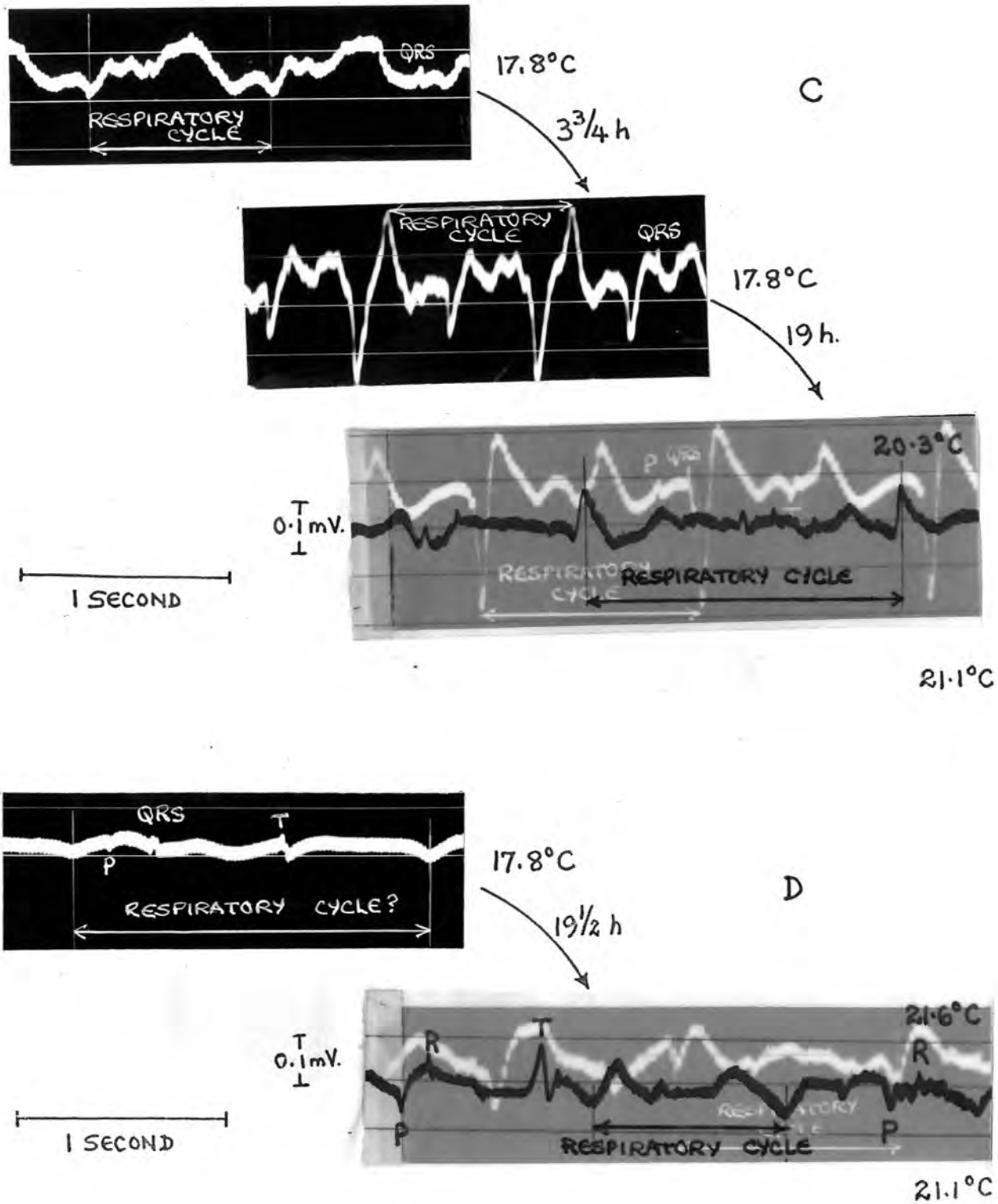

RESPIRATORY CYCLE ?
T
T
QRS
P
QRS
17·8°C
B
4¼ h
QRS
RESPIRATORY CYCLE
17·8°C
23¾ h.
RESPIRATORY CYCLE
T
T
P
P
QRS
QRS
0·1 mV
1 SECOND.
21·1°C.

Figure 20

(below and opposite)

Examples of respiratory activity in four fish before and after the temperature was raised from 17.8°C to 21.1°C, and at approximately 21°C during a temperature decrease from 25.8°C (superimposed; actual temperature given in brackets)

A - Fish A; B - Fish O; C - Fish R; D - Fish I.



(d) The effects of Sandoz MS 222 anaesthesia and handling on respiratory and cardiac functions.

In an early experiment where 2 fish were caught, anaesthetised and implanted with electrodes, it was observed that the opercular movements seemed accelerated for ± 1 day before they slowed down to a level which appeared to be normal. It was suggested that these elevated respiratory rates may have been because of a long-lasting effect of the anaesthetic and/or handling and a simple experiment was thus designed in order to investigate this possibility.

Four fish of standard length ± 8.2 to 9.2 cm (fish were not weighed) were caught at random from a holding tank, and each transferred to a transparent plastic chamber (as in Fig. 2, p. 12, but fish without electrodes) where they were left for 2 days to settle down. Water was aerated, and was changed twice by siphoning. The fish were then subjected to one of 4 'treatments', and then after a 5-day break subjected to a different 'treatment'. The first and second 'treatments' (I and II respectively) for the 4 fish were as follows:-

	'treatment' I	'treatment' II
Fish 1	control - nothing done	anaesthetised and handled
Fish 2	handled only	anaesthetised only
Fish 3	anaesthetised and handled	control - nothing done
Fish 4	anaesthetised only	handled only

Sandoz MS 222, freshly prepared, was used as a 150 to 160 mg/l solution in tap water at the same temperature as the water in which

the fish had been held. The fish were placed in the anaesthetic and were removed only after all respiratory movements had ceased and the animals were lying with their ventral side up.

Ventilation rates per minute were measured after initial capture and during and between the two 'treatments'. These were determined by counting the number of opercular/buccal movements in 20 or 30 second periods, and then making the required calculations. The rates were corrected for individual fish to those expected at 18° C (using the results reported in section (e)), as the water temperature varied between 17.5 and 21.0° C during the course of the experiment. Some selected results are given in graphical form in Fig. 21, and are representative of the results obtained.

Fig. 21 indicates that the opercular/buccal respiratory rate during the first day following capture and transfer to the experimental chambers was slightly elevated (points marked B) when compared to the respiratory rate the next day (points marked C). These values are significantly different in two of the fish (Fish 1 and 2) from respiratory rates measured during the treatments in the same fish as indicated by the 1.96 X S.E. values. During early anaesthesia and during the few minutes of recovery from anaesthesia, the respiratory rates were markedly elevated and the amplitude of the movements were also increased; during the period of complete anaesthesia, however, respiratory movements became erratic and slow, then stopped completely (between the arrows, Fig. 21). Handling in combination with anaesthesia produced similar results, but handling on its own caused no significant change in opercular/buccal respiratory rate when compared with the control fish.

These simple experiments showed that Sandoz MS 222 only caused marked alterations in opercular/buccal respiratory rate during the

P. T. O. for pages 56 and 57

(Figure 21)

Legend for Figure 21

(opposite; please rotate through 90°)

The effect of initial capture and handling, Sandoz MS 222 anaesthesia, and later handling on the buccal/opercular respiratory rate in three Tilapia mossambica.

All readings have been adjusted to represent readings at 18.0°C.

All points represent the mean of 2 or 3 readings.[1.)]

(I) or (II) - before, during or after the first (I) or second (II) 'treatment'

·····o··· - Fish 1 (II); anaesthetised and handled.

—●— - Fish 1 (I); control.

—·—▲—·— - Fish 2 (I); handled only.

----o---- - Fish 4 (I); anaesthetised only.

(see the superimposed sheet)

B - mean of 2 readings on day following initial capture, i.e. 2 days before 'treatment' I.

C - mean of 2 readings on day before 'treatment'.

D - mean of 2 readings 1½ to 1 h before 'treatment'.

↑ - 'treatment' carried out at this point.

↘ ↘ - period of complete anaesthesia between these arrows: all respiratory movements stopped.

Graphs fitted by eye in each case.

1.) Points with a central spot (e.g. ⊕) do not represent a mean of two readings, but represent the mean of all readings after any one 'treatment'. Vertical bars give the 1.96 X S.E. limits.

Figure 21.

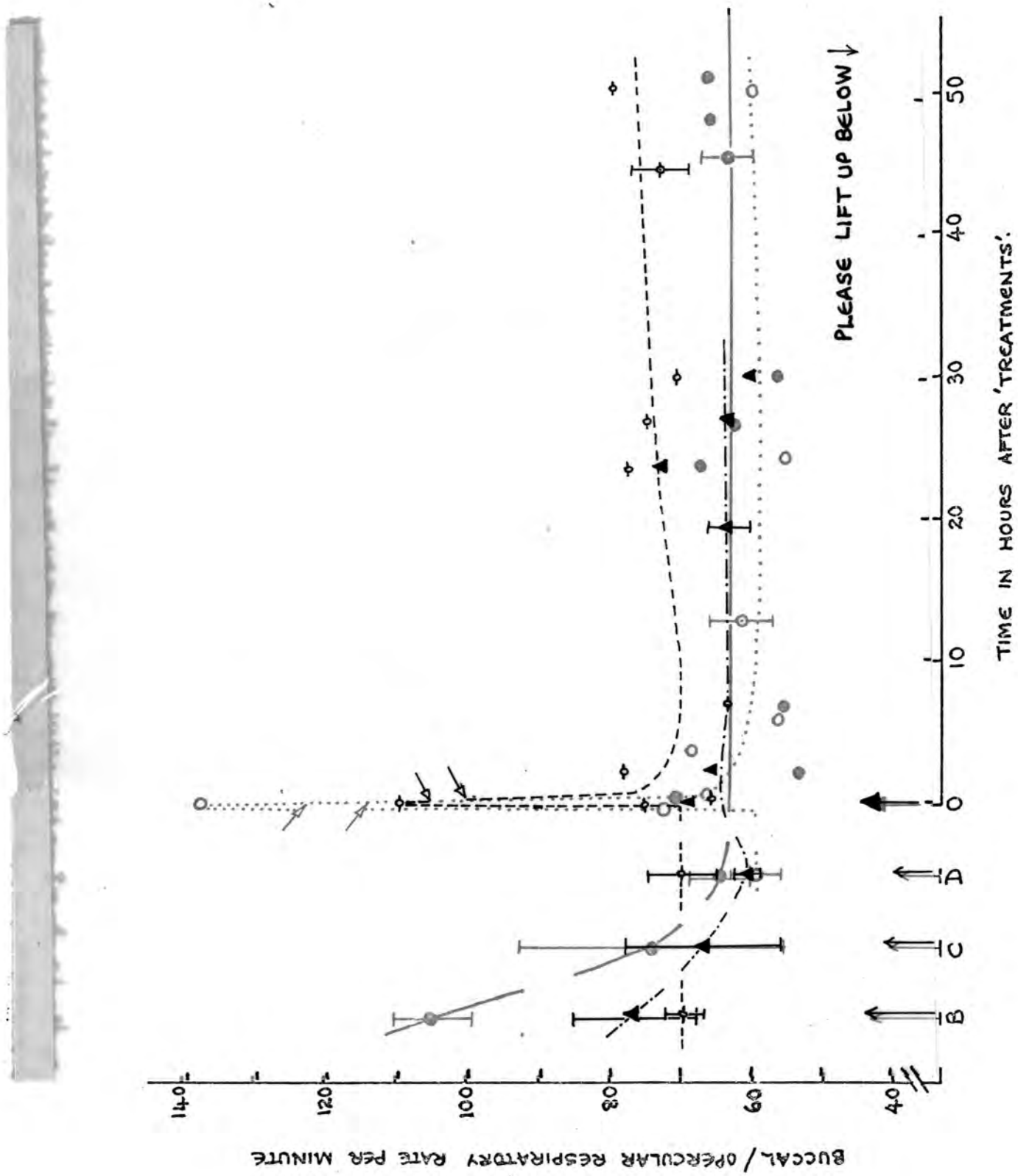
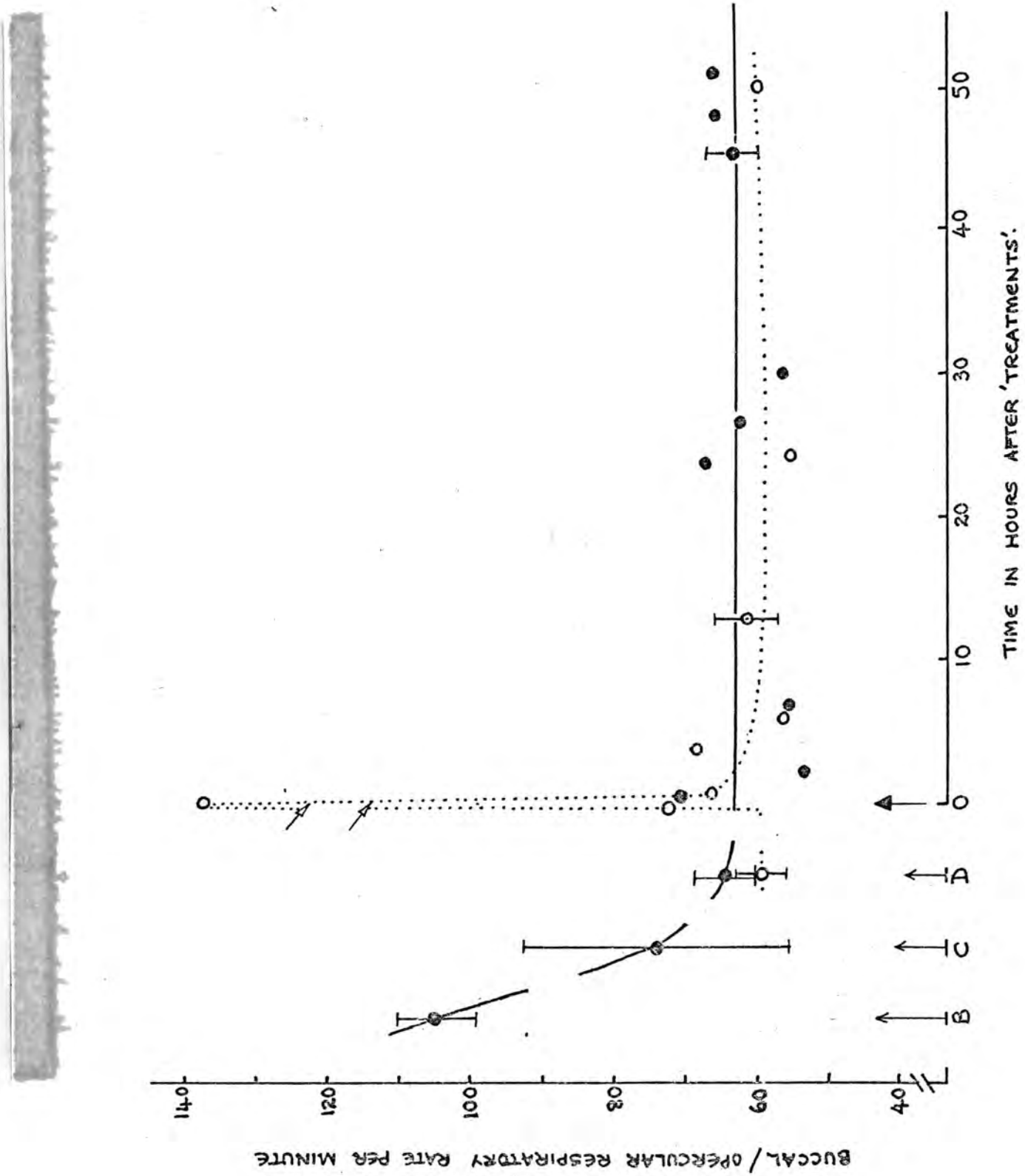

BUCCAL / OPERCULAR RESPIRATORY RATE PER MINUTE
140
120
100
80
60
40
A
C
B
0
10
20
30
40
50
TIME IN HOURS AFTER 'TREATMENTS'.
PLEASE LIFT UP BELOW

Figure 21.


BUCCAL / OPERCULAR RESPIRATORY RATE PER MINUTE
140
120
100
80
60
40
A
B
C
D
0
10
20
30
40
50
TIME IN HOURS AFTER 'TREATMENTS'.

period when the anaesthetic was present, and for the few minutes immediately after transfer back to tap water. Handling was without effect except when this was associated with initial fish capture from the holding tank, and it is suggested that the stress of initial capture and handling was responsible for the slightly raised respiratory rates seen prior to 'treatment' I. In the first few days of the experiment reported in section (g) there is also a suggestion of raised opercular/buccal respiratory rate following capture of the fish which was closely followed by Sandoz MS 222 anaesthesia. This can be seen both in Fig. 27 and in section VI(c). These experiments also showed a similar but smaller post-handling increase in some heart functions in two of the fish, as there is a slight decrease in both the P-R and R-R intervals in this post-handling period (Fig. 28 and section VI(c)). This will be discussed more fully in section (g).

It is also of interest that in a few early experiments fish were re-anaesthetised to correct electrodes or their leads, and in none of these cases were post-anaesthesia elevations in respiratory or heart functions noted.

My experiments provide no information as to the effect of whole-fish anaesthesia on heart rate or other cardiac functions as no suitable recordings were available from the anaesthesia or immediately post-anaesthesia periods. However, some fish were dissected under Sandoz MS 222 anaesthesia, and ECGs were recorded from the exposed heart (see above section (b)). These ECGs have been compared to those recorded from recovered fish with implanted electrodes held at approximately the same temperature. The results indicate that under anaesthesia the R-R interval is lengthened proportionately more than the P-R or R-T intervals (the R-R also changes more extensively when compared to the P-R or R-T under normal conditions in this fish (see p. 133 of the Discussion)),

thus the duration of diastole is increased to a greater extent than the duration of systole during the general heart slowing as a result of Sandoz MS 222 applied directly to the heart.

In order to avoid unnecessary explanations later, the next three sections ((e), (f) and (g)) require an introduction over and above that given in Part I. The following thee sections report the results of the temperature experiments in the order in which they were done.

The early experiments (section (e)) were designed to establish the way in which both the respiratory and cardiac functions measured varied during slow temperature changes (2.0 to 2.5°C per day approximately) over the range 18 to 36°C. Considerable or even complete thermal acclimation may be expected at these slow temperature changes (Hughes & Roberts, 1970), and Long et al (1961) suggest that 20° to ±35°C are adaptive temperatures for T. mossambica, i.e. temperatures to which this fish can adapt completely. These experiments, however, gave rather thin results, and few ECGs were recorded except from two of the nine experimental animals.

Two fish with implanted electrodes which showed ECGs on traces recorded from them were selected for the next experiment (section (f)). Both heart and respiratory functions were studied using a more sudden experimental temperature decrease (7.5° C in 2 h, i.e. approximately 1°C / 15 min). This rate of temperature change might allow some small degree of thermal acclimation during the change (Hughes & Roberts, 1970). The temperature decrease was from approximately 25.6 to 18.1°C, i.e. from well within the normal temperature range for this species to the upper border of the lower sub-lethal range which Long et al (1961) give as 20°-16°C or lower. As the fish were only held at 18.1°C for about 2¼ days, acclimation to this low temperature was probably very incomplete. The results of these experiments suggested, however, that it was important to repeat and extend the experiments. This was done using six fish in the last experiment (section (g)).

In these last experiments, 2 different temperature decreases were used, the 1st. from 25.8 to 17.8 °C, an 8 °C decrease very similar to that used in the previous experiment, and the 2nd. from 21.1 to 14.1 °C, a decrease of 7 °C. In both cases the fish were held at the lower temperature for 4 days. The 2nd. temperature decrease, although of similar magnitude to the 1st., has as its lower limit a temperature falling within the temperature range where physiological malfunction has been reported for this fish as already mentioned in Part I. Most particularly Allanson (1966) has found that some fish at 14 °C show kidney disruption and vacuolation after 3, 5 or 9 days at this temperature. It is also important to bear in mind that the last experiments were performed in summer (January), whereas the previous experiments (sections (e) and (f) were done in winter or early spring (July to September).

Acclimation to cold is considered a slow process in most fish, and in a thermophile such as T. mossambica 'cold' will mean temperatures below 18 - 20 °C as discussed in Part I. Thus Spaas (1959b) suggests that the rate of acclimation to decreasing temperature (25 - 15 °C) is slower than a rate of 1 °C/3 days for 3 cichlids he studied, Haplochromis melland̲i, T. melanopleura and T. macrochir, and Allanson & Noble (1964) suggest that 20 days is required for cold acclimation after a 25 - 15 °C transfer in T. mossambica. However, the 4-day period at 17.8 or 14.1 °C in these experiments may have been long enough for acclimation tendencies to be seen in those functions which start acclimating first as the most rapid acclimation changes after any initial lag period may occur during the earlier period at a new temperature (Freeman (1950) Fig. 6, Peak et al (1967) Fig 2, and Doudoroff (1942)). However, Wells (1935b) gives oxygen consumption data for Fundulus parvipinnis that indicate that the lag period before acclimation may be very lengthy in this species. Similarly in the mudminnow Umbra lima there is a stabilisation or lag

period of 4 - 5 days before oxygen consumption acclimation starts (Figs. 1 and 2 of Hanson & Stanley (1970)).

The 7 or 8°C decreases used in the present experiments is a much smaller temperature decrease than that experienced in the field under monsoon conditions (for e.g. in Vietnam). In the northern regions of Vietnam, during the winter monsoons, Long et al (1961) reports Bruzon (1930) as follows:- ".. the water temperature averages barely 23.9°C, and between December and March it may drop to 7 - 8°C...". The rate of temperature change during monsoons as recorded by Long et al (1961) is ± 1°C in 12 - 15 min. Elsewhere in Vietnam temperature decreases of 15°C or more have been reported in winter where the upper, initial temperature is approximately 30°C (Long et al, 1961); the lower limit is therefore ± 15°C, similar to the lowest experimental temperature used in the last experiments (section (g)).

Thus, although the lower experimental temperatures used are representative of lower sub-lethal temperatures for this species, the temperature decreases used in sections (f) and (g) of the present study are similar to monsoon conditions only in rate of change, not in extent, and the minimum experimental temperatures used (14.1°C) are higher than minimum monsoon temperatures in general.

(e) The 18° to 36°C experiments with particular reference to the opercular/buccal respiratory rate.

Electrodes were implanted in the gular region of 9 fish (mean weight 45.0 gm) which were then subjected to the following temperature régime after holding at an acclimation temperature of approximately 22°C for at least 2 days:- The temperature was lowered to 17.8°C and held there for 24 h. The temperature was then raised by approximately 2°C each day over a period of 2 h leaving the experimental fish at the new, higher temperature for approximately 22 h. In this way the temperature was raised to 36.0°, held there for a day, and then lowered by increments of approximately 2.5°C per day (again over a period of 2 h), the fish then being left at this new and lower experimental temperature until the next day. The temperature was, in this way, lowered to 25.0°C. Experiments were discontinued at this point because the electrodes had been implanted for over 3 weeks and were causing damage to the fish and, in a number of cases, results could not be readily recorded.

Two of the nine fish showed occasional ECGs on the oscillograph traces, and most fish gave electrical records of the opercular/buccal respiratory activity. It was, however, not possible except in a few cases, to find any record of ECGs in the two fish mentioned above at temperatures higher than 25.0°C; these few results are included in Figs. 42 and 43 (pp. 113 and 114) of section (g), and will therefore not be discussed here.

The opercular/buccal respiratory rates at any temperature during the temperature increase were not noticeably different from those at the same temperature during the temperature decrease (probably because of more or less complete acclimation), therefore the results recorded over temperature intervals of approximately 1°C were pooled and a graph

drawn to indicate the trends (these results are given in full in section VI (c)), and mean values have been calculated. These trends are illustrated in Fig. 22 (graph 2, p. 66) together with the results of an early preliminary experiment on 2 fish (graph 1, mean weight 30 gm), and of an experiment on 6 fish (graph 3, mean weight 68.6 gm) which is reported more fully in section (g). I have also included (graph 4) the mean results of a rough student experiment using 10 small ($\pm$ 14 gm) $\pm$ 18°C acclimated fish where temperature increased rapidly between 15.0° and 35.0°C (approximately 1°C in 5 min). This rapid change is seen to have excluded thermal acclimation to a large extent when comparing the graphical results, as curve 4 is much steeper than curves 1, 2 and 3 (Fig. 22). In the latter three cases, considerable thermal acclimation may have taken place, and the opercular/buccal respiratory rates have probably decreased at the higher temperatures.

Curves 2 and 3 are probably not significantly different from each other as the figures for the 1.96 X S.E. about the mean of the individual plots overlap considerably. Size differences may also account for a large proportion of the difference between curve 4 (smaller fish) and the other 3 curves (larger fish), as it has been found that respiratory functions as studied here are speeded up in smaller animals (see section (g), p. 89). Size differences may also account for the smaller differences between curves 1, 2 and 3 (mean fish weights 30.0, 45.5 and 68.6 gm respectively). Also as curves 1, 2 and 4 are derived from winter or early spring fish, whereas curve 3 is derived from summer fish, if the ventilatory rate shows any seasonal variation with higher rates in cool season fish as compared to warm season fish at the same temperatures, the lower values for fish of curve 3 (summer fish and larger fish) may be accounted for.

Fig. 22 also indicates that the opercular/buccal respiratory

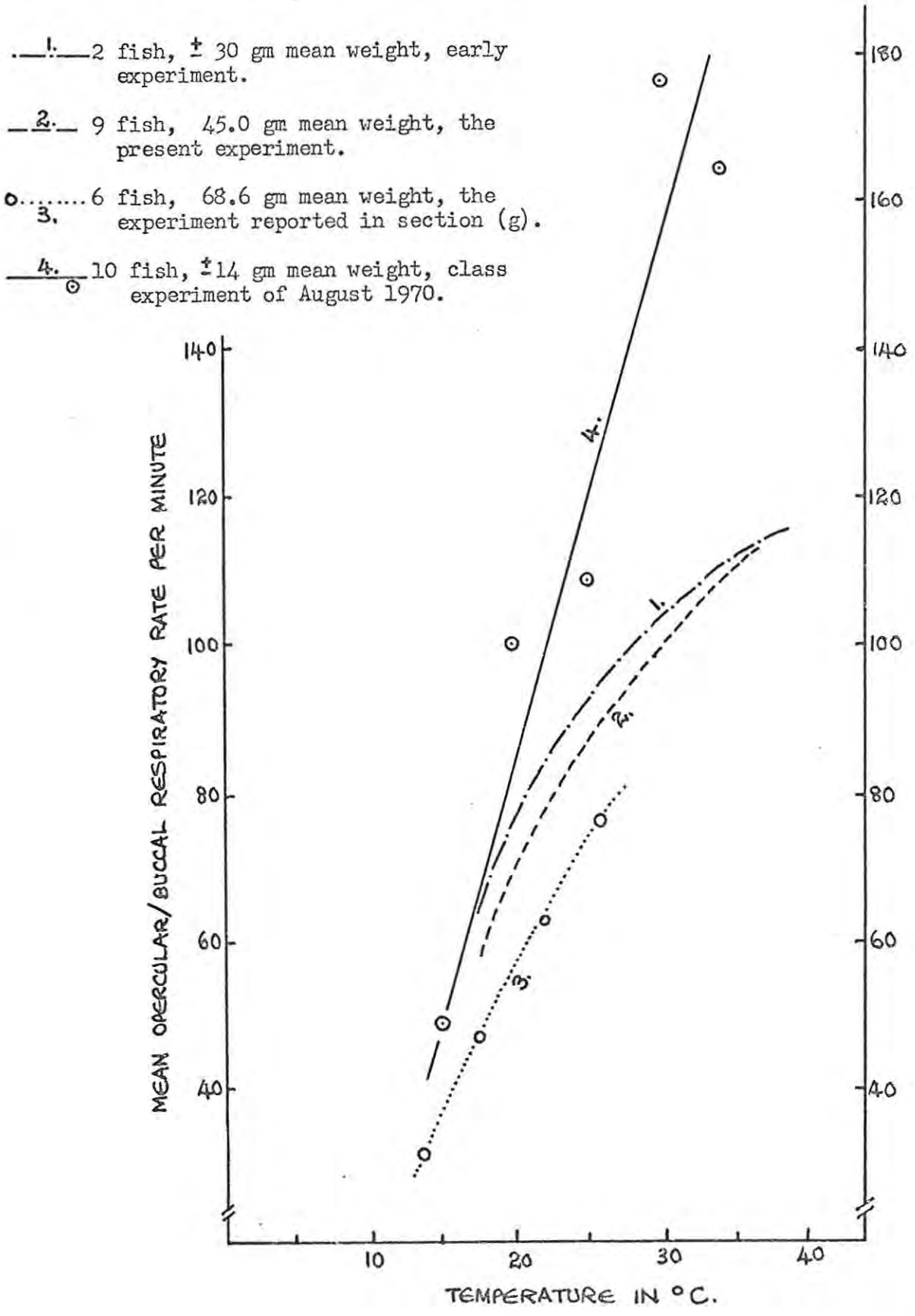
rate changes unevenly with temperature, and in particular it does not increase so rapidly at higher temperatures approaching the upper lethal limits for the species (about 39 °C). Thus the graph of respiratory rate against temperature is slightly curved particularly at high temperatures. The Q_{10} values (corrected for temperature intervals less than 10.0 °C as described in section (g), p. 77) have been calculated for the nine fish of the present experiments. The mean Q_{10} for the 25.8 to 35.8 °C range is 1.25, whereas for the 17.8 to 25.8 °C range it is 1.61. Q_{10}s calculated from the slope of the semi-logarithmic R-T (rate-temperature) plot give the following values for graphs 1 and 2 of Fig. 22, and for the mean of the two graphs:-

Experimental Temperature	Q_{10} - values		
	Graph 1	Graph 2	Mean of graphs 1 and 2
20 °C	1.87	1.71	1.79
25 °C	1.40	1.50	1.45
30 °C	1.18	1.30	1.24
35 °C	1.08	1.15	1.12

These results indicate that the Q_{10} values of the ventilatory rate tend to decrease with an increase in temperature over the temperature range used. Results reported in section (g) for the data represented by graph 3 of Fig. 22, confirm this.

Figure 22.

The mean opercular/buccal respiratory rate with change in temperature during a number of experiments. The full results of curves 2 and 3 are given graphically in section VI(c). All curves have been fitted by eye.



(f) Experiments over the temperature range 18.1° to 25.6°C with particular reference to cardiac function.

Two fish were held at 25.6 ± 0.6°C for more than 7 days before being subjected to the following temperature changes (acclimation to this temperature was thus probably complete):- The temperature was decreased rapidly (within 2 h) from 25.6 to 18.1°C approximately, and was held at 18.1 ± 0.1°C for the next 2½ days. The temperature was then raised slowly, by increments, over a period of 3¼ days to 24.5°C.

Recordings of electrical respiratory activity were not obtained, but ECGs were regularly recorded. The changes in the cardiac intervals P-R, R-T and R-R with temperature are illustrated in Figs. 23 to 25. In Fig. 23 the R-R interval has been indicated together with the heart rate. The heart rate was calculated from the R-R interval as follows:-

$$\frac{60}{\text{R-R interval in s}} = \text{heart rate per minute}$$

Thus the R-R interval and heart rate are inversely related. Fig. 24 illustrates changes in the P-R interval, and Fig. 25 changes in the R-T interval. The two fish used in this experiment were Fish VI and VIII.

The results show a number of interesting features. As is to be expected, when the temperature was lowered, all cardiac functions showed an immediate slowing; the cardiac intervals were therefore all lengthened. This means that heart rate, the rate of conduction from one heart chamber to another as well as the rate with which the myocardium contracts and relaxes again were all slowed. When the temperature rose again through stages at 19.3, 21.0 and 22.0 to 24.5°C, the few results obtained suggest that all cardiac functions accelerated once more (Figs. 23 to 25).

contd. on p. 70.

Figure 23.

Changes in heart rate and the R-R interval during the experimental temperature changes.

—●— - Fish VII; —×— or —○— - Fish VIII.

All curves have been fitted by eye.

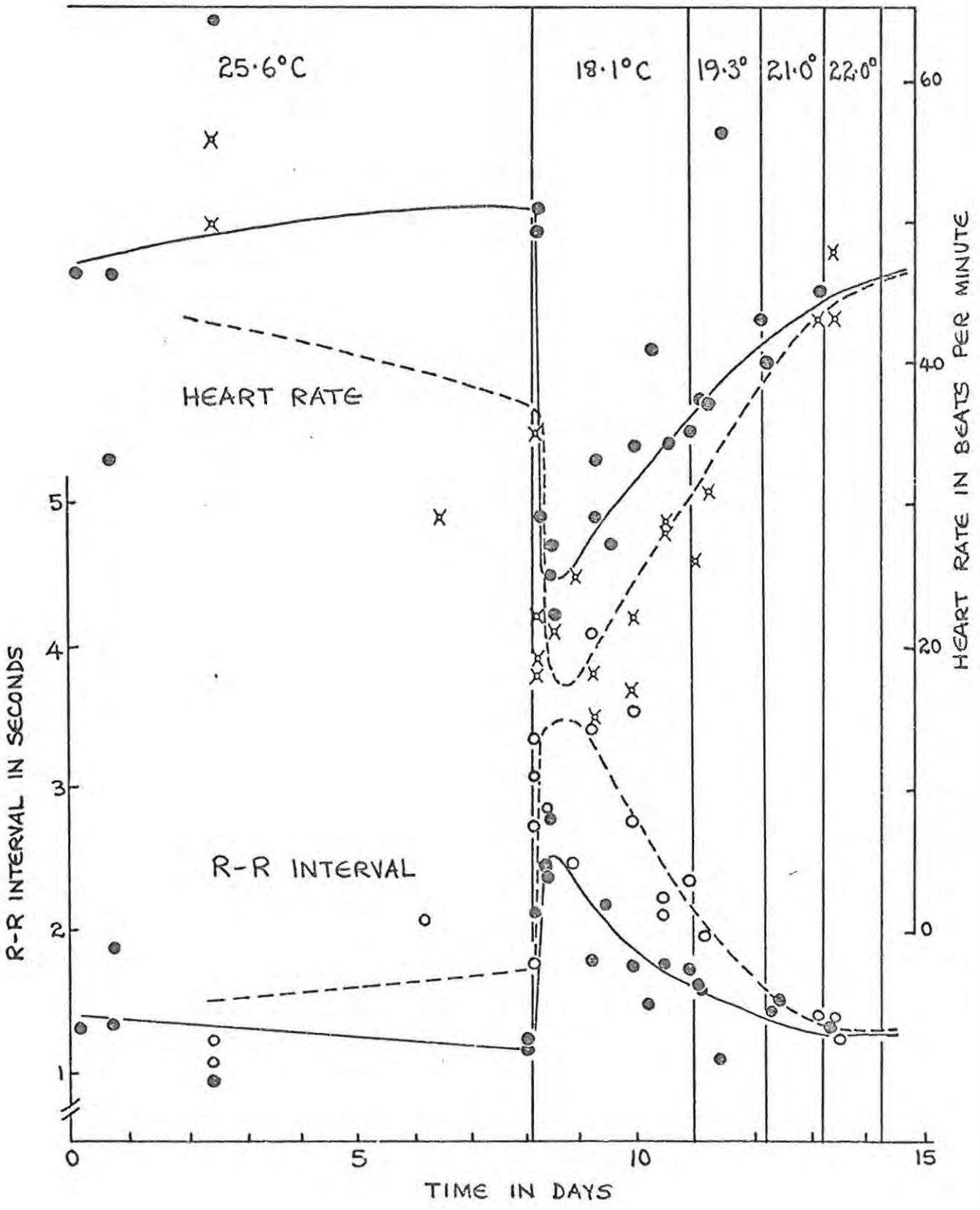


Figure 24

Changes in the P-R interval during the experimental temperature changes. Curves fitted by eye.

- Fish VII; - Fish VIII.

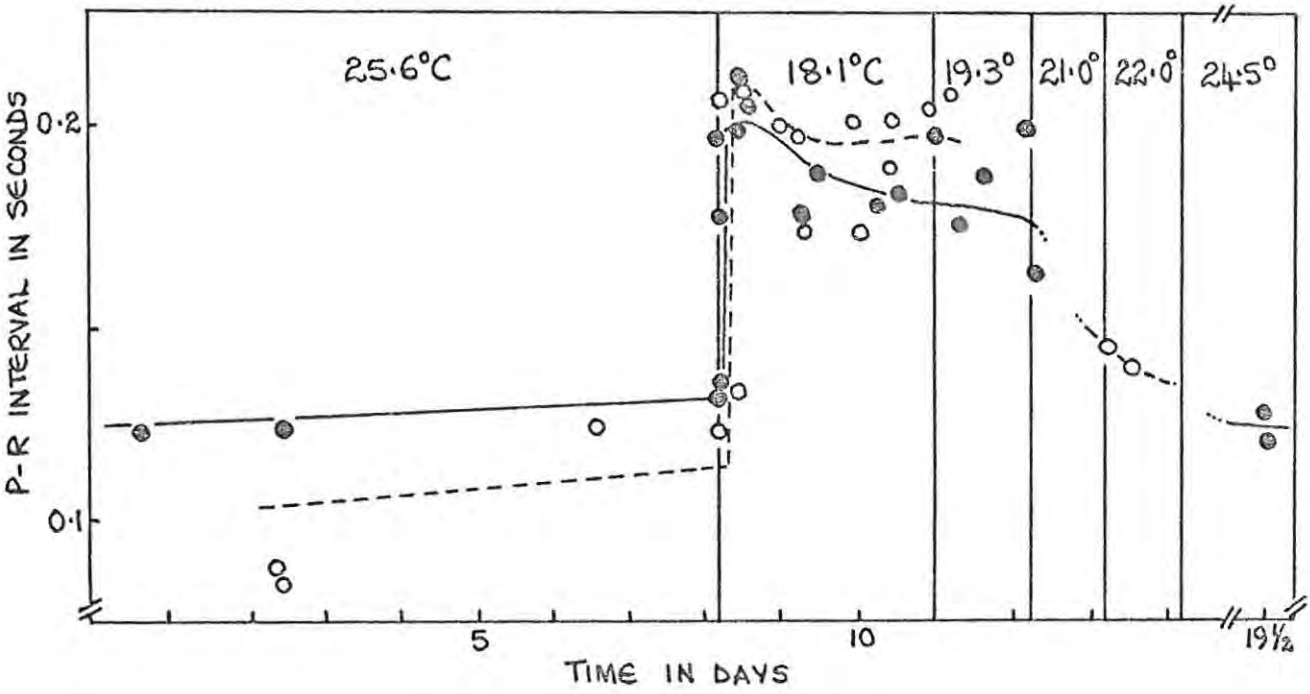
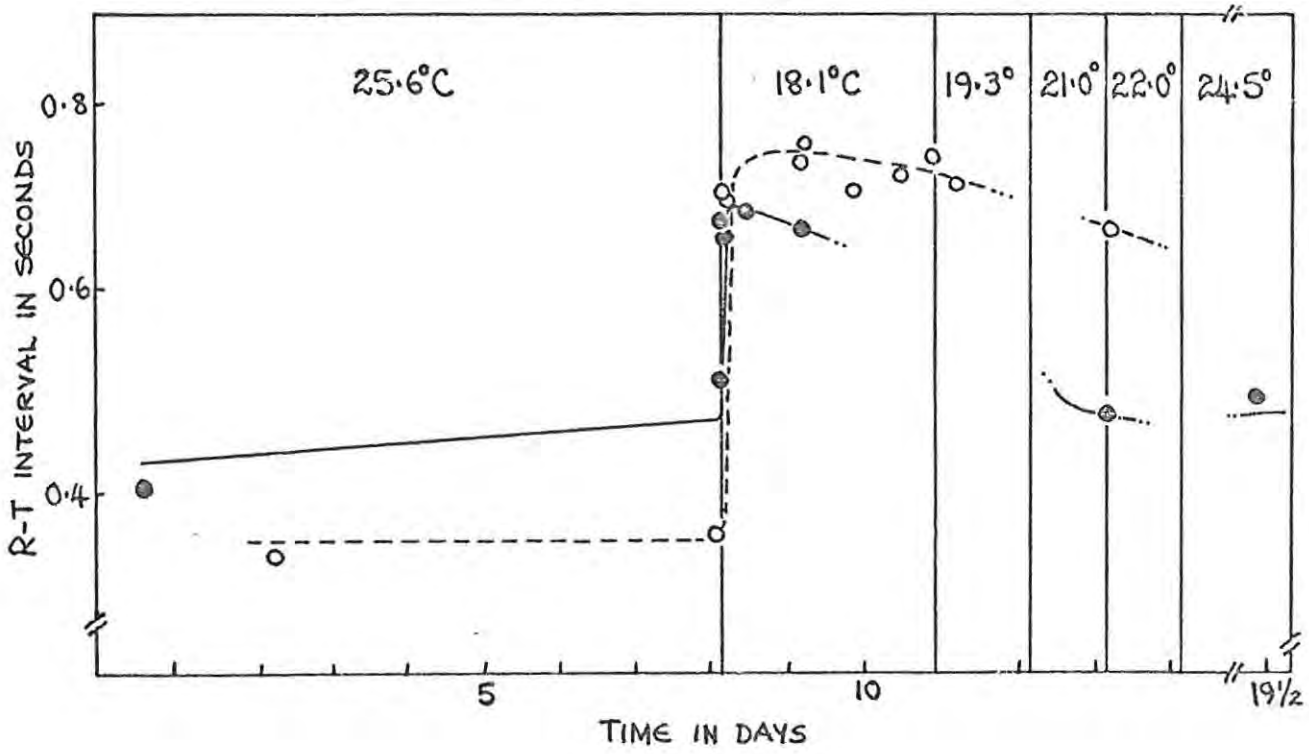


Figure 25

Changes in the R-T interval during the experimental temperature changes. Curves fitted by eye.

- Fish VII; - Fish VIII.



It can be seen that the R-R interval and heart rate did not, however, remain constant with time while the temperature remained at 18.1°C, but the R-R interval tended to shorten gradually, and the heart rate therefore to increase. The very sparse results do not indicate a similar trend in the P-R and R-T intervals.

In order to determine whether the changes in the heart rate and in the R-R interval are significant changes or not, the time period at 18.1°C was arbitrarily divided into two periods as follows:-

period D - the first 7 h the fish were at 18.1°C

period E - the remaining period at 18.1°C, i.e. from $7\frac{1}{2}$ h to $2\frac{1}{2}$ days.

In Table 4 the mean values for the three cardiac intervals measured have been calculated for the period at 25.6°C, and for the two arbitrary periods at 18.1°C, periods D and E, for the two experimental fish. This Table also indicates the % of the cardiac cycle occupied by the cardiac interval in each case. Thus the R-R interval represents the cardiac cycle, and therefore occupies 100% of it; the P-R and R-T intervals, however, occupy a smaller and variable % of the cycle.

Table 4 also illustrates that all cardiac intervals lengthen when the temperature is decreased from 25.6 to 18.1°C; for e.g. the average values for the R-R interval in Fish VII change from 1.25 to 2.21 s, and those for the P-R interval for this same fish from 0.130 to 0.203 s, the 18.1°C value taken from period D in both cases. However, if the figures for these intervals during period D are compared to those during period E we see that the R-R interval has shortened with time while the fish was at 18.1°C from 2.21 to 1.75 s, i.e. has shortened by 0.46 s, whereas the P-R interval for this same fish has shortened from 0.203 to 0.184 s, a decrease of 0.019 s.

Table 4.

Average values for ECG intervals at 25.6 ± 0.6°C and 18.1 ± 0.1°C for Fish VII and VIII, and the relative length of these intervals as a % of the cardiac cycle time.

Experimental temperature and, in case of 18.1°C, period for which fish were at this temperature	Mean times of the cardiac intervals in seconds and the % of the cardiac cycle occupied by these intervals					
	R-R interval		P-R interval		R-T interval	
	FISH VII	FISH VIII	FISH VII	FISH VIII	FISH VII	FISH VIII
25.6 ± 0.6°C	1.25 100%	1.80 100%	0.130 10.4%	0.110 6.1%	0.450 36.0%	0.340 18.9%
18.1 ± 0.1°C for first 7 hours (period D)	2.21 100%	3.39 100%	0.203 9.2%	0.185 5.5%	0.669 30.3%	0.705 20.8%
18.1 ± 0.1°C for 7½ hours or longer (period E)	1.75 100%	2.76 100%	0.184 10.5%	0.200 7.3%	0.659 37.6%	0.725 26.3%

These absolute values for the amount of change are with little meaning as, for e.g., the R-R interval is nearly 10 times larger than the P-R interval. If these values are given as a % of the interval in period D, we find that the R-R interval has shortened by 20.8% and the P-R interval by 9.4%. These relative or % changes in the three cardiac intervals between periods D and E are given in Table 5.

Thus the R-R interval changes to a greater extent than do the P-R or R-T intervals, and the change in the R-R is also in a constant direction in the two experimental fish.

Referring again to Table 4, it is also clear that if the R-R interval decreases in a more marked way than do the other intervals when the fish were held at 18.1°C, these latter intervals will therefore come to occupy a greater proportion of the cardiac cycle. Thus the % of the cardiac cycle occupied by both the P-R and R-T intervals are larger in period E than in period D.

The P-R interval together with the R-T interval as measured in the present experiments represent approximately the period for which the myocardium remains contracted (excluding any sinus venosus component, if present). Hence these two periods together represent electrical cardiac systole, and the remaining time $\{$R-R minus (P-R plus R-T)$\}$ represents the period of electrical cardiac diastole. Thus if the cardiac cycle time shortens markedly during the experimental period at 18.1°C as compared to the period of systole, the relative proportion of the cardiac cycle occupied by systole becomes greater, and the period of diastole shorter. This is illustrated in Table 6 where the % of the cardiac cycle occupied by systole is shown.

Thus while the temperature remains at 18.1°C the % of the cardiac

contd. on p. 75

Table 5.

% changes in the cardiac intervals between period D (fish at 18.1°C for first 7 hours) and period E (fish at 18.1° for remaining time) for Fish VII and VIII.

A positive percentage indicates that the time occupied by an interval during period E is longer, i.e. the interval has increased with time; a negative percentage indicates that the interval has decreased with time.

Experimental fish	Change in cardiac intervals at 18.1°C as a % of the values for the intervals during period D		
	R-R	P-R	R-T
Fish VII	-20.8%	-9.4%	-1.5%
Fish VIII	-18.6%	+8.1%	+2.8%

Table 6.

Average values for the duration of cardiac systole at 25.6 ± 0.6°C and 18.1 ± 0.1°C for Fish VII and VIII, and the % of the cardiac cycle occupied by systole.

Values were calculated only from readings where R-R, P-R and R-T were measured concurrently, and the number of readings averaged are given in brackets. The % of the cardiac cycle is followed by the 1.96 x S.E. of the mean value.

Experimental temperature and, in case of 18.1°C, period for which fish were at this temperature	Mean duration of systole (P-R plus R-T) in seconds, and % of cardiac cycle occupied by systole ± 1.96 x S.E. of the mean.	
	Fish VII	Fish VIII
25.6 ± 0.6°C	0.58 46.6%	0.45 25.0%
18.1 ± 0.1°C for first 7 hours (period D)	0.87 (4) 42.0 ± 3.9%	0.89 (2) 27.0 ± 1.4%
18.1 ± 0.1°C for 7½ hours or longer (period E)	0.74 (2) 47.5 ± 0.5%	0.90 (7) 38.6 ± 8.4%

cycle occupied by systole increases by 8.6% and 7.3% in Fish VII and VIII respectively. These represent significant changes as is shown by the 95% confidence limits of the means (Table 6).

With reference to Table 4 again, it is interesting that in Fish VII the % of the cardiac cycle occupied by the cardiac intervals P-R and R-T are virtually the same for the 25.6°C period and for the period after $7\frac{1}{2}$ h (period E) at 18.1°C. This similarity may mean that, after some time has elapsed when this fish was at 18.1°C, cardiac acclimatory changes had occurred such that the proportional times occupied by these measured phases of the heart function resembled those at higher, more normal temperature for this species. This aspect will be discussed more fully in the next section.

By measuring the cardiac intervals R-R, P-R and R-T, some idea is obtained of the time that has elapsed between two discrete electrical events. By then converting these intervals as described above for the R-R interval to the rates of the various activities, rates of the same or different activities can be compared at the same or different temperatures, and the Q_{10} and Arrhenius μ values can thus be calculated. Thus

$$\frac{60}{\text{R-T interval in s}} = \text{the rate of ventricular activity in arbitrary units}$$

and

$$\frac{60}{\text{P-R interval in s}} = \text{the speed of conduction between the atrium and the ventricle, also in arbitrary units.}$$

Table 7 gives the Q_{10} values calculated for the 7.5°C interval (25.6 to 18.1°C) for the various cardiac rates. The formula used for calculating these Q_{10} values makes a correction for the use of any temperature interval less than or greater than 10°C (Prosser et al,

Table 7.

Q_{10} values for the rates of the various cardiac functions calculated over the 7.5°C interval between 25.6 and 18.1°C for Fish VII and VIII using values both for period D (first 7 hours at 18.1 ± 0.1°C) and period E ($7\frac{1}{2}$ hours or longer at 18.1 ± 0.1°C).

Period at 18.1 ± 0.1°C used in the calculation of the Q_{10} values	Q_{10} for the different cardiac functions					
	Heart rate:: 1/R-R interval		Rate atrio-ventricular conduction:: 1/P-R interval		Rate ventricular contraction and repolarisation:: 1/R-T interval	
	FISH VII	FISH VIII	FISH VII	FISH VIII	FISH VII	FISH VIII
18.1 ± 0.1°C for under 7 h (period D)	2.14	2.33	1.81	2.02	1.70	2.65
18.1 ± 0.1°C for $7\frac{1}{2}$ h or longer (period E)	1.57	1.77	1.60	2.22	1.67	2.75

1952) and is as follows:-

$$Q_{10} = K \text{ raised to the power of } \left\{\frac{10}{t_1 - t_2}\right\}$$

$$\text{where } K = \frac{K_1}{K_2}.$$

K_1 and K_2 are the rates of any activity at the higher and lower temperature respectively; t_1 is the higher and t_2 the lower temperature.

The Q_{10} has been calculated for the 7.5°C temperature interval using the mean intervals (P-R etc.) during both periods D and E at 18.1°C. Table 7 also indicates that the Q_{10} values for the rates of the three cardiac activities measured all fall in the range of 1.57 to 2.75, with Fish VIII showing rather raised Q_{10} values (2.65 to 2.75) for the speed of contraction and relaxation of the ventricle as compared to the other fish, and to other aspects of heart activity in the same fish. A more relevant point to the above discussion, however, is that the only obvious differences in Q_{10} values between those calculated using rates from period E and from period D are for the Q_{10} values of heart rate. In the two fish the Q_{10} values of 2.14 and 2.33 respectively using rates from period D are substantially larger than the values of 1.57 and 1.77 respectively using rates from period E. This is another indication that heart rate is probably acclimating to the lower temperature by speeding up, whereas other cardiac functions remain slowed.

These findings, based on very limited results, suggested that this experiment should be repeated, and a larger number of fish held at the lower temperature for a longer period to try and establish more firmly whether this possible heart-rate acclimation is a feature of the cardiac physiology of this species at about 18.1°C. This experiment, together with a second temperature-decrease experiment, is reported in the following section.

(g) Repeat and extended experiments using temperatures between 25.8° and 14.1°C.

Six electrode-implanted fish showing consistent or occasional ECGs were selected together with a control fish (anaesthetised as were the others, but not operated upon). The fish were of standard length 11.4 - 14.6 cm and weight 49.8 - 88.0 gm. After these fish had been at approximately 22°C for a 1 - 2 day period, they were together subject to the following temperature régime (Fig. 26):- Fish were held at 25.8 ± 0.1°C for a minimum of 3 days and then the temperature was lowered rapidly (within 2 h) to 17.8° ± 0.2°C where it remained for 4 days. Then the temperature was raised fairly steadily over 17 h to 21.1°C where it remained for 7 h, followed by a rapid decrease to ± 14°C, again within 2 h. The temperature was maintained at 14.1 ± 0.1°C for the remaining 4 days of the experiment. Thus 2 rapid temperature decreases of 8° and 7°C respectively were made.

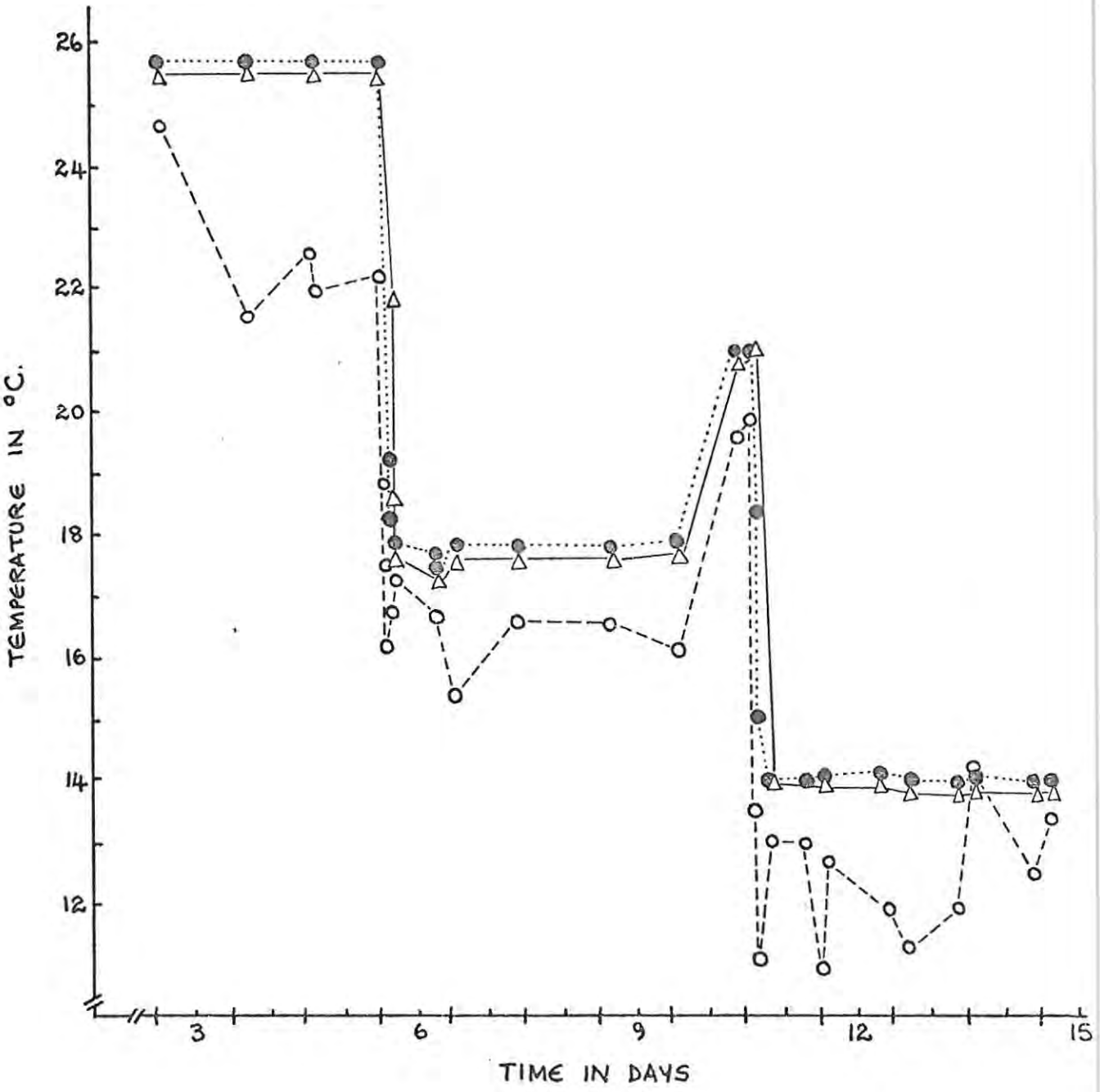
As high-temperature acclimation is often very rapid (Love, 1970), it was considered likely that fish were well acclimated to 25.8°C, and possibly even to 21.1°C after only 7 h at this temperature following 4 days at 17.8°C and some days before that at 25.8°C, as this 25.8°C stay may have resulted in a long-lasting warm acclimation. Also these fish were summer animals (January).

Only in the case of the R-R interval was there an indication of diurnal changes. The R-R was slightly increased during the night and early morning, (from ± 0.00 to ± 10.00 h) in fish held at 14.1 or 17.8°C, but not in 25.8°C held fish. Full results are given in section VI(c). The mean results of all 6 fish have been considered together for each experimental temperature and mean values calculated; trends from the individual plotted results have also been drawn (section VI(c)), and these features are given in Figs. 27 and 28. The results indicate similar responses to temperature change of the respiratory and cardiac functions

Figure 26

The temperature of the water in the plastic container of Fish V, of the water-bath, and of the air in the constant temperature room during the course of the experiment.

Fish V container; water bath; air.



studied as were reported in section (f). In the cases of the ventilatory functions, the data for 25.8°C has been plotted as 2 separate points - for the 1st. and 2nd. 2-day periods (period M and L respectively). For Fish I and A period M was shortly after electrode implantation and initial handling, so the increased respiratory rate may be, in part, a reflection of this (section (d) above). The other 4 fish had been subjected to initial handling, anaesthesia and electrode implantation some days previously, and the increased ventilatory rates during the earlier period M (as seen very clearly in Fig. 31 for Fish T, R and O; Fish V is an exception in this regard) may be due to the increase from ± 22.0°C to 25.8°C. This temperature change may also be of importance for the increased respiratory rates in period M in Fish I and A. Thus the lower respiratory rates during the later period at 25.8°C may be an indication of a thermal acclimation and/or of a settling down after an initial handling and operative stress. Increases in the P-R and R-R interval during the period at 25.8°C are also seen (Fig. 28) but are insignificant.

The mean results of the cardiac and opercular/buccal respiratory functions (Figs. 27 and 28) are given together with an estimate of the 95% confidence limits in each case. These limits indicate that in some cases the variability about the mean is greater, whereas in other cases it is smaller. Some possible reasons for this variability were thus investigated.

It was considered likely that smaller fish [1.)] would, as in the case of the opercular/buccal respiratory functions (see section (e) above), have more rapid cardiac functions than would larger fish, and

---

1.) As all fish used were caught from a rapidly-breeding natural population (see section II (a)), it was considered likely that fish of similar size would be of approximately the same "physiological age", i.e. at a similar stage in their physiological development, although males are often larger than females.

P.T.O. for pages 81 and 82

(Fig. 27)

Legend for Figure 27

(opposite; please rotate through 90°)

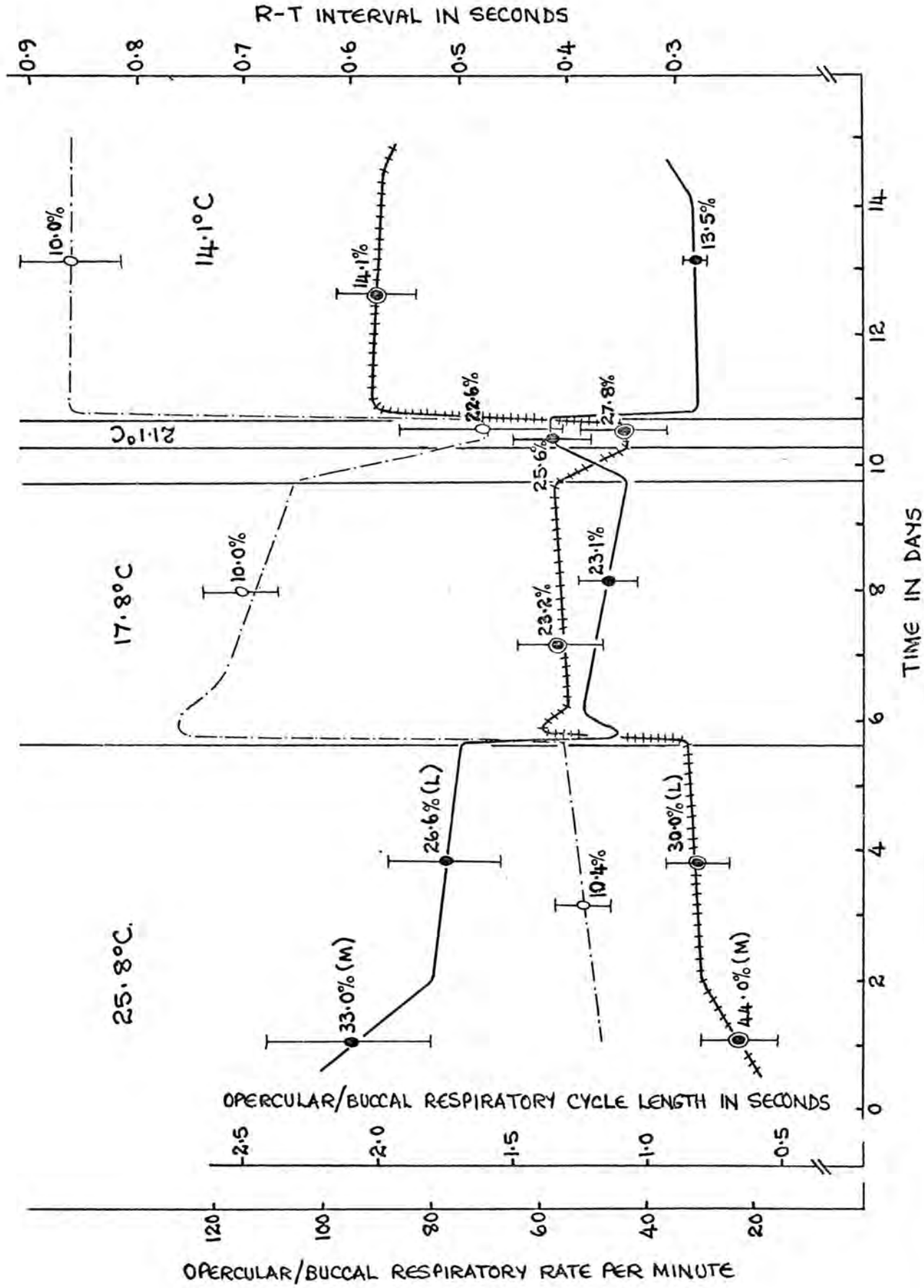
Diagrams of the changes in the R-T cardiac interval, the opercular/buccal respiratory cycle length and the opercular/buccal respiratory rate for the six experimental fish as the temperature was changed as indicated over a period of 14 days. The mean values at the main experimental temperatures are given (position on the X-axis within any experimental temperature is arbitrary) as well as the 95% confidence limits around these means; the 95% confidence limits are also recorded as a % of the mean in each case. The respiratory functions are plotted both for the first 2 days or part thereof at 25.8°C (period M), and for the later 2 days at this temperature (period L), instead of for the whole period at 25.8°C. Refer to section VI(c) for the complete set of plotted points from which these graphs were fitted by eye.

—●— - opercular/buccal respiratory rate

—·—○—·— - R-T interval

+++++◉+++ - opercular/buccal respiratory cycle length.

Figure 27.


R-T INTERVAL IN SECONDS
0·9
0·8
0·7
0·6
0·5
0·4
0·3
14·1°C
10·0%
14·1%
13·5%
22·6%
27·8%
21·1°C
25·6%
17·8°C
10·0%
23·1%
23·2%
25·8°C.
26·6% (L)
10·4%
30·0% (L)
33·0% (M)
44·0% (M)
TIME IN DAYS
0
2
4
6
8
10
12
14
OPERCULAR/BUCCAL RESPIRATORY CYCLE LENGTH IN SECONDS
2·5
2·0
1·5
1·0
0·5
120
100
80
60
40
20
OPERCULAR/BUCCAL RESPIRATORY RATE PER MINUTE

P.T.O. for pages 83 and 84

(Fig. 28)

Legend for Figure 28:

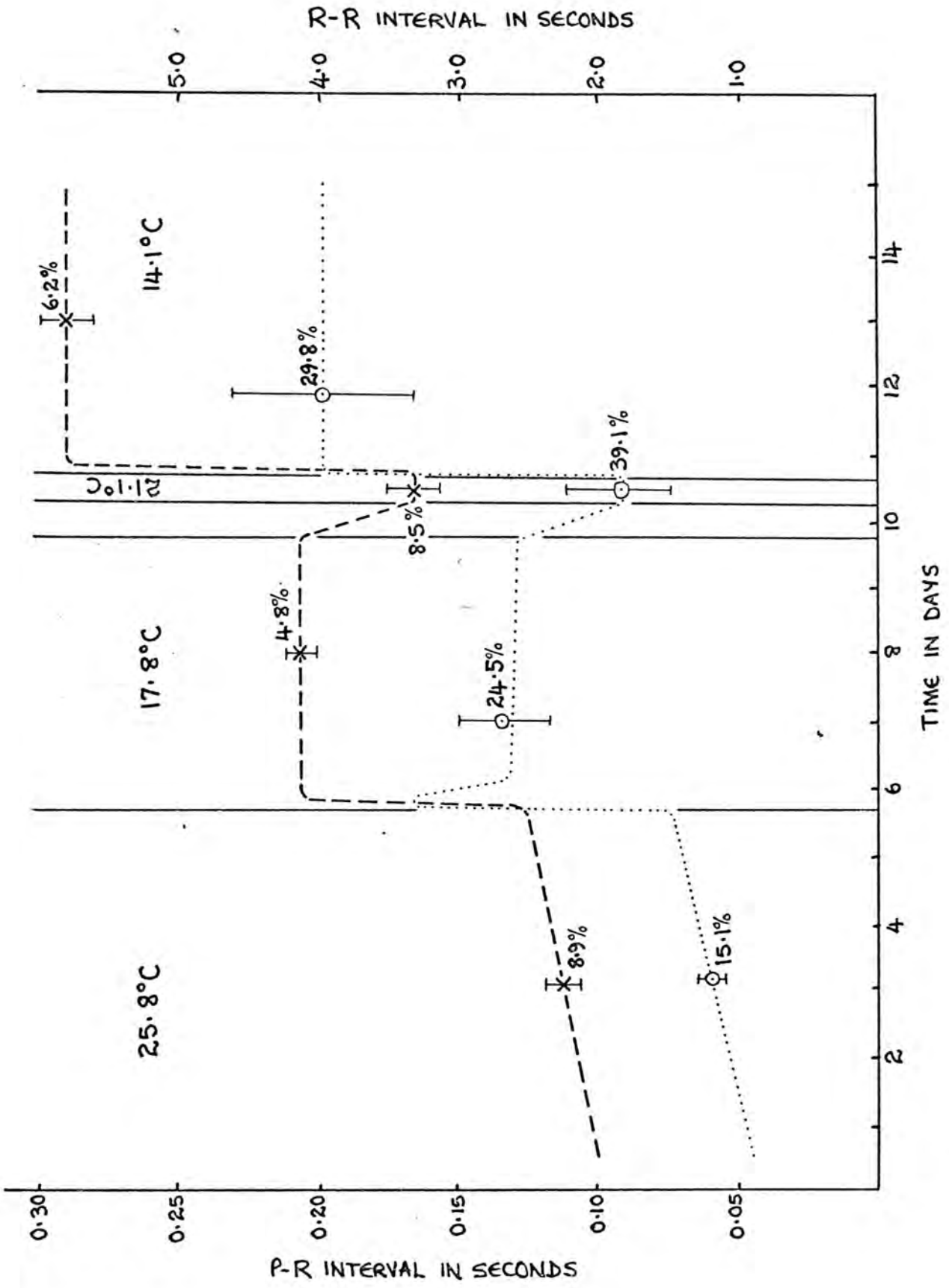
(opposite; please rotate through 90°)

Diagram of changes in the values of the R-R and P-R intervals for the six experimental fish as the temperature was changed as indicated over a period of 14 days. The mean values at the main experimental temperatures are given (position on the X-axis within any experimental temperature is arbitrary) as well as the 95% confidence limits around these means; the 95% confidence limits are also given as a % of the mean in each case. Refer to section VI(c) for the complete set of plotted points from which these graphs were fitted by eye.

..⊙..... - R-R interval

`\x_/ - P-R interval

Figure 28.


R-R INTERVAL IN SECONDS
5·0
4·0
3·0
2·0
1·0
14·1°C
6·2%
29·8%
39·1%
10·1°C
8·5%
17·8°C
4·8%
24·5%
25·8°C
8·9%
15·1%
0·30
0·25
0·20
0·15
0·10
0·05
P-R INTERVAL IN SECONDS
2
4
6
8
10
12
14
TIME IN DAYS

that the response of different size fish to temperature insofar as these cardiac and respiratory functions are concerned, may also be different. This latter has been suggested for most rate functions by Rao & Bullock (1954). Thus as fish of weight 49.8 - 88.0 gm were used in this experiment, fish size could introduce a source of variation into the results. Fish weight varies in a fairly regular way with fish length in this species in the range of fish size used in these experiments as can be seen from data published in the literature, from data of a colleague, and from my data (Fig. 29). Only in the case of very small fish (<8 cm total length) does fish length change very rapidly in comparison with fish weight. As it was thus unimportant whether fish weight or length be used as a measure of fish size, the cardiac intervals were plotted against fish weight for the experimental temperatures (Figs. 30 and 31).

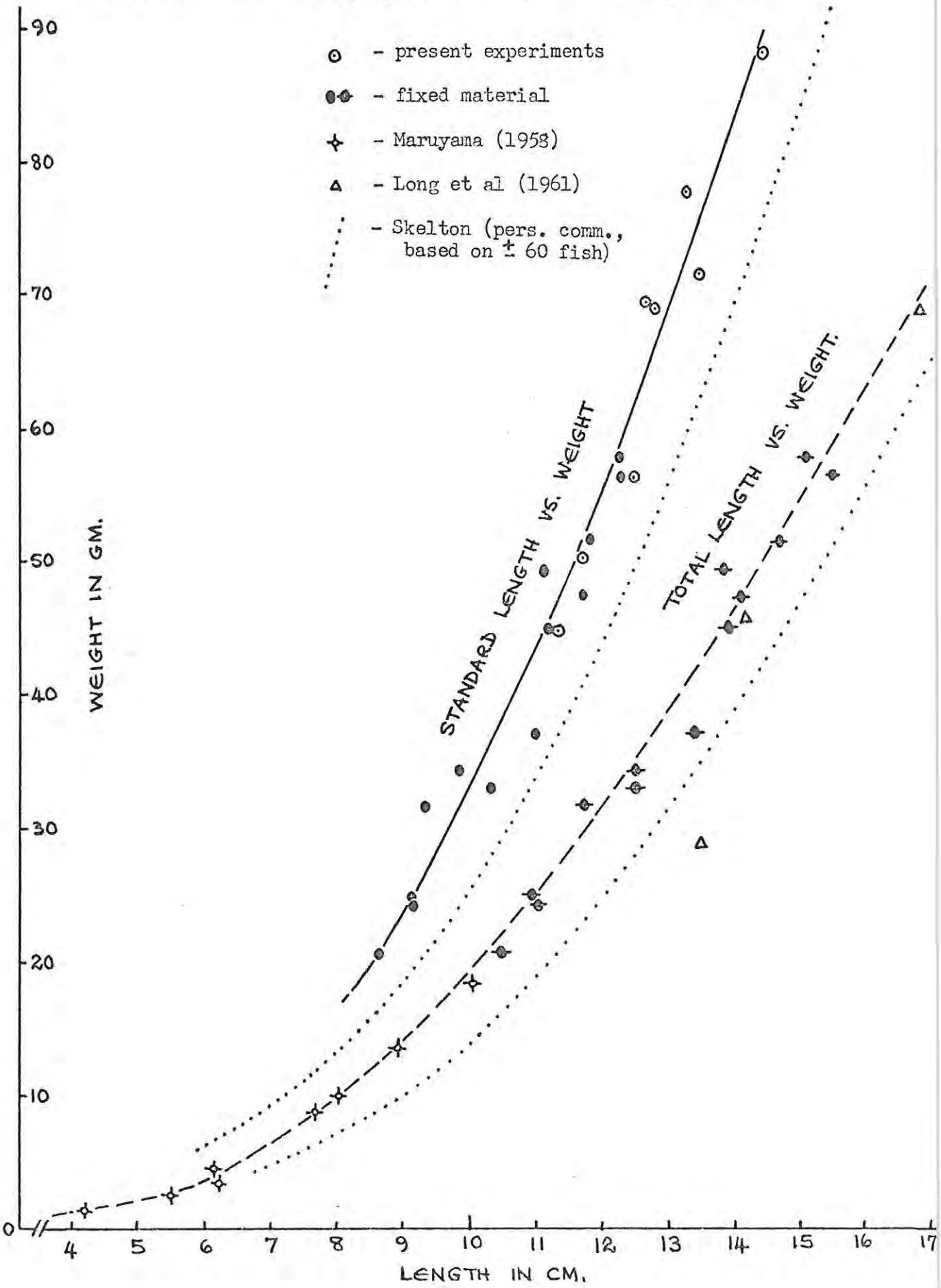
Fig. 30 illustrates that the R-T interval, and even more particularly the P-R interval, remain fairly constant at any one temperature irrespective of the size of fish used, as already illustrated in Figs. 27 and 28 as the confidence limits about the means for these intervals are small ($\pm$4.0 - 13.0% of the mean values of the respective intervals), an exception here being the R-T interval at 21.1 °C. Also the individual fish Q_{10} values for these intervals show no particular relationship to fish size.

The R-R interval and QRS duration are, on the other hand, more variable, and this can be seen both in Fig. 28 (R-R interval) and in Fig. 30 (R-R and QRS, on the right and left sides). Although the 95% confidence limits for the R-R interval represent 15.1 - 39.6% of the mean values, this variation does not seem to be related in a consistent way to fish weight, except perhaps at 14.1 °C where R-R tends to decrease with increase in fish size. This relationship is not a significant one, however, as the slope of the regression line calculated for

contd. on p. 91.

Figure 29.

Weight of T. mossambica in relation to standard length and total length using data from the literature and measurements on fresh and fixed fish. Curves have been drawn by eye to indicate the trends present.



P.T.O. for pages 87 and 88

(Fig. 30)

Legend for Figure 30.

(below and opposite)

Opposite: The R-R, P-R and R-T intervals for individual fish plotted against the weight of the fish in gm for 25.8, 17.8 and 14.1°C. Vertical lines indicate the individual fish. A regression line for the R-R interval at 14.1°C is drawn, and the formula given for the line.

⊙ ○ ● R-R interval at 25.8, 17.8 and 14.1°C respectively

⊡ □ ■ P-R interval at 25.8, 17.8 and 14.1°C respectively

△ △ ▲ R-T interval at 25.8, 17.8 and 14.1°C respectively

Below: The QRS complex duration for individual fish plotted against fish weight in gm for 25.8 and 14.1°C. Vertical lines indicate the individual fish.

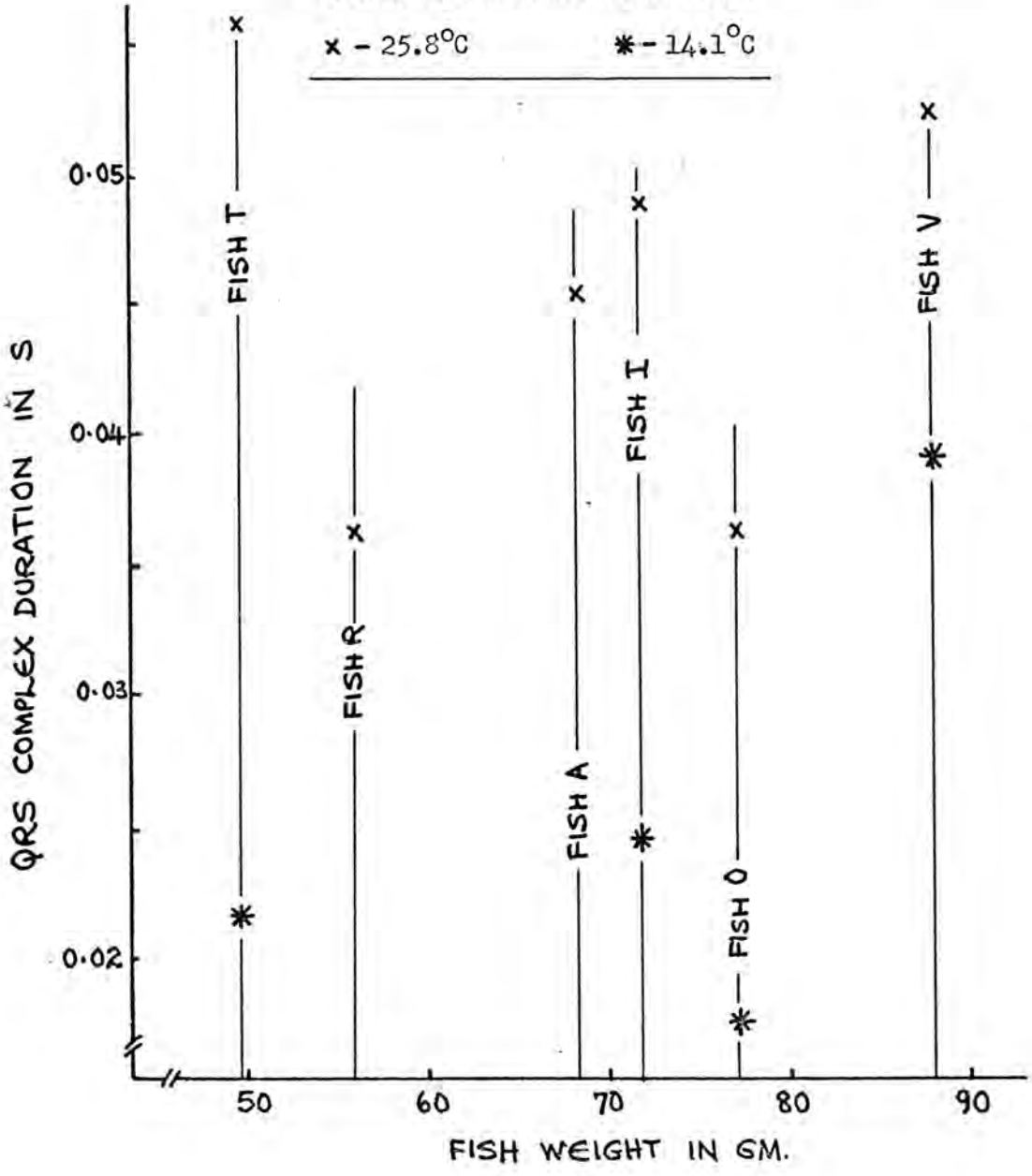
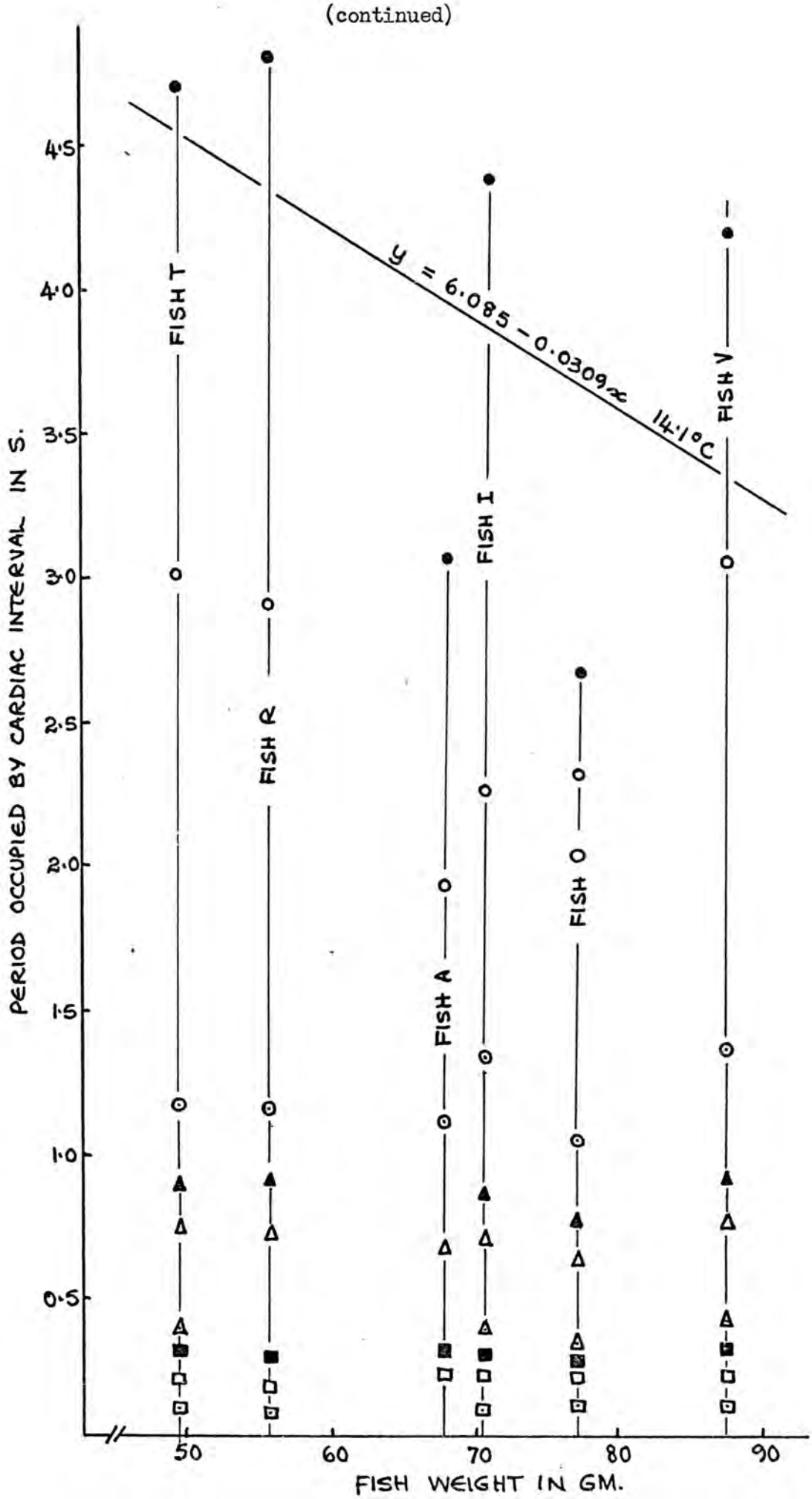


Figure 30.

(continued)



P.T.O. for pages 89 and 90

(Fig. 31)

Legend for Figure 31.

(opposite)

The opercular/buccal respiratory rate and the corresponding respiratory cycle duration (the superimposed sheet) for the individual experimental fish plotted against the weight of the fish in gm for the experimental temperatures 25.8, 21.1, 17.8 and 14.1°C. The readings obtained for the initial period at 25.8°C (period M) and for the later period at this temperature (period L) have been plotted separately. Vertical lines indicate the weights of the individual fish.

✱ - 25.8°C; period M

● - 25.8°C; period L

⊙ - 21.1°C

◆ - 17.8°C

○ - 14.1°C

Regression lines which have been calculated for each temperature, have been drawn in, and the relevant formula is given in each case.

Figure 31

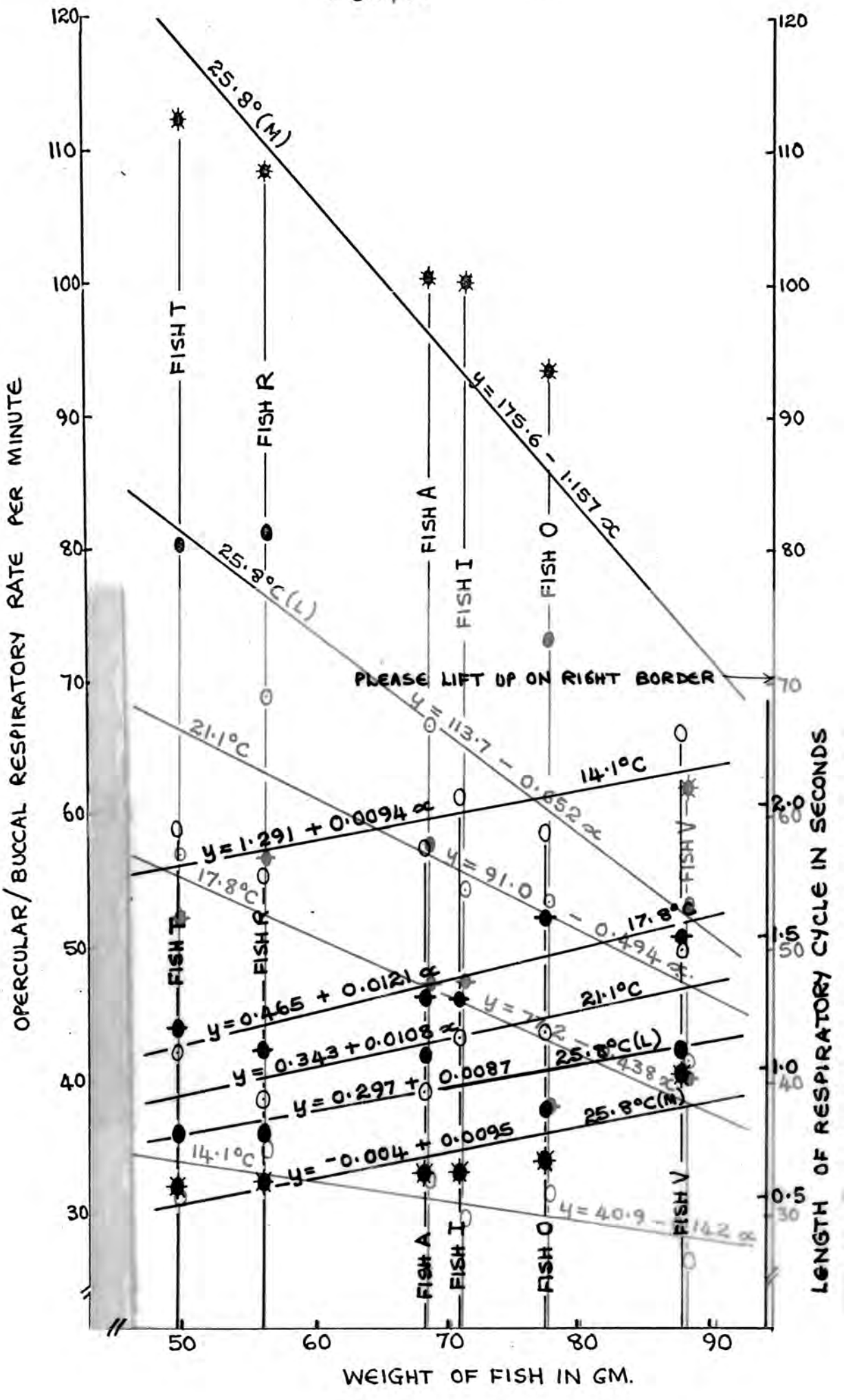
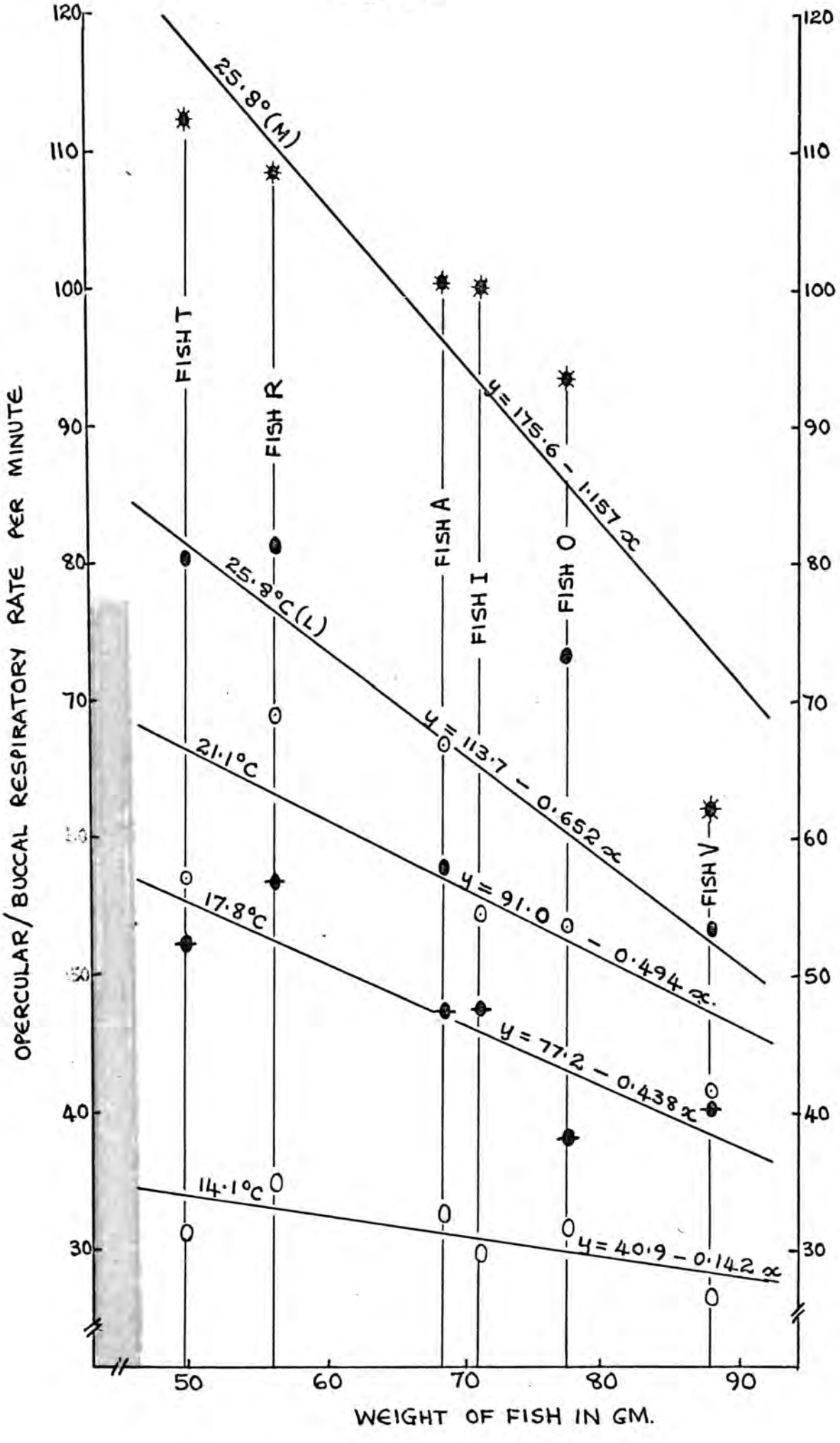

OPERCULAR/BUCCAL RESPIRATORY RATE PER MINUTE
LENGTH OF RESPIRATORY CYCLE IN SECONDS
WEIGHT OF FISH IN GM.
25.8°(M)
y = 175.6 − 1.157 x
25.8°C(L)
y = 113.7 − 0.652 x
21.1°C
y = 91.0 − 0.494 x
17.8°C
y = 76.2 − 0.438 x
14.1°C
y = 40.9 − 0.142 x
y = 1.291 + 0.0094 x
y = 0.465 + 0.0121 x
y = 0.343 + 0.0108 x
y = 0.297 + 0.0087
y = −0.004 + 0.0095
FISH T
FISH R
FISH A
FISH I
FISH O
FISH V
PLEASE LIFT UP ON RIGHT BORDER

Figure 31


OPERCULAR/BUCCAL RESPIRATORY RATE PER MINUTE
WEIGHT OF FISH IN GM.
25.8°C (M)
y = 175.6 - 1.157 x
25.8°C (L)
y = 113.7 - 0.652 x
21.1°C
y = 91.0 - 0.494 x
17.8°C
y = 77.2 - 0.438 x
14.1°C
y = 40.9 - 0.142 x
FISH T
FISH R
FISH A
FISH I
FISH O
FISH V

for these values (Fig. 30) is not significantly different from a horizontal line ($P < 0.40$). Information from many more animals would be needed in order to clarify this relationship. Q_{10} values calculated for heart rate and the ventricular depolarisation rate ($:: \frac{1}{QRS}$) do, however, show a correlation with fish size (just significant in the case of the ventricular depolarisation rate, $P < 0.05$) as smaller fish tend to have higher Q_{10} values than larger fish for these functions. This data is presented in Fig. 32 (p. 94) together with the ventilatory data.

In contrast to the cardiac intervals, the ventilatory rate and cycle length do indicate changes in relation to fish size in these experiments (Fig. 31). Both indicate that these respiratory functions tend to be accelerated in smaller fish as compared to larger fish at all experimental temperatures used. In the case of the ventilatory cycle length, the regression lines calculated for the plots seem to be parallel and thus of similar slope at the different temperatures, but in the case of the ventilatory rate they seem to be of a progressively steeper slope with an increase in temperature, reaching a maximum for period M at 25.8°C (first 2 days at this temperature). These apparent slope differences are, however, purely a function of the way in which a rate function is related to the interval from which it has been derived. This contention is also supported by the close similarities between the ventilatory rate and the ventilatory cycle length regression line t and P values at the same temperature (Table 8). Only in the cases of the early period at 25.8°C (period M), and of the period at 17.8°C, are the slopes of the regression lines both for the ventilatory rate and for the ventilatory cycle length significantly different from horizontal lines (P values of < 0.05 or 0.02). However, from a consideration of Fig. 31 and Table 8, in particular of the 17.8°C results

Table 8

Probabilities that regression lines calculated for values of the opercular/buccal respiratory functions at different temperatures, plotted against fish size. (Fig. 31), are significantly different from horizontal lines, together with the relevant t values and degrees of freedom (n-2)

An asterisk (*) indicates a significantly small P value

Both the earlier 2-day and later 2-day periods at 25.8°C are represented (periods M and L respectively).

Temperature °C	n-2	Opercular/buccal respiratory function	t value	P value
25.8(M)	4	cycle length	2.974	<0.05 *
		rate/min	4.116	<0.02 *
25.8(L)	3	cycle length	2.112	<0.20
		rate/min	2.196	<0.20
21.1	4	cycle length	2.170	<0.10
		rate/min	1.950	<0.20
17.8	4	cycle length	3.366	<0.05 *
		rate/min	3.513	<0.05 *
14.1	4	cycle length	2.038	<0.20
		rate/min	1.959	<0.20

where the plotted points were closely positioned around the regression line, it seems clear that if more experimental fish had been used, the regression lines (although probably of similar slope to those drawn here) would probably have proved to be more significantly different from a horizontal line in all cases. Thus the above indicates, as does the information presented in Fig. 22 (p. 66), that there may well be a significant variation in opercular/buccal respiratory function rates with fish size over a large temperature range in this species.

It is also clear that the Q_{10} values for these ventilatory functions in the experimental temperature range would tend to increase with a decrease in fish weight, as can be seen in Fig. 32. This tendency is significant for the opercular/buccal respiratory values ($P<0.02$), just significant for the ventricular depolarisation rate ($P<0.05$), and is not significant, because of great variability in the results as discussed earlier, for the heart rate ($P<0.10$). Thus these results indicate a size-related variation in ventilatory function which is accentuated at higher temperatures, in particular during the early period at 25.8°C which is, in many cases, concurrent with an initial handling stress. Therefore we can suggest that the variation in the magnitude of the 1.96 X S.E. values about their means for the cardiac and opercular/buccal respiratory functions studied at the different experimental temperatures may be partly due to the differential responses to temperature of the different size fish only in the case of the opercular/buccal respiratory functions.

Returning to Figs. 27 and 28 (pp. 82 and 84 respectively), it can be seen that during the first temperature decrease (25.8 - 17.8°C) there is a slowing of all cardiac functions and of the ventilatory rate (see Fig. 48, p. 121 for photographs). Once again, as reported in section (f), the heart rate (or its reciprocal the R-R interval) shows an acclimatory response during the time the fish are held at 17.8°C. This change

Figure 32.

Q_{10} values for the (i) ventricular depolarisation rate (14.1 - 25.8°C range only), (ii) opercular/buccal respiratory rate, and (iii) heart rate (mean of all 6 temperature intervals in both the latter) plotted against fish weight in those fish which gave complete results. See section VI(c) for the full respiratory results. Regression lines and relevant formulae have been calculated and are given. Vertical lines indicate the individual fish.

● - ventilatory rate ⊙ - heart rate

* - ventricular depolarisation rate

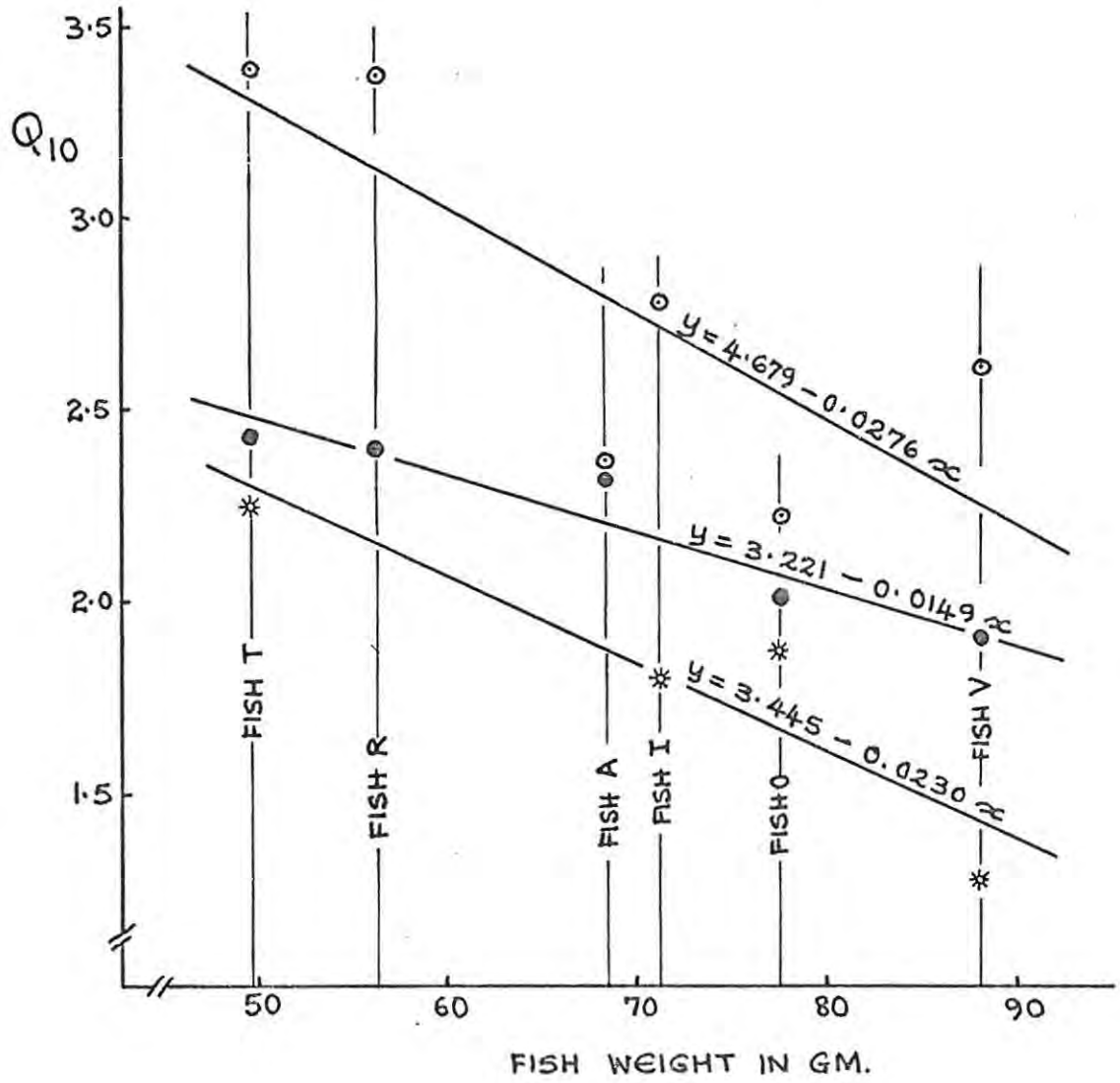
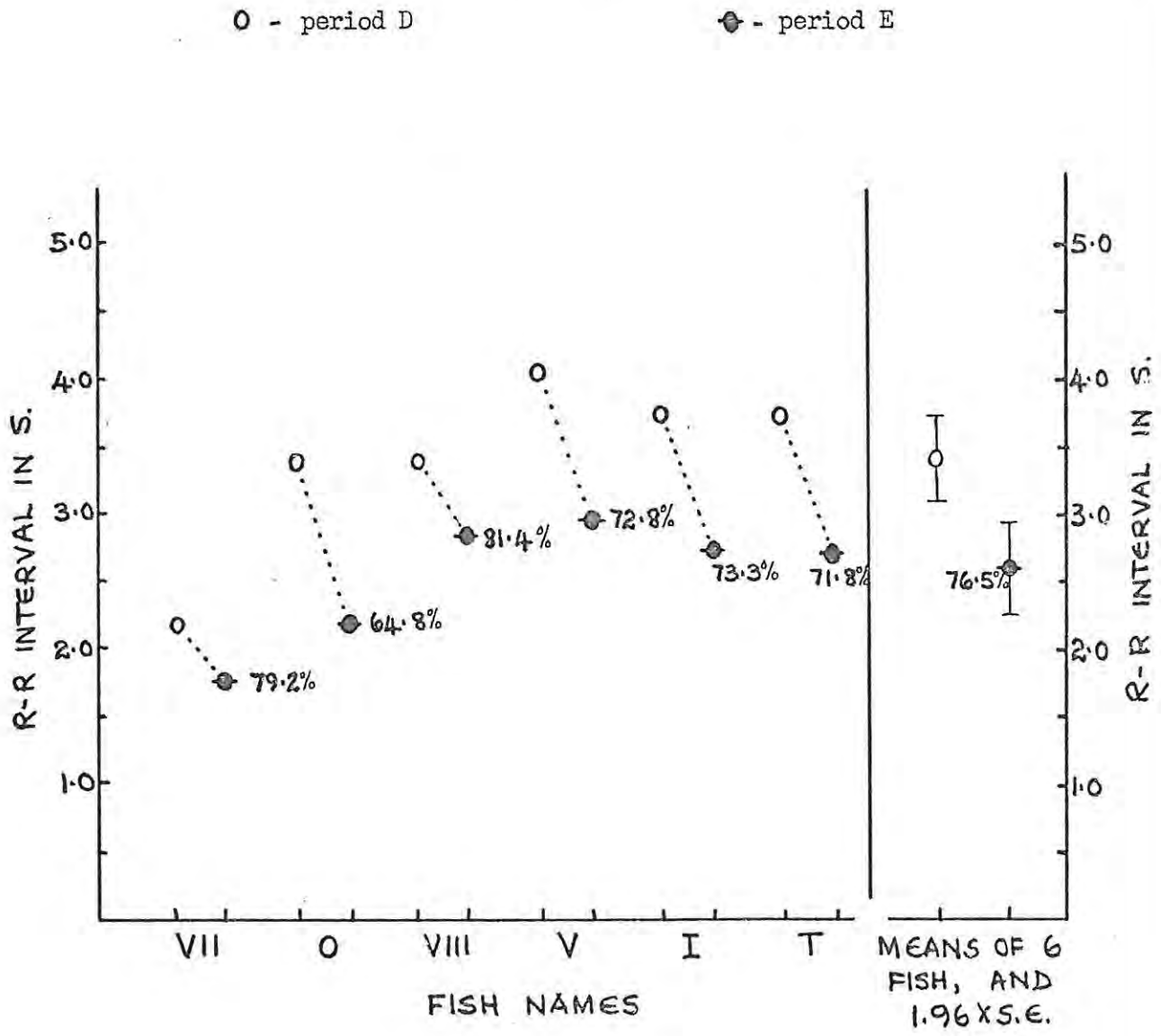


Figure 33.

Mean R-R interval values in period D (first 7 h at ± 18°C) and in period E ($7\frac{1}{2}$ h or longer at ± 18°C) for the 2 fish of an earlier experiment (Fish VII and VIII, see section (f)) and for the 4 fish of the present experiments which gave adequate readings. The mean value for all 6 fish is also given together with the 95% confidence limits (1.96 X S.E.). In each case the value in period E is also given as a % of the value in period D.

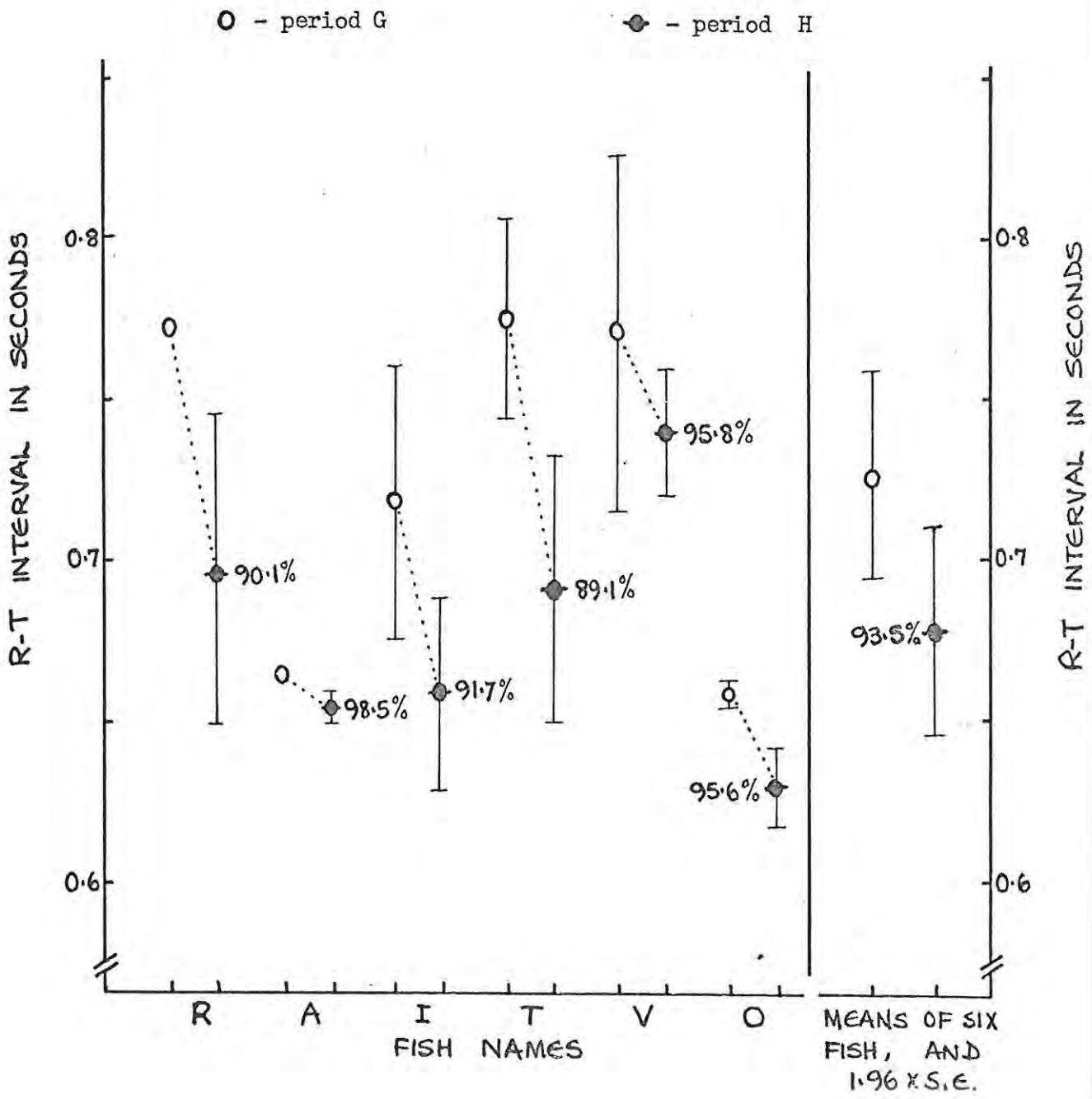


in the R-R interval is a significant one as can be seen in Fig. 33 where the mean values for each of 6 fish are given for the arbitrary periods D and E as defined in the previous section (page 70). The 6 fish used in this analysis were 4 from the present experimental series (the other 2 gave no results in period D), and the 2 fish from the previous experiment (at 18.1°C). In each case the mean value for R-R in period D is higher than that during period E for any one fish. Using the method of paired comparisons, the probability that this pattern will be found in other fish under similar experimental conditions is high ($P < 0.01$). Also there is no overlap between the average 95% confidence limits for the 2 periods at 17.8/18.1°C (Fig. 33). The mean heart rate for the 6 fish in period D is 17.6/min, whereas in period E it is 23.2/min. The R-R value in period E has been calculated as a % of the value in period D which is taken as 100%. These %s are given in Fig. 33, and the mean period E value for the 6 fish is 76.5%. As Fig. 28 and the full results in section VI (c) indicate, the R-R value remains relatively stable for the remaining time at 17.8°C after these initial changes. It is also of interest to note that the 2 fish from the earlier winter experiment show a smaller acclimatory response as compared to the summer fish of the present experiment (Fig. 33), also the average R-R interval is lower in these winter fish in both periods D and E (2.80 and 2.26 respectively) than for the summer fish (3.70 and 2.65 respectively) suggesting a seasonal variation in heart rate. More extensive experiments would be necessary to certify this finding.

These more extensive experiments also indicate that the R-T interval appears to acclimate to a small extent with time at 17.8°C (Fig. 27). (The earlier experiments on 2 fish have few and variable results for the R-T interval at 18.1°C (p. 71)). This R-T acclimation appears to take a much longer time, and is less extensive during the 4-day experimental period than in the case of the R-R interval. It can be demonstrated in

Figure 34.

Mean R-T interval values together with their 95% confidence limits in period G (first $1\frac{1}{4}$ days at 17.8°C) and in period H ($1\frac{3}{4}$ days or longer at this temperature) for each of the 6 fish of the present experiment. The mean values for all fish in each period is also given together with their 95% confidence limits. In each case the value in period H is given as a % of the value in period G. A dotted line joins the values in periods G and H for any one of the fish.



a similar way to the R-R acclimation if the period at 17.8°C is divided into 2 arbitrary periods as follows:-

period G - fish at 17.8°C for under $1\frac{1}{4}$ days

period H - fish at 17.8°C for $1\frac{3}{4}$ days or longer.

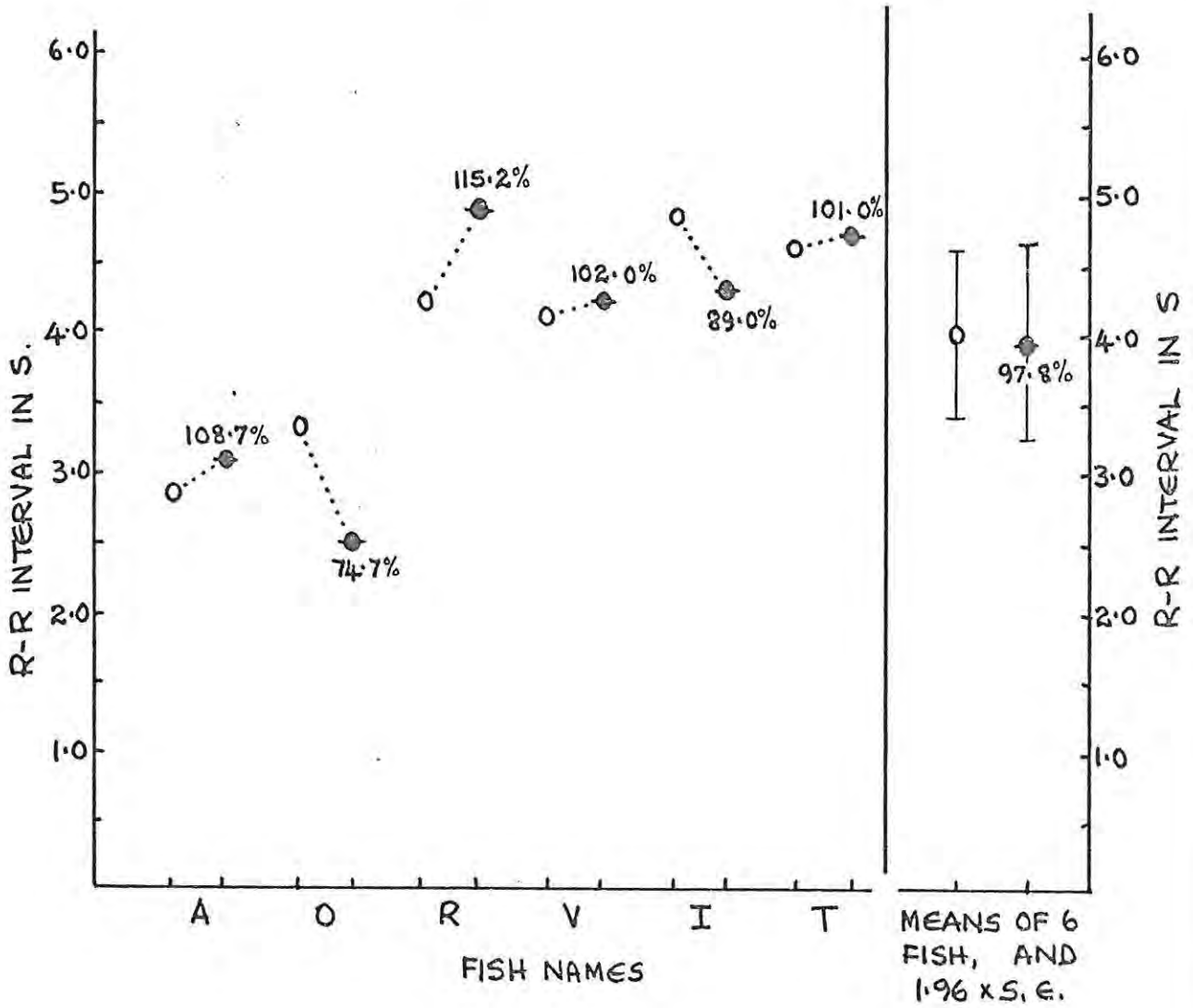
Mean results for individual fish during these 2 periods are given in Fig. 34, together with the mean of all fish for each period and the relevant 95% confidence limits. In each and every fish the mean value during period G is always higher than that during the later period H, and the probability that this pattern will be found in other fish has been found to be high ($P < 0.01$) using the method of paired comparisons. It is clear that the mean of the means also shows this pattern, but the 95% confidence limits for the individual fish and for the means of all fish for the 2 periods often show considerable overlapping. If the mean R-T values for any one fish during period G is taken as 100% and the value during period H is given as a % of this, the values are variable, and the mean for all fish is 93.5%. This small decrease in R-T with time at 17.8°C is, in contrast to the rapid R-R decrease which is followed by a plateau (Fig. 28), probably not complete by 96 h (4 days) as can be seen from Fig. 27 and in section VI(c) where the full results are given.

The second temperature decrease (21.1 - 14.1°C) caused all respiratory and cardiac functions studied to slow down even more than at 17.8°C, and these features are also seen in Figs. 27 and 28. Unlike the 25.8 - 17.8°C decrease, there was no indication of any change in any cardiac function with time during the 4 days that the fish were at this lower temperature. For e.g. there is no consistent difference between the mean R-R value during the first 7 h at 14.1°C and the later period at this same temperature for the individual fish as can be seen in Fig. 35. Thus there is a large overlap between the 95% confidence

Figure 35.

Mean R-R interval values during the first 7 h at 14.1°C (period J) and during the later period K ($7\frac{1}{2}$ h or longer) at this same temperature. Mean values for each of the 6 fish are given as well as the mean values for all 6 fish together with their 95% confidence limits (1.96 X S.E.) In each case the value in period K is also given as a % of the value in period J.

O - period J ⊖ - period K



limits about the means of all of the fish in the 2 arbitrary periods as can be seen on the right hand side of this Figure.

The magnitude of the QRS complex (as total height of the QRS on the recordings converted to mV) was also measured a number of times for individual fish at all the experimental temperatures, as has already been mentioned in section (b), where QRS complex height in relation to electrode position and fish size was also discussed. Although individual fish show considerable variability, the mean QRS heights show only small variations with temperature as can be seen in Fig. 36.

The results given in Fig. 36 may indicate a tendency for the electrical field surrounding the heart and therefore probably the ventricular muscle potentials to decrease slightly at temperatures below about 18°C. This interpretation is, of course, based on the assumption that the myocardium is functionally a syncytium with complete depolarisation occurring at each beat. The slightly lower QRS voltages at 14.1°C are thus another indication of a change in cardiac function at this lower temperature, and the tendency for the QRS voltages to be fairly constant over the 17.8 - 25.8°C range may indicate a stabilisation of the potentials generated by the ventricular myocardium over the normal temperature range for this fish. The QRS complex heights showed no indication of acclimation during the 96 h (4 day) stay at 17.8°C in contrast to the R-R and R-T intervals; however a 44% increase compared to earlier and later readings was recorded at 13 h (average of the 3 fish which gave complete results; see section VI (c) for complete results).

The duration of the QRS complex, measured a few times in each recording where possible, gave mean values of 0.026 ± 0.007 at 25.8°C, and 0.048 ± 0.008 at 14.1°C (see Fig. 30). The Q_{10} value for the 14.1 - 25.8°C range for the inversely related rate of ventricular activation

contd. on p. 103

Figure 36.

Mean QRS complex heights in mV for the six fish of the present experimental series for three of the experimental temperatures (14.1, 17.8 and 25.8°C), together with their 95% confidence limits. As two of the fish (Fish I and A) gave no results at 21.1°C, no point was plotted for this temperature.

The complete results from which this Figure was drawn is given in section VI(c).

A curve has been fitted by eye.

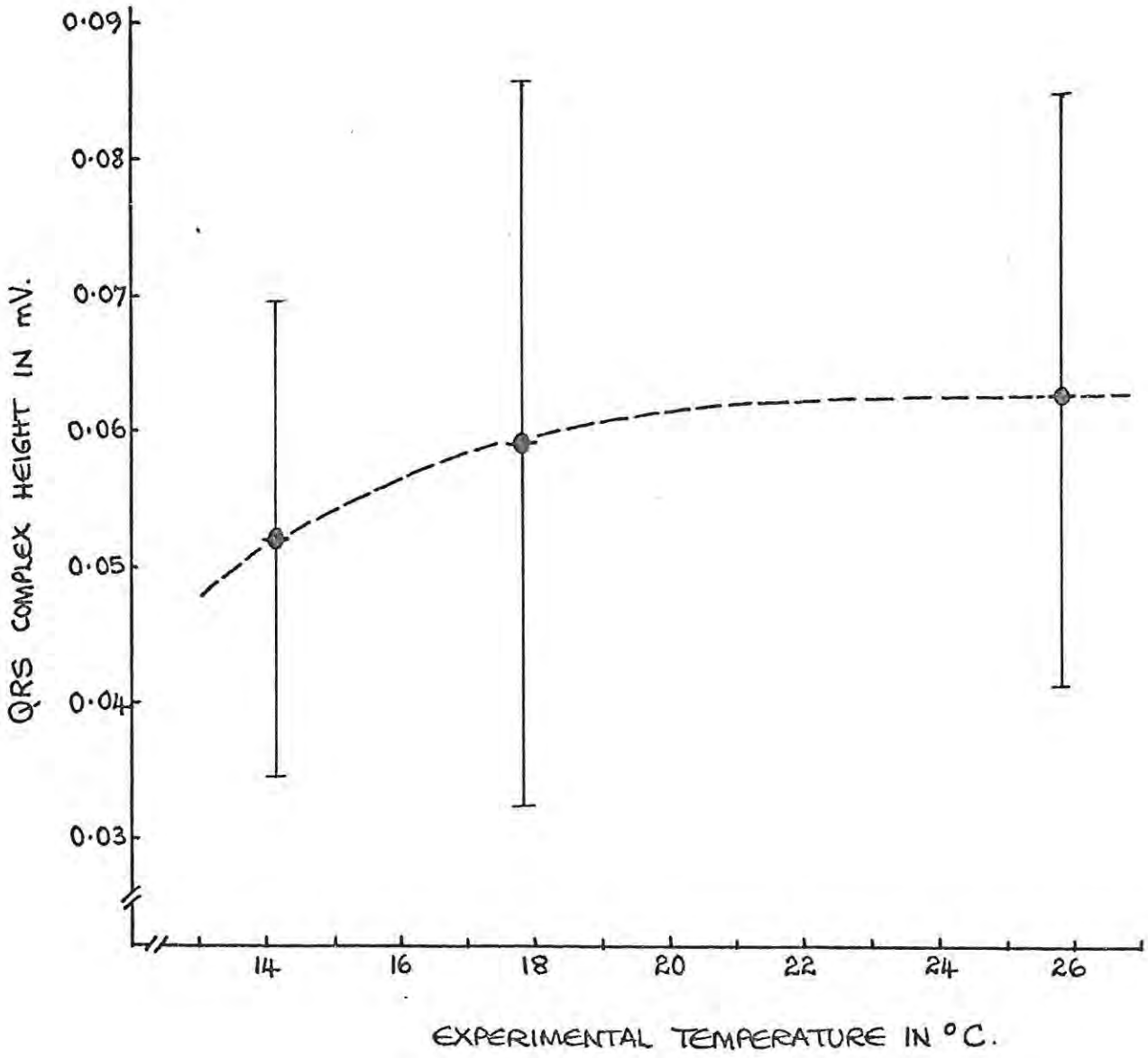


Figure 37.

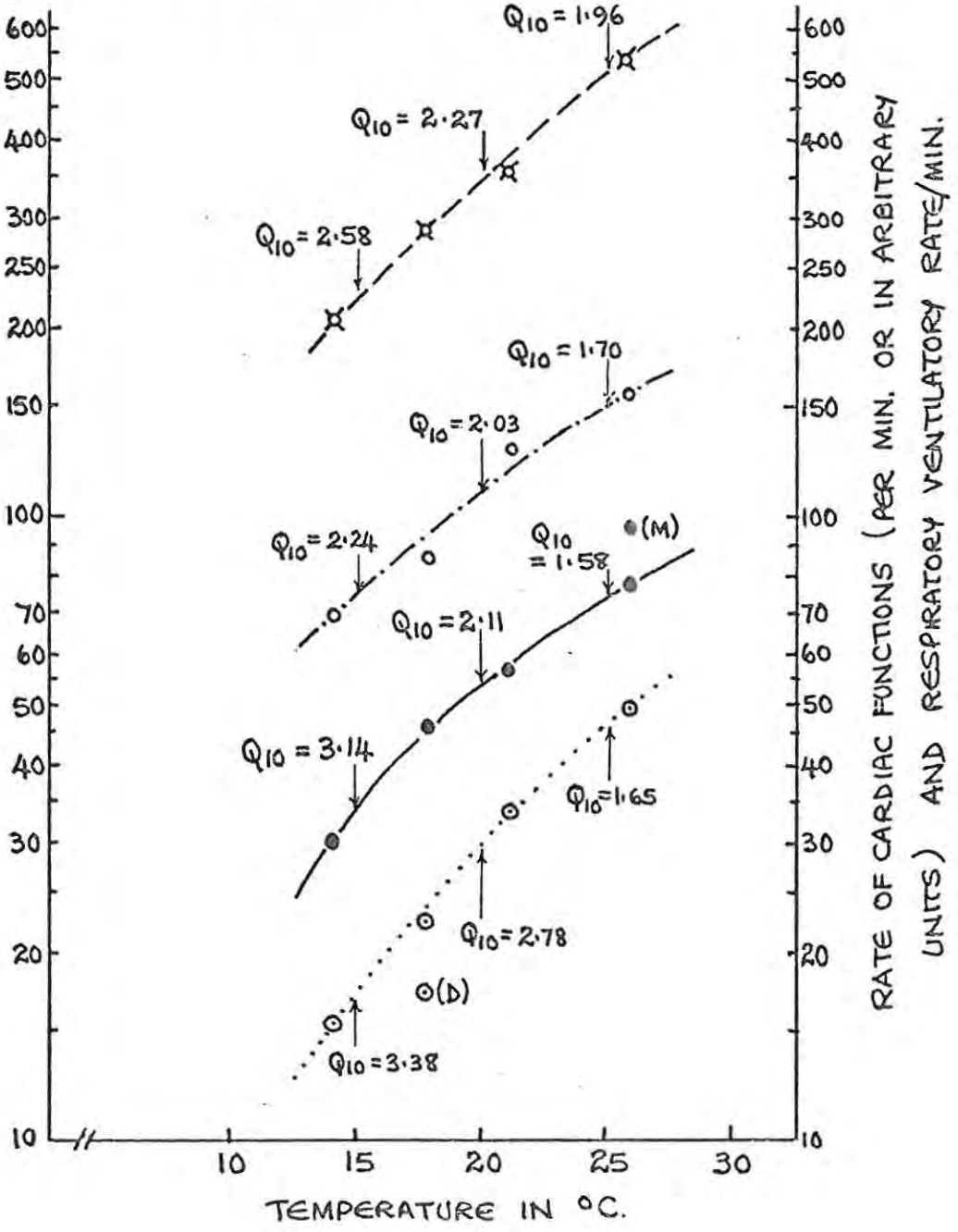
Cardiac function rates (excluding ventricular depolarisation rate) and the ventilatory rate plotted on a logarithmic scale against the experimental temperatures. The mean rates (see footnote, p. 104) were plotted and a smooth curve drawn to fit these points by inspection. Q_{10} values, calculated from the tangents to the curves at 15, 20 and 25°C are given.

..⊙.. heart rate :: $\frac{1}{R-R}$; value for period D at 17.8°C is also plotted.

—·o·— rate of ventricular functions :: $\frac{1}{R-T}$.

—x— rate of conduction from atrium to ventricle :: $\frac{1}{P-R}$

—•— ventilatory rate; value for period M at 25.8°C is also plotted.



or depolarisation ($:: \frac{1}{QRS}$) is 1.70.

The R-R, P-R and R-T intervals were converted to rate functions as described on pp. 67 and 75 and their mean values and those of the opercular/buccal respiratory rate have been plotted (on a log. scale) against experimental temperature in Fig. 37. These results have been utilised for the calculation of Q_{10} values at 15.0°, 17.5°, 20.0°, 22.5° and 25.0°C from the tangents to the slopes of the curves at these temperatures. The results are also used for the calculation of Q_{10} values from all the temperature intervals available, using the method described on p. 77. These results are given in full in section VI(c).

The Q_{10} values suggest, as is to be expected for biological phenomena with a lower thermal limit at which they cease, that the Q_{10} for an activity tends to increase as the temperature decreases towards a lethal limit. This tendency can be seen in Fig. 38 where the mean Q_{10} values of all activities measured (using the mean values of an activity at any one temperature) over the 6 possible temperature intervals are indicated, together with their midpoint. These midpoints have been taken as being indicative of the centre of the temperature ranges over which this Q_{10} value is operative, and have hence been used as plotted points for the calculation of a regression line which is, however, not significantly different from a horizontal line ($P < 0.10$). It does indicate a tendency for Q_{10} values to increase as the temperature decreases. However, if the mean Q_{10} values for these same activities as calculated from the semi-logarithmic plots of Fig. 37 are plotted, and a regression line drawn, this line is steeper and has a slope that is significantly different from a horizontal line ($P < 0.001$).

The Q_{10} values also suggest that those for heart rate seem

elevated when compared to those for the other rates measured. This feature is illustrated in Fig. 39 where the mean Q_{10}s of the 6 possible temperature intervals and their 95% confidence limits are plotted for each of the activities (excluding the ventricular activation rate), using only the mean rates[1.] of any one activity as is the usual practise unless otherwise stated. It is clear from Fig. 39 that the Q_{10} and Arrhenius μ values (see below) for heart rate are significantly higher than those for the other activities, with a mean Q_{10} of 2.86 as compared to mean Q_{10}s of 2.27, 2.22 and 2.18 for the ventilatory rate, the rate of conduction from the atrium to the ventricle and the rate of ventricular functions respectively; the heart rate Q_{10} is also much higher than that for the ventricular activation rate (Q_{10} = 1.70, see p. 103). This Figure also indicates that the rate of depolarisation and conduction spread between the atrium and the ventricle (:: $\frac{1}{P-R}$) has very consistent Q_{10} and μ values when compared to the other rate functions. Similarly mean Q_{10} values of each activity are plotted as calculated from the semi-logarithmic plots of Fig. 37, and these indicate similar trends, although heart rate Q_{10}s are not quite so elevated as compared to the value calculated from the discrete temperature intervals (2.62 as compared to 2.86). This means thus that heart rate will tend to decrease more quickly as the temperature drops, or increase more rapidly as the temperature rises, within the experimental temperature range, when compared to the other rates studied.

The Arrhenius μ, expressed in cal per mole (calories per mole), is derived from the following formula (Prosser, et al, 1952; Hoar, 1966):-

$$\mu = \frac{4.58 \log_{10} (K_2 - K_1)}{1/T_1 - T_2}$$

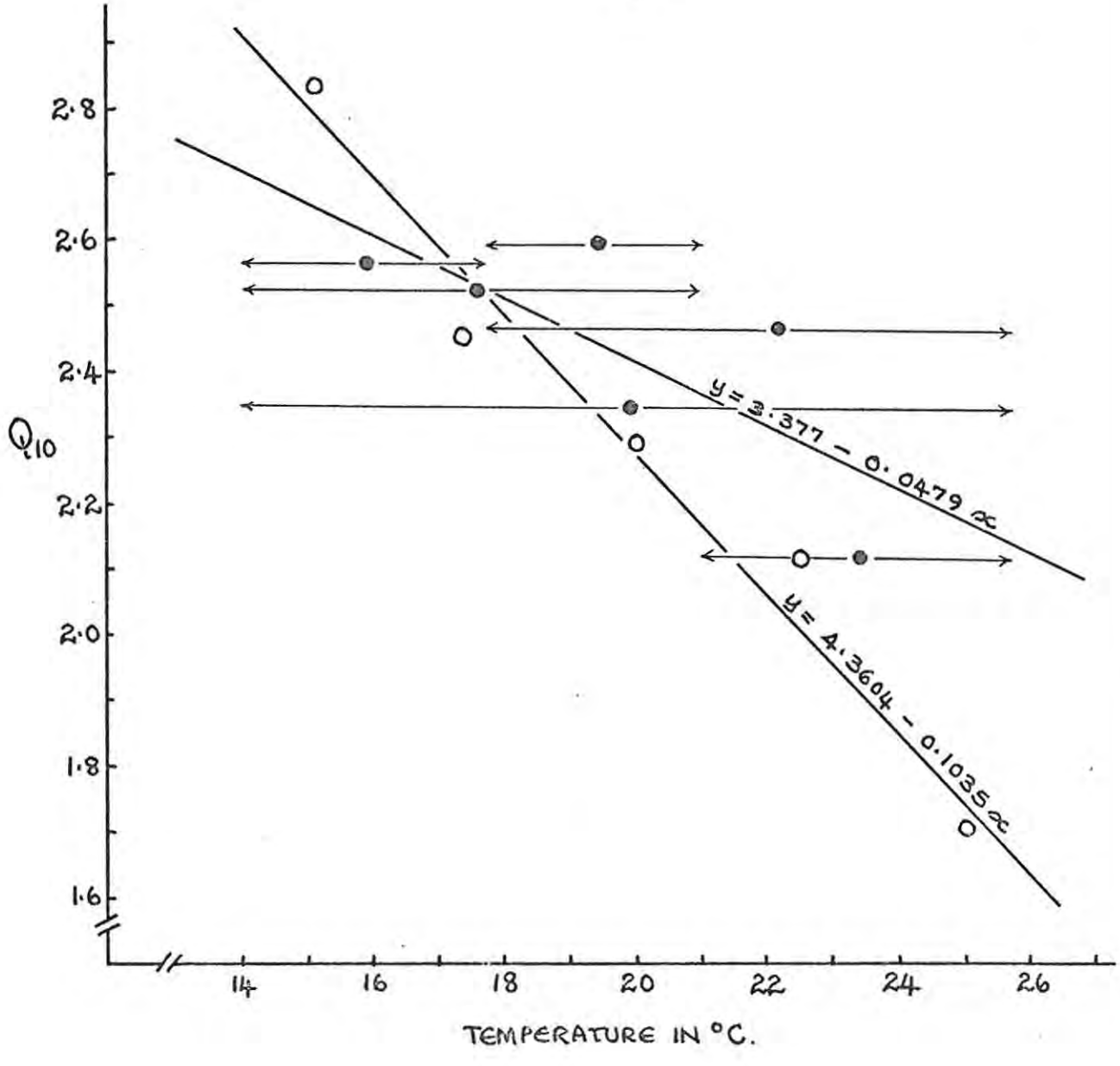
where K_1 and K_2 are the rates of activities at the 2 experimental tem-

---

1.) The use of the term 'mean rates' excludes those means calculated from an arbitrarily chosen period only, with the exception of the later 2 days at 25.8°C (period L) for the ventilatory functions which is taken as the mean for this particular rate at this temperature.

Figure 38.

Mean Q_{10} values for rates studied (excluding the rate of ventricular depolarisation (:: $\frac{1}{QRS}$), wherever results from all experimental fish were complete) plotted against experimental temperature: (a) Q_{10}s are indicated as horizontal arrows over the relevant temperature range for each of the 6 possible temperature ranges studied; the midpoint of each arrow is plotted (dark circle), and these plots are used for the calculation of a regression line. (b) Q_{10}s are given as calculated from semi-log. rate-temperature plots (Fig. 37) for 15.0, 17.5, 20.0, 22.5 and 25.0°C (light circles); a regression line has been calculated for these plots. Section VI(c) gives the complete data from which this Figure was constructed.



peratures (t_1 and t_2), and T_1 and T_2 are these temperatures respectively on the absolute scale. Thus μ is a function of the slope of the graph resulting from the plotting of an activity as a logarithm (to the base 10 in this case) against the reciprocal of the absolute temperature. The Arrhenius μ values change in a manner closely comparable to the way in which the Q_{10} values vary for the same activity, as has already been suggested by the information presented in Fig. 39. This relationship has also been stressed by Rao & Bullock (1954) who suggest that "the expression mu value may be substituted wherever the former (i.e. Q_{10}) is used ... except when values for Q_{10} are given." (Underlined part my insertion). In fact μ and Q_{10} values as calculated from the same data show a linear relationship as is seen in Fig. 40.

The logarithms to the base 10 of the various rate functions have thus been plotted against the reciprocal of the absolute temperature in Fig. 41, and the calculated Arrhenius μ values are given. These indicate the same trends as shown by the Q_{10} values as calculated from the discrete temperature intervals, as given in section VI(c).

Parenthetically it should be noted here that I was unable to calculate the μ values in the usual way from my limited number of experimental temperatures. This more generally used method is to plot, as individual points, values of an activity over a slowly changing temperature range. Parallel lines are then drawn to enclose the points, and a series of differently sloping pairs of parallel lines usually result (for e.g. see Fig. 10, Crozier, 1926). The slopes of these lines allow the required μ values to be calculated.

In order to investigate whether the opercular/buccal respiratory and cardiac functions studied are significantly different at the adjacent experimental temperatures, the means of these functions together with

contd. on p. 109.

Figure 39.

Q_{10} (dark circles) and Arrhenius μ (light circles) values (mean of the 6 possible temperature ranges) together with their 95% confidence limits, for the ventilatory rate and the cardiac rates studied (excluding the ventricular depolarisation rate :: $\frac{1}{QRS}$). Section VI(c) gives the complete data from which the information presented here was taken. Q_{10} s as calculated from the semi-log. rate-temperature plots of Fig. 37 are also given for comparison in each case (crosses).

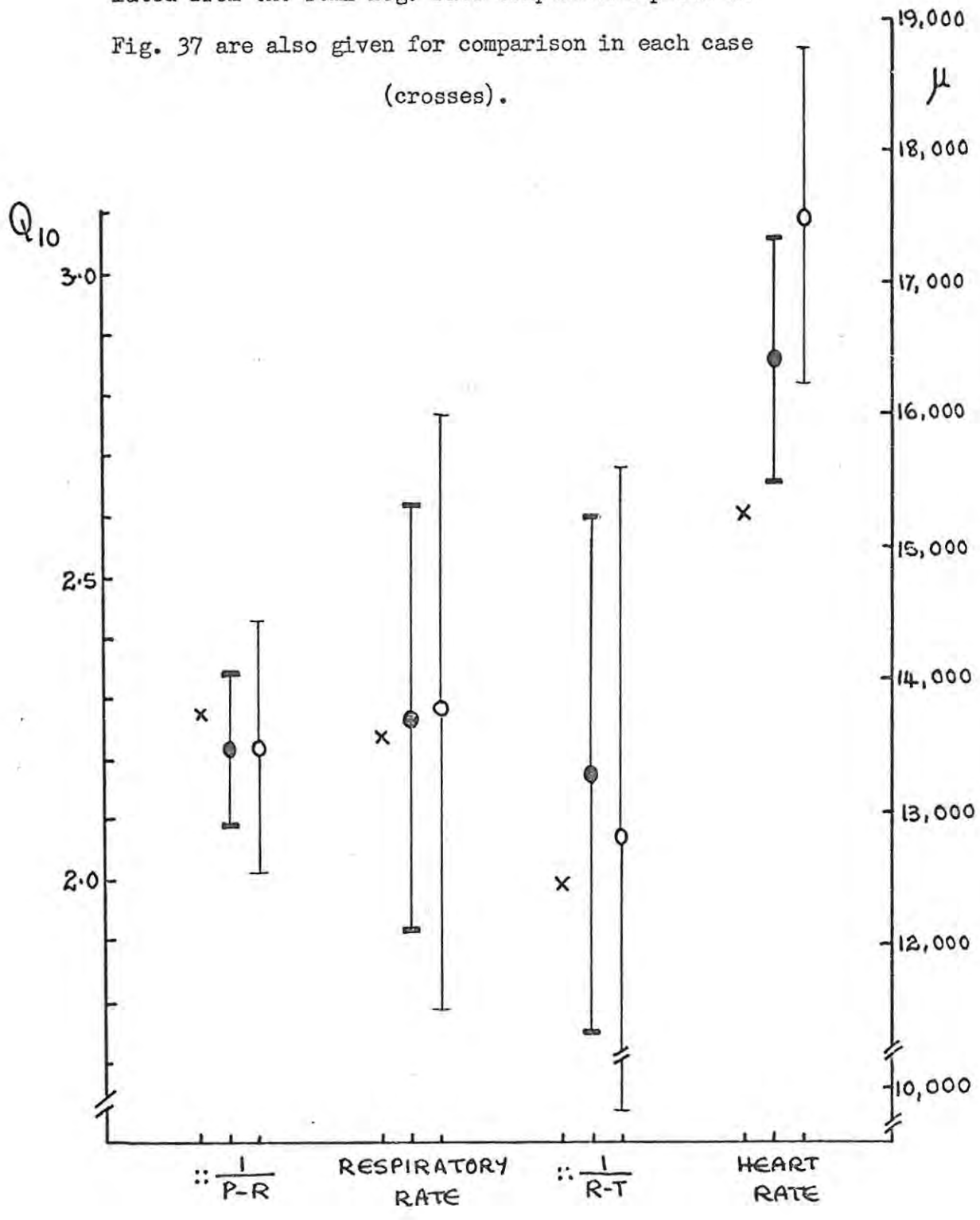
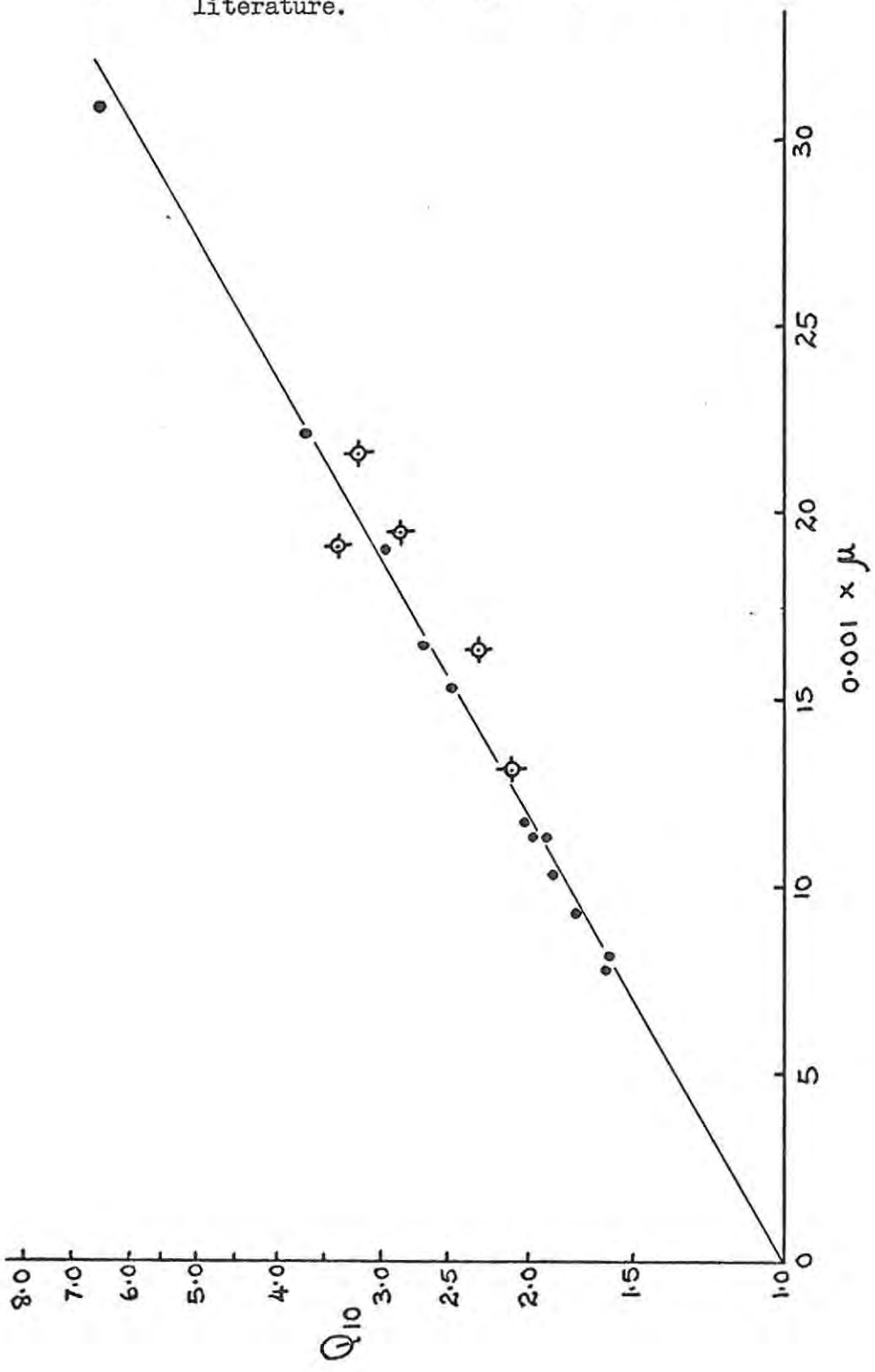


Figure 40.

Q_{10} values (represented on a Log.$_{10}$ scale) plotted against Arrhenius μ values as calculated from the same experimental data. The graph has been fitted by eye.

● - from experimental data of this investigation.

⊙ - from data giving both Q_{10} and μ in the literature.



their confidence limits as presented in Figs. 27 and 28 are re-drawn in Figs. 42 - 45, both for the intervals measured (on the left hand sides) and for the related rate functions (on the right hand sides). (The ventilatory rate vs. temperature plot and graph has already been given and discussed, see Fig. 22, and p. 62). The 95% confidence limits are also given as a % of the mean in each case and, in the case of an interval and its related rate function at the same temperature, these show close similarities in 10 of the 16 cases (ratio of one % to the other 0.95 - 1.05). In the other 6 cases (indicated in Figs. 42, 44 and 45), the % values were not so comparable, but no explanation of this fact can be offered.

Curves have been fitted by eye in Figs. 42 - 45 as linear regression lines were considered to be unsuitable and invalid, because these could only be drawn for small sections of the experimental temperature range and remain within the 95% confidence limits about the means in all cases. It was not considered practicable to attempt calculating the formulae for non-linear regression lines to fit the plotted points as the calculations are extremely involved. The curves drawn for the different cardiac or respiratory intervals (on the left hand sides) tend to approach the Y axis asymptotically at the lower end of the temperature scale, a feature related to to the fact that as a function ceases at low temperatures, the time taken for that function becomes inordinately long. The rate curves for the cardiac functions (Figs 42 - 44, right hand sides) appear to become asymptotic with the Y axis at the upper end of the temperature scale also, with the R-T interval as a possible exception (see later).

Figs. 42 - 45 indicate that where the variability in a function is small, as for e.g. for the R-T and P-R intervals and their related

contd. on p. 112.

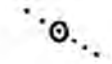
P.T.O. for pages 110 and 111

(Fig. 41)

Legend for Figure 41,

(opposite)

The ventilatory rate and the rates of the cardiac activities measured (excluding ventricular depolarisation rate), as logarithmic values to the base 10, plotted against the reciprocal of the absolute temperature. Graphs have been drawn by joining only points derived from the mean rates (see footnote on p. 104) and the corresponding Arrhenius μ values are given for the intervals between adjacent temperatures. The full results of the μ values calculated for all available temperature intervals are given in an Appendix (see section VI(c)).



K is proportional to $\frac{1}{R-R}$; i.e. K is heart rate; values for periods D and E at 17.8°C are also plotted.

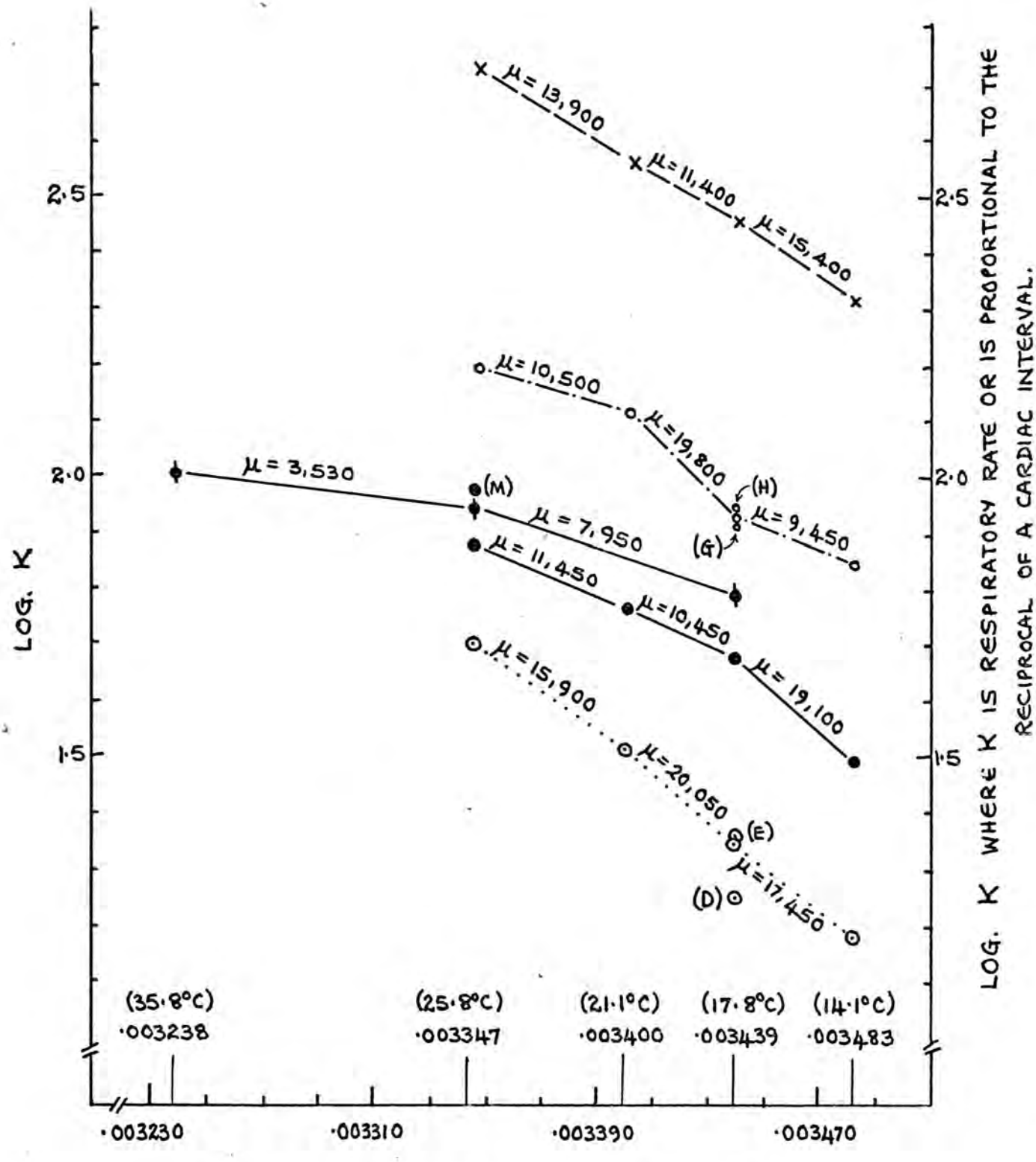
K is proportional to $\frac{1}{R-T}$; values for periods G and H at 17.8°C are also plotted.

K is proportional to $\frac{1}{P-R}$.

K is respiratory ventilatory rate for the present experimental series; the value during period M at 25.8°C is also plotted.

K is respiratory ventilatory rate for the earlier 1967-1968 experiments.

Figure 41.


LOG. K
2·5
2·0
1·5
μ = 13,900
μ = 11,400
μ = 15,400
μ = 10,500
μ = 19,800
μ = 3,530
(M)
(H)
μ = 9,450
(G)
μ = 7,950
μ = 11,450
μ = 10,450
μ = 19,100
μ = 15,900
μ = 20,050
(E)
μ = 17,450
(D)
(35·8°C)
·003238
(25·8°C)
·003347
(21·1°C)
·003400
(17·8°C)
·003439
(14·1°C)
·003483
·003230
·003310
·003390
·003470
LOG. K WHERE K IS RESPIRATORY RATE OR IS PROPORTIONAL TO THE RECIPROCAL OF A CARDIAC INTERVAL.
THE RECIPROCAL OF THE ABSOLUTE TEMPERATURE
(CORRESPONDING VALUE IN °C FOR THE EXPERIMENTAL TEMPERATURES IN BRACKETS.)

rate functions (Figs 43 and 44), and the 95% confidence limits at adjacent experimental temperatures are well separated from each other, and hence the functions concerned are clearly significantly different in magnitude at the 2 temperatures concerned. If the variability is larger, however, as in the case of the respiratory rate, R-R interval etc., the 95% confidence limits do approach, but only in the cases of the respiratory opercular/buccal functions at 17.8 and 21.1°C (Fig. 45) is there actually an overlap. In these instances it can be suggested either that there is a significant difference at the 2 temperatures but the variability within the 6 fish (size-correlated in these particular cases as discussed earlier) masks this and causes the overlap, or that there is no significant difference. The method of paired comparisons was therefore used to compare the values of the ventilatory functions at the 2 temperatures, and the P values of < 0.02 and < 0.01 (for ventilatory cycle length and ventilatory rate respectively) indicated that these respiratory functions were indeed significantly different at the 2 temperatures at greater than the 95% level. Thus in all cases the functions studied were significantly different at adjacent experimental temperatures which represented temperature intervals of as little as 3.3°C (17.8 - 21.1°C).

The rate function vs. temperature graphs (R-T graphs in the literature, not to be confused with the R-T interval !) on the left hand sides of each of the Figs. 42 - 45 would have been able to give an indication of the low temperature at which the various functions might be expected to cease if lower experimental temperatures e.g. 10°C had been used. The results that have been obtained do not provide reliable extrapolations below about 10°C, but do suggest that the ventilatory functions may well cease at a higher temperature than the various cardiac functions

contd. on p. 117.

Figure 42.

Mean values for the R-R interval (left) and the heart rate (right) at the four experimental temperatures together with the 95% confidence limits about the mean in each case. The confidence limits are also given as a % of the mean next to each point. At 14.1 and 17.8°C these limits as a % are rather different when the rate function is compared to its related interval. These are indicated by A_1 & A_2 and B_1 & B_2 respectively, and the relevant ratios are as follows:-

$$\frac{A_1}{A_2} = 1.27 \; ; \qquad \frac{B_1}{B_2} = 1.18.$$

Four plots have been included from one fish of an early experiment (section (e)), and graphs have been fitted by eye to indicate the trends present.

Present experiment - ⊙ ; section (e) - ✸

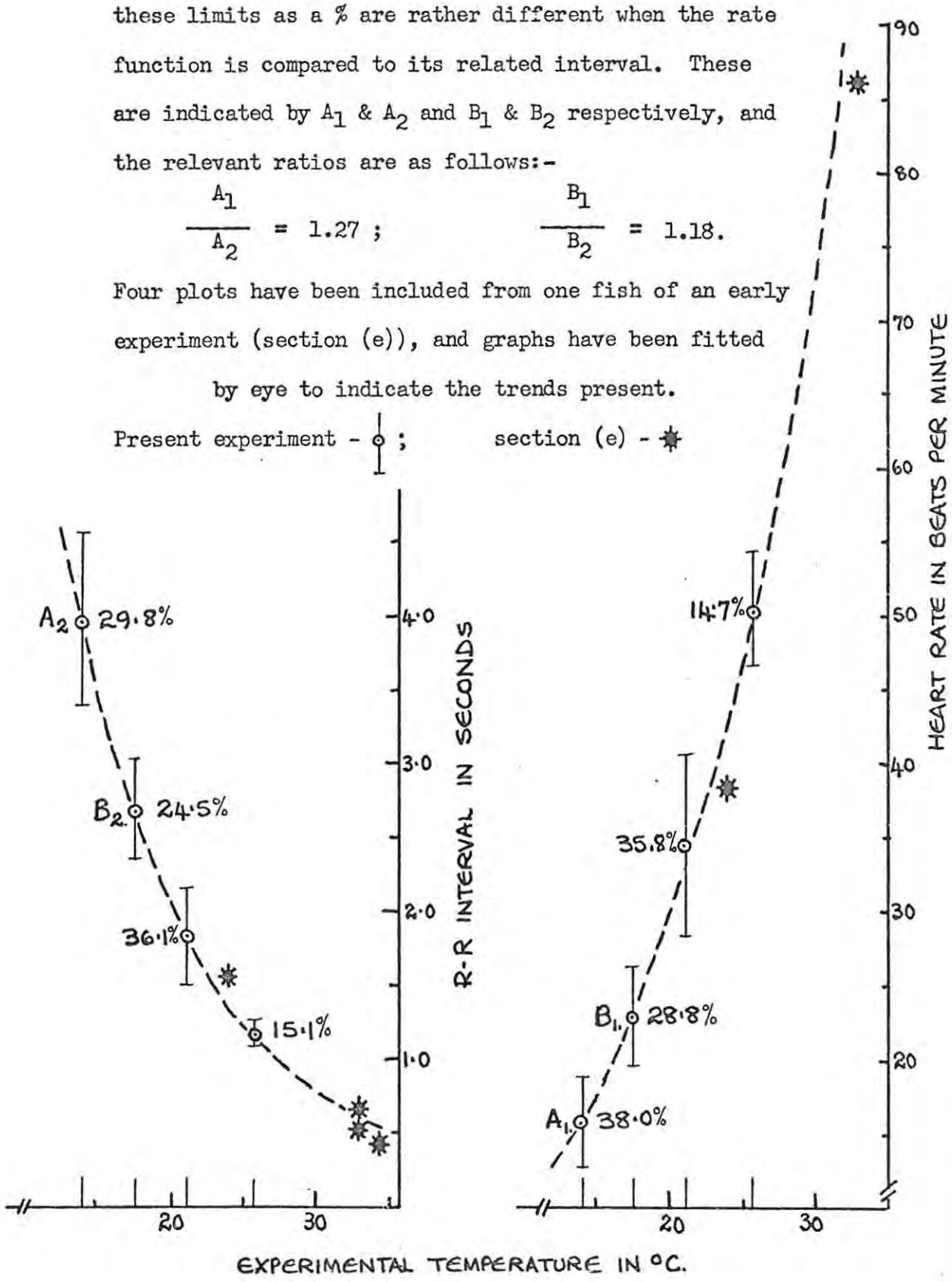


Figure 43.

Mean values for the P-R interval (left) and the rate of conduction from the atrium to the ventricle (right) at the four experimental temperatures together with the 95% confidence limits about the mean in each case. The confidence limits are also given as a % of the mean next tc each point. Three plots have been included from one fish of an early experiment (section (e)) and graphs have been fitted by eye to indicate the trends present.

Present experiment plots - ⊥ ; section (e) plots - ✕

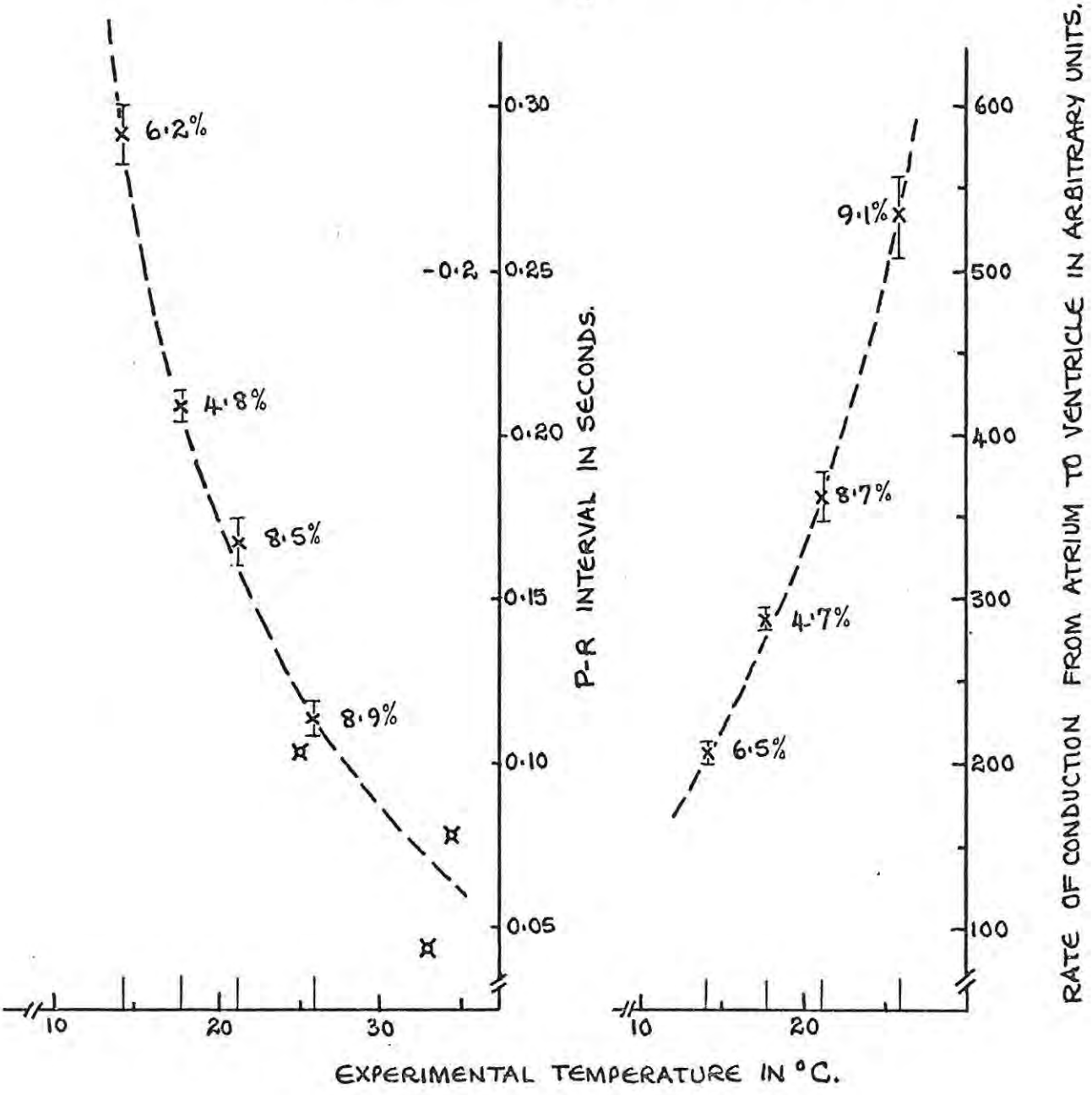


Figure 44.

Mean values for the R-T interval (left) and the rate of ventricular functions (right) at the four experimental temperatures together with the 95% confidence limits about the mean in each case. The confidence limits are also given as a % of the mean next to each point. At 14.1 and 21.1°C these limits as a % are rather different when the rate function is compared to its related interval. These are indicated by A_1 & A_2 and B_1 & B_2 respectively, and the relevant ratios are as follows:-

$$\frac{A_1}{A_2} = 1.08 \; ; \qquad \frac{B_1}{B_2} = 1.10.$$

Graphs have been fitted by eye to indicate the trends present.

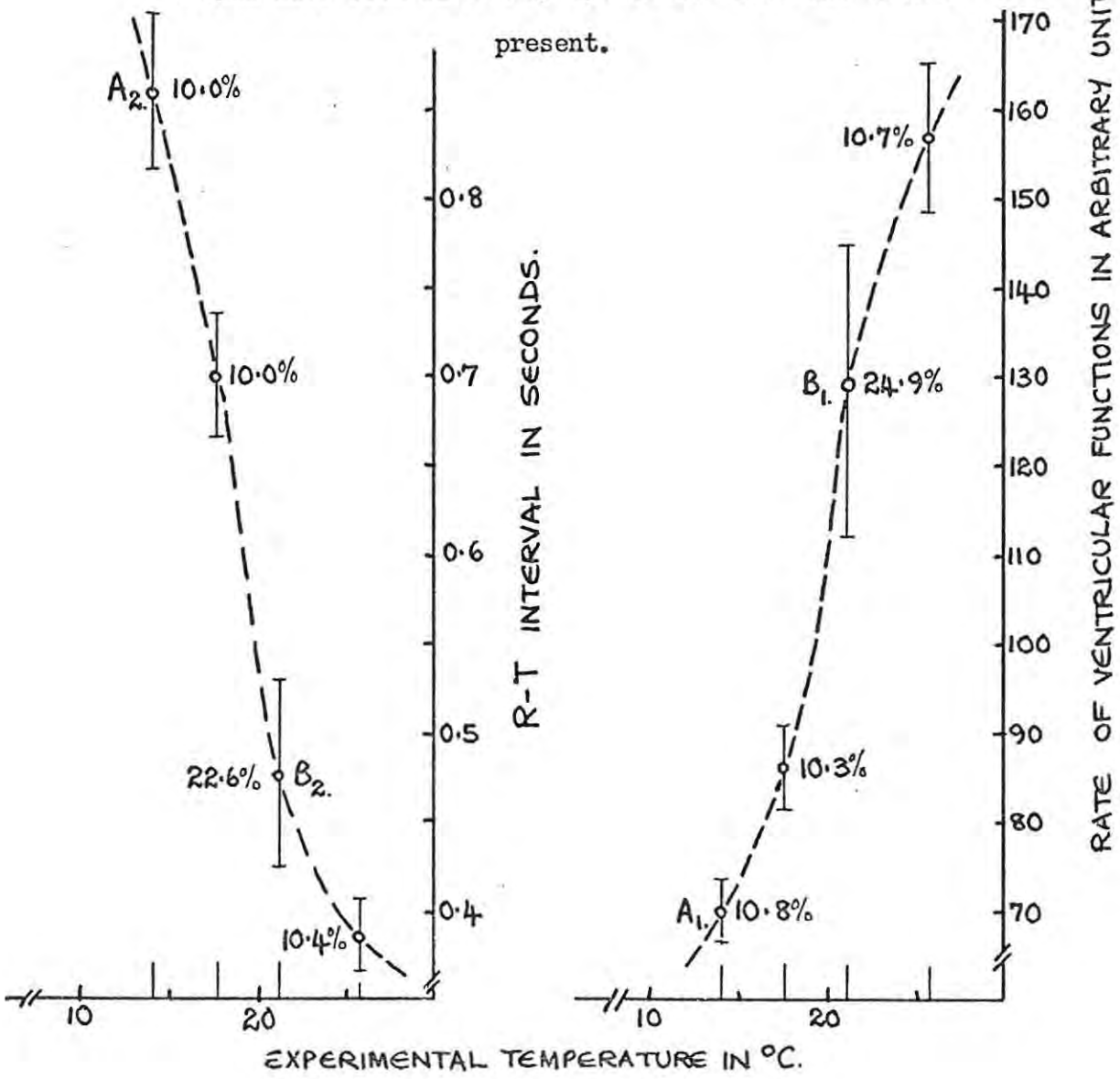
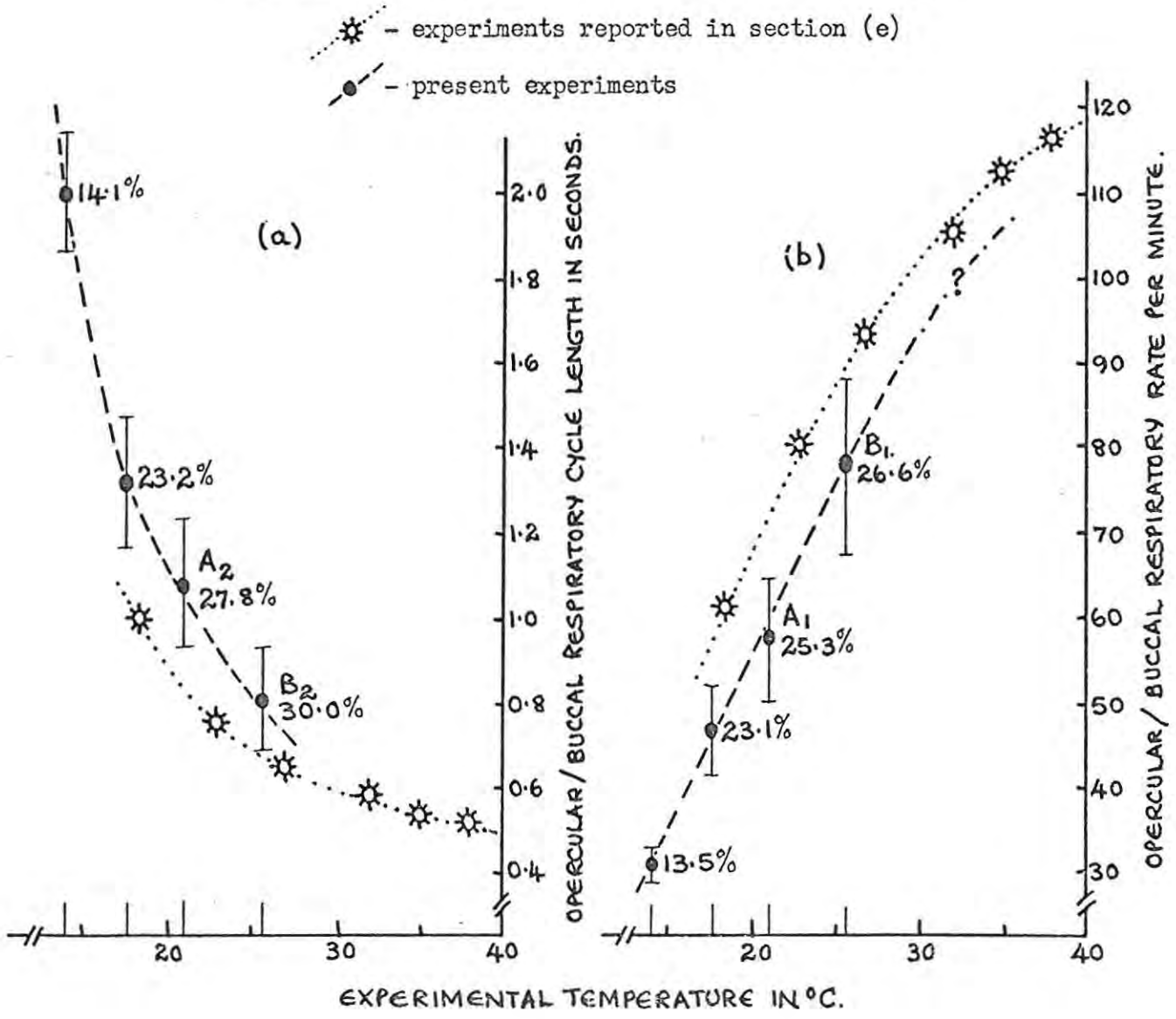


Figure 45.

Mean values for the ventilatory cycle length (a) and its related rate (b) for the 6 fish of the present experiment (mean weight 68.6 gm) at the 4 experimental temperatures together with their 95% confidence limits. These limits are also given as a % of the mean in each case, and these %s are usually closely comparable in (a) and (b) at the same temperature (ratio between the 2 between 0.95 and 1.05). However at 21.1 and 25.8°C this is not so, and the ratios in these cases are as follows:-

$$21.1^{\circ}C: \frac{A_1}{A_2} = 0.91; \quad 25.8^{\circ}C: \frac{B_1}{B_2} = 0.89$$

In addition 6 points have been plotted for the 9 fish (mean weight 45.0 gm) of the earlier experiment reported in section (e). Graphs have been fitted by eye.



studied as discussed again later. These Figs. also give some insight into the changes of the various cardiac intervals and the ventilatory cycle time with temperature and indicate that at temperatures which approach the upper lethal temperatures (± 38°C) for this species, there is a distinct flattening of the curve only in the case of the ventilatory cycle length, as seen also in Fig. 45 for the smaller fish utilised in the earlier experiment reported in section (e). This tendency is also noticeable, but to a small extent only, in one of the cardiac functions - the R-T interval - at about 25.8°C (Fig. 44).

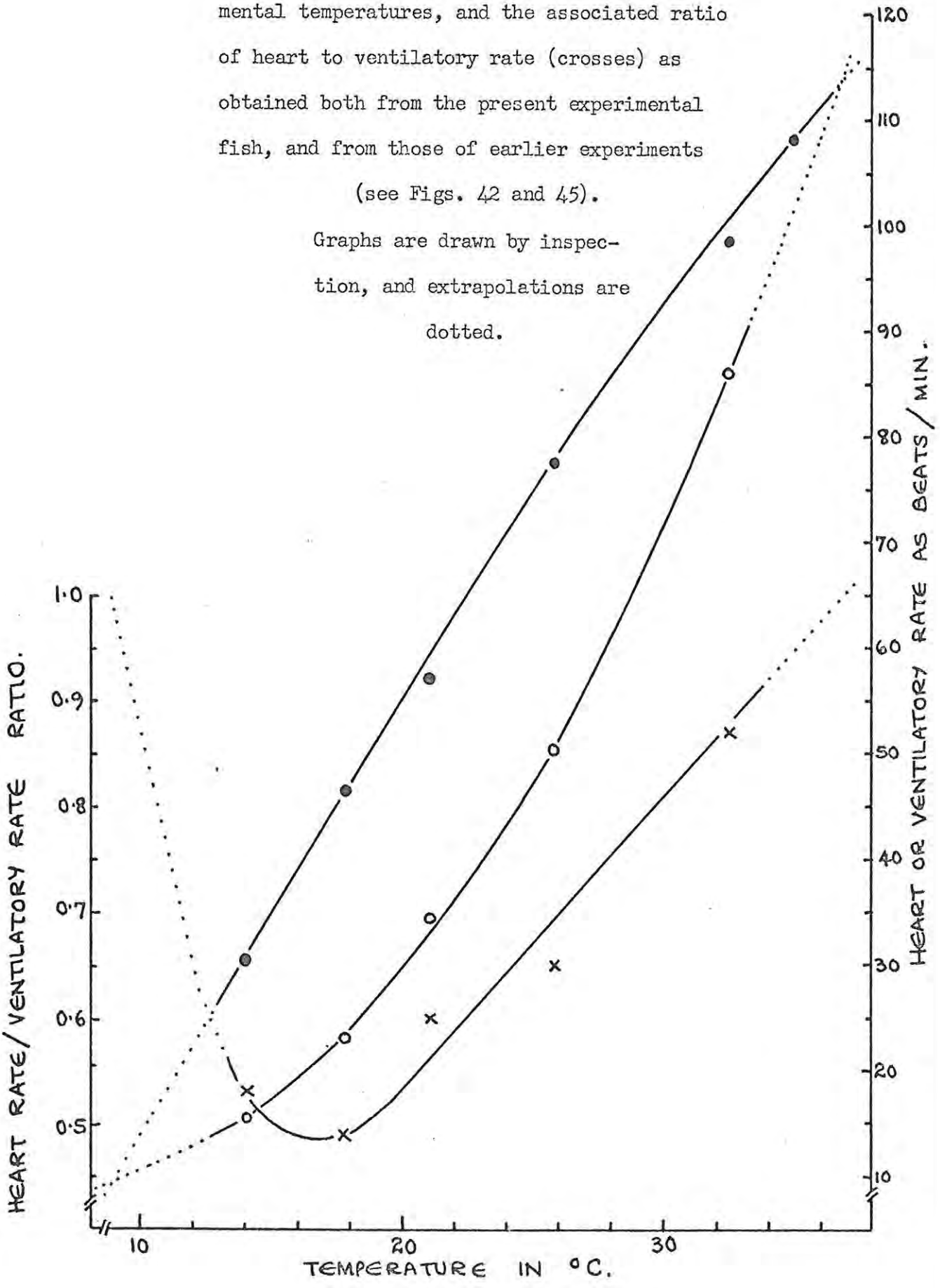
The relationship between the ventilatory beat and the heart beat is an interesting one, as a cardio-respiratory synchrony is well known in many elasmobranchs, and sometimes in teleosts particularly under stressful conditions such as anoxia. This synchrony is probably of functional significance as it allows the most rapid gill water flows and blood flows to coincide. The mean ventilatory and heart rates have been plotted against temperature together (Fig. 46) as well as the ratio of the heart rate to the ventilatory rate with temperature, in order to investigate whether there are general trends towards a cardio-respiratory synchrony at any temperature in this fish or not. These graphs illustrate the raised Q_{10} values for heart rate as compared to the ventilatory rate (steeper R-T graph), and that a possible 1:1 ratio between the 2 rates may be a fairly general phenomenon only at very high (± 37°C) and very low (± 9°C) temperatures, i.e. where the extrapolations of the R-T graphs for heart and ventilatory rate (dotted lines) intersect (Fig. 46). However, on the odd occasion, 1:1 ratios were observed in one or two of the traces recorded at 21.1°C.

In order to ascertain whether the heart beat may tend to occur at any particular region(s) of the ventilatory cycle, P deflection positions

Figure 46.

Heart rates (light circles) and ventilatory rates (dark circles) at the different experimental temperatures, and the associated ratio of heart to ventilatory rate (crosses) as obtained both from the present experimental fish, and from those of earlier experiments (see Figs. 42 and 45).

Graphs are drawn by inspection, and extrapolations are dotted.



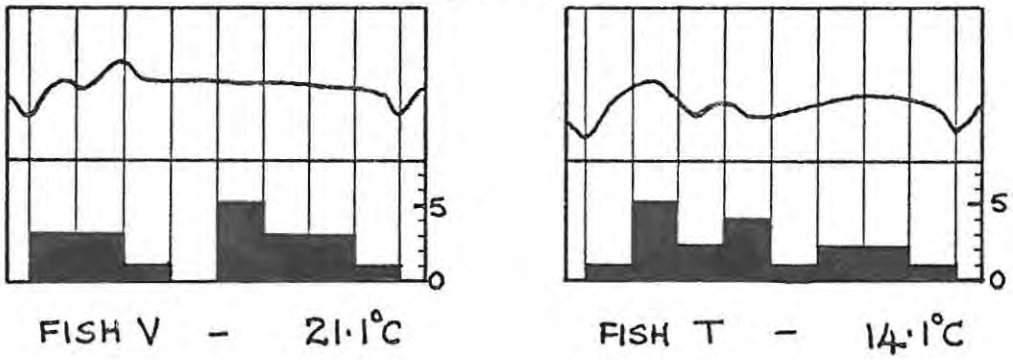
relative to the electrical recording of the ventilatory cycle of movements (see section (c), p. 45) were recorded at the main experimental temperatures in a number of cases where both the ECG and ventilatory recordings were clear enough. The methodology used here is basically that used by Hughes & Umezawa (1968), and also by Berger et al (1970) when studying ventilation-wing beat relations in birds. Some examples are given in Fig. 47. These records often indicated a very temporary tendency for the heart beat to start in particular regions of the ventilatory cycle (the mouth opening or closing etc. stages were not correlated with the electrical recordings, unfortunately), but no permanent tendency was seen, not even at 14.1°C where, because of the very low ventilatory rate and small movements, possible hypoxic stress may be expected. Also the 1 m lengths of film exposed at any one time for any one or pair of fish did not allow sufficient readings to be made so as to establish whether these tendencies were significant at any one time or not.

To help summarise the results of this last experiment, selected photographic results from Fish R and T which were considered fairly representative of the 6 fish, are presented in Fig. 48 for a few of the experimental temperatures in the order in which they were experienced. (N.B. the scale on the time or X axis used for the 14.1°C records as shown is much smaller than for the other temperatures because of the long periods between ECGs, etc.). These photographs illustrate the various aspects discussed above such as increasing length of intervals with temperature decrease, and also that when the temperature drops rapidly from 21.1 - 14.1°C or from 25.8 - 17.8°C, the T deflections may become more pronounced in some fish, as seen in photographs (ix) and (iii), the latter recorded at 20.3°C during the 25.8 - 17.8°C decrease, and indicating marked differences in this respect from photograph (viii)

contd. on p. 123.

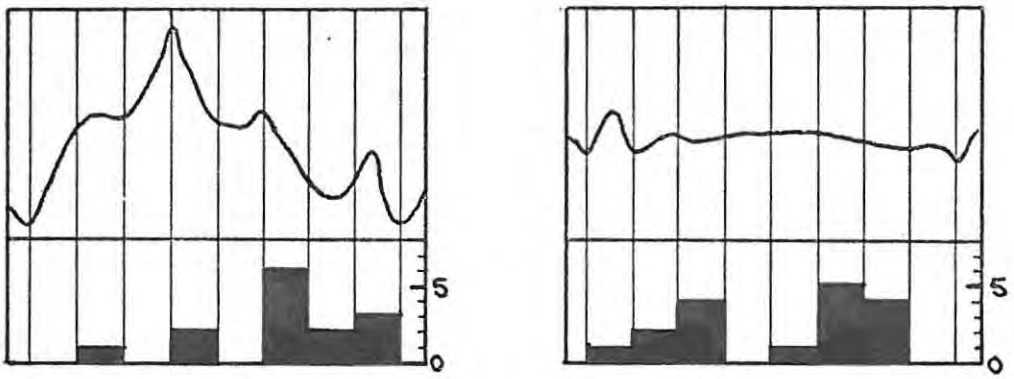
Figure 47.

Drawings of the electrical changes caused by a ventilatory cycle in a few of the experimental fish with the frequency of the occurrence of the P deflection of the ECG at different phases of the ventilatory cycle indicated as a histogram. Fish name, and the temperature at which the recording was made, is given in each case. The ventilatory pattern is shown on top in each case.



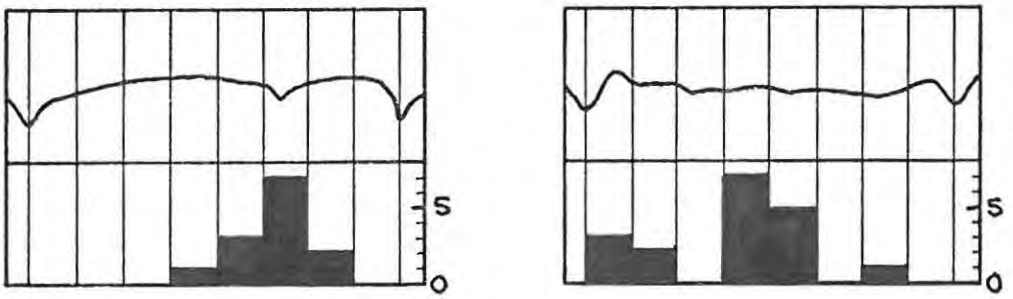
FISH V - 21·1°C

FISH T - 14·1°C



FISH R - 21·1°C

FISH R - 14·1°C



FISH O - 21·1°C

FISH O - 14·1°C

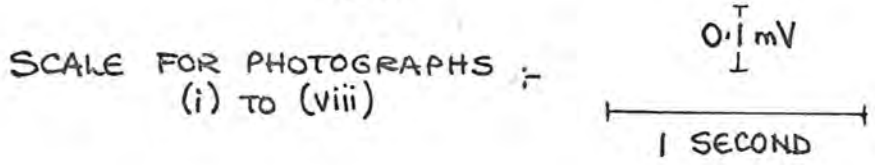
P.T.O. for pages 121 and 122

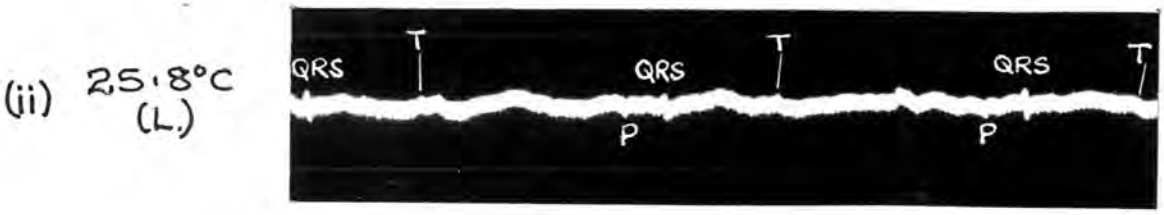
(Fig. 48)

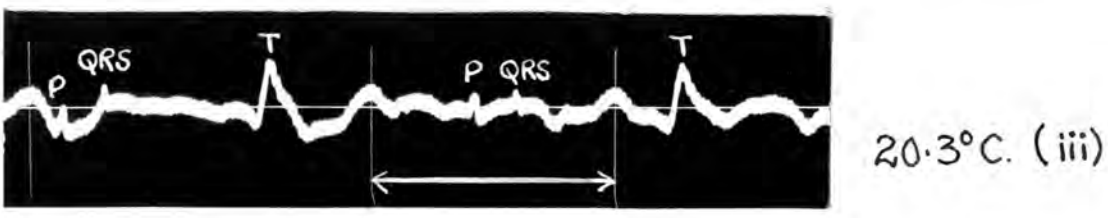
Figure 48.

(Below and opposite)

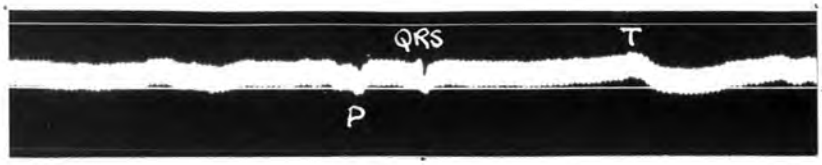
Oscillographic recordings showing ECGs (also ventilatory muscle potentials in some cases) from Fish R and T during the experimental temperature changes. The photographs are presented in the order in which they were recorded ((i) - (x)) and those from Fish R are displaced to the left, those from Fish T to the right. ECG deflections are labelled, and, where visible, a ventilatory cycle is marked by a horizontal arrow. The temperature is given and, where the period at a temperature has been arbitrarily subdivided for study purposes, these are also given if they apply.





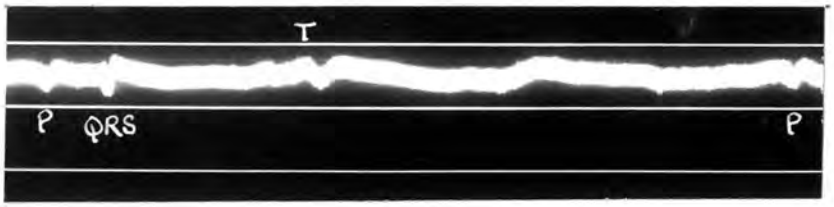


(iv) 17.8°C (D)

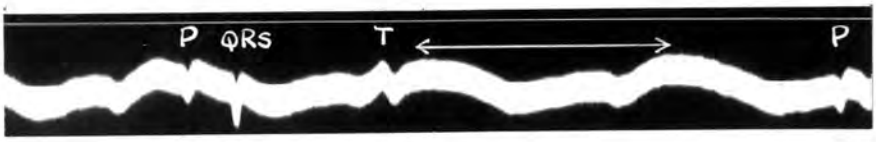


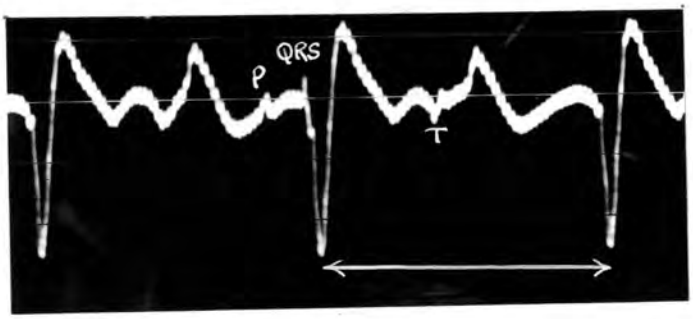
17.8°C (D) (v)

(vi) 17.8°C (E)



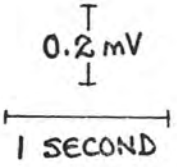
(vii) 21.1°C

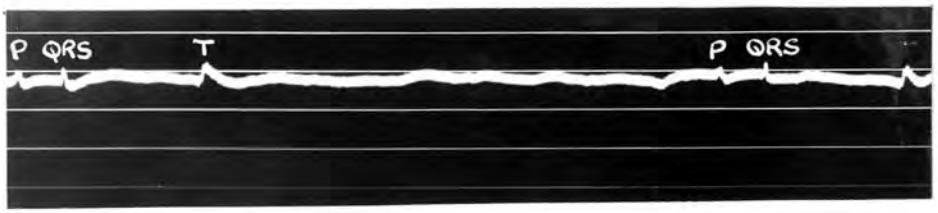




21.1°C (viii)

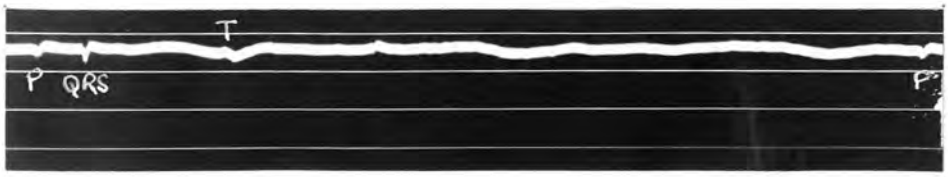
SCALE FOR PHOTOGRAPHS :- (xi) and (x).





14.1°C (ix)

(x) 14.1°C



recorded at 21.1°C where the temperature had just been raised. The T-hump height and duration are often difficult to discern because of the marked ventilatory activity, but both seem not to be so marked. No diminution in T-hump prominence was noted at 14.1°C, but, in some fish, actually an increase during the 4-day stay at this temperature.

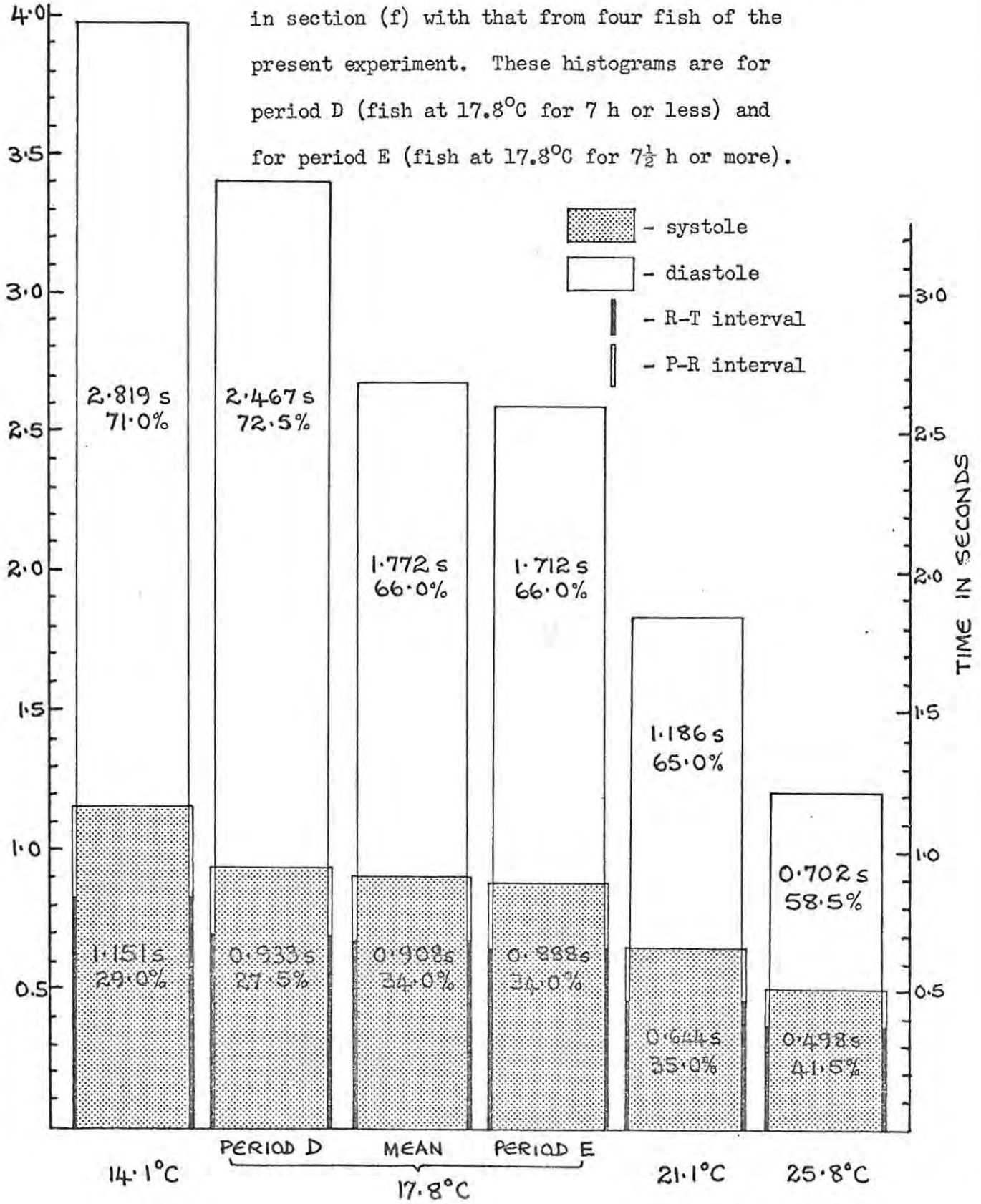
Finally, the over-all changes in heart function with temperature can best be summarised by a histogram indicating not only the times occupied by the cardiac cycle (R-R interval duration), but also the times for which the heart remains active (electrical systole, approximately equal to the P-R plus the R-T interval), or inactive (electrical diastole, approximately equal to the R-R interval minus the period of electrical systole). These are illustrated in Fig. 49 which includes the durations of the P-R and R-T intervals.

As illustrated in Fig. 49, and also in Fig. 48 utilising discrete examples, the main features accompanying a decrease in temperature over the experimental temperature range are as follows:-

1. The over-all duration of a cardiac cycle increases.
2. The component parts of a cardiac cycle, electrical systole and diastole, also increase, as do the P-R and R-T intervals which comprise electrical systole.
3. The Q_{10} values for systole and diastole over the 25.8 - 14.1°C temperature interval are 2.05 and 3.49 respectively, indicating that diastole increases more rapidly than does systole as the temperature decreases. Therefore systole occupies, in general, a progressively smaller % of the heart cycle (41.5% at 25.8°C; 29.0% at 14.1°C). (The only exception to this is that during the first few h the fish were at 17.8°C (period D), systole occupied

Figure 49.

Line histograms of mean R-T and P-R interval lengths, and block histograms of mean lengths of systole and diastole (in s and as a % of the cardiac cycle) for 6 fish at the experimental temperatures used. Two additional histograms have been drawn for 17.8°C, incorporating information from the two fish of the earlier experiment reported in section (f) with that from four fish of the present experiment. These histograms are for period D (fish at 17.8°C for 7 h or less) and for period E (fish at 17.8°C for 7½ h or more).



an even smaller % of the cardiac cycle than at 14.1°C). Consequently both the P-R and R-T intervals occupy smaller and smaller proportions of the cardiac cycle as the temperature decreases over the experimental temperature range e.g. P-R : R-R = 0.095 at 25.8°C but only 0.073 at 14.1°C (see Fig. 50, p. 129). The 2 components of systole have, however, a fairly consistent relationship to each other over the experimental temperature range, the P-R : R-T ratio varying between 1 : 2.86 and 1 : 3.41 in random fashion.

4. Diastole occupies a progressively larger % of the cardiac cycle time (58.5% at 25.8°C ; 71.0% at 14.1°C). Again, period D at 17.8°C is an exception as diastole occupies a larger % of the cardiac cycle than at 14.1°C.

5. After the 8°C temperature decrease (25.8 - 17.8°C), as discussed earlier, there is a cardiac acclimation involving an R-R interval shortening, mainly a result of a shortening of diastole. This acclimation means, therefore, that the relative period of systole increases from 27.5 to 34.0% of the cardiac cycle time while the fish remained at 17.8°C, this acclimation being complete in less than a day, and being more marked in summer fish.

This last point is, perhaps, one of the more interesting findings of the present experiments. It indicates that the % of the heart cycle occupied by systole (the systolic duration fraction) changes with time and approaches the situation found at more normal temperatures, because of a possible acclimation in the form of a shortening of electrical diastole. No similar changes were observed during the 4-day experimental period at 14.1°C, however.

IV. Discussion.

(a) Heart structure and electrocardiographs.

The heart of T. mossambica and the arrangement of the chambers of the heart are closely similar to other higher teleost fish, including the large atrium with a volume similar to that of the ventricle, the non-contractile elastic bulbus arteriosus, and the 2 atrio-ventricular valves (Satchell, 1971). Shrivastava (1965), however suggests that most teleosts have 4 such valves, often arranged in 2 unequal pairs. Some features of the heart of T. mossambica are, on the other hand, probably different to those of some or many other teleosts. These include (i) the arrangement of the atrio-ventricular and ventriculo-bulbar apertures in close proximity; this is also seen in Salmonids, (Satchell, 1971); (ii) the longitudinal folds on the inner wall of the bulbus arteriosus, reported also for Clarias batrachus by Shrivastava (1965); (iii) the occasional presence of asymmetrically arranged atrial lobes (the eel usually has the left sides of both atrium and ventricle better developed (Oets, 1950); and (iv) the apparently non-contractile nature of the sinus venosus, as no oscillograph deflections could be obtained even from electrodes in direct contact with this chamber; thus either pacemaker potentials originating here are too small to be recorded, or pacemaker potentials are associated with the atrial musculature. The importance of the bulbus in maintaining blood flow during a large proportion of systole, particularly at higher heart rates as found by Randall (1968) for the lingcod Ophiodon elongatus, is relevant here.

The heart of vertebrates is composed of distinct cells of 2 main types, muscle elements and pacemaker/conductile elements (Purkinje fibres). Fish heart muscle elements may be smaller, on average, than their mammalian counterparts (Randall, 1962) and, unlike other vertebrates, intercalated discs are absent where adjacent muscle elements join (Couteaux & Laurent, 1957). Notwithstanding the myocardial elements of fish together form

a functional unit, as in other vertebrates, particularly as the transmission of electrical potentials from one cell to the next seems in no way impeded by the intercellular boundary (Schaefer & Haas, 1962; Prosser & Brown, 1961) Thus the variation in the voltages recorded from the ventricle (the QRS complex height) at different temperatures could hardly be because of uneven or partial recruitment of the myocardial elements. Also the generally low ECG voltages recorded from Tilapia and from other teleosts as compared to other vertebrates (see Table 9) are probably not related to the smallness of the individual elements, but rather to the small relative and absolute size of the teleost heart (0.10 to 0.21% of the body weight, Lagler et al (1962) and the distance of the electrodes from the heart (Table 9). The fact that, in the present investigations, a "bipolar" lead system was utilised, may also be of importance as Serfaty & Reynaud (1956) have shown that more prominent and clear deflections were usually obtained using a "unipolar" system in tench when compared to a "bipolar" one.

It is not surprising that the stages of the ECG can be accurately correlated with changes in the trans-membrane action potentials in individual ventricular cells for a particular animal, as shown by Omura et al (1967) for rats, turtles and frogs. These authors similarly describe parallel alterations in the Q-T interval and the transmembrane action potentials after heparin injection into these animals. Therefore we can suggest that, for the individual experimental fish used in this investigation, the R-T interval as measured bears a more or less consistent relationship to the duration of the action potential in the individual cells of the ventricle.

The ECG also informs us of the rate of impulse conduction over the heart. The QRS indicates the rate of ventricular conduction, and the degree of synchronisation of the myocardial elements of the ventricle. The QRS duration varies between 0.020 and 0.055 s in the Tilapia mossambica studied here, and Kisch (1948) reports values of 0.04 to 0.06 s

Table 9.

Approximate QRS voltages as recorded from intact and dissected animals both from the present investigations and as recorded in the literature. It can be seen that, in many cases, for the same animal or animal group QRS magnitudes are 2 to 10 times larger when the recording electrode is near the heart (ϕ) than when it is implanted subcutaneously or placed on the skin surface at some distance from the heart (0).

Experimental animal	Investigator or Information source	Position of the recording electrodes	Approximate QRS voltage in mV
Mustelus canis (Elasmobranch)	Kisch (1948) (Fig. 17)	ϕ On ventricle, dissected fish	1.40 - 4.50
Anguilla vulgaris (Teleost)	Oets (1950)	0 skin surface in intact fish	0.11 - 0.40
		ϕ pericardium dissected fish	0.70 - 1.00
Pleuronectes flesus (Teleost)	Oets (1950)	ϕ on ventricle, dissected fish	0.15 - 0.20
Tilapia mossambica (Teleost)	present investigations.	0 gular muscle in intact fish	0.01 - 0.17 (average 0.03 - 0.09)
		ϕ on heart, dissected fish	0.30 - 0.70
Osphronemus goramy (Teleost)	Remorov (1964)	0 subcutaneous muscles in intact fish	0.50
Xenopus laevis (Amphibian)	Furman (1960)	various positions ϕ near heart in intact toad	0.50 - 3.00
	present investigations (section VI (a))	ϕ on heart, dissected toad	0.40 - 0.50
Gaecilia guentheri (Amphibian)	Peters & Mullen (1966)	ϕ near heart in intact animal	0.30 - 1.00
Canis (Mammal)	Schaefer & Haas (1962)	ϕ on heart, dissected animal	15.00 - 25.00
Homo sapiens (Mammal)	Schaefer & Haas (1962)	0 skin surface in intact man (leads V1, V2, V3 and V4)	1.50 - 2.50

for those teleosts he studied. This latter author suggests that this interval is short in fish as compared to mammals (0.093 s in Homo), but Robertson et al (1966) report values of 0.12 to 0.60 for 66 - 96 cm salmon at 11.0 - 15.5°C. These findings are of interest as fish are not known to have Purkinje fibres which are specialised for impulse conduction. Thus the small size of the fish heart (particularly in smaller fish) and a possibly rapid conduction within the ventricular myocardium itself, may therefore compensate adequately except in large fish such as the salmon mentioned above which have hearts weighing between 6 and 16 gm (Robertson et al, 1966).

The P-R : R-R ratio is normally considered as an indicator of the rate of atrio-ventricular conduction, and also of the extent of the atrio-ventricular junction delay. The smaller the above ratio, the smaller the delay, and the less advanced the nodal delay tissue responsible for it. This P-R : R-R ratio in the T. mossambica of the present investigation varied between 0.073 and 0.095 (see Fig. 50). Other vertebrates have higher values, amphibians included (Furman, 1960), and Peters & Mullen (1966) report ratios of 0.172 to 0.200 for Cecilia guentheri. Thus the heart of T. mossambica and other fish contains little nodal delay tissue (i.e. "transitional cells" of Martinez - Palomo et al, 1970) between atrium and ventricle. This is an interesting and puzzling feature in view of the similar volumes of atrium and ventricle, and of the fact that the contraction of the atrium is responsible for ventricular filling to an extensive degree (Randall, 1968), unlike the tremendous atrial through-flow prior to atrial contraction in mammals.

The R-T : R-R ratio in Tilapia is also markedly less than in Caecilia and other vertebrates, being 0.216 to 0.324 (see Fig. 50) as

---

1.) Kisch (1948), however, reports long Q-T intervals for fish as compared to mammals; his use of intact but anaesthetised fish may explain his finding.

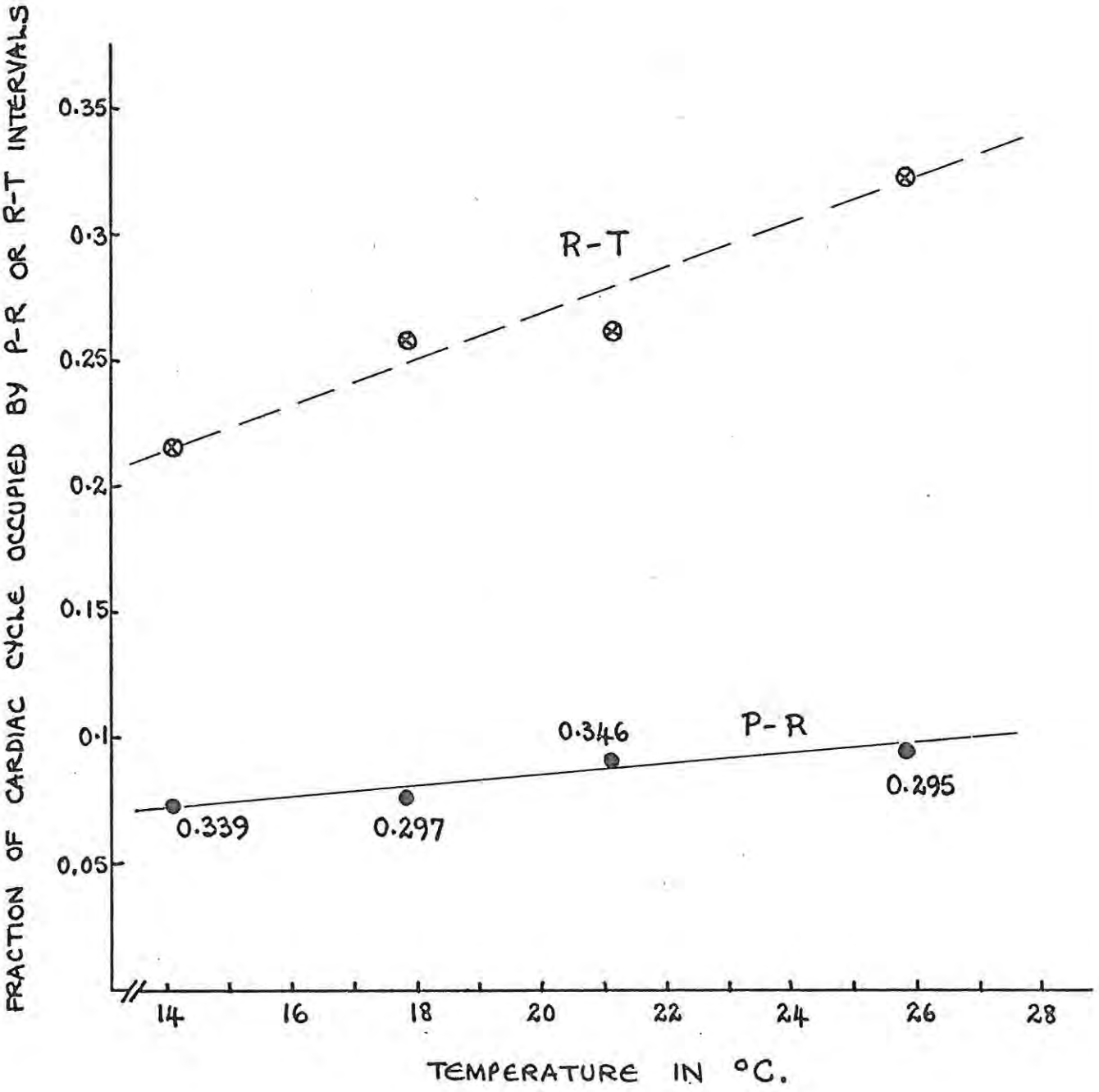
compared to 0.57 to 0.67 in Caecilia (Peters & Mullen, 1966). Thus, because of the small P-R : R-R and R-T : R-R ratios in Tilapia, it is suggested that electrical diastole in this fish is rather long, and the phases of electrical systole {= R-T plus P-R} are rather short, when compared to other animals. This feature is also a function of temperature (Fig. 50) and it seems clear that both the P-R : R-R and R-T : R-R ratios increase with temperature increase, suggesting both a more efficient atrial-ventricular blood transfer and ventricular contraction at higher temperatures.

The P-R : R-T ratio in T. mossambica is fairly consistent at all experimental temperatures (Fig. 50), and that the P-R and R-T intervals respond in a similar way to temperature (Fig. 37, p. 102) is probably the reason for this. The atrial cells of fish repolarise more rapidly than do the ventricular cells, e.g. Jaeger (1965) found that 80% of atrial cell repolarisation occured by 0.194 s and 80% of ventricular cell repolarisation by 0.470 s. These times give an atrial : ventricular repolarisation time ratio of 1 : 4.2, a ratio similar to the P-R : R-T ratios of 1 : 2.9 to 1 : 3.4 found in Tilapia, and to P-R : R-T ratios for other fish e.g. 1 : 1.88 in catfish, 1 : 2.5 to 1 : 5.00 for 5 spp. teleost (Labat, 1966) and 1 : 3.33 for salmon (Robertson et al, 1966). These suggest that the small potentials associated with atrial repolarisation will tend to be hidden by the QRS complex, a feature probably found in all fish (Satchell, 1971) and in other vertebrates (suggested for Caecilia by Peters and Mullen, 1966), as it is obviously important that the atria remain contracted until but not beyond the onset of ventricular contraction.

Labat (1966) suggests that only positive P, R and T deflections should be expected in normal fish (Fig. 9, p. 28) and Satchell (1971) suggests the R as +ve, and the T wave as most often +ve. Satchell's (op. cit) Fig. 3 indicates a -ve P wave for an elasmobranch fish, and

Fig. 50.

The fraction of the cardiac cycle occupied by the P-R and R-T intervals (i.e. the P-R : R-R and R-T : R-R ratios) at the 4 main experimental temperatures, and the trend of these with temperature change is indicated by lines fitted by eye. The P-R : R-T ratio at each temperature is also given as a figure next to each P-R : R-R plot.



Schaefer & Haas (1962) suggest that most animals have -ve T waves. As indicated in Table 1 (p. 37), 5 of the 6 fish of the last experiment always have a +ve T deflection (the 6th fish shows both +ve and -ve deflections), 4 fish have +ve R deflections (one has -ve, the last both +ve and -ve) and 4 fish have -ve P deflections (one +ve, the last both +ve and -ve). Thus it is clear that in these apparently normal fish, no absolute consistency in the direction of the main ECG deflections is seen, a feature also reported by Robertson et al (1966) for salmon with pectoral fin electrodes, and other authors also report teleost ECG's of pattern dissimilar to Labat's typical teleost ECG (Oets, 1950; Hoar, 1966 in his Fig. 5.10). As summarised in Fig. 14 (p. 39 - 40) the sign of the deflections obtained in the present investigation may be correlated with the position of the electrodes in relation to the heart, and may also reflect the direction of movement of polarisation waves across atrium and ventricle. Thus a standardisation of electrode positions in relation to the heart may be a necessary requirement before we can generalise and give a "typical" teleost ECG.

The interpretation of the QRS complex in this work has been to name the R deflection that deflection of the QRS complex that is most prominent (see pp. 30 - 31). The correct interpretation of the QRS is clearly difficult in some teleosts, for Kisch (1948) reports that the Q - deflection is seldom visible, whereas Satchell (1971) suggests "Q is always a negative deflection, ... the S deflection is usually feebly developed." Also only one deflection may be recorded if interference is bad (see Fig. on pp. 25-6 in Remorov, 1964).

The P-deflection, as recorded here in T. mossambica, never showed double peaks etc. such as have been recorded by Oets (1950) and Serfaty & Reynaud (1956). Thus the auricles of the atrium form an electrical unit in Tilapia. The recording of mirror-image ECGs from electrodes placed on left and right sides of the exposed heart in Tilapia parallels similar observations by Kisch (1948), and further support the

above contention that a generalised teleost ECG can only be suggested if electrode position is constant.

The consistency of the P-R and R-T intervals at the same experimental temperature, in any one fish or even within the group of fish used is very marked, the R-R being very variable in contrast (Table 10). Labat (1966) indicates consistency in both the P-R and Q-T intervals (his Table 1), and Peters & Mullen (1966) have found the QRS and Q-T (less so the P-R) to be consistent as measured in different specimens of Caecilia guentheri. These latter authors consider the greater P-R variation as a possible artifact related to the anaesthetic used. The P-R was the most consistent interval measured in Tilapia, however, and the nearly complete lack of nodal delay tissue in teleosts, as suggested above (p. 129) may be the reason for this. However, Xenopus laevis has a very stable SV - PR interval (Furman, 1960) when compared to the QRS duration or the Q-T interval, and shows an atrio-ventricular delay (although no nodal delay tissue has been found in this species).

The tremendous P-R and/or R-T constancy while there is a large change in R-R during the recording period (Table 10) is paralleled by similar observations on Xenopus (Furman, 1960) and on crocodilians (Huggins et al, 1970); and Thauer (1965) finds Q-T (or S-T) changes do occur in hypothermic, anaesthetised dogs, but are much smaller than R-R alterations; QRS and P-Q changes were hardly noticeable. Thus in the heart of Tilapia, as in other animals, factors affecting pace-maker depolarisation rate are often without effect on the rate of impulse conduction across the heart; in fact increases in R-R can be accompanied by slight decreases in P-R or R-T as readily as by slight increases (Table 10). This conclusion is supported by the observations of Omura et al (1967) that the transmembrane action potentials can be experimentally shortened without any effect on heart rate; similarly

Table 10.

Values of the P-R, R-T and R-R intervals as recorded during earlier (A) and later (B) stages of recordings are given in seconds for 6 instances where marked heart rate changes were observed during a recording. In each case the % change from A to B is also given, and the number of intervals averaged is given in brackets where this is known. The last column gives the average change from A to B for all instances, and without reference to sign ($|\bar{x}|$)

ECG interval	Stage and % change from A to B	Fish name and experimental temperature						$\lvert\bar{x}\rvert$ as %
		Fish O 25.8°C	Fish V 25.8°C	Fish V 14.1°C	Fish T 14.1°C	Fish I 14.1°C	Fish O 14.1°C	
P-R	A	-	0.126	0.297 (15)	0.280 (10)	0.303 (10)	0.291 (26)	4.80
	B	-	0.140	0.288 (10)	0.290 (11)	0.315 (8)	0.284 (18)	
	x = % change	-	+ 11.0%	- 3.0%	+ 3.6%	+ 4.0%	- 2.4%	
R-T	A	0.50	-	1.090 (10)	1.111 (10)	0.990 (9)	0.933 (12)	2.24
	B	0.50	-	1.080 (6)	0.997 (9)	0.990 (8)	0.932 (14)	
	x = % change	0.0%	-	- 0.9%	- 10.2%	0.0%	0.1%	
R-R	A	1.91 (33)	2.13	5.45 (14)	3.81 (9)	4.59 (8)	3.01 (35)	23.00
	B	2.17 (51)	1.24	6.31 (11)	2.92 (14)	3.68 (10)	3.71 (16)	
	x = % change	- 14.0%	- 42.0%	+ 15.8%	- 23.3%	- 19.8%	+ 23.1%	

temporary R-R changes led to no appreciable R-T alteration. Also Randall (1966) found that vagal stimulation in goldfish and tench, resulting in reduction in heart rate, caused no changes in the spatial relationships of the P, QRS and T waves of the ECG

Although the sinus venosus of T. mossambica is apparently non-contractile (see above), a pacemaker area in the sinus venosus region can be demonstrated in this fish using the heat method of Gaskel (a heated glass rod placed near the sinus venosus caused the exposed heart to increase its rate of beat). This observation is difficult to correlate with the suggested movement of the atrial depolarisation front from the extremities of the auricles in a dorsal direction towards the main atrial chamber (Fig. 14, pp. 39 - 40), as it would be expected that the sinu-atrial pacemaker would initiate a depolarisation wave at the sinu-atrial junction. However, an extremity to main atrial chamber depolarisation front, if it was accompanied by an isotonic muscle contraction, is of obvious functional significance in emptying the atrial auricles.

The movement of the ventricular depolarisation front from base to apex or from atrio-ventricular node downwards and backwards in a ventro-posterior direction (Fig. 14, pp. 39 - 40) has also been reported for fish (Randall, 1968; Noseda et al, 1963), for Caecilia guentheri (Peters & Mullen, 1966) and also possibly for Xenopus laevis as suggested by the graphical results of Furman (1960). The direction of ventricular contraction in trout (observed visually) appears to be from apex to base (Randall, 1968); this may be so in all teleosts. Thus the direction of the electrical depolarisation (together with isometric muscular contraction?) is in a direction opposite to that of the direction of observable isotonic contraction during the ejection phase of the ventricle. The ventricular repolarisation front in T. mossambica moves in an opposite direction to the

depolarisation front in this chamber (Fig. 14, pp. 39 - 40). This situation is commonly found in fish and other animals e.g. Caecilia guentheri (Peters & Mullen, 1966).

The electrode positions used in this investigation (Fig. 1b, p. 10) are essentially a reversed lead I, with electrodes to left and right of the heart. In view of the suggested approximately antero-posterior direction of ventricular depolarisation, other electrode positioning would obviously be more suitable e.g. one electrode anterior, the other posterior to the heart, and would probably give not only bigger ECG deflections, but also more consistent results. Such electrode positioning on a long term basis which will allow a stable harnessing of the fish, is desirable but has not, to my knowledge, been achieved. Robertson et al (1966) record considerable variability in their results from pectoral leads (lead I arrangement) in salmon which they blame on a variety of causes. I believe the cause is their electrode placement. Serfaty & Reynaud (1956) illustrate larger deflections (Traces 1, 2, 3, Figure on p. 124) where one electrode is just posterior to the heart and the other in the water when compared to recordings where both electrodes are situated in the precordial region (Traces 4, 5, 6, Figure on p. 124). These authors suggest that they achieved better traces in the former cases because of less muscular interference; this interpretation seems invalid as all traces indicate background oscillations (due to muscular activity from surrounding muscles?) of similar magnitude.

Variability in recordings obtained even a few hours apart (Figs. 12 & 13, pp. 34 & 36) may be caused by slight shifts in electrode position in relation to the heart, as Oets (1950) and Peters & Mullen (1966) report that very small changes in the position of the electrodes relative to the heart can cause very large changes in the ECG voltages recorded. Clear visibility of the ECG may also be masked by potentials from surrounding muscles, particularly the muscles operating the respiratory pump as

these can cause large deflections that all but obscure the ECG (see photograph (viii), Fig. 48, pp. 121 - 122). Interference from these sources was probably minimal in most of the present experiments as recordings were only made when fish were quiet, as Serfaty & Reynaud (1956) have found that the ventilatory potentials decreased in the 4 spp. of teleost they investigated when the animals were at rest. Similarly Labat (1966) reports that "parasitic" deflections from the voluntary muscles disappear when the fish rests. Variation in the appearance of the ECG may also be caused by changes in both the composition and/or the volume of the pericardial fluid, as was seen after the first human transplant when aberrant ECGs were recorded from the transplanted heart which was now situated in an over-big pericardial sac. In a few of the experimental fish used in this investigation, where pericardial penetration by the electrodes or their leads occurred this may be a factor to consider as a cause of variability.

In our T. mossambica (weight of 48 to 90 gm), no size-related features were noted in the cardiac intervals or in the height of the QRS deflections, with the possible exception of a tendency towards a slightly lower heart rate at 14.1°C in smaller fish when compared to larger fish (Fig. 30, p. 88). Most fish and other animals show an opposite trend, i.e. smaller animals have a more rapid heart rate. However, Labat (1966) reports that cardiac frequency varied little with fish length in the 6 species of teleost he studied. Only in the Japanese killifish (Oryzias) is the same trend shown as seen here in our experimental Tilapia at 14.1°C as small fish and larvae have lower heart rates than adults (Yamamoto, 1931, and Matsui, 1940). However, at the warmer experimental temperatures used in these investigations, the use of the differently sized fish in the 48 to 90 gm range was without noticeable effect on the features measured.

(b) Handling and anaesthesia stress.

In the present investigations the temperature changes themselves were probably not rapid enough to cause a stress situation; however the handling of fish for the first time, or after a long break undisturbed in a big tank ('initial handling') was investigated (pp. 54 - 59) and found to cause an increase in the ventilatory rate for up to 24 h. Fry (1957) also reports that fish are slow to reduce their metabolic rate after activity or excitement, and Wells (1935a) allowed 24 h to elapse before measuring "normal metabolism" following any disturbance of the environment in Fundulus. An increased ventilatory rate after stress has been found in one of the 3 spp. of teleost investigated by Marvin & Heath (1967) and a readily provoked increase in oxygen consumption is a well-known indicator of excitement or of increased activity in fish (Fry, 1957, and Spaas, 1959a).

Anaesthesia with Sandoz M2 222 on its own did not cause a similar disturbance, as ventilatory functions were only markedly affected during and immediately after the anaesthesia period when the fish appeared darkened, a sign of stress. Similar increases in ventilatory rate are reported for Hydrocynus under Sandoz MS 222 anaesthesia by Begg (1969), and our results are closely similar to those of Shelton & Randall (1962) using the tench (Tinca tinca).

The effects of the operative procedures used (e.g. the implantation of electrodes, pp. 7 to 11), were not specifically investigated, but the increased heart and ventilatory rates in the post-implantation period (Figs. 27 and 28, pp. 81 - 84) suggest that the actual operation may contribute to stress, but that the increased temperature (in all fish) may be a more likely cause of the increased rates observed; 'initial handling' plus operative stress may contribute in the two fish handled more recently.

Heart rate is also reported to change after handling. In the tench there are large increases in heart rate, whereas in the trout there is a decreased rate (Randall & Smith, 1967). The data from T. mossambica in the present experiments are contradictory. After electrode implantation, and with a 22° to 25.8°C temperature increase, an increased heart rate is recorded as compared to those recorded later at the same temperature, as mentioned above. However, on two of three occasions when fish appeared excited or stressed during the experiments, heart rate was lower or the same immediately following or during the stress than under more normal conditions at the same temperature; on only one occasion was the heart (and ventilatory) rate elevated (Table 11).

Table 11.

Respiratory and ventilatory rates of fish under stress, or of abnormally active fish (i) as compared to average/rates of the same fish at the same temperature (II). A dash indicates that no readings were available.

	Fish Name	Stress cause, or symptoms seen.	Temperature.	Heart rate per min.		Ventilations per min.	
				I	II	I	II
1.	O	@ Very active before recording.	25.8°C	57.4	36.4	-	-
2.	O	Very darkly pigmented, ? due to fungus infection.	14.1°C	22.5	22.9	31.7	30.4
3.	A	Very active before recording.	25.8°C	-	-	79.3	55.0
4.	T	Out of water and handled to replace just before recording.	17.8°C	19.9	25.2	52.2	62.0

@ See Fig. 54 for photograph of part of this recording.

Fish O when apparently stressed (increased pigmentation, 2. above in Table 11) showed no changes in heart or ventilatory rate. Table 11 thus suggests that periods of over-activity (1. and 3., Table 11) were accompanied by decreased heart and ventilatory rates (but perhaps also by an increased ventilatory amplitude and/or an increased cardiac stroke volume),

whereas periods of removal from water and handling (4., Table 11) were accompanied by increased rates.

c) Diurnal cycles.

Wells (1935a) reports that there is no indication of a diurnal rhythm in metabolic functions of Fundulus, but diurnal cycles of activity have been reported for other species e.g. Polyakov (1940) reports an oxygen consumption rhythm, and Siegmund (1969) has found that gill beat rate has a diurnal cycle for 3 spp. of teleost fish, and decreased considerably during rest phases.

As pointed out in the Introduction (pp. 1 - 2), young T. mossambica have a diurnal cycle of activity in the field (Donnelly, 1969b), as also found by Maruyama (1958). The latter author describes resting fish, at night, as motionless and deeply pigmented on the bottom or sides of the dam. Only when water temperatures increase and/or sunlight penetrates the water do the fish migrate to 'nurseries' or to the water surface to feed etc.

This diurnal pattern of activity in T. mossambica is also readily observed in the laboratory, even under constant temperature conditions and in a confined space. Late night or early morning (up to 10.00 a.m.) recordings often indicated smaller amplitude ventilatory movements and very slightly decreased heart rate (e.g. 14.4 beats/min at 14.1°C, experiments reported in section III (g)) as compared to later in the day at the same temperature (15.5 beats/min at 14.1°C; see section VI (c) for the full results). This pattern thus correlates with the above mentioned data from other teleosts, although the difference between day and night readings were not significant. The slight day-night differences in heart rate were not observed at 25.8°C (see section VI (c)), perhaps because such warm temperatures are more normally found during the day in

shallow or surface waters.

d) Ventilation, respiration, temperature and fish size.

The oscillographic traces show ventilatory muscle activity (pp. 45 - 53) similar to that reported by other authors, e.g. Marvin & Heath (1967). Although these deflection patterns were not correlated specifically with the phases of the ventilatory cycle, they nevertheless have provided information about the length of the cycle. An indication of the amplitude or strength of the respiratory movements is also given by the height of the oscillographic deflections, assuming that the same muscles are usually involved.

Sumner & Wells (1935) also report that the ventilatory rate is strongly correlated with the depth of the ventilatory movements in Gillichthys and Fundulus, a correlation I have found only in general in the present experiments as there were some marked exceptions at 21.1°C and 25.8°C (see below p. 142). It can be suggested that increases in the ventilatory rate and/or amplitude, as recorded oscillographically, indicate increased ventilatory volumes, i.e. the volumes of water passing through the branchial chambers. This relationship can be inferred from opercular movement traces of carp in conjunction with ventilatory volume values which were recorded simultaneously (Peyraud, 1965), and Spitzer et al (1969) reports an increase in ventilation volume as mediated via rate and/or amplitude increases under hypoxia. Such ventilation volumes will, in general, be proportional to the amount of blood-water exchange occurring, in particular to the oxygen-uptake of the gills, other factors[1.] being more or less constant. As we are concerned with some

---

1.) Such factors as catecholamine, adrenaline and haemoglobin concentrations of the blood, rate of gill blood flow, gill blood distribution, water flow patterns between the gill lamellae, water oxygen content, etc., are of importance for any accurate assessment of the relation between ventilation volume and, for e.g. oxygen uptake.

aspects of metabolic functions as they relate to ventilatory and cardiac activity, this oxygen-exchange factor is of interest, and we can therefore suggest a probable tendency towards greater oxygen uptake at higher temperatures as ventilation rate increases (Fig. 22, p. 66, and Fig. 27, pp. 81 - 82); a lower uptake at lower temperatures.

There is a tendency for the ventilatory rates of the summer animals (Fig. 22, p. 66, curve 3) to be lower at all experimental temperatures than those of winter animals of slightly smaller size, (Fig. 22, curves 1 and 2). The size difference may also be partly responsible for the differences, as will be discussed below (p. 144). Wells (1935b) reports a similar seasonal variation in oxygen consumption in Fundulus parvipinnis with higher consumption recorded in the coldest months although measured at the same experimental temperatures.

The 3.3°C overnight temperature increase from 17.8° to 21.1°C resulted in a marked increase in the amplitude of the ventilatory movements in most of our experimental fish (Table 3, p. 49, and Fig. 20, pp. 52 - 53) as compared to the same fish at the same temperature during the previous temperature decrease. However, when the temperature was increased from ± 22.0°C to 25.8°C at the start of the experiment (a ± 3.8°C change), no ventilatory amplitude "overshoot" was seen (Table 3, p. 49) but, in this case, a marked increase in ventilatory rate is recorded as compared to later at the same temperature (99.5 ± 14.5 beats/min in period M as compared to 77.5 ± 10.3 beats/min in period L, see Fig. 37, p. 102). Peyraud (1965) also reports an "overshoot", in his case in respiratory water volume in carp, recorded when the temperature increased from 2° to 7°C. In our experiments, both the ± 22.0° to 25.8°C and 17.8° to 21.1°C changes probably both cause a marked increase in ventilation volume, but by different means. These differences may reflect the fact that the 17.8° to 21.1°C change follows a warm-acclimation period at

25.8°C (Fig. 27, pp. 81-82), thus the fish are essentially warm-adapted and are not under conditions of handling etc. stress. However, the fish had not had any recent high temperature experience when they were transferred to 25.8°C at the start of the experiment, although they had recently been under a feeding régime (p. 6) and were also fed at 25.8°C although little food was taken (p. 11). Feeding may cause an increase in metabolism for some days, although Wells (1935a) did not find this to be so in Girella nigricans. Thus the different ventilation responses to the increased temperature in these 2 cases may, therefore, reflect the different thermal, handling and feeding histories.

The rapid stabilisation and/or acclimation responses of the ventilatory rate within about 48 h after the 3.3° or ± 3.8°C temperature changes is comparable to known acclimation responses in ventilatory rate in this and other species during temperature increases of similar or greater magnitude. (The early experiments (section III (e)), using 2.0°C increases, do not indicate similar overshoots, as the temperature changes were smaller). Thus Peak et al (1967) report that T. sparmanni requires 10 to 14 days for ventilatory rate acclimation after a 13°C change (16° to 29°C), Meincke (1970) has shown that Tinca tinca requires 4 days after a 12.5°C change (13.5 to 26.0°C) and Freeman (1950) reports similar results for Carassius after a 7°C change (20.0° to 27.0°C). Meuwis and Heuts (1957) found a 100% increase in the ventilatory rate of carp when fish were transferred from 32° to 36°C water (4°C change near the upper lethal limit for this fish). Only 1½ days were required for 80% of the later accommodation in ventilatory rate to take place. Donnelly (1969a) notes that ventilatory rates were extremely rapid for about 10 days when 2 - 4 cm T. mossambica were transferred to water at 36.5°C. Acclimation was considered complete after 15 days.

The 4-day periods after the 2 temperature decreases of 8.0° and

and 7.0°C (25.8° to 17.8°C, and 21.1° to 14.1°C respectively, section III (g)), were probably not long enough to allow ventilatory rate acclimation (if this occurs at all at lower temperatures) as acclimation to lower temperatures takes longer (Bullock, 1955). However, Freeman (1950) found that 88% of the acclimatory increase in oxygen consumption of Carassius took place in 4 days after a 15°C temperature decrease (from 27° to 12°C). The very slow and slight ventilatory movements at 14.1°C do not change at all during the 4-day period at this temperature, suggesting a very low ebb in metabolic activity at this temperature, and the rising ventilatory rate Q_{10} values (Fig. 37, p. 102) suggest that this trend continues and gets worse as temperatures drop further (discussed below p. 154 ff.)

Another point of interest is the ventilatory rate variations with fish size[1.] as seen in Fig. 22, p. 66 and in Fig. 31, pp. 89 to 90. At low temperatures (14.1° and 17.8°C), the differences in ventilatory rate with fish size are apparently small. As the temperature increases, the smaller fish have ventilatory rates that are increasingly faster than these of the larger fish. However, as explained on pp. 91 and 93, when the ventilatory cycle length is considered instead, or if larger numbers of experimental fish had been used, it is likely that the ventilatory rates would always be significantly higher in smaller fish at temperatures between 14° and 35°C.

Long et al (1961) indicates a similar variation in ventilatory rate with changing fish size (his Table 4), and his data are plotted together with the mean data from the six fish of our last experiment (section III (g))

1.) Fish size was given as fish weight in gm in the present experiments, and the relationships between standard length, total length and weight as given in Fig. 29 (p. 86) are closely comparable to the data for the same measurements as given by Moe (1969) for the red grouper Epinephelus morio.

in Fig. 51, the data from our fish being given as two plots, one for the mean results of the 2 smaller fish (mean weight 53.0 gm), the other for the mean results of the 4 larger fish (mean weight 76.3 gm). This Figure shows that the ventilatory rate results of the present experiments are similar to but lower than those reported by Long et al (1961) in the 10° - 15°C to 25° - 30°C temperature range, but both sets of results clearly show the tendency for smaller fish to have a more rapid ventilatory rate than larger fish. As Long et al (1961) do not give data as to acclimation temperatures, time of year, etc., it is difficult to suggest reasons for the differences in the 2 sets of results. However, as Long et al (1961) also report that cold death occurs between 5.5° and 8.1°C in their fish, whereas cold death has been observed at between 7° and 9°C in fish acclimated to ± 22°C in our laboratories, it is possible that their fish were, in general, more cold adapted than ours. These authors also report that ventilation movement are more regular in the larger than in the smaller fish (fish weight varied between 30 and 90 gm), a comment we could not substantiate in the fish we used (fish weight 49 to 90 gm). Sumner & Doudoroff (1938) also report that small fish have a more rapid respiratory rhythm, but there are also reports of larger fish having the more rapid ventilatory rate e.g. in Cyprinus carpio (Meuwis & Heuts, 1957) if fish No. I and II of Fig. 4 (larger fish, average weight 2,000 gm) are compared to the other fish in the study (smaller fish, <320 gm).

The greater ventilatory rate in smaller T. mossambica as compared to larger fish, may be related to the fact that in fish and in organisms in general, oxygen consumption/gm/h is higher in smaller than in larger animals (Job, (1969a and 1969b) for T. mossambica; Prosser et al (1952) for organisms in general, and Gerald & Cech (1970) for a large number of fish and other aquatic organisms). Gerald & Cech (1970) also show that ventilation volume/kg decreases with increasing size, a feature they also

P.T.O. for pages 146 and 147

(Figure 51)

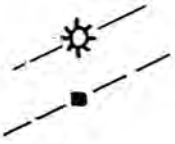
Figure 51.
(opposite)

Mean ventilation rates/min for T. mossambica as given by Long et al (1961) (his Table 4), and as determined in the present investigations, plotted against the approximate experimental temperatures. Fish of different weight groups are plotted separately, and curves have been drawn by eye to indicate the trends present. Vertical bars indicate the total range of ventilation rates as given by Long et al (1961).

Key to plots of Long et al's (1961) data: Number of fish averaged in an approximate way are given in brackets:

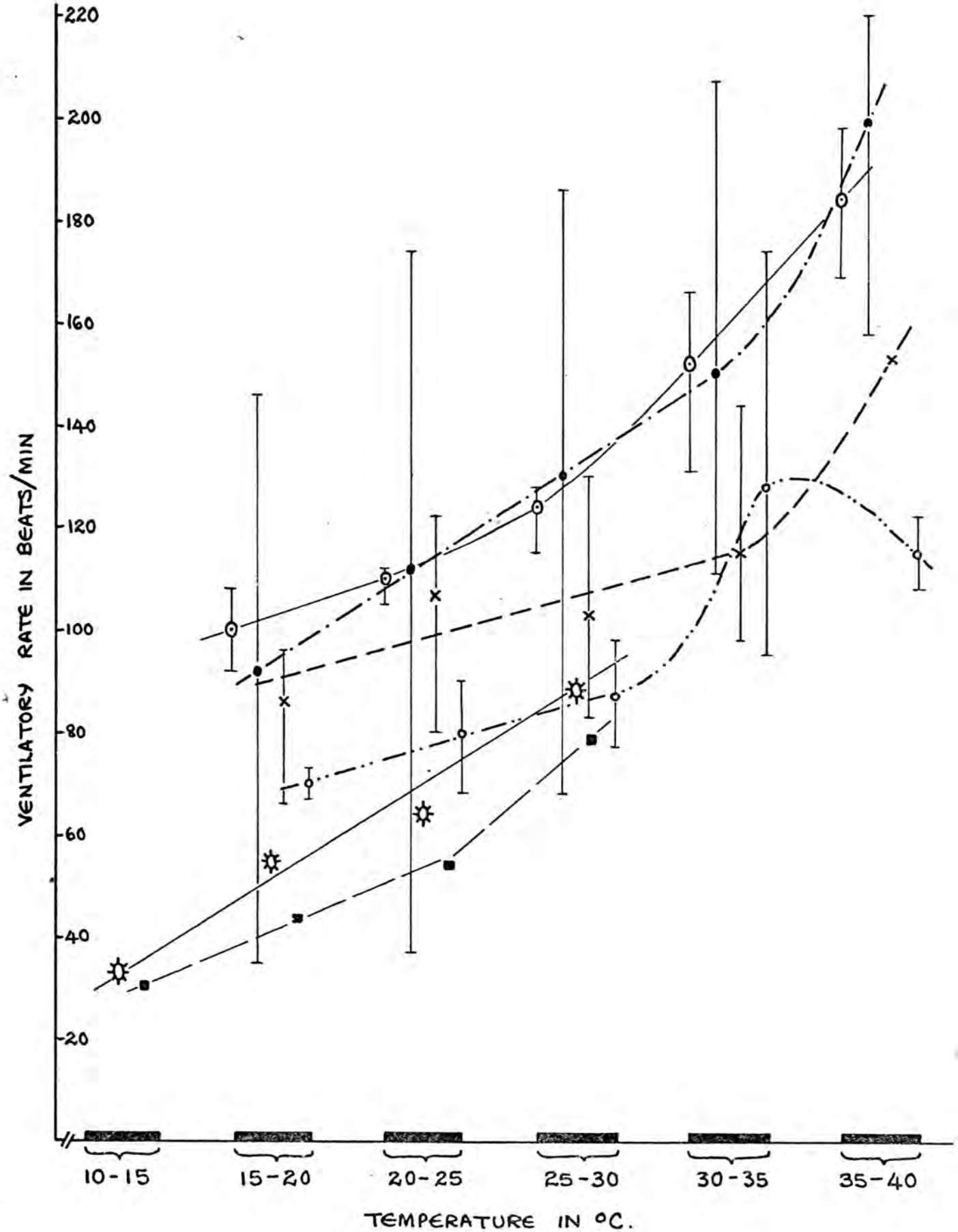
- < 30 gm fish (4)
- 30 to 60 gm fish (21)
- 60 to 90 gm fish (19)
- > 90 gm fish (6)

Key to plots of present experimental data:
Number of fish averaged are given in brackets:



- 53.0 gm (average) (2)
- 76.3 gm (average) (4)

Figure 51


220
200
180
160
140
120
100
80
60
40
20
VENTILATORY RATE IN BEATS/MIN
10-15
15-20
20-25
25-30
30-35
35-40
TEMPERATURE IN °C.

illustrate for Ictalurus of 3.5 to 9.5 gm. The fact that smaller fish have a larger square area of gill lamellar surface/gm body weight (Fry, 1957), is also an important point to remember in these considerations.

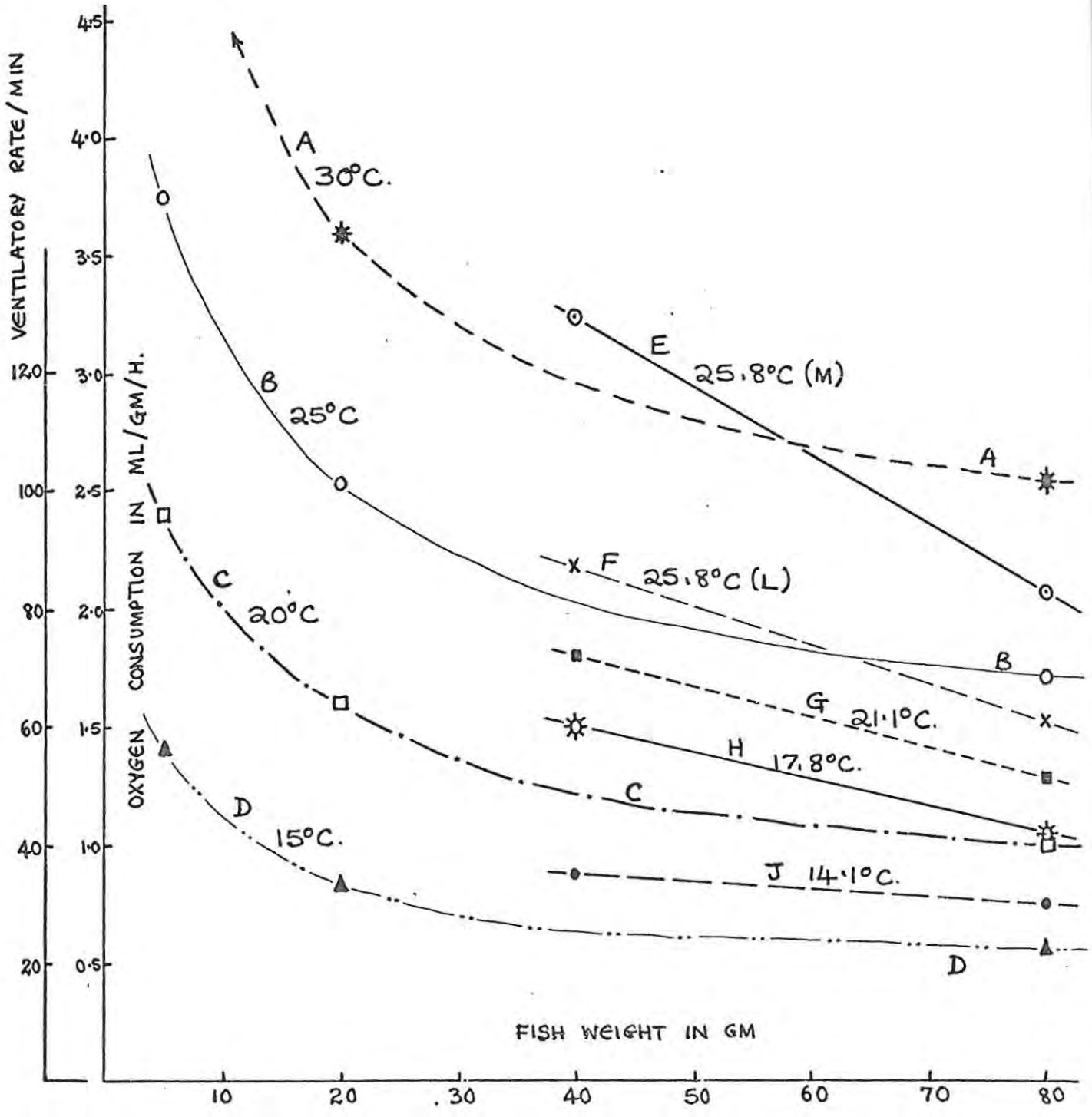
Thus the observed increased ventilatory rates of smaller animals, together with the increased ventilation volume/gm and the increased gill lamellar surface area/gm may comprise an important part of the machinery responsible for the increased oxygen consumption/gm seen in smaller fish.

These ventilatory rate differences with fish size in the 49 - 88 gm range used are of particular interest in the light of what is known about other size-related features in T. mossambica. In particular it is important to consider the oxygen consumption data for 5, 20 and 80 gm fish as presented by Job (1969a, 1969b). This author, using 30°C-acclimated animals, studied the oxygen consumption of fish immediately after transfer to temperatures between 15 and 40°C under fresh water (0.4°/ooS), 50% sea water (12.5°/ooS) and 100% sea water (30.5°/ooS) conditions. The relevance of this work is clear for cold-temperature studies on T. mossambica in view of the fact that cold death and cold-coma are far more marked and occur at higher temperatures in fish held in fresh water than in fish held in water of higher TDS (see p. 5c).

The fresh-water data in Table 1 of Job (1969a, p. 123) and in Table 1 of Job (1969b, p. 224) have been modified so as to be presented as oxygen consumption in ml/gm/h, and are given in graphical form in Fig. 52, together with the ventilatory rate data from the present experiments (abscissa scale different) as derived from Fig. 31 (pp. 89 - 90). It can be seen that there is a general similarity of response by both the oxygen consumption and the ventilatory rate in the different sized fish to the different temperature conditions, and also that the

Figure 52.

The oxygen consumption of fresh-water T. mossambica as recorded at temperatures of 15°, 20°, 25° and 30°C from Job, 1969a and 1969b; curves A, B, C, and D), and the ventilatory rates of our experimental fish at the four main experimental temperatures used (25.8°, 21.1°, 17.8° and 14.1°C using data from both period M and period L at 25.8°C; curves E, F, G, H and J), plotted against fish weight.



oxygen consumption per gm decreases with an increase in fish weight as will be discussed more fully below.

This data from Job (1969a, 1969b) can also be used, in conjunction with the ventilatory data from the present experiments and with the ventilatory data provided by Long et al (1961, his Table 4, p. 449) for the same species, to calculate the approximate oxygen consumption per ventilatory beat in fresh water under different temperature conditions (Fig. 53). The formula used is as follows (modified from Stroganov, 1956):-

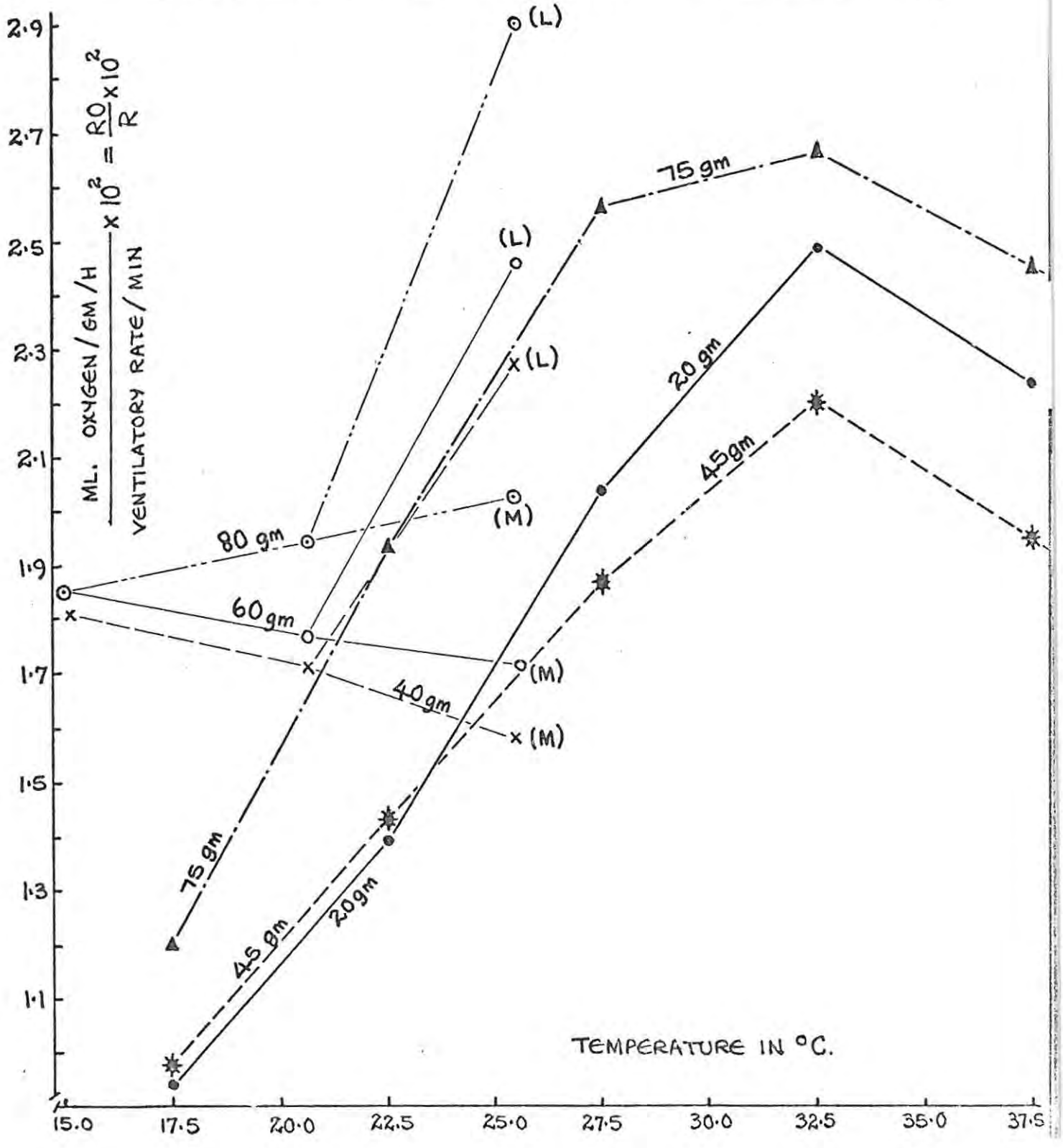
$$\frac{RO}{R} = \frac{\text{oxygen consumption in ml/gm live weight/h}}{\text{ventilatory rate/min}} \times 10^2$$

where the oxygen consumption (RO) includes both oxygen absorbed from the medium through the buccal cavity and gills, and through the general body surface (Stroganov, 1956). This value RO/R is a measure of the efficiency of oxygen uptake from the medium, and, as has been found by Stroganov (1956) for the tiny mosquito fish (Gambusia affinis holbrooki), it is often (but not necessarily) true that higher RO and RO/R values are indicative of a better adaptation to the cold or a better ability to survive cold conditions.

Fig. 53 indicates that larger fish have a more efficient oxygen absorption per ventilatory beat and at all temperatures studied i.e. 14.0 to 37.5°C, except in the case of the 20 gm fish in comparison to the 45 gm fish above 22.5°C. The efficiency of oxygen uptake per ventilatory beat seems maximal at about 27.5 to 37.5°C (using the data of Long et al, 1961), but declines markedly below these temperatures in all sized fish. If the results of the present experiments are considered (using only the earlier period at 25.8°C, period M, to represent the 25.8°C period, see Fig. 27, pp. 81 - 82), this tendency of the RO/R values to decrease is not seen below 25.8°C. If the later period at

Figure 53.

Oxygen consumption of T. mossambica of various weights per ventilatory beat (using a formula modified from Stroganov, 1956; see p. 150) as calculated from data of Job (1969a, 1969b) in conjunction with data from the present experiments (fine graphs) or from the experiments of Long et al (1966) (heavy graphs) plotted against the experimental temperature. Two plots for 25.8°C are given when our data is utilised, i.e. from the earlier (M) and later (L) periods at this temperature.



25.8°C (period L) is utilised, however, there is a decrease in the RO/R values below 25.8°C, in close agreement with RO/R values determined using Long et al's (1961) data. It is very important to stress, however, that such use of data from different authors has a distinct disadvantage as different (or even unknown) temperature acclimation regimes have been used, fish are used at different seasons and are drawn from different populations etc. However, these combined results do indicate that T. mossambica in fresh water has a maximally efficient absorbing system between approximately 25.0 and 38.0°C, again an indication of the thermophilic nature of this species. The data also suggest that, in general, larger fish have a slightly more efficient oxygen absorbing system per ventilatory beat than do smaller fish at all temperatures in the range of fish size used.

Other factors in relation to size may, however, be of greater importance. In 50% sea water, Job (1969a) finds the increase in oxygen consumption with increased size is in direct proportion to the increase in weight of the fish. Thus b values (representing the slopes of the double logarithmic plot of oxygen consumption of the whole animal against the wet weight of the animal, (Zeuthen, 1953, and Prosser & Brown 1961)) are approximately 1.0 (average 0.9999) and are consistent except at 35° and 40°C where slightly lower b values are found (0.988 and 0.915 respectively). However, in 100% sea water, b values are lower than in the case of 50% sea water fish (b = 0.874 on average), although consistent at different temperatures, and, of greatest relevance, the fresh water fish have even lower b values, values that are not at all consistent at different temperatures and increase with temperature increase (b = 0.638 at 15°C, b = 0.826 at 40°C); the regression line calculated for a plot of these b values against temperature is significantly different from a horizontal line ($P<0.001$). Thus, in fresh water, less and less metabolic energy is available to animals as they grow and as the

temperature decreases (see Fig. 53). b values for fish in the literature, suggest that higher b values are found under temperature conditions to which the animals have become adapted on an acclimatory or evolutionary basis. Thus the fresh water fish Fundulus adapted to and held in sea water at 10° to 12°C, have increasing b values as the temperature drops from 22° to 12°C (Wells, 1935a), an interesting feature in this normally thermophilic fish. The Cichlid fish Etroplus, indigenous to the monsoon lands of India and Ceylon, has a b value of 1.00 at 0°C, but a lower b value at 35°C (b = 0.67) (Prosser & Brown, 1961). Thus a fish which has evolved in areas where temperatures commonly plummet to 7° or 8°C may have adapted to this through evolution by means of raised b values at these low temperatures. The high b values for T. mossambica in 50% sea water also correlate with Job's (1969a) finding that in both 20 and 80 gm fish, highest oxygen consumptions are found in 50% sea water, giving this fish greatest "scope for activity" at this salinity (Fry, 1947, in Job, 1969a, p. 124).

The above results suggest that T. mossambica under fresh water conditions has less energy available for the various physiological processes such as ventilation, osmoregulation, motility, digestion etc. i.e. fresh water inhibits metabolism to some extent, more so under low temperature conditions. This interpretation seems preferable to the suggestion that more energy is required in water of low TDS for osmoregulation, etc.[1.] Conversely, in water of higher TDS, it is not that less

---

1.) The probable breakdown of osmoregulation in fresh water at low temperatures (see pp. 2, 5c - 5d) may reflect a situation that is detrimental to the whole function and survival of the fish (Bok, 1968, and Allanson et al, 1971), and may involve not only metabolism but also hormones e.g. prolactin-like hormones are important for blood ion regulation in fresh water T. mossambica at normal temperatures (Dharmamba et al, 1967). Also such a blood dilution as is reported for T. mossambica must have a limit at which various physiological functions which depend on blood ion homeostasis, will stop. Whether blood dilution continues as temperatures drop further below 11°C is not known, but investigations are continuing in this Department. To what extent the lowered blood chloride etc. at 11°C is adaptive (needs less energy for regulation to this level, Prosser et al, 1970), or detrimental, is not known at present.

energy may be required for osmoregulation, but rather that more energy is available under these conditions as metabolism is boosted. A similar but smaller boost occurs in fresh water T. mossambica as the temperature rises. Wikgren (1953) reports similar metabolic boosts in fresh water in the crucian carp and in the lamprey where these animals are under a marked osmotic stress. This author suggests that the "higher energy output in fresh water as compared to that in saline water is not primarily dependent on the osmoregulatory strain. An increase in the basal metabolism may possibly be involved."

R-T (rate-temperature) graphs (Fig. 22, p. 66, and Fig. 45, p. 116) indicate a fairly consistent increase in ventilatory rate with temperature, but with a slight decrease in the slope of the R-T graphs as temperature increases. This is also reflected in the progressively lower Q_{10} and Arrhenius μ values at the higher temperatures (p. 65 and Fig. 37, p. 102), a feature found in all the rate functions studied in these experiments (Fig. 38, p. 105). Long et al's (1961) data suggest a slightly different pattern, however, (see Fig. 51 above), as in all fish size groups there is an increase in the slope of the R-T graphs above 30 or 35°C, followed in the case of the largest fish (>90 gm) by a decrease in ventilatory rate. A similar decrease in ventilatory rate above 35°C is reported by Denzer (1968) for young T. nilotica.

The Q_{10} values for the ventilatory rate data of the present experiments, as well as that obtained from a semi-logarithmic plot of the data presented in Table 4 of Long et al (1961) for their 30 to 90 gm T. mossambica, are given in Fig. 54. This Figure indicates that the summer T. mossambica of the present experiments (curve 1) give higher Q_{10} values than the winter animals (curve 2), and than the Q_{10} values derived from Table 4 of Long et al (1961) (curve 3). Thus although the summer animals of the present experiments were, on average, larger fish (average weight 68.6 gm) than those used in the earlier winter

P.T.O. for pages 155 and 156

(Figure 54)

Figure 54
(opposite; please rotate through 90°)

Q_{10} values for ventilatory rate, oxygen consumption, and cardiac function rate data as derived from various of the present experiments on T. mossambica, and from other published work on the same species and on other fish and on animals in general as plotted against temperature.

Key to the curves in the Figure:-

A. Those on the lower graph:

- 1. Ventilatory rate, summer T. mossambica, present experiments (section III(g)); average weight 68.6 gm.
- 2. As in 1., winter fish; average weight 42.0 gm.
- 3. Ventilatory rate T. mossambica, Long et al (1961); combined 30 to 60 and 60 to 90 gm weight groups.
- 4. Rate of oxygen consumption, winter cunner (Tautogolabrus), from Table 5, Scholander et al,(1953).
- 5. As in 4., but summer cunner.
- 6. Ventilatory rate, Carassius auratus, Freeman (1950).
- 7. Metabolic rate, Gambusia affinis holbrooki, mean of fish in various physiological states, Table 1 Stroganov (1956); <1 gm
- 11. Oxygen consumption T. mossambica held in 50% sea water, Job (1969a, 1969b); weight 80 gm.
- 13. Ventilatory rate winter to early spring T. mossambica, class experiment at Rhodes University; average weight 14 gm.

B. Those on superimposed graph:

- 8. Oxygen consumption for number of temperate poikilotherms, after Krogh, from Tables, Scholander et al, 1953.
- 9. Oxygen consumption, T. mossambica in fresh water, Job (1969a, 1969b); weight 80 gm.
- 10. As in 9, but weight 20 gm.
- 12. Heart rate, summer T. mossambica, present experiments (section III (g)); average weight 68.6 gm.
- 14. Rate of ventricular functions; rest as in 12.
- 15. Rate of atrio-ventricular conduction; rest as in 12.

(Continued opposite)

Figure 54.

—•— - 16. Rate of diastolic functions; rest as in 12.

- 17. Rate of systolic functions; rest as in 12.

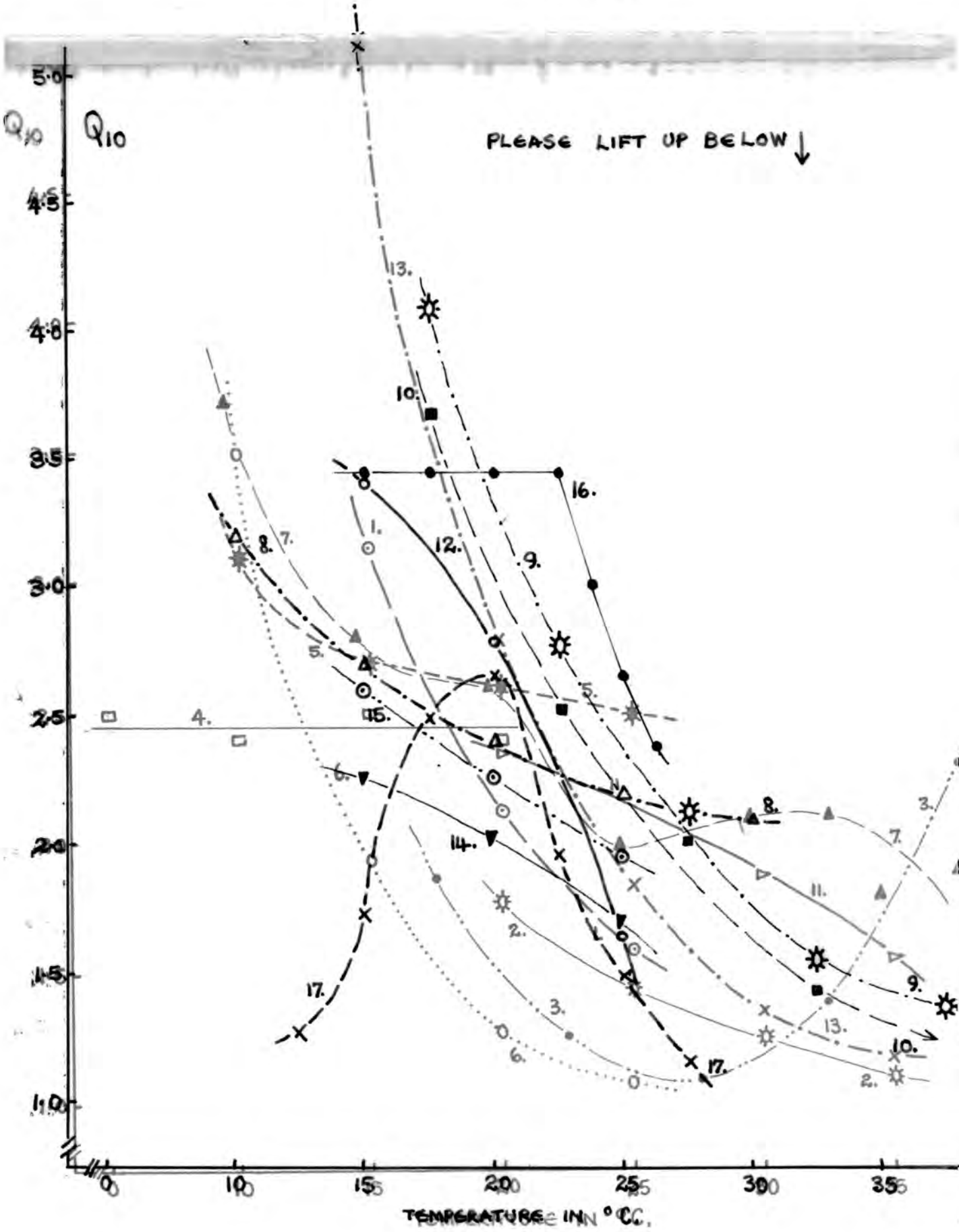
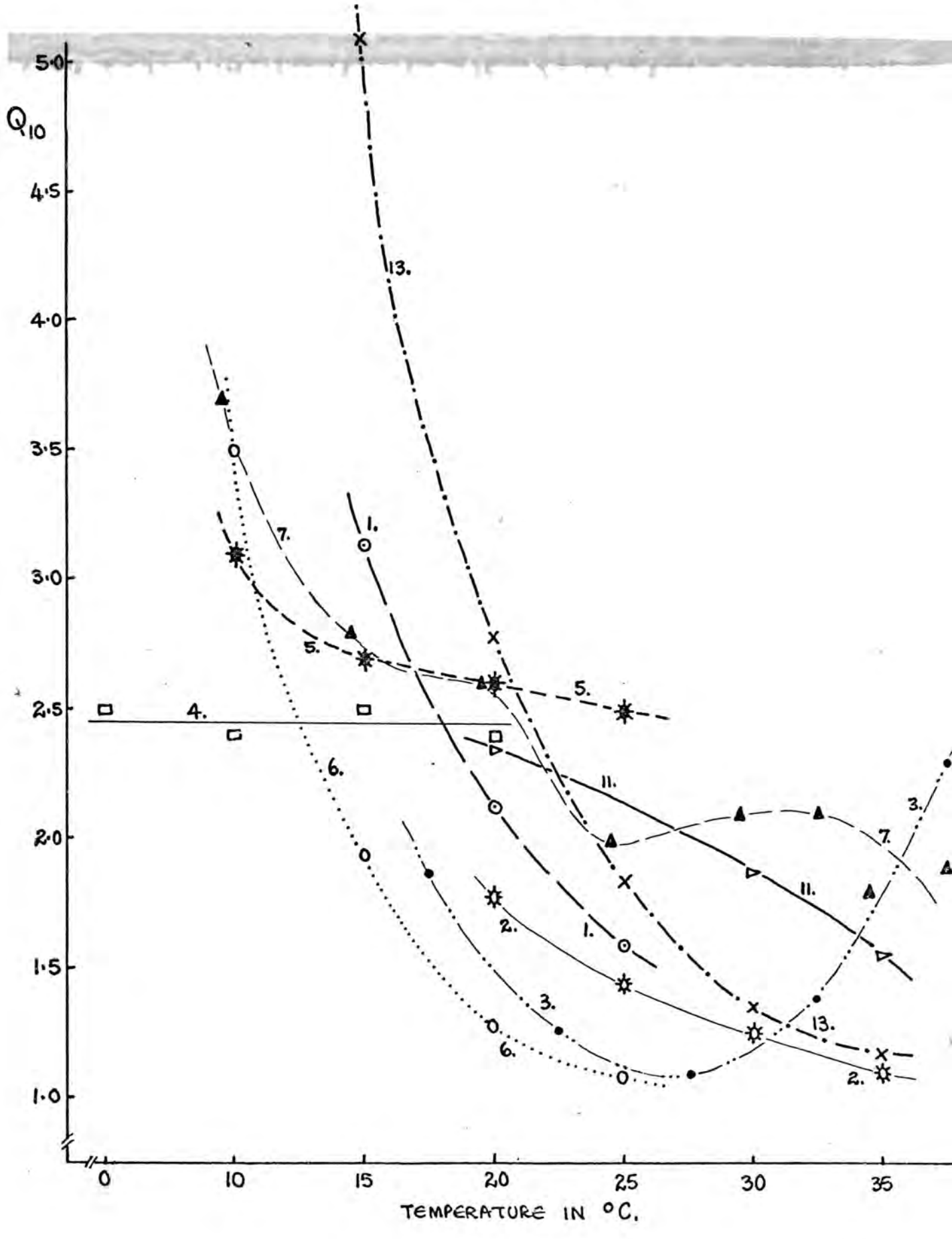


Figure 54.

—•— - 16. Rate of diastolic functions; rest as in 12.

-×- - 17. Rate of systolic functions; rest as in 12.



experiments (average weight 42.0 gm), they still give higher Q_{10} values over the same temperature range, contrary to the normal Q_{10} - size relationship for ventilatory rate (see above). The results of the class experiment on small (average weight = 14 gm) winter T. mossambica under conditions of acute ventilatory rate measurements (curve 13, see also Fig. 22, p. 66) give higher Q_{10} values at all temperatures than for the other winter fish (curve 2) and than for the large summer fish (curve 1), probably because they are very small fish, and were under conditions of more rapid temperature change (1°C in 5 min approximately). The comparison with Long et al's (1961) data suggest once again that their fish were more cold-adapted than those used in the present experiments because of the lower Q_{10} values for similar sized fish. Similarly winter cunner, Tautogolabrus, (curve 4 Fig. 54) have lower (and more consistent) Q_{10} values than summer fish (curve 5) (Table 5, Scholander et al, 1953). These findings are similar to those of Wells (1935a) who found that Mystus had ventilatory rate Q_{10} values of 2.1, and could withstand cooler temperatures than Barbus with a Q_{10} of 2.8 under the same experimental conditions, and Rao & Bullock (1954) suggest that Q_{10} values increase with an increase in the adaptation temperature. The results in Fig. 54 also suggest that the slope of the Q_{10} vs. temperature graph is steeper for summer than for winter animals of similar size both in Tilapia (compare curves 1 and 2) and for Tautogolabrus (curves 4 and 5).

The winter fish (curve 2) of the present experiments give a continually decreasing Q_{10} value with temperature increase even at 30° and 35°C, probably because of the very slow temperature changes used (p. 63). This interpretation is in agreement with a report by Suhrmann (1955) who found that Q_{10} values for carp in the upper range of temperature fell with warm acclimation. Morris (1965b) reports a similar finding for a catfish, Ictalurus natalis. Long et al's (1961) results suggest

an increase in Q_{10} above about 30°C (curve 3), probably because temperature changes were sudden, although no data is given on this point; a later experiment reported by these authors indicate a rapid temperature increase (their Table 5, p. 450) thus a similar method may have been used in the case under consideration. However, the small T. mossambica of the class experiment (curve 13) gave no such Q_{10} increase at higher temperatures, although rates of temperature change were fairly rapid. This again suggests that Long et al's (1961) fish may have been more cold-adapted than those held under laboratory conditions here (20° to 22°C).

Thus in T. mossambica there is a pattern of Q_{10} increase with temperature decrease for the ventilatory rate closely comparable to the pattern seen in Carassius (curve 6, Fig. 54, after Freeman, 1950), Gambusia (curve 7, after Stroganov, 1956), summer Tautogolabrus (curve 5), and to the generalised curve for temperate animals as suggested by Krogh (curve 8, from Table 5 of Scholander et al, 1953) for goldfish, frog, decerebrate toad and mosquito. The oxygen consumption data for fresh-water T. mossambica of Job (1969a) suggests Q_{10} values which change in a similar way with a temperature change (curves 9 and 10, Fig. 54), and rather different to the pattern obtained from 50% (curve 11) or 100% sea water animals. This pattern of Q_{10} change with temperature change in fresh water T. mossambica is quite different from the pattern obtained from arctic and tropical animals (Fig. 5, Rao & Bullock, 1954) thus indicating that T. mossambica is not a tropical but rather a temperate fish when held in fresh water, and the tendency for the ventilatory rate Q_{10} data to be higher in summer than in winter animals also indicates that T. mossambica may show a seasonal pattern of response to temperature for this activity. However as even winter T. mossambica have a fairly steep R-T curve, and have continually increasing Q_{10} values

as the temperature decreases (as in other temperate forms, this suggests not only a drastic drop in metabolism and ventilatory movements[1.] but in nervous[2.] and other activities when the temperature drops below 15° to 12°C. Thus the Q_{10} (or R-T) curves of Fig. 54 suggest that fresh water T. mossambica cannot survive moderately low temperatures even as well as other fairly cold-sensitive forms e.g. Carassius or Gambusia[3.] because the R-T curves for T. mossambica are steeper or further to the right than for these other fish. Also the Q_{10} variations with temperature in 80 gm T. mossambica held in 50% sea water (curve 11, Fig. 54) indicate clearly that there is not a rapid fall-off in oxygen consumption in this medium as there is in fresh water, once again indicating why this animal is more tolerant of cold in a saline medium.

When considering the cardiac functions in comparison to the ventilatory rate, only the ventricular depolarisation rate shows a significant Q_{10} increase with decrease in fish size (Fig. 32, p. 94) although heart rate shows a similar but not statistically significant trend. These results are also included in Fig. 54.

Thus for some of the rate functions in fresh-water T. mossambica there is a tendency for the Q_{10}s to increase with a decrease in fish size

---

1.) Alexander (1967), on the other hand, points out that ventilatory movements of fish are very gentle and slow under conditions of good oxygenation and when fish are at rest.

2.) Early siting experiments also indicate a progressive reduction of central nervous activities as ± 20° to 22°C - acclimated T. mossambica go into chill coma:- At ± 9.7°C balance is lost, swimming ceases at ± 8.7°C, and ventilation stops at ± 7.8°C. Roots & Prosser (1962) indicate a similar progressive cessation of nervous activity in goldfish as the temperature decreases.

3.) Gambusia can survive several months at 10°C if temperature decrease is slow (Stroganov, 1956), and Carassius can survive temperatures of ± 5°C (Roots & Prosser, 1962).

in the size range above about 14 to 30 gm, whereas the Q_{10}s of oxygen consumption show an opposite trend in this size range (Job, 1969a). The independent nature of different rate functions as stressed by Bullock (1955) is thus demonstrated. Thus small fish may gain from a metabolic point of view at low temperature and the apparent loss from a ventilatory rate point of view is probably irrelevant to the over-all ability of the gills to acquire sufficient oxygen because of regulation of other features controlling ventilation volume or oxygen absorption (see footnote p. 141). Some authors report an increasing Q_{10} with an increase in fish size e.g. Morris (1962)[1.] and Meuwis & Heuts (1957), whereas other authors report the reverse e.g. Hasan & Qasim (1960), Sumner & Lanham (1942) and Wells (1935a). It is interesting to note that both 50% and 100% sea water T. mossambica indicate hardly any Q_{10} variation with fish size alteration (Fig. 60, and Job, 1969a), and it would be interesting to investigate whether the same is true for the heart and ventilatory rate functions in these media.

(e) Heart functions and temperature.

Our results have indicated not only the general pattern of response of various cardiac functions of T. mossambica to temperature over the range of $\pm$ 14° to 26°C, but have also suggested that changes of some heart functions occur in time at 17.8°C after a decrease in temperature from 21.1°C.

The general responses of heart rate and the rates of atrio-ventricular conduction, ventricular depolarisation time and the rate of ventricular functions to temperature as illustrated in Figs. 42 to 44 (pp. 113

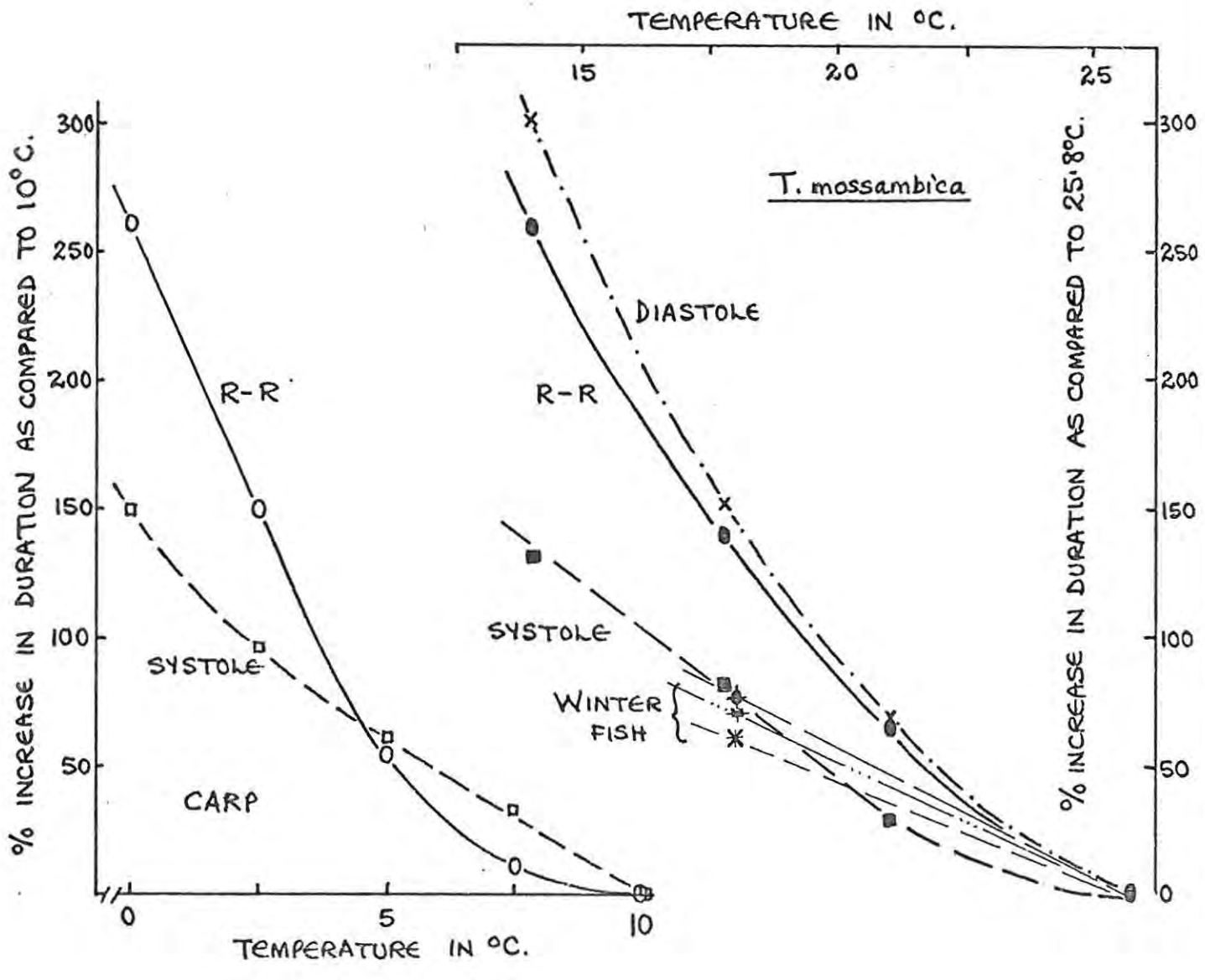
1.) The results of Morris (1962) actually suggest (his Table 1) that only for the $\pm$ 27°C acclimated Aequidens portalagrensis (a Cichlid) is this increase in Q_{10} with increase in fish size undoubtedly true. The low (22°C) and high (32°C) temperature acclimated fish tend to show an opposite trend.

to 115) and in Fig. 49 (p. 124), indicate that all rate functions of the myocardium are affected by temperature in the same sort of way. However the extent to which they are affected are clearly dissimilar as are indicated by the rates of change with temperature (Q_{10} and μ values) as shown in Fig. 37 (p. 102), and more dramatically perhaps in Fig. 54 (pp. 155 to 156) where Q_{10} values at different temperatures are given for heart rate (curve 12), atrio-ventricular conduction rate (curve 15) and for the rate of ventricular functions (curve 14). These illustrate the markedly different response of heart rate compared to the other two rate functions, the Q_{10} values for heart rate rising steeply as temperatures decrease (curve 12), levelling off a little by 15°C, whereas both of the other rate functions have Q_{10} values that rise only slowly with decreasing temperature. The T-hump length, where roughly assessed, showed no dramatic lengthening with the temperature decreases we used, and the R-T (Q-T) interval also did not show the radical lengthening as shown by Labat (1966). Possibly the temperatures we used were not low enough, and it may well be that T. mossambica would succumb because of other reasons before such temperatures were reached. However, the results of Labat (1966) do show a similar trend to those of our own when the R-R interval and the period of systole are compared (Fig. 55) as in both instances the R-R interval increase with temperature decrease tends to be more extensive than those of systole (i.e. they would have higher Q_{10} values). It is interesting to note that Labat (1966) does not appear to have commented on this, but has only emphasized Q-T and T-hump changes, both representing periods that are normally considered as part of systole (see p. 31).

These strong reductions in heart rate while systolic functions do not slow so rapidly cause a marked increase in the rest period (diastole) over the temperature range under consideration. The rate of increase

Figure 55.

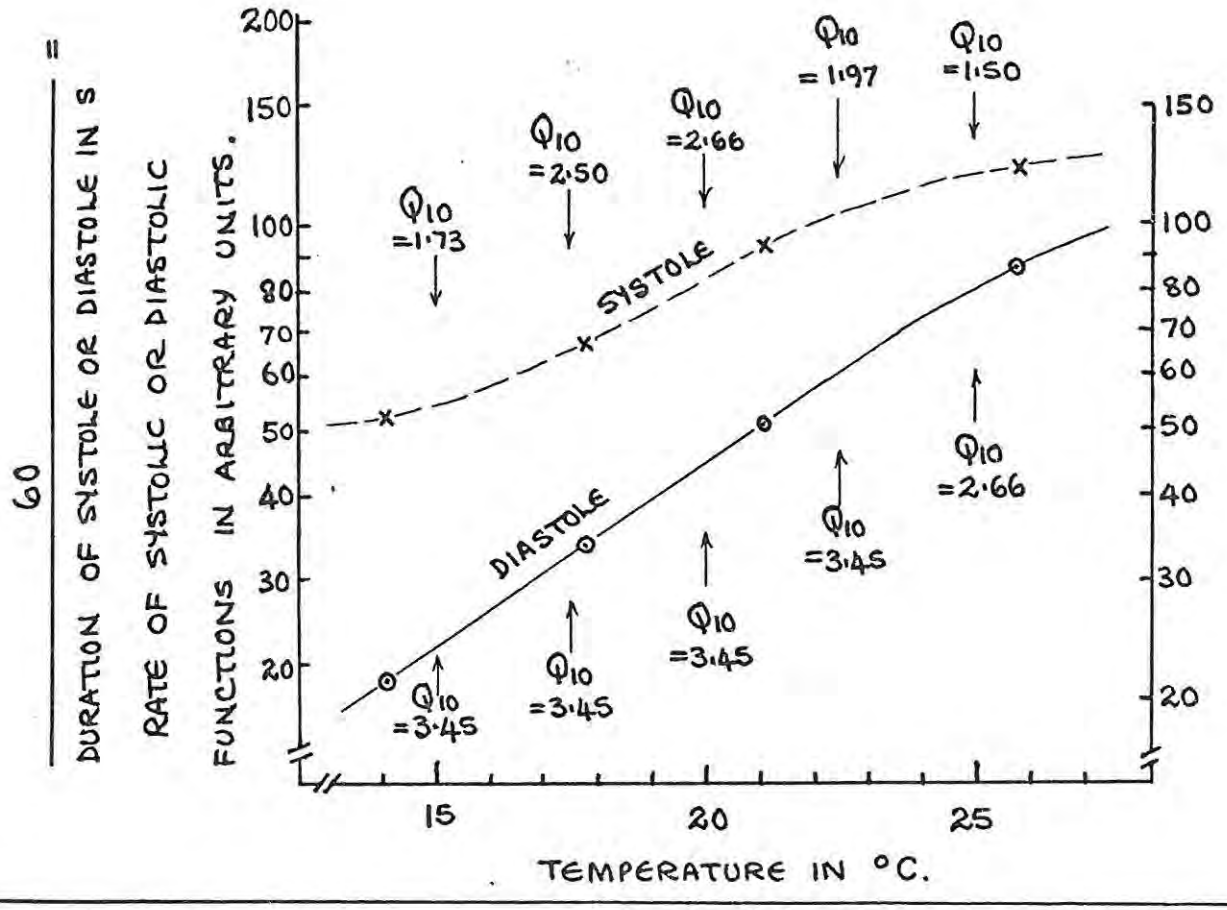
% increases in the cardiac cycle length (O plots) and the durations of systole (□ plots) and diastole (X plots) in relation to the value of these at the highest experimental temperature used as calculated from the present experimental results for T. mossambica (solid plots) and from the results of Labat (1966) for the carp (open plots). The T. mossambica results for winter fish (section III (f): cross through the plots) and for summer fish (section III (g)) are both given. Curves have been drawn by eye in some cases to indicate the trends present.



of diastole with temperature change i.e. the Q_{10} of diastolic change [1], thus increases rapidly to a high value over the ± 26° to 22°C temperature range (curve 16, Fig. 54, pp. 155 to 156) and Q_{10} values remain more or less consistantly high down to ± 14°C (Fig. 56) at Q_{10} = 3.45. Systole, on the other hand, shows a different pattern

Figure 56.

The rates of systole and diastole as plotted on a logarithmic scale vs. experimental temperature for the 6 fish of the experiment reported in Section III (g). Q_{10} values as calculated from the slope of this semi-log. plot, are given for a number of temperatures.



1.) Diastole represents the period of cardiac rest, thus the shorter this period, the more rapid the rate of recovery processes. Hence Q_{10} values can be calculated.

of change with temperature (Fig. 56) and Q_{10} values are lower than for diastole (mean Q_{10} value for 25.8° to 14.1°C = 2.05, whereas for diastole mean Q_{10} over same range = 3.27). The Q_{10} values for systole at different temperatures are also given in Fig. 54, and show a region of elevated values between ± 16° and 22°C, the progressively lower values at higher temperatures being similar to the pattern in all other rate functions studied (Fig. 38, p. 105).

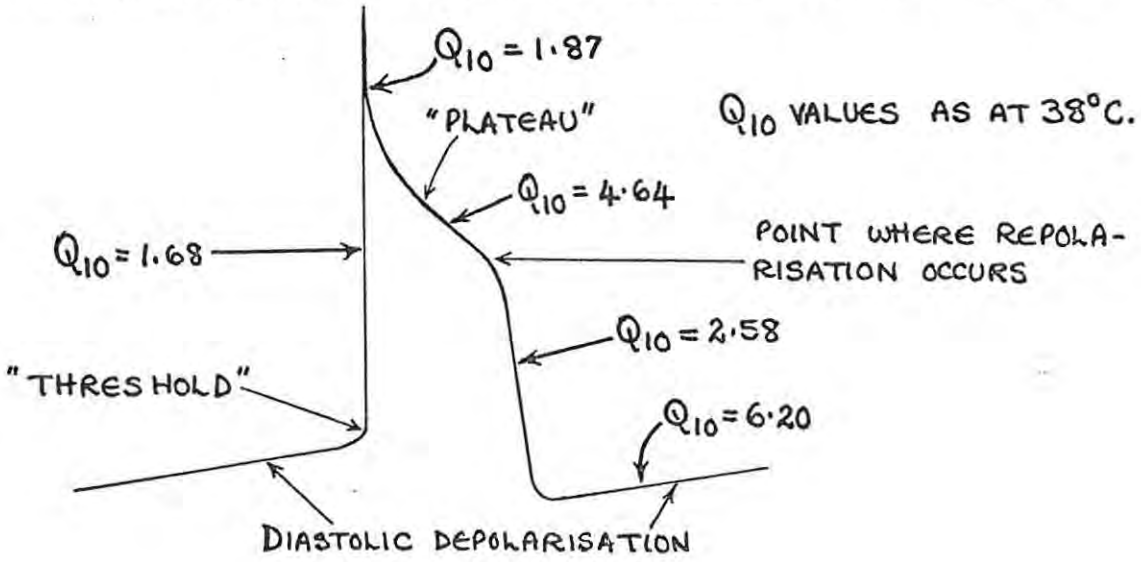
Thus it is clear that the high Q_{10} of heart rate and diastole at low temperatures on the one hand, and the lower Q_{10}s of the other two rate functions and systole (which they together comprise) on the other, represent two rather different processes of cardiac activity in T. mossambica which may be of tremendous functional significance. For example as the temperature decreases, a larger and larger % of the cardiac cycle is occupied by resting phases of the heart[1.], smaller %s by active phases (Fig. 49, p. 124) and thus the ability of the bulbus arteriosus to feed back pressure energy to the blood by means of its elastic wall (p. 25) also cannot continue throughout the lengthening resting phase; thus blood pressures fall off to zero for greater and greater proportions of the cardiac cycle time. The possibility that this may result in reduced kidney filtration cannot be excluded, and thus the reduced urine volume at lower temperatures (Minshull, 1967) in T. mossambica might be partially explained. Also, with lowered heart rate and thus a possible decrease in cardiac efficiency such as a reduction in cardiac output, venous return may be impaired (causing the above reduction in stroke volume) which may be secondary to tissue space flooding and oedema (Berne, 1954, reporting

---

1.) Electrical diastole is taken to be roughly equivalent to mechanical diastole.

Prec and co-workers). Oedema and resulting tissue damage is reported by Allanson (1966) for T. mossambica at temperatures below about 14°C. Alternatively the heart rate may be insensitive to increasing venous pressures, i.e. where "intrinsic rate regulation" as described by Jensen (1970) for the hagfish and for some teleosts, does not occur. It must, however, be stressed that the basic operation of the heart as an effective pump does not seem impeded at temperatures near 14.1°C. There is sufficient time for atrial and ventricular contraction and emptying, and the myocardial contractions result in normal ECG traces which suggest that the potentials produced by the myocardium of the ventricle (related to QRS complex height) are probably adequate for competent muscle activation as the decrease in QRS height with temperature decrease is extremely slight in extent (Fig. 36, p. 101). The total spike height recorded from Purkinje fibres of sheep and calf (Coraboeuf & Weidmann, 1954) when redrawn from Fig. 2, p. 34 of these authors, suggests a similar pattern of QRS voltage decrease below about 25°C (a slight decrease as temperatures are raised above 30°C is also suggested by the data of these authors).

The variations in the Q_{10} values for the different phases of the cardiac cycle can be compared with Q_{10} values for various phases of the action potential as recorded from fibres of sheep and calf heart (Fig. 4, Coraboeuf & Weidmann (1954)), as illustrated below:



Thus very high Q_{10} values are found for the slow phases of the action potential i.e. a.) the plateau, corresponding to the period for which the cardiac fibres remain depolarised i.e. for which the atrial and, of greater relevance, the ventricular muscle remains contracted, (i.e. the Q-T (or R-T) interval approximately including the T-hump); and b.) the slow diastolic depolarisation with an even higher Q_{10} of 6.20 (corresponding to the period of diastole). Thus although our results have not indicated any markedly raised Q_{10} for the rate of ventricular functions (? because of the method of R-T measurement, see pp. 31 and 33), these are stressed by Labat (1966); our results do indicate the very temperature-sensitive diastolic period.

The rather sudden elevation of the voltages of the QRS complex $\pm$ 13 h after the temperature decrease to 17.8°C (see section VI (c) for complete results) cannot be explained very readily. Similarly the fact that there is a tendency for heart rates to decrease with a decrease in fish size only at 14.1°C (Fig. 30, pp. 87 - 88) does not correlate with what is known about the response of T. mossambica to cold from a ventilatory rate point of view (Fig. 31, pp. 89 to 90). Similarly early siting experiments suggest that average cold-coma temperatures for 9 smaller fish (10 to 20 gm) were hardly distinguishable from those of 18 larger animals (38 to 50 gm; 10.2 $\pm$ 3.7°C as compared to 9.9 $\pm$ 2.4°C respectively).

The heart functions at 17.8°C suggest that both heart rate (involving mainly a shortening of the diastolic period) and, at a slower rate and to a smaller extent, ventricular functions, tend to acclimate by accelerating (Figs. 33 and 34, pp. 95 and 97). It is interesting that these acclimations involve sections of the heart cycle with high Q_{10} values (see above), and, in both cases, sections where there is a slow change in membrane permeability leading to

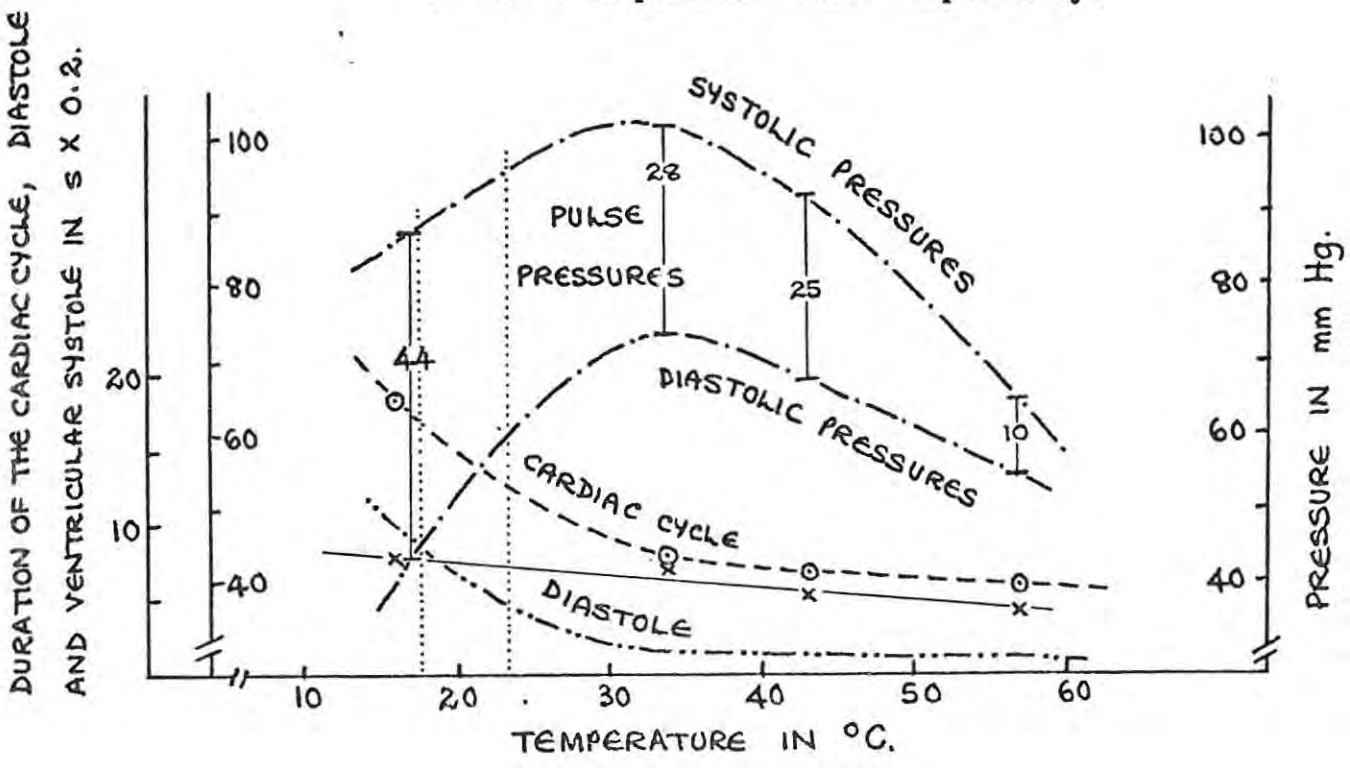
"threshold" or to a point where membrane repolarisation occurs (see diagram p. 165). These features suggest that at 17.8°C the heart becomes more efficient with time (see pp. 123 to 125). In particular the proportion of the cardiac cycle time occupied by systole increases, thus the proportion of the cardiac cycle during which adequate arterial blood pressures are maintained, is increased. Thus although similar proportions of the cardiac cycle are occupied by systole when fish are first cooled to 17.8°C or to 14.1°C, at 17.8°C the acclimation of the R-R interval causes a marked change in this situation which does not occur at 14.1°C. However the possibility that the heart retained some heart rate acclimation from the earlier stay at 17.8°C, cannot be excluded. The inability of the rate of the 14.1°C fish heart to acclimate within the 4-day experimental period may thus be because acclimation had already occurred and had not been lost. Alternatively, of course, no acclimation of heart rate (or of the rate of ventricular functions) may ever occur at temperatures below ± 14.5°C. More extensive experiments would be required to clarify the situation.

The possible mechanism of heart rate increase after some time at 17.8°C may, on the one hand, be because of a reduction in vagal tone, as is found to be the operative factor in the increased heart rate of winter carp (Labat, 1966, his Fig. 56). Heart rate acclimation is reported for other fish (Randall & Smith, 1967; Hart, 1957 and Tsukuda, 1961), but the possible reasons are not given. Although a sympathetic innervation of the teleost heart is not usually advocated, reports of adrenalin - containing cells within the teleost heart and of acceleratory or other positive effects of catecholamines, are presented in the literature (Gannon & Burnstock, 1969; Randall, 1968 and Jofre & Izquierdo, 1967). Randall & Stevens (1967) also report that the presence of catecholamines in the blood of fish are effective as heart accelerators when vagal cholinergic action is abolished. Shelton &

Randall (1962), commenting on early results of theirs using Tinca, suggest that "there may be some cardio-accelerator fibres in the vagus if not elsewhere" (p. 249). These possibilities are of interest as Berne (1954), using hypothermic dogs used artificial stimulation to increase heart rate (his Fig. 2, p. 92). The collective data in this Figure is redrawn in graphical form (Fig. 57), and it is interesting that small increases in heart rate (e.g. from 17.6 to 23.2 beats/min as found in our fish at 17.8°C) result in a marked improvement in diastolic pressures in the hypothermic dog, and in a reduction

Figure 57.

The relationship between aortic pressure (diastolic and systolic), pulse pressures and the rate of heart beat in hypothermic dogs when artificial stimulation was used to elevate heart rate, as taken from Berne (1954). The duration of the cardiac cycle, diastole, and of ventricular systole are also given. Curves have been fitted by eye to indicate the trends present. The 2 dotted vertical lines indicate 17.6 and 23.2 beats/min, the average heart rates found in our fish at 17.8°C in periods D and E respectively.



in pulse pressures. Systolic pressures are also elevated. Thus if similar alterations in blood pressure accompany the heart rate increase in our 17.8°C fish, they would be of great functional significance. Metabolic increases as a possible cause of the above acclimations are discussed in (f) below.

(f) Cardio-respiratory correlations.

Although both ventilatory rate and heart rate are intimately associated with metabolic rate in fish and animals in general (see Part I), and although the general responses of these two basic functions (ventilation and cardiac function) to temperature in this study have been shown to be basically comparable as is to be expected (Figs. 42 to 45, pp. 113 to 116, and Fig 37, p. 102), it is of interest that particular differences emerge, as have done within the cardiac functions themselves (see (e) above). In particular no indication of any ventilatory acclimation to low temperatures were indicated, although overshoots of either rate or ventilatory movement amplitude were noticed after temperature increases (pp. 142 to 143). However it is possible that there were changes in respiratory volume etc. at 17.8°C or 14.1°C which would not be detected by the methods employed.

The cardiac and respiratory activities may also be correlated in a temporal sense both in teleosts e.g. the tench (Shelton & Randall, 1962) and, more particularly, in elasmobranchs. This type of cardio-respiratory synchrony may be of functional significance if maximal blood and water flows co-incide in and out of the gill lamellae respectively, and if such flows actually are effective in improving oxygen uptake by the blood. Some measurements (Fig. 47, p. 120) and general impressions suggest that, under the conditions used, no sign of cardio-respiratory synchrony is found in T. mossambica, even at 14.1°C, possibly related to the fact that blood flow through the gills is likely

to be fairly even due to the elastic rebound properties of the bulbus. Fig. 46 (p. 118) indicates that, if the extrapolations suggested for heart and ventilatory rate are valid, cardio-respiratory synchrony might occur at higher ($\pm$ 36°C) and at lower ($\pm$ 9°C) temperatures, temperatures that are indeed equal to or close to the lethal temperatures for T. mossambica. Such a synchrony might be of significance at these temperatures. The ventilatory and heart rate curves and their relationship to each other (Fig. 46, p. 118) are closely comparable to similar data given by Tsukuda (1961) for Oryzias and Lebistes over the normal temperature range and encroaching slightly into the sub-normal range for these fish. Tsukuda (1961) gives values of between 0.5 (at 10°C) and 1.0 (at 30°C) for the relationship $\frac{\text{heart rate}}{\text{ventilatory rate}}$ which are similar to those for T. mossambica as given in Fig. 46 (p. 118).

The possible relationship of both cardiac and ventilatory activity to metabolism cannot be readily assessed, and although oxygen consumption data of Job (1969a, 1969b) could be compared in a limited sense to ventilatory data, no information is available other than the heart rata data as to whether a metabolic adaptation to temperatures of $\pm$ 18°C occurs or not. If such metabolism increases occurred, these may affect the activity of the cardiac pace-maker and of other myocardial elements and thereby may, in fact, be the cause of the cardiac acclimations observed.

V Conclusions.

1. Fresh water Tilapia mossambica, obtained from North End Lake in Port Elizabeth, were utilised for a series of experiments on aspects of respiratory and cardiac function in relation to temperature. The basic structure of the heart of this fish was also investigated as background to the functional investigations.

2. The form of the electrocardiograph (ECG) recorded from T. mossambica oscillographically was discussed both in relation to the position of the implanted recording electrodes, and to what is known in general about the teleost ECG. It was concluded that a "typical" teleost ECG can only be suggested if electrode positions are standardised.

3. The oscillographic tracings of ventilatory movements as recorded have been discussed, and variations in the ventilatory functions with size of fish and with temperature are given.

4. Possible effects of initial handling, later handling, and MS 222 anaesthesia have been investigated. Only initial handling causes long term alterations in ventilatory functions which may last for a day.

5. Small temperature increases and decreases in the 18° to 36°C range cause a reversible alteration in ventilatory rate without overshoots or any sign of acclimation. This rate function has a Q_{10} of about 1.5 which increases with a temperature decrease.

6. More extensive temperature increases of from ± 22.0 to 25.8°C or of 17.8° to 21.1°C cause a temporary "overshoot" in ventilatory rate or amplitude (as recorded oscillographically).

7. Rapid temperature decreases (3° to 4°C /h) from 25.8° to 17.8°C and from 21.1° to 14.1°C resulted in no observable ventilatory rate or

amplitude change within 4 days, but both heart rate, and, to a smaller extent and more slowly, the rate of the ventricular functions, became more rapid during the early part of the 4 days after the temperature decrease to 17.8°C; a similar change was not seen after the decrease to 14.1°C. The possible functional significance of these acclimatory changes at 17.8°C are discussed and related to other known aspects of the response of T. mossambica to cold. No information is available such that these cardiac acclimations can be related to any metabolic acclimation at this temperature, although such a relationship may exist.

8. Cardiac functions such as the heart rate, rate of atrio-ventricular conduction, rate of ventricular functions, rate of ventricular depolarisation and height of the QRS complex have been studied from the various deflections of the ECG. The Q_{10} values for all cardiac rate functions have been determined for the 14° to 26°C range, and heart rate has been found to have a noticeably higher average Q_{10} value than the other rate functions. This feature is related to the high Q_{10} value of the rate of diastolic repolarisation ($Q_{10} = 3.27$) as compared to an average Q_{10} of the various rate functions occurring during systole which have an average Q_{10} of 2.05. The Q_{10} values of cardiac functions all tend to increase with decreasing temperature over the temperature range studied.

VI Appendices.

(a) Suitability of recording equipment for electrocardiographs: trials using Xenopus heart.

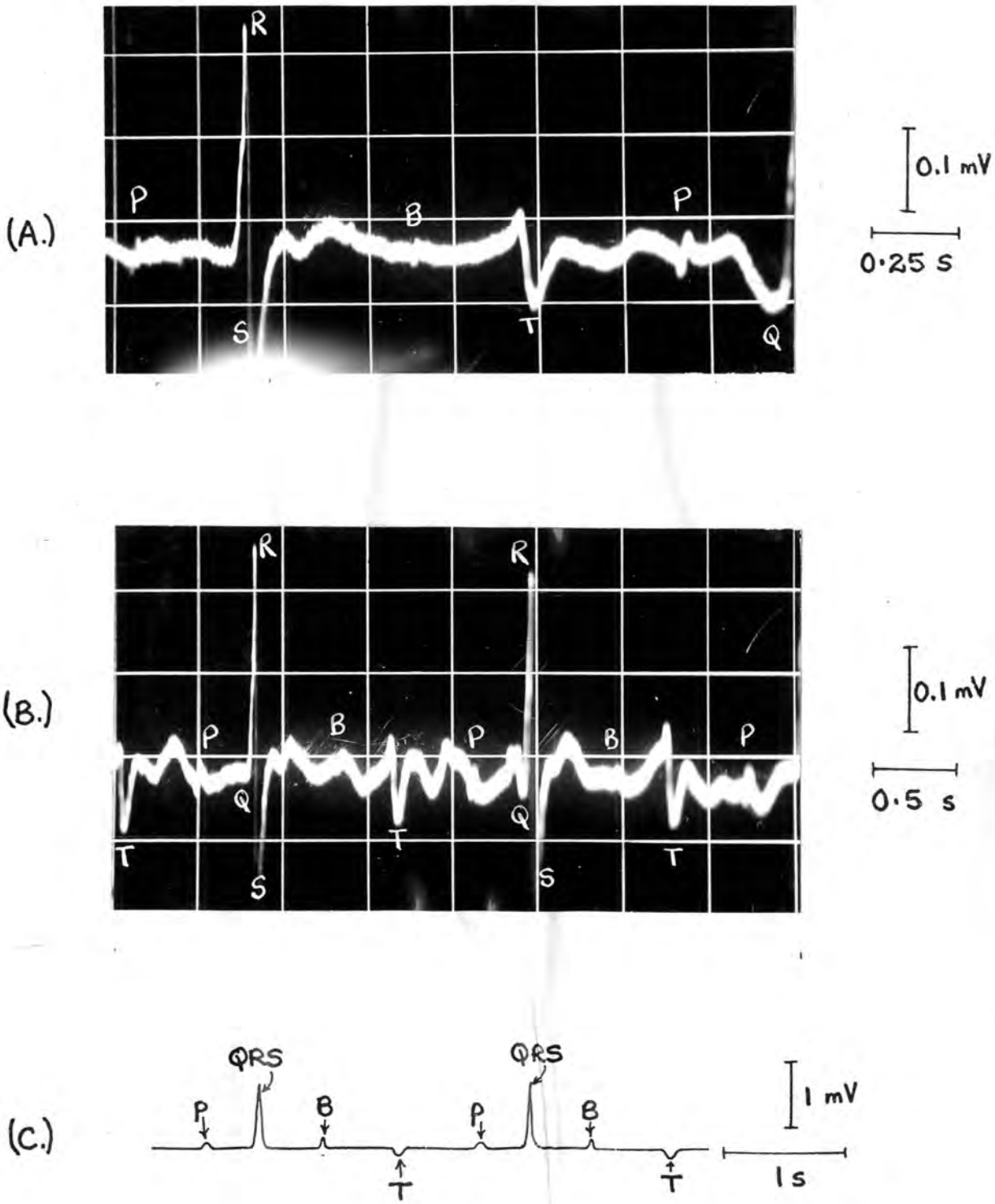
South African clawed toads Xenopus laevis of weight similar to the experimental fish used (35 to 50 gm) were decerebrated and pithed, and the heart was exposed from the ventral surface. Electrodes, as used to record ECGs from fish, were held manually such that the +ve electrode was in contact with the ventricle, thus the electrode placement corresponded roughly to the V2 position used by Furman (1960). ECGs were recorded at room temperature (= 20°C), and 2 examples are given in Fig. 57(a). These ECGs compare favourably with ECGs recorded from the same species by Furman (1960) at 15° to 25°C as can be seen from Fig. 57(a).

These results suggest that the experimental technique utilised provides a fully detailed ECG of the platanna, and is therefore suitable for use with animals of similar size such as our experimental T. mossambica.

Figure 57(a).

ECGs as recorded from pithed Xenopus laevis (A) and (B) as compared to ECGs recorded from the same animal by Furman (1960) (C) using implanted electrodes.

B - depolarisation of the bulbus arteriosus.



(b) Respiratory opercular-buccal movements and their recording as an electrical phenomenon.

During very early stages of the preparatory work for the recording and interpreting of ECGs from T. mossambica, difficulty was encountered in correctly interpreting the various deflections recorded oscillographically notwithstanding the kind advice of Dr W. H. Craib. It was thus considered imperative to actually check which of the deflections were caused by the contractions of the various muscles associated with the ventilatory movements. This was simply done by visually comparing observed opercular movements of a fish in a temporal sense with deflections on the oscilloscope screen in an electrode-implanted fish.

While one observer recorded the opercular or buccal movements (whichever was easier) on moving paper film, the other observer similarly recorded when the large oscillographic oscillation cycles occurred. Both observers recorded on the same strip of moving paper film, one immediately above or below the other (see Fig. 58). The film was analysed, and the relationship between buccal movement cycle rate on the one hand and the oscillographic oscillation rate on the other, was determined as a ratio between the rates of the two cycles (Table 12, p. 177). Table 12 clearly shows the very close relationship in a temporal sense between these two cycles, thus it was concluded that the deflections described in section III(c) above were indeed associated with the ventilatory muscle de- and repolarisations and were thus not associated with the heart contractions. Subsequent more careful recordings demonstrated ECGs, often together with the ventilation records.

Although the above results made it possible for us to use later oscillographic deflections of similar form as representative of the ventilatory cycle, etc., there were a number of occasions where visually observed ventilatory rates did not correlate very well with oscillographic

Figure 58 (a)

Diagram illustrating the simultaneous recording of visually observed opercular/buccal movement cycles and of oscillographic beam oscillations, where pencils are used by 2 independent observers to record the completion of each cycle, pencil marks being made along the same vertical line as paper film is moved across the back of a Cossor oscilloscope camera.

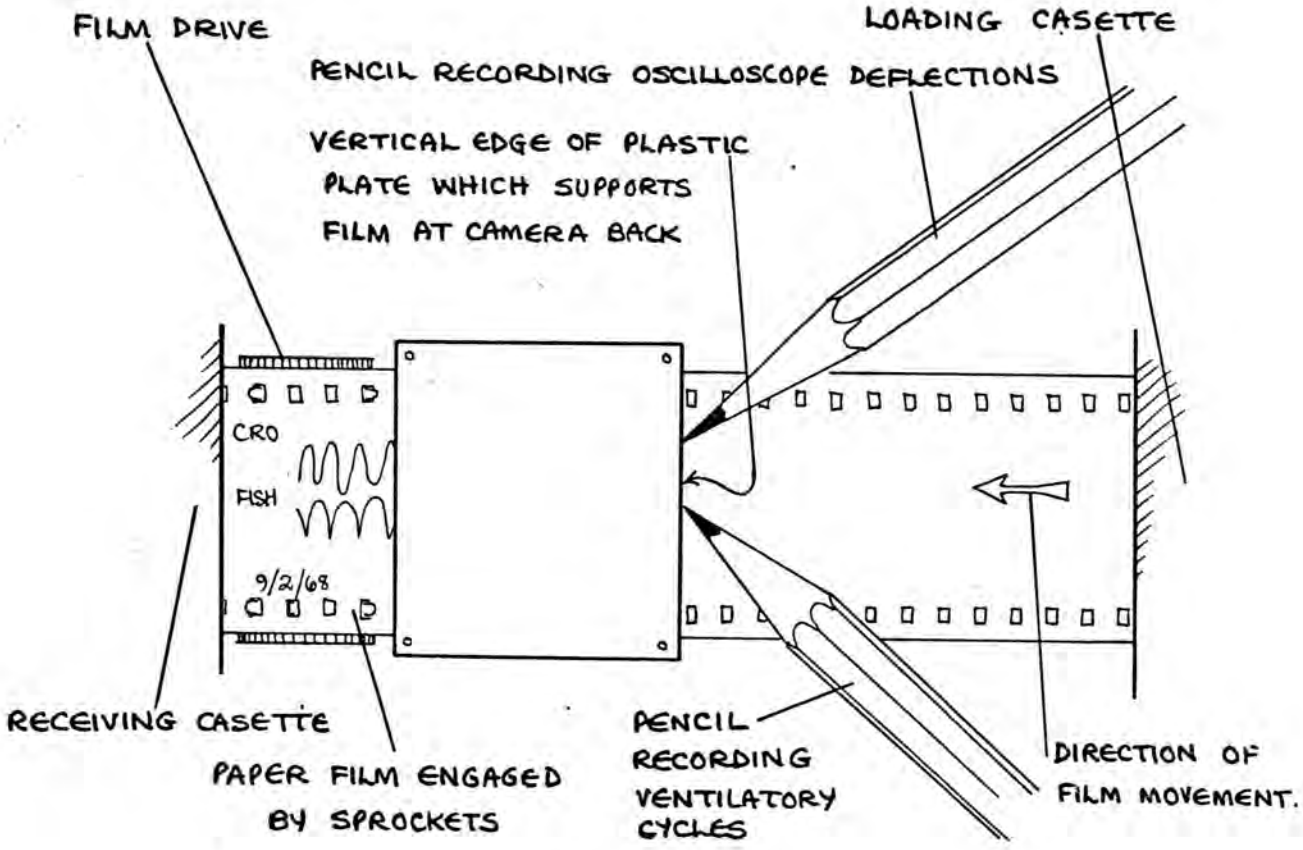


Figure 58 (b)

An example of a paper film recording as described in (a) above.

Table 12

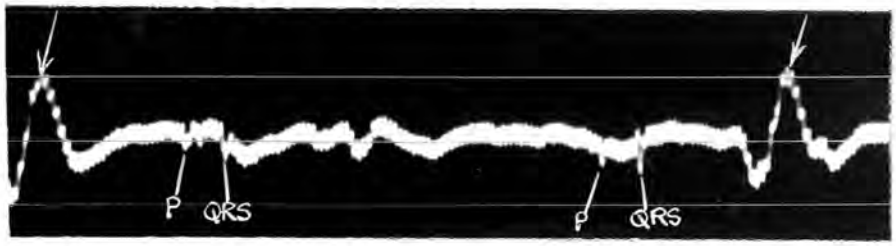
The numbers of complete cycles of ventilatory activity observed visually in arbitrary time periods (x) and the number of oscillographic cycles observed in the same time (y) together with the ratio $\frac{y}{x}$.

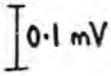
Number of complete ventilatory cycles = x	Number of complete oscillographic cycles = y	$\frac{y}{x}$
40	40	1.00
18	17	0.94
48	48	1.00
57	57	1.00
43	44	1.02

records of immediately before or after. Thus in later experiments both oscilloscope and visual observations were utilised and a mean of the two used in the calculation of results. No consistent relationship was observed between the two classes of observations, e.g. one always faster than the other of by a set amount. Thus variations in ventilatory rate within a short time (1 to 4 min) may be the reason for these variations at least in part. Also where rather larger and regular deflections (Fig. 18B, p. 47 and Fig. 59(a)) were recorded, it was not known whether these were of ventilatory origin or not, and the distance between them (from 2 to 6 seconds) seemed too great for such an interpretation. Cases where extensive bodily movement was occurring or where 50-cycle or other extraneous electrical disturbance was prevalent produced entirely different results when compared to these more regular deflections of unknown origin (Fig. 59(b)).

Figure 59 (a)

Oscillograph trace recorded from Fish T at 25.8°C showing large, regular deflections (arrows) of unknown nature that occurred every 2 to 6 seconds in this recording.





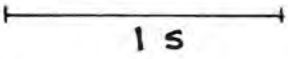
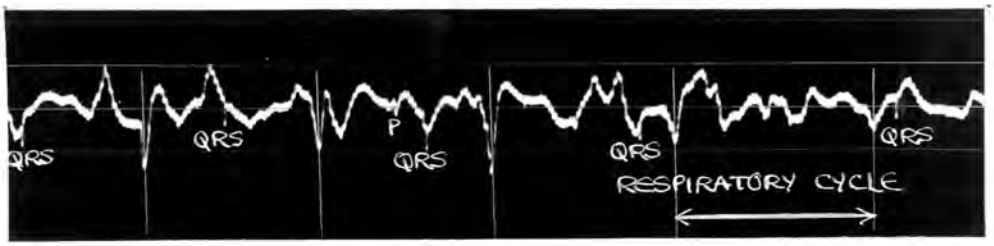
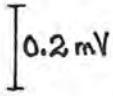
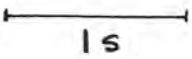


Figure 59 (b)

Oscillograph trace recorded from Fish O at 25.8°C when the fish was in an excited and disturbed state. Note the irregularities superimposed on the ventilatory cycle and oscillations. A few QRS complexes are clearly visible.







(c) Complete results of Part III.

The complete results of certain portions of section III, given either in graphical or tabular form, are presented below in the following order:-

P. T. O. for pages 180 and 181

(Figure 60)

Legend for Figure 60

(opposite)

Full results for the graphs labelled Tilapia 2 and Tilapia 3 in Figure 22 for the opercular/buccal respiratory rate changes with temperature over the range 14.1 to 36.0°C. (The Tilapia 3 values at 25.8°C are those only for period L - i.e. after the fish had recovered from any initial handling stress.) Each small point represents the mean value at that temperature for any one fish. Each large point represents the mean for all fish of the experimental group at that temperature and the 95% confidence limits are also given for these points. Graphs have been fitted by eye.

KEY

Fish of the 1967 - 1968 experiments (see Section III (e))

Fish of the January 1969 experiments (see Section III (g))

Figure 60

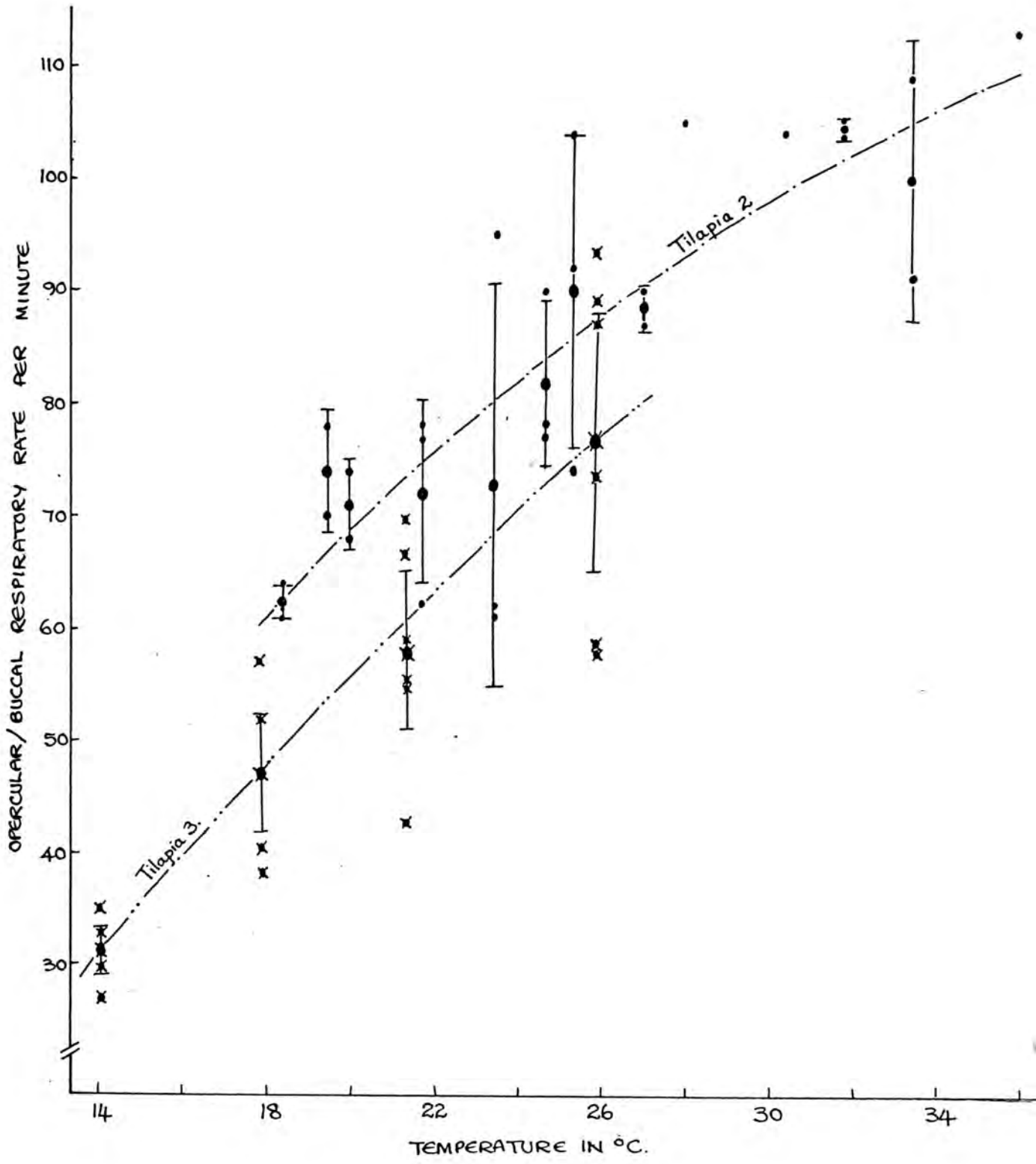

OPERCULAR/BUCCAL RESPIRATORY RATE PER MINUTE
110
100
90
80
70
60
50
40
30
14
18
22
26
30
34
TEMPERATURE IN °C.
Tilapia 2.
Tilapia 3.

Figure 61

Full results (six fish) of the changes in opercular/ buccal respiratory cycle length during the experiment resported in Section III(g) as the temperature was changed as indicated below. Mean values for individual fish (smaller dots) and for all six fish (large encircled dots) are given, the latter with 95% confidence limits. A graph of the overall trends has been fitted by eye.

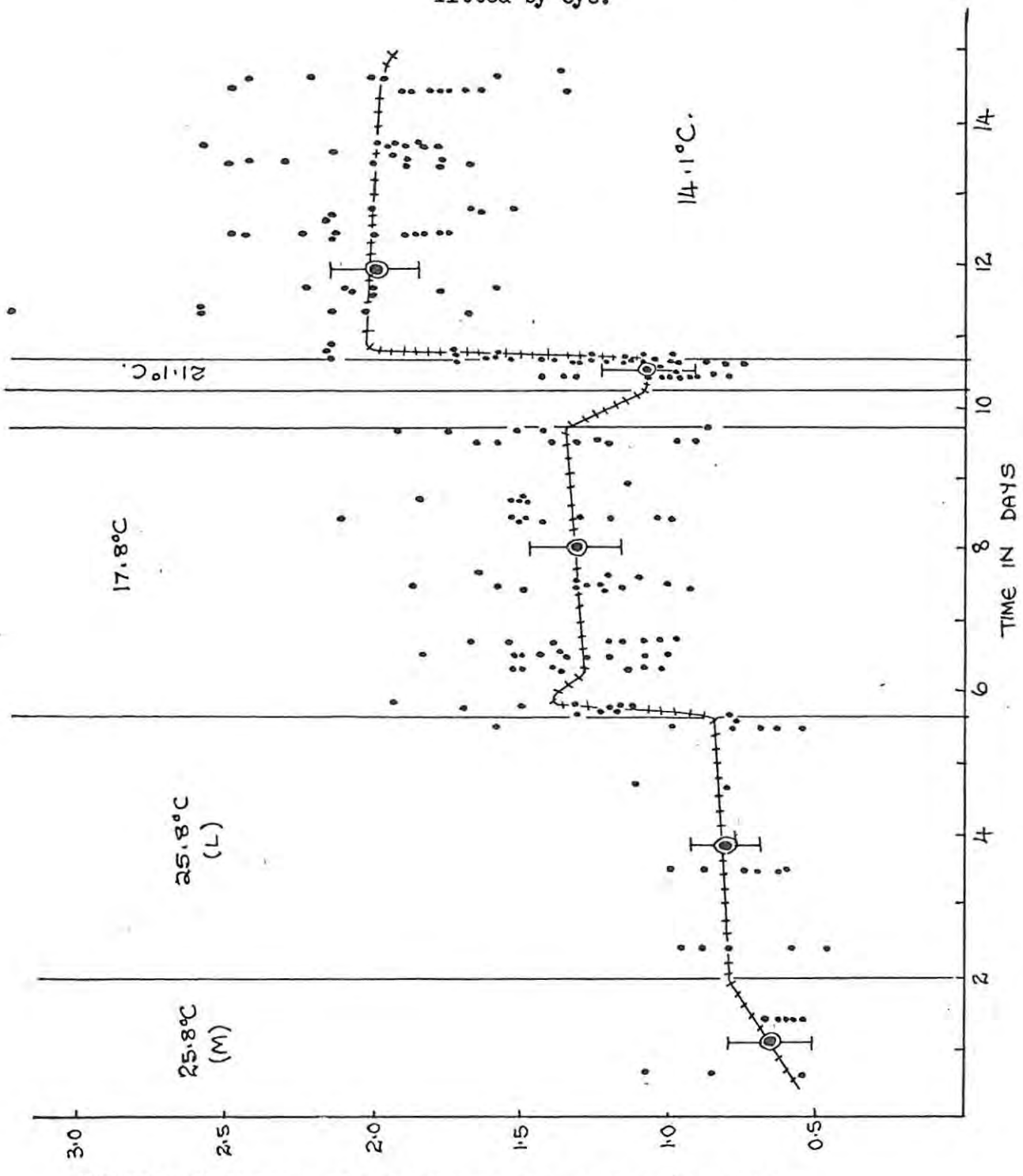


Figure 62

Full results (six fish) of the changes in opercular/buccal respiratory rate during the experiment reported in Section III(g) as temperature was changed as indicated below. Mean values for individual fish (small dots) and mean values for all six fish (large dots) are given, the latter with 95% confidence limits. A graph of the overall trends has been fitted by eye.

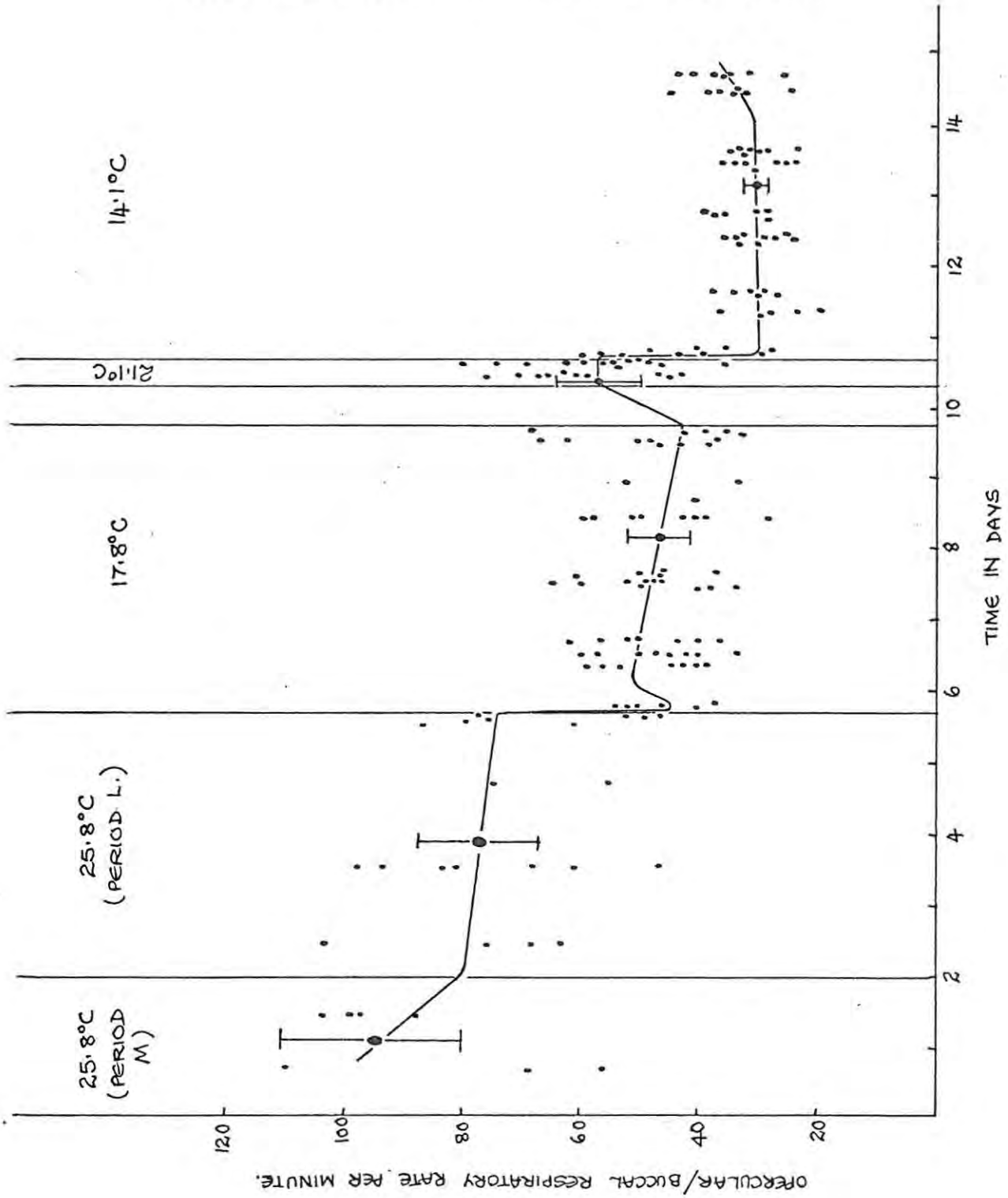


Figure 63

Full results (six fish) of the changes in the R-R interval during the experiment reported in Section III (g) as the temperature was changed as indicated below. Mean values for individual fish (small circled dots) and mean values for all six fish (large circled dots) are given, the latter with 95% confidence limits. A graph of the overall trends has been fitted by eye.

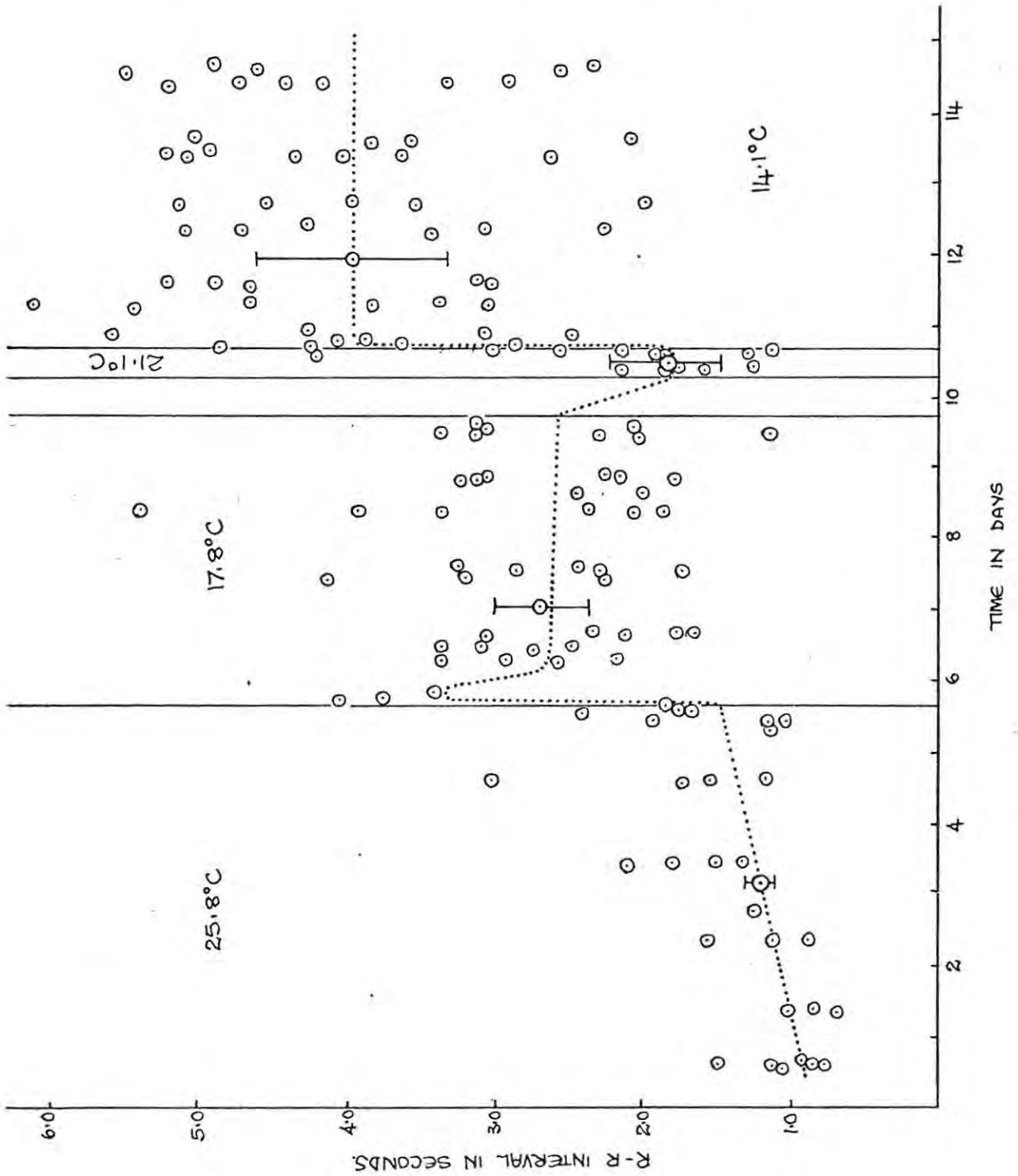


Figure 64

Full results (six fish) of the changes in the P-R interval during the experiment reported in Section III (g) as the temperature was changed as indicated below. Mean values for individual fish (small crosses) and mean values for all six fish (large crosses) are given, the latter with 95% confidence limits. A graph of the overall trends has been fitted by eye.

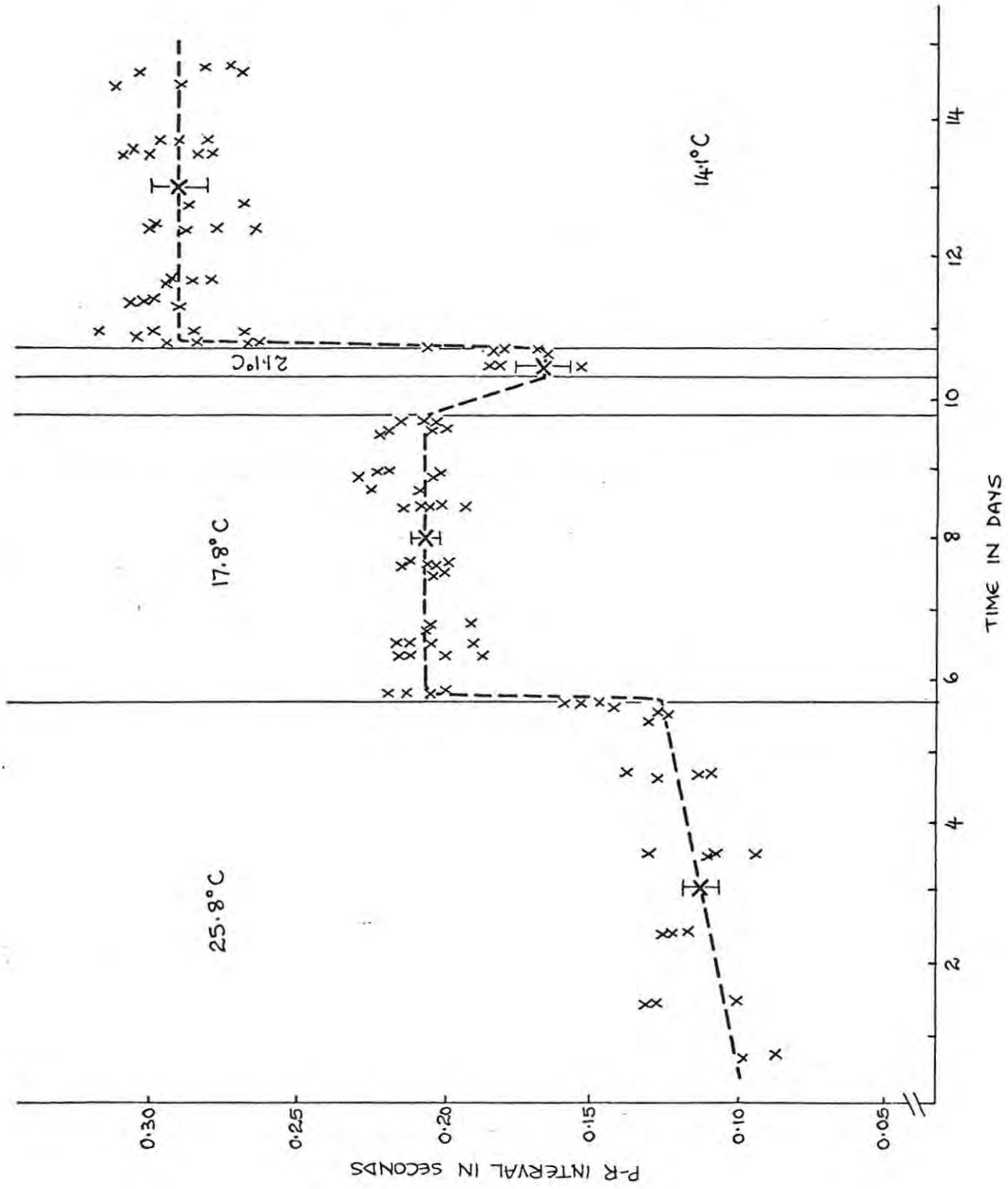


Figure 65

Full results (six fish) of the changes in the R-T interval during the experiment reported in Section III(g) as temperature was changed as indicated below. Mean values for individual fish (small circles) and mean values for all six fish (larger circles) are given, the latter with 95% confidence limits. A graph of the overall trends has been fitted by eye.

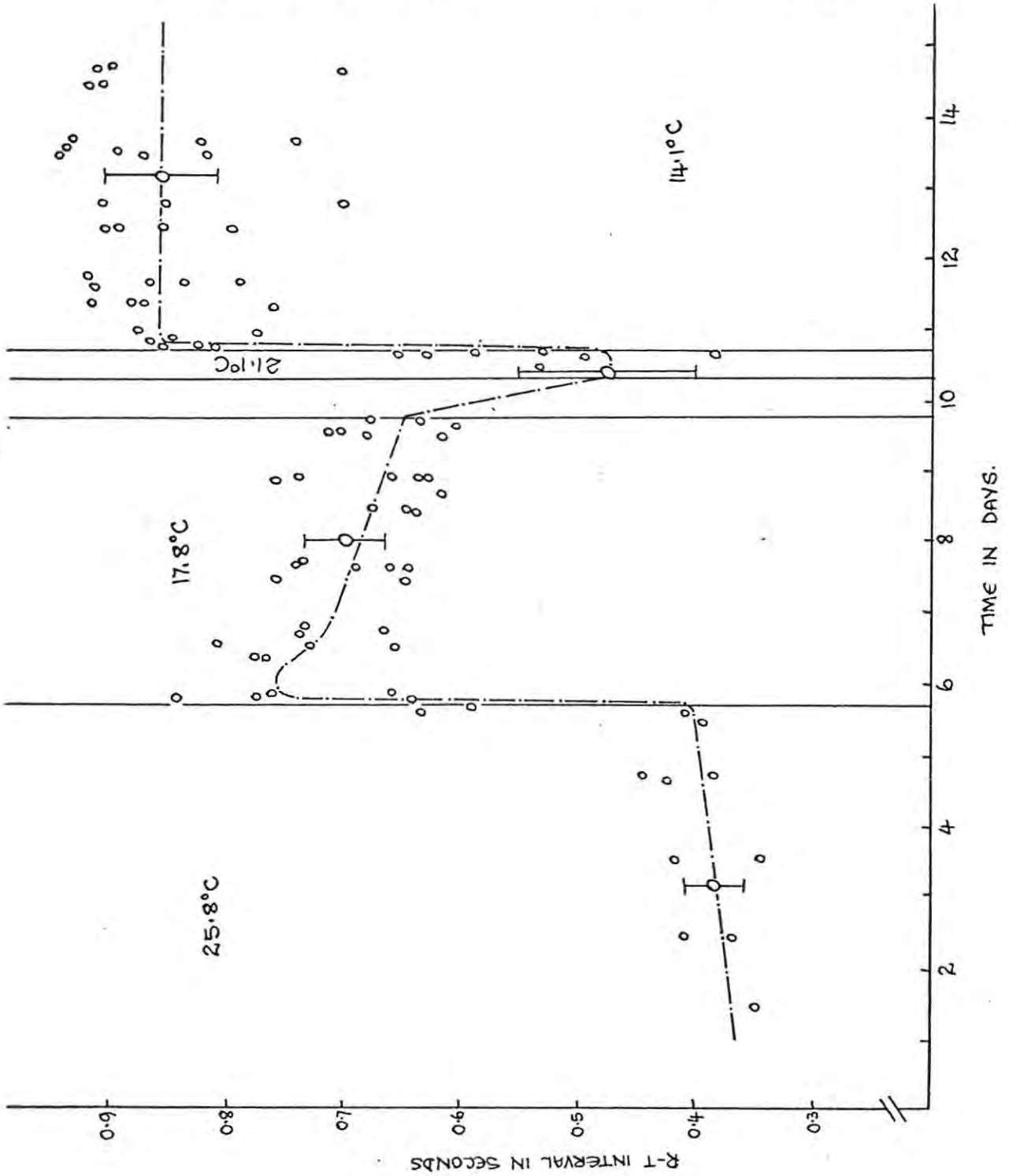


Figure 66.

Plots of the R-R duration in the 6 individual fish of the experiment reported in section III (g) as recorded between midnight and 10.00 a.m. (on the left) and between 10.05 a.m. and 10.00 p.m. (on the right), for 14.1°, 17.8° and 25.8°C. The position of the plots on the X axis is arbitrary. Means ($\bar{x}$) of each series are given as a horizontal line, and the value of the mean is given above in each case together with the 1.96 x S.E. values.

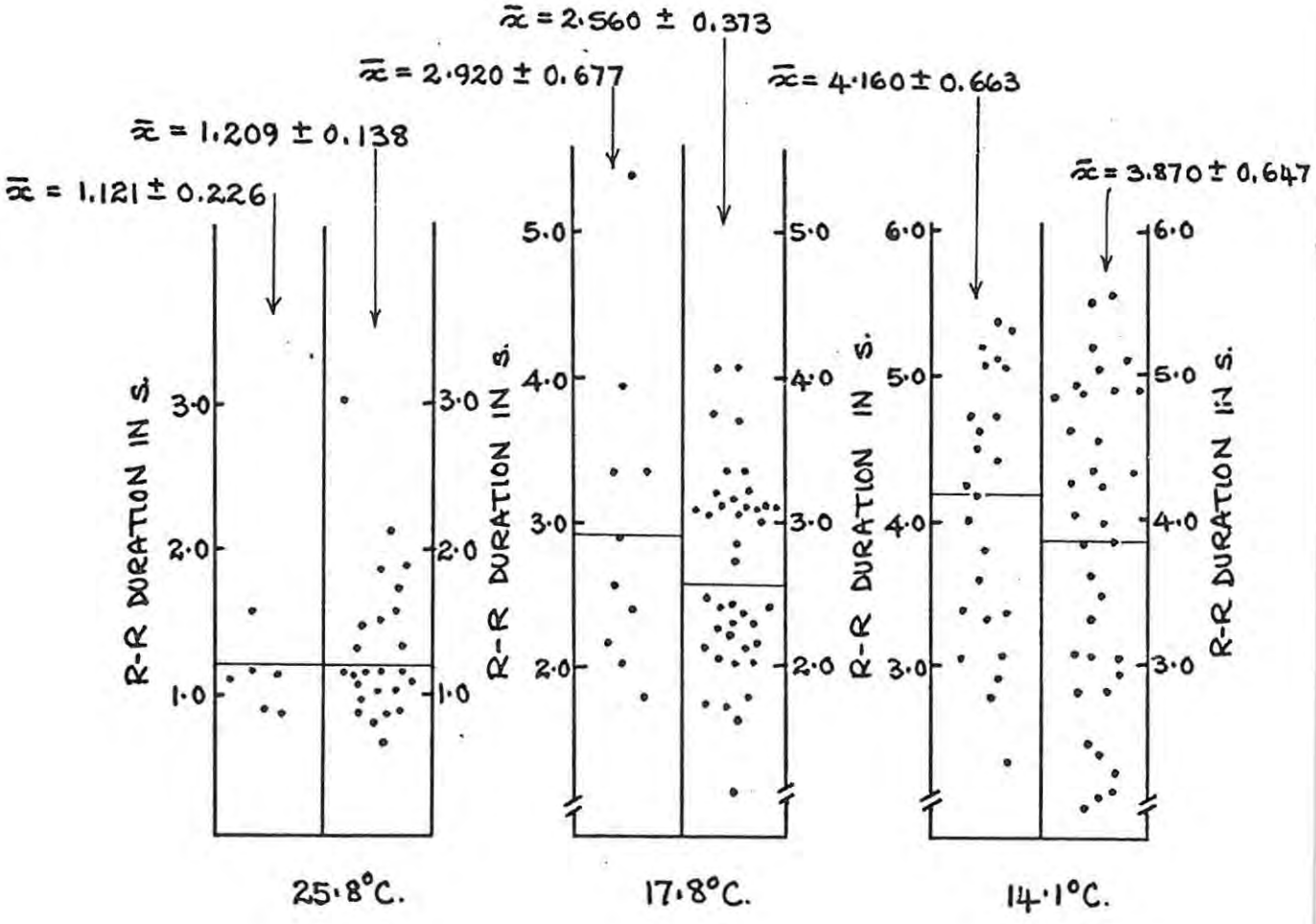


Table 13

Full Q_{10} results for ventilatory rates of the individual fish over the various temperature intervals.

Temperature interval	Fish T 49.8 gm	Fish R 56.2 gm	Fish A 68.5 gm	Fish I 71.2 gm	Fish O 77.6 gm	Fish V 88.0 gm
25.8°(M) - 14.1°C	2.98	2.63	2.60	2.82	2.52	2.06
25.8°(L) - 14.1°C	2.23	2.06	1.63	--	2.05	1.82
25.8°(L) - 21.1°C	2.10	1.98	--	--	1.94	1.69
21.1° - 17.8°C	1.30	1.78	2.96	1.50	1.42	1.11
17.8° - 14.1°C	3.87	3.72	2.71.	3.55	1.67	3.06
25.8°(L) - 17.8°C	1.83	1.56	1.28	--	2.25	1.43
21.1° - 14.1°C	2.36	2.64	2.76	2.36	2.12	1.90

Table 14.

Mean QRS voltages for individual fish at the main experimental temperatures together with the means and 1.96 x S.E. of 4 and 6 fish at each temperature. The number of recording means that have been averaged is given in brackets.

Fish name	14.1°C	17.8°C	21.1°C	25.8°C
I	0.039 (8)	0.039 (7)	-	0.047 (3)
A	0.036 (11)	0.018 (2)	-	0.050 (1)
R	0.066 (10)	0.047 (6)	0.068 (2)	0.023 (3)
T	0.049 (10)	0.054 (11)	0.080 (3)	0.071 (8)
V	0.094 (10)	0.125 (13)	0.100 (2)	0.109 (11)
O	0.035 (10)	0.071 (11)	0.042 (3)	0.076 (7)
Mean of 6	0.061 ± 0.022	0.074 ± 0.030	-	0.070 ± 0.030
Mean of 4	0.052 ± 0.018	0.059 ± 0.027	0.073 ± 0.021	0.063 ± 0.022

Table 15.

Variations in the QRS voltages in 3 of the experimental fish with the passage of time at 17.8°C.

Fish	QRS height in mV after indicated number of hours.				
	@ ± 1 h	± 13 h	@ ± 18 h	@ >30 h	Mean of 3 items marked @
T	@ 0.051	0.082	@ 0.054	@ 0.055	0.053
O	@ 0.085	0.126	@ 0.085	@ 0.081	0.084
V	@ 0.120	0.164	@ 0.123	@ 0.122	0.122
Total	0.256	0.372	0.262	0.258	0.259
Mean	0.085	0.124	0.087	0.086	0.086

Table 16

Full Q_{10} results (means of all 6 fish) for ventilatory rates and cardiac functions over various temperature intervals. Where values from period D or E at 17.8°C, G or H at 17.8°C, and M or L at 25.8°C are used instead of values from the whole period at these temperatures, this is indicated by means of the period letter in brackets.

Temperature interval	Ventilatory rate	Heart rate	Atrio-ventricular conduction rate	Rate of ventricular functions
21.1° to 25.8°C	(M) 2.96 (L) 1.96	2.46	2.22	1.59
17.8° to 21.1°C	1.86	(D) 7.48 (E) 2.91	1.98	(G) 3.60 (H) 2.91
17.8° to 25.8°C	(M) 2.46 (L) 1.89	(D) 3.65 (E) 2.64	2.12	(G) 2.23 (H) 2.05
14.1° to 25.8°C	(M) 2.65 (L) 2.31	2.79	2.24	2.00
14.1° to 17.8°C	3.12	(D) 1.55 (E) 3.12	2.51	(G) 1.60 (H) 1.92
14.1° to 21.1°C	2.45	3.04	2.24	2.34

Table 17.

Full Arrhenius μ results (means of all 6 fish) for ventilatory rates and cardiac functions over various temperature intervals. Where values from period D or E at 17.8°C, G or H at 17.8°C, and M or L at 25.8°C are used instead of values from the whole period at these temperatures, this is indicated by means of the period letter in brackets.

Temperature interval	Ventilatory rate	Heart rate	Atrio-ventricular conduction rate	Rate of ventricular functions
21.1° to 25.8°C	(M) 18,850	15,900	13,900	10,500
	(L) 11,450			
17.8° to 21.1°C	10,450	(D) 31,350	11,400	(G) 21,550
		(E) 18,000		(H) 18,050
17.8° to 25.8°C	(M) 15,600	(D) 22,450	13,000	(G) 13,900
	(L) 11,550	(E) 16,800		(H) 12,450
14.1° to 25.8°C	(M) 16,750	17,600	13,800	11,950
	(L) 13,650			
14.1° to 17.8°C	19,100	(D) 7,430	15,400	(G) 7,880
		(E) 19,350		(H) 11,000
14.1° to 21.1°C	15,050	18,700	13,500	14,300

VII Acknowledgements.

A sincere thanks go to Prof. B.R. Allanson for his great patience, encouragement, and helpful criticism. Also grateful thanks to my colleagues for time spent in useful discussions. Thanks also to technical staff for their help with apparatus, etc., and to Mr. R.H.M. Cross for helping with photographic printing. Sincere thanks go to my two patient typing aides, Mrs. Lorna Wallace and Mrs. Lorraine Richardson. Last, but not least, thanks to my "three boys at home" and to my father for their patience, understanding and continued encouragement.

Grateful acknowledgement of financial aid for the purchase of equipment go to both the Council of Rhodes University, and to the South African Council for Scientific and Industrial Research.

VIII Bibliography.

Alexander, R. McN. (1967) Functional design in fishes. Hutchinson University Library, London.

Allanson, B.R. (1966) A note on histological changes in Tilapia mossambica exposed to low temperatures. Limnol. Soc. Sth. Afr. Newsl. No. 7 : 16 - 19

———, Bok, A and van Wyk, N.I. (1971) The influence of exposure to low temperature on T. mossambica Peters (Cichlidae). II Changes in plasma osmolarity, sodium and chloride ion concentrations. J. Fish Biol. 3 : 181 - 185

——— and Cross, R.H.M. (1970) Ultrastructural changes in renal proximal tubule cells of T. mossambica Peters following exposure to low temperatures. Septiéme congres international de microscope électronique, Grenoble, : 611 - 612

———, Ernst, M.H. and Noble, R.G. (1962) An experimental analysis of the factors responsible for periodic fish mortalities during winter in bushveld dams in the Transvaal, South Africa. Biological Problems in Water Pollution. Third Seminar : 293 - 298

——— and Noble, R.G. (1964) The tolerance of T. mossambica (Peters) to high temperatures. Trans. Am. Fish. Soc. 93 (4) : 323 - 332

Badenhuizen, T.R. (1967) Temperatures selected by Tilapia mossambica (Peters) in a Test Tank with a Horizontal temperature Gradient. Hydrobiologia 30 (3-4) : 541 - 554

Begg, G.W. (1969) The use of MS 222 (Tricaine methanesulphonate) in anaesthetising Hydrocynus vittatus and Tilapia melanopleura prior to packaging and transport by air over long distances. Limnol. Soc. Sth. Afr. Newsl., 13 : 22 - 26

Berger, M., Roy, O.Z. and Hart, J.S. (1970) The co-ordination between respiration and wing beats in birds. Z. vergl. Physiol., 66 : 190 - 200

Berne, R.M. (1954) Myocardial function in severe hypothermia. Circulation Res., 2 : 90 - 95

Bok, A.H. (1968) The effects of exposure to low temperature on the serum chlorides of T. mossambica (Peters).
Honours Project, Rhodes University. (unpublished).

Boyer, D.R. (1966) Comparative effects of hypoxia on respiratory and cardiac function in reptiles.
Physiol. Zool., 39 : 307 - 316

Bruzon, E. (1930) Le climat de l' Indochine (Hanoi)
as quoted in Long et al, (1961)

Bullock, T.H. (1955) Compensation for temperature in the metabolism and activity of poikilotherms.
Biol. Rev., 30 : 311 - 342

Coche, A.G. (1967) Fish culture in rice fields. A world wide synthesis.
Hydrobiologia 30 (1) : 1 - 44

Coraboeuf, E. and Weidmann, S. (1954) Temperature effects of the electrical activity of Purkinje fibres.
Helv. Physiol. Pharmac. Acta 12 : 32 - 41

Couteaux, R. and Laurent, P. (1957) Étude au microscope électronique du coeur de l'anguille: observations sur la structure du tissu musculaire de l'oreillette et son innervation.
C.R. Acad. Sci., Paris, 245 : 2097 - 2100

Covino, B.G. (1957) Some observations on ventricular fibrillation in acute hypothermia.
Cold Injury, 5th Conference : 135 - 158

Crozier, W.J. (1926) The distribution of temperature characteristics for biological processes: critical increments for heart rates.
J. gen. Physiol., 9 : 531 - 546

Denzer, H.W. (1968) Studies on the physiology of young Tilapia.
FAO Fish. Rep., 44 (4) : 357 - 366

Dharmamba, M., Handin, R.I., Nandi, T. and Bern, H.A. (1967) Effect of prolactin on fresh water survival and on plasma osmotic pressure of hypophysectomised Tilipia mossambica.
Gen. comp. Endocr., 9 : 295 - 302

Donnelly, B.G. (1969) Observations on the temperature preferenda of three cichlid fishes, T. mossambica (Peters), T. sparrmannii (A. Smith) and Hemihaploc hromis philander (M. Weber).
Honours Project, Rhodes University. (Unpublished)

——————— (1969) A preliminary survey of Tilapia nurseries on Lake Kariba during 1967/1968.
Hydrobiologia 34 (2) : 195 - 206

Doudoroff, P. (1942) The resistance and acclimatization of marine fishes to temperature changes. I Experiments with Girella nigricans (Ayres).
Biol. Bull. mar. biol. Lab., Woods Hole, 83 : 219 - 242

Freeman, J.A. (1950) Oxygen consumption, brain metabolism and respiratory movements of goldfish during temperature acclimatization, with special reference to lowered temperatures.
Biol. Bull. mar. biol. Lab., Woods Hole, 99 : 416 - 422

Fry, F.E.J. (1957) "The Aquatic Respiration of Fish."
In: Brown, M.E. (Editor). The Physiology of Fishes I Ch. 1. : 1 - 63
Academic Press, N.Y.

Fukusho, K. (1968) The specific difference of temperature responses among cichlid fishes genus Tilapia.
Bull. Jap. Soc. scient. Fish., 34 : 103 - 111

Furman, K.I. (1960) The electro-cardiogram of the South African Clawed Toad (Xenopus laevis) with special reference to temperature effects.
S. Afr. J. Med. Sci., 25 : 109 - 118

Gannon, B.J. and Burnstock, G. (1969) Excitatory adrenergic innervation of the fish heart.
Comp. Biochem. Physiol., 29 (2) : 765 - 773

Gerald, J.W. and Cech, J.J. (1970) Respiratory responses of juvenile catfish (Ictalurus punctatus) to hypoxic conditions.
Physiol. Zool., 43 (1) : 47 - 54

Goodrich, E.S. (1958) The heart - Pisces; In: Studies on the structure and development of vertebrates : 540 - 542
Dover Publications, N.Y.

Grassé, P.P. (1954) Coeur des Poissons - Morphologie. Traité de Zoologie, XII : 888 - 894

Guyton, A.C. (1966) Textbook of Medical Physiology. W.B. Saunders & Co.

Hanson, R.C. and Stanley, J.G. (1970) The effects of hypophysectomy and temperature acclimation upon the metabolism of the central mudminnow, Umbra limi (Kirtland) Comp. Biochem. Physiol. 33 : 871 - 879

Hart, J.S. (1957) Seasonal changes in carbon dioxide sensitivity and blood circulation in certain fresh water fishes. Can. J. Zool., 35 (2) : 195 - 200

Hasan, R. and Qasim, S.Z. (1960) Studies on fish metabolism. I. The effect of temperature on the heart rate. Proc. Indian Acad. Sci. Section B LIII (5) : 230 - 239

Houston, A.H. and Madden, J.A. (1968) Environmental temperature and plasma electrolyte regulation in the carp, Cyprinus carpio. Nature, Lond., 217 : 969 - 970

———————, Reaves, R.S., Madden, J.A. and De Wilde, M.A. (1968) Environmental temperature and the body fluid system of the fresh-water teleost. I: Ionic regulation in thermally acclimated rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol., 25 : 563 - 581

———————, Madden, J.A. and De Wilde, M.A. (1970) Environmental temperature and the body fluid system of the fresh-water teleost. IV. Water-electrolyte regulation in thermally acclimated carp, Cyprinus carpio. Comp. Biochem. Physiol., 34 (4) : 805 - 818

Huggins, S.E., Hoff, H.E. and Peña, R.V. (1970) The respiratory - Heart rate response in Crocodilian Reptiles. Physiol. Zool., 43 (1) : 10 - 18

Hughes, G.M. and Umezawa, S.I. (1968) On respiration in the dragonet Callionymus lyra L. J. exp. Biol. 49 (3) : 565 - 582

——————— and Roberts, J.L. (1970) A study of the effect of temperature changes on the respiratory pumps of the rainbow trout. J. exp. Biol., 52 (1) : 177 - 192

Jaeger, R. (1965) Action potentials of myocardial fibres of the fish heart. Naturwissenschaften 52 (16) : 482 - 483

Jankowsky, H.D. (1968) Versuche zur adaptation der Fische im normalen temperaturbereich. Helgolander wiss. Meeresunters., 18 (3) : 317 - 362

Job, S.V. (1969a) The respiratory metabolism of Tilapia mossambica (Teleostei). I. The effect of size, temperature and salinity. J. mar. Biol., 2 : 121 - 126

——— (1969b) The respiratory metabolism of Tilapia mossambica (Teleostei). II. The effect of size, temperature, salinity and partial pressure of oxygen. J. mar. Biol., 3 (3) : 222 - 226

Jofre, I.J. and Izquierdo, J.A. (1967) The concentration of adrenaline and noradrenaline in the brain and the heart of the goldfish, Carassius auratus. J. Pharm. Pharmac., 19 : 340 - 341

Jubb, R.A. (1961) An illustrated guide to the freshwater fishes of the Zambesi River, Lake Kariba, Pungwe, Sabi, Lundi and Limpopo Rivers, pp. 132 and 4 - 9 Stuart Manning, Bulawayo.

Kisch, B. (1948) Electrographic investigations of the heart of fish. Expl. Med. Surg., 6 : 31 - 62

Kropp, B.N. (1947) The effect of temperature on the rate and variation of opercular movement in Fundulus diaphanus diaphanus. Can. J. Res., D. 25 : 91 - 95

Labat, R. (1966) Electrocardiologie chez les poissons téléostéens: Influence de quelques facteurs écologiques. Annls. Limnol. 2 (1) : 1 - 175

Lagler, K.F., Bardach, J.E. and Miller, R.R. (1962) Ichthyology. John Wiley & Sons, Inc. N.Y.

Long, L.Q., Giay, N.D. and Vinh, N.Q. (1961) The first results of the study of the temperature action on the fish Tilapia mossambica (Peters) acclimatised in Vietnam since 1951. Zh. obshch. Biol., 22 (6) : 444 - 451 (In Russian)

Martinez-Palomo, A., Alanis, J., and Benitez, D. (1970) Transitional cardiac cells of the conductive system of the dog heart. J. Cell Biol., 47 (1) : 1 - 17

Maruyama, J. (1958) An observation on Tilapia mossambica in pond referring to the diurnal movement with temperature change. Bull. Freshwat. Fish. Res. Lab., Tokyo 8 : 25 - 32

Marvin, D.E. and Heath, A.G. (1967) Cardiac and respiratory responses to gradually induced hypoxia in 3 spp. of fresh-water fish. Amer. Zool., 7 (4) : 730 (seen in abstract only)

Matsui, K. (1940) Temperature and heart beat in fish embryo, Oryzias latipes. I. The relation of temperature coefficient of heart beat to embryonic age. Sci. Rep. Tokyo Bun. Daig. 5 (B) (83) : 39 - 51

Meincke, K.F. (1970) The influence of temperature on the ion and water content in blood and plasma in tissues of Tinca tinca. Mar. Biol. (Berlin) 6 (4) : 281 - 290

Meuwis, A.L. and Heuts, M.J. (1957) Temperature dependence of breathing rate in carp. Biol. Bull. mar. biol. Lab., Woods Hole. 112 (1) : 97 - 107

Minshull, J.I. (1967) A preliminary investigation into the meaning of laboratory diuresis in the fish T. mossambica (Peters) and the effects of exposure to low temperatures on urine volume and composition. Honours Project, Rhodes University (unpublished)

Moe, M.A. (1969) Biology of the red grouper Epinephelus morio (Valenciennes) from the eastern Gulf of Mexico. Florida Dept. Natural Resources Professional Papers Series Number 10.

Morhardt, J.E. and Morhardt, S.S. (1970) Correlations between heart rates and oxygen consumption in rodents. Am. Zool., 10 (4) : 516, Abs. No. 256.

Morris, R.W. (1962) Body size and temperature sensitivity in the Cichlid teleost fish, Aequidens portalagrensis (Hensel). Am. Nat., 96 (886) : 35 - 50

——— (1965) Thermal acclimation of metabolism of the yellow bullhead Ictalurus natalis (Le Sueur). Physiol. Zool., 38 (3) : 219 - 227

——— (1965a) Thermal acclimation in Eleotrid fish. Carassiops compressus (Kreft) Am. Nat. 49 (905) : 81 - 87

Noseda, V., Chesa, F. and Marchetti, R. (1963) Vectorcardiogram of Anguilla anguilla L. and Pleurodeles waltlii Mich. Nature, Lond., 197 : 816 - 818

Oets, J. (1950) Electrocardiograms of fishes Physiol. comp. Oecol., 2 (2) : 181 - 186

Omura, Y, Lehr, D, and Goldman, W. (1967) Correlation of ECG changes with alterations of the transmembrane action potential of single cardiac cells in the in situ heart under various experimental conditions. Bull. N.Y. Acad. Met., 43 (12) : 1219

Owen, R.B. (1969) Heart rate, a measure of metabolism in blue-winged teal. Comp. Biochem. Physiol., 31 (3) : 431 - 436

Parvatheswararao, V. (1968) Initial adaptive responses to thermal stress in freshwater teleosts. I. Oxygen consumption of whole animal. Proc. Indian Acad. Sci., 68 B (Supp) : 225 - 231

Peak, M.J. Fuller, N.R. and Peak, J.G. (1967) Temperature acclimation in Tilapia sparrmarnii: Some effects of thiourea. Zool. Afr., 3 (1) : 95 - 104

Peyraud, C. (1965) Recherches sur la regulation des mouvements respiratoires chez quelques teleosteens: analyse d'un reflexe opto-respiratoire. Ph.D. Thesis, University of Toulouse.

Peters, J.A. and Mullen, R.K. (1966) Electrocardiography in Caecilia guentheri (Peters) Physiol. Zool., 39 (3) : 193 - 201

Polyakov, Yu, D. (1940) Daily rhythm of oxygen consumption of tench fry. Byulleten Moskovskogo Obschchestra Ispytatelei Prirody. Otdel Biologicheskii, 49 (1)

Precht, H, Basedow, T, Bereck, R, Lange, F, Thiede, W, and Wilke, L. (1966) Reactions and adaptations of poikilothermal animals after a change of adaptation temperature and the time course. Helgolander Wiss, Meeresunters., 13(4) : 369 - 401

Prosser, C.L., Brown, F.A., Jahn, T.L., Bishop, D.W., and Wulff, V.J. (1952) Comparative animal physiology. W.B. Saunders Company. Philadelphia & London.

Prosser, C.L. (1964) Perspectives of adaptation: theoretical aspects.
In: Handbook of physiology : Section 4 : Adaptation to the environment : 11 - 26
American Physiological Society, Wash. D.C.

Prosser, C.L., Mackay, W. and Kato, K. (1970) Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures.
Physiol. Zool., 43 (2) : 81 - 89

Randall, D.J. (1962) Effect of anaesthetic on the heart and respiration of Teleost fish.
Nature, Lond., 195 (4840) : 506

——————— (1966) The nervous control of cardiac activity in the tench (Tinca tinca) and the goldfish (Carassius auratus).
Physiol. Zool. 39 (3) : 185 - 192

——————— (1968) Functional morphology of the heart in fishes.
Am. Zool. 8 : 179 - 189

———————, and Smith, J.C. (1967) The regulation of cardiac activity in fish in a hypoxic environment.
Physiol. Zool. 40 (2) : 104 - 113

———————, and Stevens, E.D. (1967) The role of adrenergic receptors in cardiovascular changes associated with exercise in salmon.
Comp. Biochem. Physiol., 21 (2) : 415 - 424

Ras, K.P. and Bullock, T.H. (1954) Q_{10} as a function of size and habitat temperature in poikilotherms.
Am. Nat., 88 (838) : 33 - 44

Remorov, V.A. (1964) Electrocardiogram recording in long-term experiments;
In: Techniques for the investigation of fish physiology : 25 - 26
(Israel Program for Scientific Translation), Jerusalem.

Robertson, O.H., Krupp, M.A., Thompson, N., Thomas, S.F., and Hane, S. (1966) Blood pressure and heart weight in immature and spawning Pacific salmon.
Am. J. Physiol. 310 : 957 - 964

Roots, B.I. and Prosser, C.L. (1962) Temperature acclimation and the nervous system in fish.
J. exp. Biol. 39 : 617 - 629

Satchell, G.H. (1971) Circulation in fishes
(Cambridge University Press, Cambridge)

Schaefer, H. and Haas, H.G. (1962) "Electrocardiography". Ch. 13 In: Handbook of Physiology. Section 2 : CIRCULATION : 323 - 415 Vol. I. American Physiological Society, Wash. The Williams & Wilkins Co., Baltimore.

Schamroth, L. (1966) An introduction to electrocardiography. (2nd Edition) Blackwell Scientific Publications, Oxford and Edinburgh.

Scholander, P.F., Flagg, W., Walters, V., and Irving, L. (1953) Climatic adaptation in arctic and tropical Poikilotherms. Physiol. Zool., 26 : 67 - 92

Serfaty, A. and Reynaud, P. (1956) Electrocardiographe du poisson dans son milieu naturel. Bull. Soc. Zool. Fr., 81 : 121 - 126

Shelton, G. and Randall, D.J. (1962) The relationship between heart beat and respiration in teleost fish. Comp. Biochem. Physiol., 7 : 237 - 250

Shrivastava, Anil K. (1965) Studies on the morphology and physiology of the heart of Clarias batrachus (Linn) I. The gross anatomy of the heart. Zoo Anz. 175 (4/6) : 310 - 316 (Seen in Abstract only)

Siegmund, R. (1969) Locomotor activity and rest periods in indigenous fresh-water fish (Pisces, Percidae, Cyprinidae). Biol. Zbl., 88 : 295 - 312 (Seen in abstract only)

Singh, G.P. (1960) The structure of the heart of some fresh-water teleosts. Indian J. Zootomy 1 (1) : 1 - 25

Solomon, K. and Allanson, B.R. (1968) The effects of exposures to low temperatures on the serum protein of T. mossambica (Peters) Comp. Biochem. Physiol., 25 : 185 - 192.

Spaas, J.T. (1959a) Contribution to the biology of some cichlids. III Energy phenomena as a function of temperature. Hydrobiologia 14 (2) : 155 - 176

——————— (1959b) Contribution to the biology of some cultivated cichlidae. Temperature acclimation, lethal limits and resistance in three cichlidae. Biol. Jaarboek. Konink. Nat. Genoot. Dodonaea Gent., 27 : 21 - 38

Spitzer, K.W., Marvin, D.E. and Heath, A.G. (1969) The effect of temperature on the respiratory and cardiac response of bluegill sunfish to hypoxia. Comp. Biochem. Physiol., 30 : 83 - 90

Stroganov, N.S. (1956) Physiological adaptability of fish to the temperature of the surrounding medium. Zool. Inst. Acad. Sci. USSR, Moscow. (Translated: Israel Programme for Scientific Translations. Published by National Science Foundation (1962) Washington, D.C.).

Suhrmann, R. (1955) Weitere versuche über die temperatur-adaptation der Karauschen (Carassius vulgaris Nils.) Biol. Zentralbl., 74 : 432 - 448 In: Morris (1965a)

Sumner, F.B. and Doudoroff, P. (1938) Some experiments upon temperature acclimatization and respiratory metabolism in fishes. Biol. Bull. mar. biol. Lab., Woods Hole. 74 (3) : 403 - 429

———— and Lanham, U.N. (1942) Studies of the respiratory metabolism of warm and cool spring fishes. Biol. Bull. mar. biol. Lab., Woods Hole, 82 (2) : 313 - 327

———— and Wells, N.A. (1935) Some relations between respiratory metabolism in fishes and susceptibility to certain anaesthetics and lethal agents. Biol. Bull. mar. biol. Lab., Woods Hole, 69 : 368 - 378

Thauer, R. (1965) The circulation in hypothermia of non-hibernating animals and men. Ch. 54 In: Handbook of Physiology Section 2 : Circulation, Vol. III : 1899 - 1916 (American Physiological Society) The Williams & Wilkins Co., Baltimore.

Tsukuda, H. (1961) Temperature acclimatization on different organization levels in fishes. J. Biol., Osaka City Univ., 12 : 15 - 45.

Wells. N.A. (1935a) The influence of temperature upon the respiratory metabolism of the Pacific Killifish (Fundulus parvi-pinnus. Physiol. Zool., 8 : 196 - 227

———— (1935b) Variations in the respiratory metabolism of the Killifish Fundulus parvipinnis, due to size, season and continued constant temperature. Physiol. Zool., 8 (3) : 318 - 336 (Seen in abstract only)

Wikgren, B.J. (1953) Osmotic regulation in some aquatic animals with special reference to the influence of temperature. Acta. Zool. fenn. 71 : 1 - 102

Yamamoto, T. (1931) Temperature constants for the rate of heart beats in Oryzias latipes. J. Fac. Sci. Univ. Tokyo (Zool.,) 2 : 381 - 388

Zeuthen, E. (1953) Oxygen uptake as related to body size in organisms. Q. Rev. Biol., 28 (1) : 1 - 12