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PRELIMINARY INVESTIGATION OF THE RESPONSES OF JUVENILE SHORTFINNED EELS (*ANGUILLA AUSTRALIS*) TO ELEVATED TEMPERATURES.

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ABSTRACT

Fully pigmented, migratory elvers (<95mm long) of the shortfinned eel *Anguilla australis* were collected from the Huntly Power Station elver by-pass on the Waikato River in March 1983. Experiments determining the critical thermal maximum (CTM) for elvers acclimated to different temperatures, and median resistance times at constant lethal temperatures are described.

The temperature at which eels become lethargic and lay on their sides was used as the CTM end point; temperatures at which regular ventilation ceased were also recorded. Elvers acclimated to mean temperatures of 20.0, 28.5 and 30.5°C had CTMs of 31.4, 38.6 and 38.5°C respectively at a heating rate of 1°C/10 mins. Acclimation to higher temperatures also increased the temperature at which elvers ceased regular gill movements (34.4, 38.9, 39.3°C).

Median resistance times for elvers acclimated to 20°C and tested at constant temperatures of 36.5, 36.0, 35.5, 35.0, 34.5 and 34.0°C were 28, 73, 144, 222, 468 and 1200 mins respectively. Upper thermal tolerance of shortfinned elvers was considerably greater than that for salmonids reported in the literature; however, the slope of the temperature median restistance-time relationship [$Y\ (^{\circ}C) = 38.9 - 1.607 \log_{10} X$ (mins)] was similar to that of salmonids and other fish species.

The significance of these results is discussed in relation to thermal discharges into the Waikato River.

1.0 INTRODUCTION

The increasing incidence of elevated temperatures in the aquatic environment, primarily as a result of thermal discharges from power-generating stations, has stimulated much interest in the biological effects of temperature on aquatic organisms. Fish and most other aquatic animals are cold-blooded and temperature plays an important, highly regulatory role in their physiology. Temperature intimately influences reproductive cycles, digestion, respiration rates and the chemical processes that occur in an animal's body. For these reasons thermal requirements are among the most difficult to define and establish. However, studies on lethal temperatures have proved useful for describing species-specific responses to temperature. This subject has been considered in a number of comprehensive literature reviews and books (e.g. Brett 1956, Fry 1967, Parker and Krenkel 1969).

With the present commissioning of the 1000 MW Huntly Power Station and proposals for a second plant on the Waikato River the need to evaluate the possible effects of heated water discharges on the ecology of the river has been highlighted (e.g. NZE 1981, WVA Tech. Publ. 21 1982). As the original operating criteria for the Huntly project were based largely on overseas literature, studies to date have examined the validity of these criteria for the freshwater shrimp *Paratya curvirostris*, a common species in the Waikato River (WVA Tech. Publ. 21 1982). The experiments described in this report form the second in a series investigating the ability of freshwater organisms to withstand the impact of thermal effluents from power stations on the Waikato River. These investigations are being conducted on selected local species, to ensure adequate protection for aquatic life.

The lower Waikato River represents a pathway for at least 12 distinct fish migrations. Of particular importance are the large numbers of juvenile eels (*Anguilla* spp) which annually migrate upriver to restock populations in the Waikato Catchment. These eels (consisting of 2 species, the longfinned *Anguilla dieffenbachii* and the shortfinned *Anguilla australis*) enter the river from the sea as small transparent "glass eels" in late winter and early spring (Jellyman 1979). Following pigmentation of the body and behavioural changes the juvenile eels (now called elvers) migrate upstream between December and February, usually passing Huntly between January and March (C.P. Mitchell pers. comm.). Tidal and lunar factors affect these migrations but they are not fully understood. The continued yearly recruitment of these juvenile eels has considerable commercial importance to the Waikato Region as the adult eel fishery is the largest in New Zealand, producting 400-500 tonnes per year with an approximate value of $1,000,000 (Todd 1981).

Little is known of the temperature tolerance of New Zealand eel elvers. Coffey et al. (1975) noted an "observed habitable temperature" of 12-30°C, and Jellyman and Coates (1976) found a constant temperature of 20-25°C optimal, with shortfins being more tolerant of warmer conditions than longfins. Upper lethal limits for glass eels of 30°C and 25°C for short and longfinned eels respectively have been recorded (Jellyman 1974). These animals could withstand a direct change of +8°C from an ambient of 15°C. Jellyman also found that the growth rate of shortfinned eels was highest at 15°C for any given food supply. The present study is a preliminary investigation of the responses and survival of shortfinned elvers *Anguilla australis* to elevated water temperatures.

There are several possible criteria for determining the extreme temperature tolerance of fish (Fry 1971). Two commonly used criteria have been employed in this study, these being:

(i) the Critical Thermal Maximum (CTM) and

(ii) thermal restistance time for elvers exposed to high constant temperatures.

Cowles and Bogert (1944) defined the CTM as "the thermal point at which locomotory activity becomes disorganised and the animal loses its abiliy to escape from conditions that will promptly lead to its death". This definition requires that the test organism be exposed to water heated at a continuous, rapid and controlled rate such that the deep-body temperature of the animal follows the change in the water temperature without substantial lag. CTM experiments have been used to examine the thermal tolerances of a variety of aquatic organisms and to explore questions about temperature acclimation, geographical and seasonal responses, the effects of body size, age and photoperiod, and to compare populations and species from different habitats (e.g. Hutchison 1961, Everich and Gonzalez 1977).

In addition to determining the CTM, the effects of exposure to high constant temperatures can be examined. The period of tolerance prior to death when exposed to a lethal constant temperature is known as the resistance time, the median resistance time being the time taken for half of a group of experimental animals to die. By virtue of its resistance time a fish in its natural habitat may be able to make short excursions into potentially lethal areas and then return to preferred temperature zones without suffering detrimental effects (Brett 1956).

2.0 METHODS

2.1 Test Animals

Shortfinned elvers were collected from the elver by-pass at Huntly Power Staton during the evening of 14 March 1983. All elvers were between 57mm and 93.5mm in length and weighed less than 0.8g.

Elvers were acclimated in covered 15 l perspex aquaria (13-15 fish/aquarium) containing 10 l of reaerated water pumped directly to the laboratory from the Hamilton City stretch of the Waikato River. The results of an analysis of this water are shown in Table 1. Water quality is likely to be better than that which organisms would be exposed to downstream of Hamilton.

Before experimentation, all elvers were acclimated for at least 7 days at 20°C $\pm$ 1°C in continuously aerated, unfiltered river water. During acclimation elvers were fed to excess every 2-3 days with live tubificid worms; however, test animals were starved for 2 days before experimentation. Tanks were siphoned after feeding to remove wastes and remaining food scraps, and approximately 50% of the water volume was replaced. Acclimation and experiments were conducted in constant-temperature rooms (20°C) with fluorescent lighting on a 12 h light/dark cycle set to instantaneously change at 0600 h and 1800 h daily.

Relatively large numbers of elvers apparently remained in good health in the laboratory and their general wellbeing was enhanced by providing aquaria with dark bottoms and short lengths of PVC pipe as refuges.

TABLE 1: CHARACTERISTICS OF UNFILTERED LABORATORY WATER PUMPED FROM THE WAIKATO RIVER, HAMILTON. SAMPLES COLLECTED 7.3.83 (1145h)

Analysis	
Conductivity	190 μS
pH	7.5
Total alkalinity	38.0 ppm
Suspended solids	2.4 g m^{-3}
Ammonia (NH_3-N)	59.0 mg m^{-3}
Nitrates (NO_3-N)	62.0 mg m^{-3}
Total Phosphate	32.0 mg m^{-3}
Dissolved reactive phosphate	6.0 mg m^{-3}
Faecal coliforms (100 ml)	80
Standard plate count (37°C/100ml)	1.5×10^4
Standard plate count (20°C/100ml)	3.3×10^4

A. australis is a hardy species, although it can be subject to epidemic diseases in the laboratory, particularly at high temperatures. Levels of parasitism are unknown. Care was taken to select only healthy active specimens for experimentation.

Some eels were reacclimated to higher temperatures for an additional 15 days. Submerged thermostats were used to establish mean temperatures of 31.0, 30.5, 28.5 and 20°C. Temperature fluctuations in the aquaria were ±1°C, except for 31.0°C where the range was ±2°C. Waikato River water was used, after filtering to reduce phytoplankton growth in the tanks. Animals were fed and the tanks cleaned every 2-3 days; replacement water was added slowly to prevent temperature reductions.

Following direct transfer from 20°C to each acclimation temperature the fish (n = 10) were monitored for any sublethal, long term effects due to acclimation at the higher temperatures. Due to deaths in the 31.0°C tank (see Results), CTM trials were conducted using only healthy eels acclimated to mean temperatures of 30.5, 28.5 and 20.0°C for 15 days.

2.2 Experimental Procedure

Critical Thermal Maxima (CTM). The apparatus used to determine the CTM consisted of a 15 l perspex test chamber immersed in a stainless steel Grant Instruments water bath (60.5 x 29.8 x 26.5cm deep) (Fig.1). Filtered, aerated river water filled both the test chamber and the water bath; mesh-covered inlets in the sides of the chamber allowed water to flow between the two, improving temperature regulation of the test chamber. Water was mixed by two stirrers which provided sufficient water circulation to achieve even heating. This was aided by aeration of the test chamber water. A calibrated mercury thermometer graduated in 0.5°C units was used to measure temperature changes. Dissolved oxygen levels, measured with a Yellow Springs oxygen probe, remained above 6 ppm. Experiments began at acclimation temperature and 5 elvers were warmed at one of two rates. Preliminary trials to determine CTM using only animals acclimated to 20°C were conducted using a heating rate of 1°C/5mins. Subsequent trials with animals acclimated to this and higher temperatures were carried out at a heating rate of 1°C/10 mins (comments on these heating rates are given in the discussion on techniques). When the CTM was reached (criteria for this given in the Results) the fish were immediately transferred back to water at their acclimation temperature and recovery monitored.

Resistance Times. Following acclimation at 20°C for at least 8 days elvers were tested at a range of constant temperatures between 34.0 and 36.5 $\pm$ 0.25°C, over a maximum period of 24 h, in the CTM apparatus previously described. Experiments were not run simultaneously, but as individual trials in the following order: 34.0, 35.0, 36.0, 36.5, 35.5 and 34.5°C.

Elvers were introduced into the test temperature in a transparent plastic bag containing approximately 200 ml of acclimation water and a thermometer. This tempering process was intended to reduce drastic temperature shock; however, bag temperature took only 5 mins to equilibriate with the experimental temperature. The eels were released after 5 mins, continuously observed and the time of death recorded for individual fish. The criterion for death was cessation of heart beat for 10 sec. After all gill movements ceased, eels were taken for short periods (<30 sec) at about 5 min intervals from the water bath and the heart examined under a stereomicroscope. Therefore, resistance time was measured from the time the eels were introduced into the lethal bath (in the bag) until cessation of heart beat.

Dissolved oxygen levels remained above 6 ppm in all experiments.

3.0 RESULTS

Acclimation Observations

During 15 day acclimation to higher temperatures, eels in the two warmest tanks (30.5 and 31.0°C) initially showed a loss of equilibrium by lying on their sides. Although this effect disappeared after the first day, those at 31.0°C always appeared lethargic compared with those at lower temperatures. This effect may have resulted from the greater stress and wider temperature fluctuations (±2.0°C) experienced by these animals. At 31.0°C elvers survived for the first 7 days after which 90% (9) eels died over the next 4 days; the 50% mortality time was approximately 10.5 days. The remaining individual survived to the end of acclimation.

Critical Thermal Maxima

During rising temperatures, *Anguilla australis* displayed a distinctive series of responses. These initially involved fast wriggling backwards (an escape response which also occurred in response to other stimuli), body curling and twisting, and hyperactivity. Continued heating produced disorientation and loss of equilibrium characterised by the eels floating or lying on their lateral or dorsal surfaces and showing deep and rapid opercular pumping. The first sign of this response was taken as the end point for the CTM determination. Although animals displayed this behaviour often showed spontaneous swimming especially in the early stages of the response, it was usually unco-ordinated, spasmodic and of short duration.

Further increases in temperature produced muscular spasms, followed by cessation of regular opercular movements and body movement. This final immobile condition could not be considered as death as the heart continued to beat and good recovery was possible when the animals were returned to acclimation temperature.

CTM values for shortfinned elvers were dependent upon heating rate and acclimation temperature (Fig.2). CTM tolerance was improved by increasing the acclimation temperature to about 28.5°C, above which no significant increase in tolerance was obtained. A second end point, the temperature at which regular ventilation stopped, was also recorded. This responded to acclimation in a way similar to the CTM (Fig.3).

Resistance Times

After a short period of hyperactivity, elvers exposed to potentially lethal temperatures (>34°C) rapidly became lethargic and disorientated, lying on the bottom of the tank and ventilating deeply. Some spontaneous activity did occur, but this was irregualr and only occurred in the early stages of the experiments.

Mortality distributions were converted to cumulative percentage of elvers dead and plotted against time on semilog X probit graph paper (Fig. 4). Lines were fitted by eye excluding values for 0 and 100% mortality. Times to 50% mortality (median resistance times) as well as those for $\pm$ 1 probit (standard deviation) were interpolated from the graphs (Table 2). Median resistance times were subsequently plotted onto semilog graph paper for each test temperature (Fig. 5). These points form a linear series with an equation of Y (^{o}C) = 38.9 - 1.607 $\log_{10}$ X (mins) (r = 0.99). This line has been plotted with similar data for other fish species from Parker and Krenkel (1969).

TABLE 2: MEDIAN RESISTANCE TIMES FOR SHORTFINNED ELVERS (*ANGUILLA AUSTRALIS*) EXPOSED TO HIGH CONSTANT TEMPERATURES (N = SAMPLE NUMBER)

Test Temperature °C	N	Median Resistance Time (mins)	± 1 Probit Limits (mins)
34.0	5	1200	792 - 1770
34.5	8	468	294 - 720
35.0	6	222	138 - 336
35.5	8	144	144 - 186
36.0	6	73	61 - 85
36.5	8	28	16 - 39

No correlation between body size and resistance time was apparent in this study. The small range of sizes of animals used may have precluded this.

In some cases transferring 'dead' animals (those in which heartbeat had stopped for at least 10s) to a recovery bath at 20°C resulted in re-establishment of regular heartbeat and gill lamellae bloodflow. Muscular and opercular movement did not reappear, but cutaneous oxygen uptake was probably sufficient to keep these animals alive for at least 18 h (after which they were killed). Swelling and deformation of the dorsal surface of the head was also apparent, and this along with body paralysis was probably due to damage of sensitive central nervous tissue.

4.0 DISCUSSION

The compilation of temperature tolerance data for fish species found in New Zealand's natural waters has an important bearing on the formulation of regulations governing power station thermal discharges. Ideally, the temperature of these discharges should not cause mortalities nor disrupt life cycle processes of aquatic organisms.

At Huntly, open water temperatures in the Waikato River are vertically well mixed, but there is small scale regional variation especially during the summer months. Annual maximum mean open water temperature can reach 24°C in February, and microclimate temperatures of up to 34°C have been recorded in marginal submerged weed beds (Coffey *et al.* 1975). Thermal discharges at the Huntly Power Station are estimated to raise the ambient river temperature by no more than 8°C at the outfall. Thermal stratification of this heated discharge water adjacent to the power station is likely to occur, with the warm water overlying the cooler ambient river water. This should restrict the immediate thermal impact to the surface water and allow fish to move to the cooler waters below. As mixing with river water occurs, the temperature elevation at a point about 450m downstream from the disharge point is expected to be only 3°C above ambient. The water right restrictions for the station prevent any thermal dischage at a river temperature of 26°C and above (WVA Tech. Publ. 21 1982).

Data obtained in this preliminary study suggest that thermal discharges from the Huntly Station during normal operations and within the requirements of its water right should not be harmful to migrating shortfinned elvers. It is apparent that *A. australis* can tolerate rapid increases in water temperature and that this tolerance can be improved by acclimation to a limited extent (Figs 2 and 3). Although lethargy and loss of equilibrium can occur within short periods at constantly rising temperatures above 31.5°C when acclimated to 20°C, recovery from these symptoms is rapid following removal to lower temperatures. Temperatures above 34.0°C result in a severe impairment of activity, and eventually death within 24h.

Elvers exposed to 30.5°C + 1°C for 15 days during acclimation appeared to remain in good health, whilst at 31.0 ± .2°C mortality occurred after 7 days (50% mortality after 10.5 days). Thus, long term tolerance may be restricted to a maximum of about 32°C.

Resistance to high constant temperatures in *A. australis* is constantly better than for some other fish species, especially the salmonids, e.g. *Salmo salar* (Atlantic salmon) and *Oncorhynchus tshawytscha* (Quinnat salmon) (Parker and Krenkel 1969) (Fig. 5). Although line equations for these species were not given by Parker and Krenkel, calculation of the slopes for the data points shown are between 1.222 and -2.107. The slope of the line for *A. australis* (-1.607) is within these limits, showing the decline in tolerance with increasing temperature is very similar in rate for different species and independent of the level of tolerance.

Brett (1956) suggested that the resistance period allows fish to make short excursions into potentially lethal temperature zones. However, resistance times determined for *A. australis* may be overestimated in view of the rate at which the eels became distressed and unable to swim when first introduced into water at high temperatures. For most of the resistance time the elvers largely remained immobile lying on the bottom of the test chamber. Thus elvers showed a sensitivity to heat stress in terms of initial behavioural responses, but had high stamina in resisting physiological death once inactive.

Elevated temperatures within the tolerance limits of a fish can result in direct physiological effects. For example, respiration, digestion, circulation and metabolic rate all increase with increases in temperature. As metabolism and activity increase so does the fish's demand for oxygen (de Sylva 1969, Chan and Woo 1978). Furthermore, the level of oxygen dissolved in water decreases with increasing water temperature. Although oxygen consumption was not measured during this study, observations of opercular pumping tended to suggest increased respiratory demands of A.australis in warmer water. Shortfinned eels normally have a pattern of shallow gill ventilation interspersed with apnoeic periods when ventilation ceases (Forster, 1981). This adaption allows for reduced metabolic cost and survival in water with very low oxygen levels. Observations of eels in this study exposed to an acute rise in temperature from 20°C to approximately 26°C showed opercular cavity pumping to become more continuous. As temperatures increased further and the elvers became hyperative or lost equilibrium, opercular pumping became rapid and exaggerated. Heightened metabolic demands arising from the hyperativity and added respiratory stresses stimulated by elevated temperatures may result in higher mortalities amongst migrating elvers.

There is evidence that temperature can affect the migratory activity of young eels of some species (Tesch 1977); however, the influence of temperature on migratory responses of New Zealand eels is unknown. Jellyman (1979) found no correlation between surface water temperatures and catches of glass eels (Anguilla spp) in the lower Waikato River. It is expected that the gradual movement of elvers upstream during the spring and early summer will acclimatise them to the warmer conditions they may be exposed to due to power station thermal discharges, even during the summer months. Thermal stratification of the river adjacent to the station may allow elvers to avoid areas of high temperatures by swimming near the bottom.

In conclusion, it appears unlikely that the presently expected rises in water temperature in the Waikato River due to the operation of the Huntly Power Station would reach levels that would be lethal to healthy juvenile shortfinned eels. These animals appear to have a considerable safety margin of tolerance for protection against abnormal, short-term elevations in temperature. However, some physiological stress may be induced in elvers approaching the oufall, although this has yet to be verified.

5.0 DISCUSSION OF TECHNIQUES USED

It has been pointed out that laboratory determined CTMs are not entirely suitable for predicting the effects of thermal effluents on aquatic life (Everich and Gonzalez 1977). This is because both time and temperature are used to determine a final end point (temperature value). Higher mean end-point temperatures can be achieved at faster heating rates because the dying rate does not keep pace with the rapid heating of the external media. Furthermore, failure to ensure that body temperature follows the temperature of the external medium can lead to an incomplete assessment of CTM as a measure of temperature tolerance. In this study the lag between water and deep-body temperature of the elvers was considered negligible in view of their small size. Also, the slower heating rates of 1°C/5 min and 1°C/10 min based on the predicted rate of thermal decay of heated water discharge into the Waikato River (Environmental Impact Statement Huntly Power Station 1972) and an approximate swimming speed for elvers of 0.2 to 0.3 m s^{-1} were intended to provide more realistic experimental conditions. It is considered unlikely, however, that field conditions at Huntly would emulate conditions used to measure or reach CTM values determined in the laboratory. While the CTM may not be the ideal tool for predicting the effects of thermal discharges on fish, it remains a simple and rapid technique for obtaining preliminary and comparative information about how different organisms respond to temperature. It provides a means to determine differences in temperature sensitivity and responses to acclimation. As yet this limited background information is unavailable for many fish and invertebrate species from New Zealand waters.

The results of this study were based on carefully controlled situations using test fish of uniform size and fed on a single diet at regular intervals. Because of the close relationship between an organism and ambient environmental temperature, more work on the effects of size, sex, diet, maturity and activity on thermal tolerance is required before we can adequately predict the impact of temperature modification on the Waikato eel migrations.

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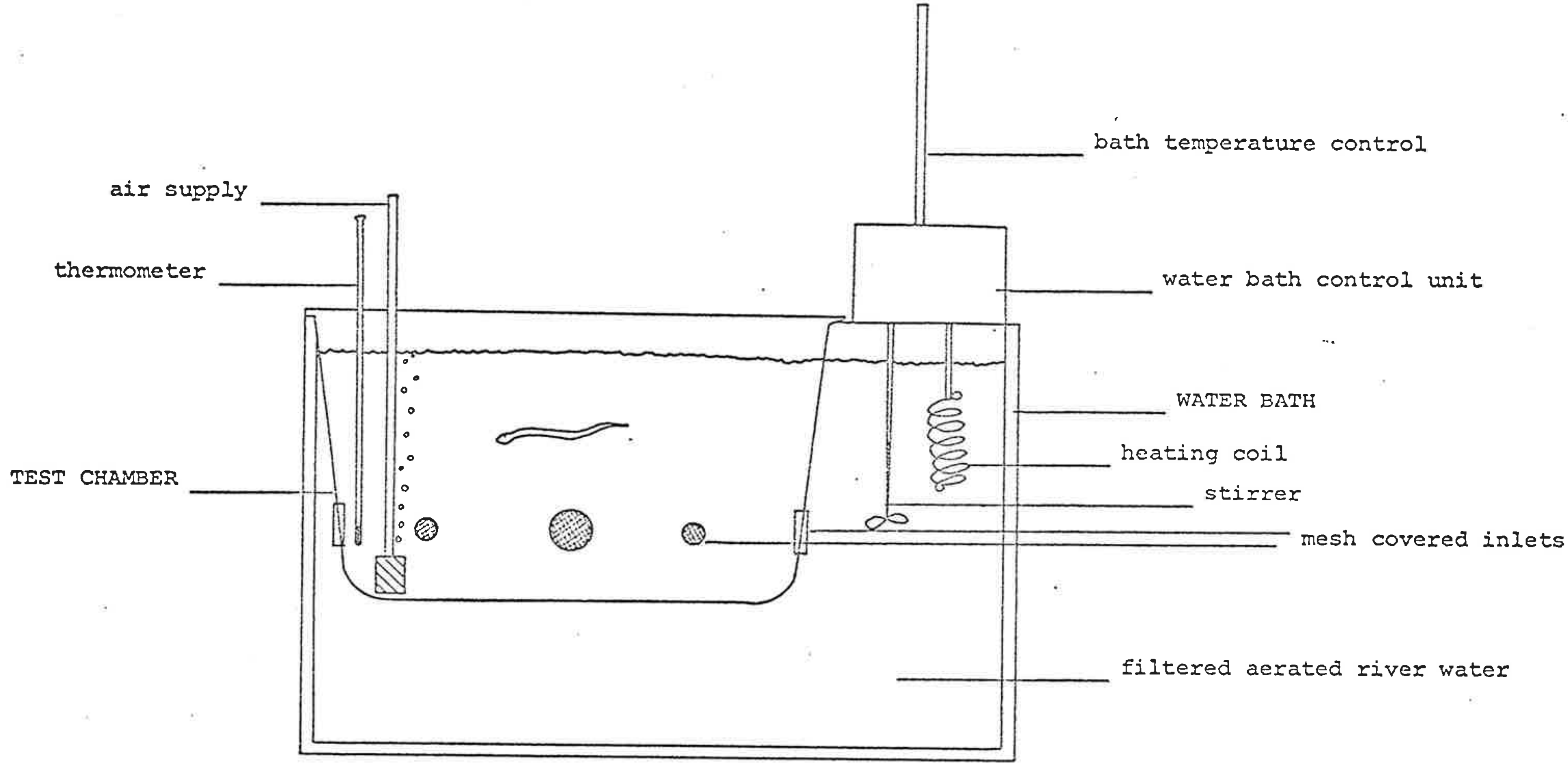

Figure 1: Apparatus used for exposing shortfinned elvers to continuously rising and high constant temperatures.

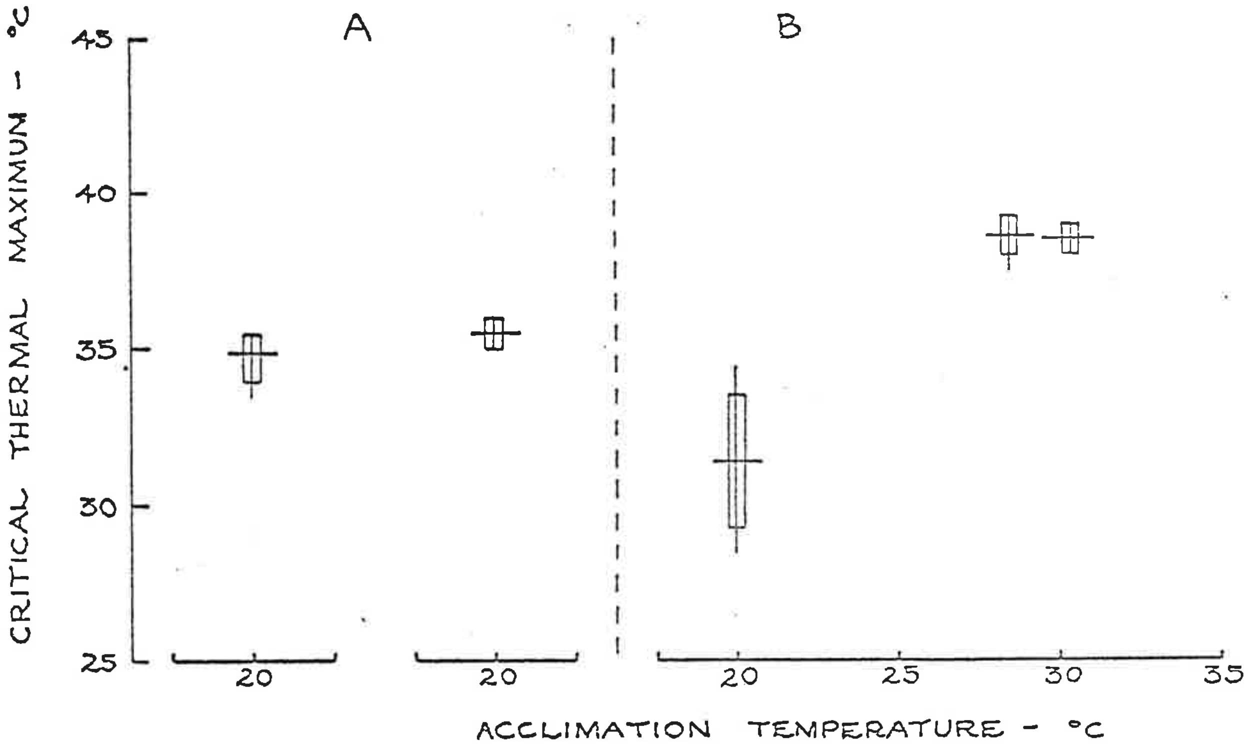

Figure 2: Critical thermal maxima for shortfinned elvers (*Anguilla australis*) at different acclimation temperatures and exposed to different heating rates. A - eels heated at 1°C/5 mins, B - eels heated at 1°C/10 mins. Horizontal bar - mean, vertical bar - range, open box - standard deviation ($n = 5$).

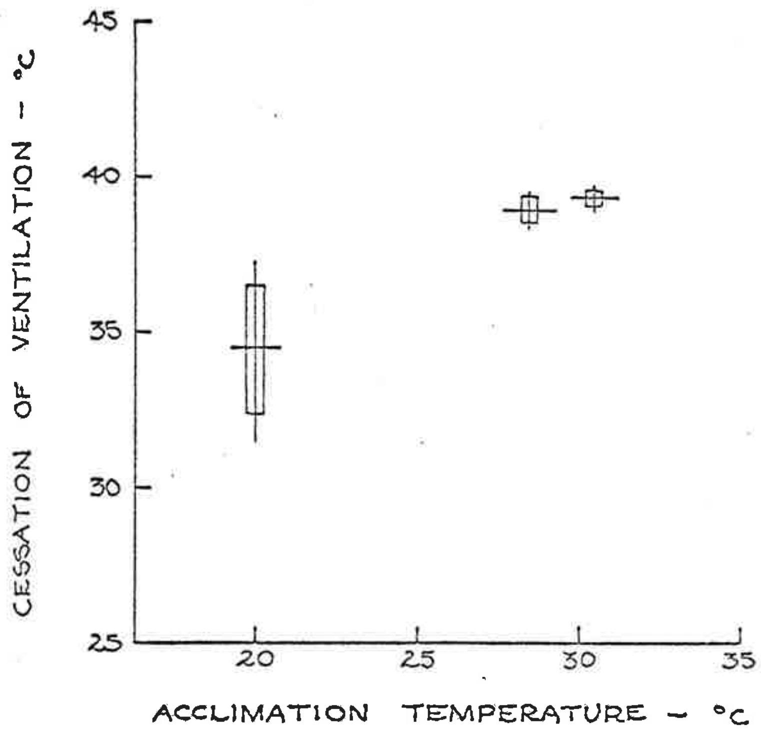

Figure 3: Effect of acclimation temperature on the temperature at which regular ventilation ceases in shortfinned elvers (*Anguilla australis*). Heating rate 1°C/10 mins. Symbols as in Fig. 2 (n = 5).

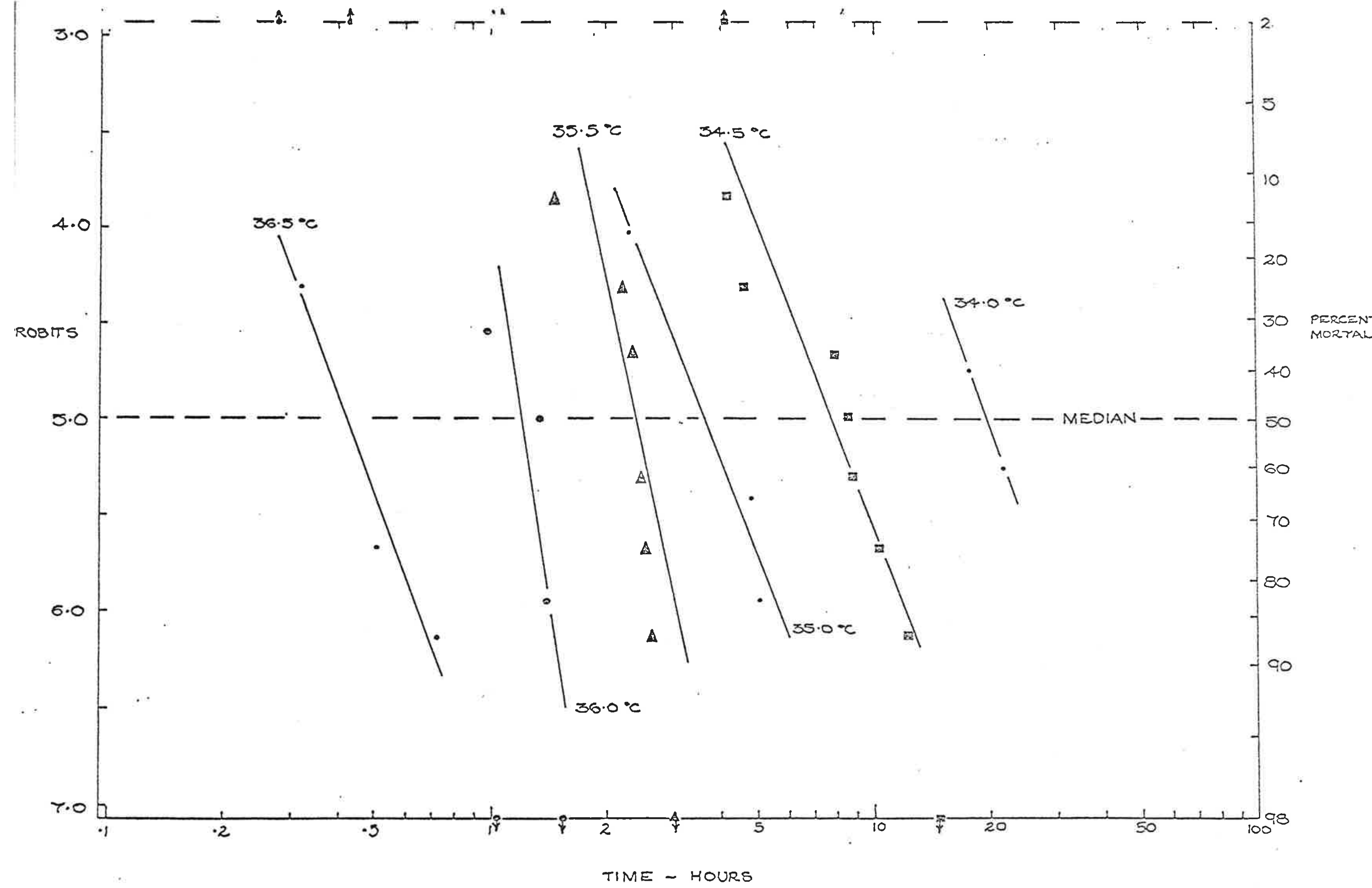

Figure 4: Time - percentage mortality lines for shortfinned elvers (*Anguilla australis*) exposed to various constant temperatures. Eels acclimated to $20^{\circ}C$.

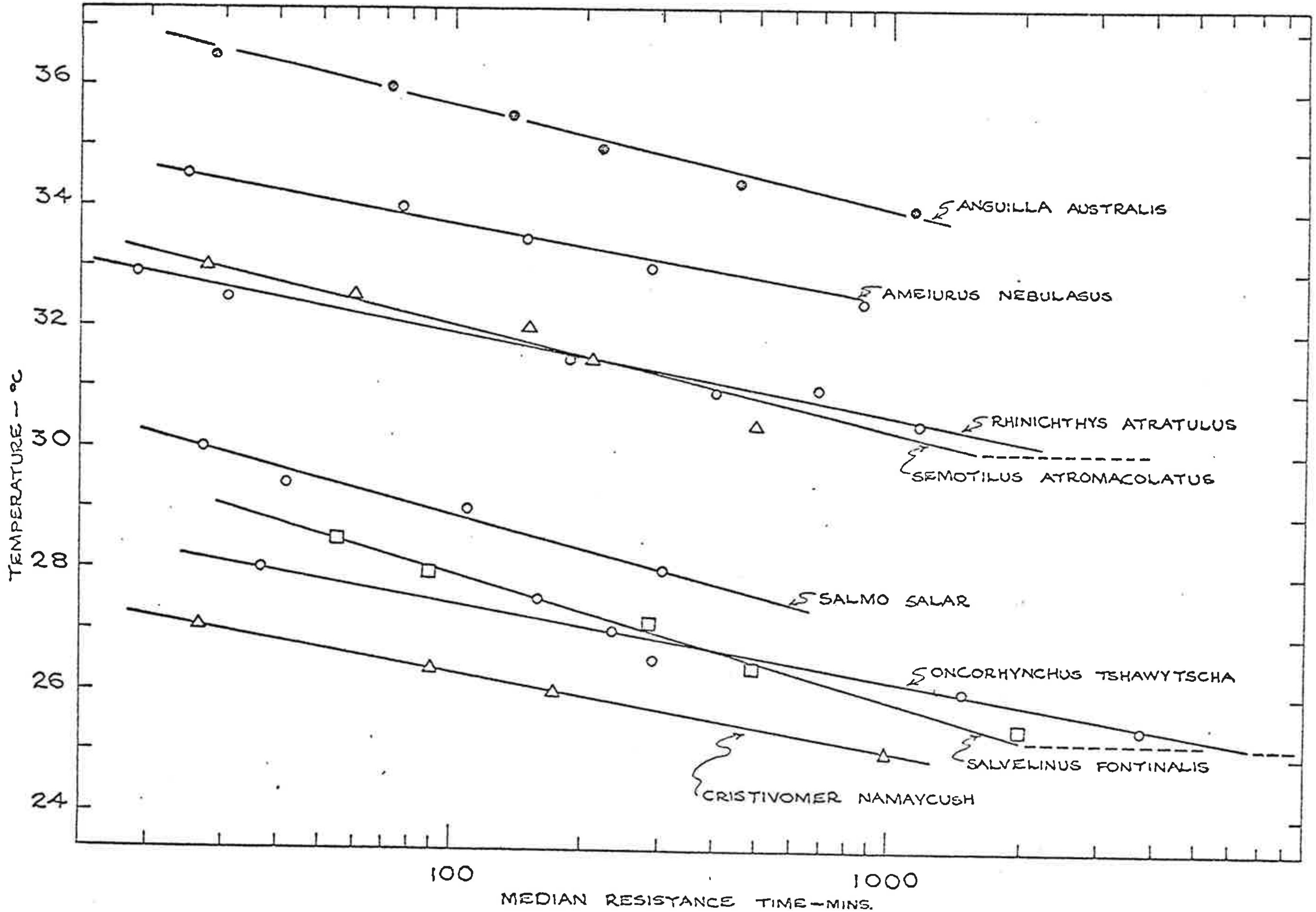

Figure 5: Median resistance times for various fish species acclimated to 20°C. Open symbols - data obtained from Parker and Krenkel (1969). Closed circles - present study.