SPECIES - SPECIFIC RESPONSES OF FRESHWATER ORGANISMS TO ELEVATED WATER TEMPERATURES

WAIKATO VALLEY AUTHORITY
TECHNICAL PUBLICATION № 29
JUNE 1984

SPECIES-SPECIFIC RESPONSES OF FRESHWATER ORGANISMS

TO ELEVATED WATER TEMPERATURES

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ABSTRACT

This study reports on continuing investigations into the potential impacts of thermal discharges from coal-fired power stations on organisms in the Waikato River.

Experiments were undertaken to rank the sensitivity of freshwater aquatic organisms to elevated water temperatures. Interspecific comparisons were made by determining a Critial Thermal Maximum (CTM) for each of 6 fish and 2 crustaceans acclimated to 12°C and/or 20-22°C. The temperature at which animals became narcotised was used as the end-point. Temperatures at which activity noticeably increased and at which swimming equilibrium was first disrupted were also noted.

CTM values ranged between 31.8°C and 38.5°C, with thermal tolerance increasing in the following order: smelt (Retropinna retropinna), freshwater crayfish (Paranephrops planifrons), freshwater shrimp (Paratya curvirostris), inanga (Galaxias maculatus), Cran's bully (Gobiomorphus basalis), common bully (Gobiomorphus cotidianus), shortfinned eel elvers (Anguilla australis) and mosquitofish (Gambusia affinis). Animals acclimated to 20°C had higher CTM's than those acclimated to 12°C, and the ranking of species was unaffected by acclimation temperature.

The temperatures at which losses in equilibrium occurred during swimming showed a similar species ranking, but were (on average) about 2°C lower than the CTM.

INTRODUCTION

The compilation of temperature tolerance data for aquatic organisms found in New Zealand's natural waters has an important bearing on the formulation of regulations governing the operation of power stations and other industries which have thermal discharges. With a fully operational station at Meremere, the present commissioning of the 1000 MW Huntly Power Station and proposals for a third on the Waikato River, the need to evaluate the possible effects of heated water discharges on the biota has been highlighted (e.g. NZE 1981, WVA 1982).

The open-cycle cooling system for the Meremere and Huntly power stations uses considerable quantities of Waikato River water in 'once-through' cooling operations. At Huntly, heat removed from the station at full production is predicted to raise the river water temperature by 8°C at the outfall structure. Following mixing, the difference between the ambient river temperature and discharge temperature is expected to be no more than 3°C (approximately 450m downstream). At present, water right restrictions for the station prevent any discharge at ambient river temperatures of 26°C or above (WVA 1982). The cooling method for the third power station planned for the lower Waikato has yet to be decided.

In an attempt to establish water temperature criteria for these projects, a working group of scientists interested in power developments on the Waikato River recommended that experiments be carried out on selected local species to determine if criteria could be formulated to provide "adequate protection" for aquatic life.

Preliminary studies on the freshwater shrimp *Paratya curvirostris* (W.V.A. 1982) and shortfinned eel *Anguilla australis* (W.V.A. 1983) have already been undertaken. Town (W.V.A. 1982) showed that summer-acclimated (22-23°C) *P. curvirostris* suffered high mortality at constant temperatures above 27.5°C. Simons (W.V.A. 1983) obtained a medium resistance time for *A. australis* elvers of 20 hours at 34°C and estimated "long term" survival occurred at 32°C and below.

For the great majority of organisms in the Waikato River little or nothing is known of their responses to changes in environmental conditions. Faced with such a variety of potential test species, we must make a basic assumption that protection of the most thermally-sensitive species will provide overall protection for the community in general. Thus, pinpointing these sensitive species will pave the way for later more detailed work on these particular animals.

This report ranks the sensitivity of a number of common local aquatic species to rising water temperatures. This information is prerequisite for any serious assessment of the possible impacts of thermal power stations on the Waikato River.

METHODS

Test Animals

Many fish species are resident and/or migratory in the lower Waikato River. Mitchell and Saxton (1983) found 9 fish species adjacent to the Huntly Power Station, with inanga, smelt, eel elvers and bullies being the most abundant. However, overall a crustacean, the freshwater shrimp, was the most abundant organism caught, making up over 80% of the total catch (n = 11044). Fish are generally regarded as being less temperature tolerant than invertebrates (Welch 1980) and are good indicators of water quality. Therefore, 6 fish species and 2 crustaceans were examined in this study (Table 1). All except Cran's bully *Gobiomorphus basalis* were selected because of their abundance in the Waikato River. Cran's bullies were being kept in the laboratory at the time and were tested to provide a comparison with results for the common bully, *Gobiomorphus cotidianus*. Some of the species tested migrate along the Waikato River.

All animals were collected from the Waikato River and/or its tributaries adjacent to or downstream of Hamilton City, between April 1983 and February 1984. Specimens were captured with sweepnets or electric-fishing equipment and transported back to the laboratory in sealed buckets or plastic bags.

Prior to exposure to elevated temperatures, all animals were acclimated at a constant temperature of 12°C or 20-22±1°C for at least 7 days. Acclimation is the regulation of an organism's physiology to selected laboratory conditions. Successful acclimation to a particular temperature assumes that the physiological processes of each species tested will be adjusted to a common baseline. Any responses to a subsequent test stimulus will therefore reflect specific genetic differences, rather than differences due to previous thermal experiences of the individual organisms established prior to capture. Thus each species will have a characteristic temperature tolerance range as a result of exposure to a particular acclimation temperature. When collection temperature varied greatly from the proposed acclimation temperature (e.g. winter collections acclimated at 20°C), the animals were adjusted gradually (1°-2°C change/day) towards the acclimation temperature and held for a further 7 days before testing.

Animals were acclimated in 15 l perspex aquaria containing approximately 10 l of continuously aerated water pumped directly to the laboratory from the Hamilton City stretch of the Waikato River. Results of an analysis of this water are shown in Table 2. During acclimation animals were fed every 2-3 days on tubificid worms (except for *Paratya* which fed on the organic film growing on surfaces within their aquaria); however, they were starved for 2 days prior to, and during experimentation. Aquaria were siphoned regularly to remove wastes and up to 75% of the water volume was replaced with water at the correct temperature. The aquaria were held in constant-temperature rooms with fluorescent lighting on a 12 hours light/dark cycle set to change at 0600 h and 1800 h daily.

Most animals remained in good health during acclimation. Deaths were uncommon except for *Retropinna retropinna* and *Galaxias maculatus* which were difficult to keep successfully in the laboratory. Dosing with a commercially available "white spot" cure was usually necessary to ensure healthy specimens. The general well-being of the animals was enhanced by providing aquaria with dark bottoms, and short lengths of PVC pipe and rocks as refuges.

TABLE 1: SPECIES TESTED

Common Name	Scientific name	
FISH:		
Mosquitofish	*Gambusia affinis*	Introduced, non-migratory
Shortfinned eel	*Anguilla australis*	Native, migratory
Common bully	*Gobiomorphus cotidianus*	Native, sometimes migratory
Cran's bully	*Gobiomorphus basalis*	Native, non-migratory
Inanga (whitebait)	*Galaxias maculatus*	Native, migratory
Common smelt	*Retropinna retropinna*	Native, migratory
CRUSTACEANS:		
Freshwater shrimp	*Paratya curvirostris*	Native, migratory
Freshwater crayfish	*Paranephrops planifrons*	Native, non-migratory

TABLE 2: 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

Experimental Procedure

Extreme temperature tolerance was determined using a Critical Thermal Maximum (CTM) technique (Hutchison 1961). Briefly, this requires a test organism to be exposed to water heated at a continuous, rapid and controlled rate such that the animal's deep-body temperature follows the change in the water temperature without substantial lag. From the small size of the animals used in this study it was assumed that this lag was negligible. The end-point (= CTM) is the temperature at which locomotory activity becomes totally disorganised and the animal loses its ability to escape from conditions that would lead to its death (Cowles and Bogert 1944). This method provides a quick and simple way of obtaining a comparative "critical" temperature value for each species and thus a means of relating interspecific sensitivity and intraspecific acclimation responses (Everich and Gonzalez 1977).

The apparatus used to determine the CTM consisted of a 15 l perspex aquarium insulated with 50 mm polystyrene and fitted with a heater/stirrer unit from a Grant water bath (Fig.1). A perspex barrier with mesh-covered ports separated the animals in the test chamber from the heating coil and stirring propellor. Circulation of water in the tank was sufficient to provide even heating and a gentle current for the fish. Rocks were placed in the test chamber to provide some refuge for test organisms. A calibrated mercury thermometer graduated in 0.5°C units was used to measure temperature changes. Aeration of the test chamber was continuous, maintaining dissolved oxygen levels well above 6 ppm.

Experiments began when the organisms were introduced to the test chamber at acclimation temperature. They were then left for at least 1 hour to become accustomed to the chamber conditions. Various numbers of animals were used (2-10, usually 3 to 6) depending on their size and activity, but care was taken to select only active and apparently healthy, specimens for testing. The water was then warmed at a constant rate of 1°C/2min. Heating was continued until animals became narcotised, failed to respond strongly to a gentle squeeze and could be lifted from the water with forceps. This incapacitated state was recorded as the end point or CTM and usually followed a period of violent activity and loss of equilibrium with the animals eventually drifting ventral side up. When each individual reached its CTM the temperature was noted and the animal immediately removed to water at 20°C and its recovery monitored. Notes on changes in activity, disorientation and other disruptions in behaviour during experimentation and recovery were made.

RESULTS AND DISCUSSION

A mean CTM value was calculated for each species based on CTMs obtained from individuals of the species over all the trials. These are shown in Table 3 along with the numbers of animals tested and their size ranges. Figure 2 presents some of this data graphically and highlights the interspecific differences in thermal sensitivity. The decreasing order of sensitivity for animals acclimated to 20°C is as follows:

Gambusia affinis
Anguilla australis
Gobiomorphus cotidianus

TABLE 3: MEAN CTM VALUES OBTAINED FOR FISH AND CRUSTACEANS FOLLOWING ACCLIMATION TO VARIOUS TEMPERATURES

Species	Acclimation temperature (°C)	Sample number	Mean CTM (°C)	Variance	*Size range (mm)
FISH					
G. affinis	12	6	34.2	2.35	16-23
	20	15	38.5	0.43	16-27
A. australis	12	13	33.4	1.01	58-108
	20	16	35.6	1.66	67-128
	21.5	12	36.0	0.39	62-88
G. cotidianus	12	10	32.7	0.35	29-65
	20	21	34.0	0.25	30-74
G. basalis	12	13	32.3	0.21	41-53
	20	5	33.9	0.24	41-64
G. maculatus	20	6	32.9	0.01	52-67
	22	16	33.8	0.20	44-64
R. retropinna	20	5	31.8	0.97	73-80
CRUSTACEANS					
P. curvirostris	12	20	28.9	0.69	4.5-6.5
	20	18	32.6	0.13	4.5-6.5
P. planifrons	12	6	28.8	2.36	12-23
	20	11	31.9	2.31	6-22

* Total length for fish species.
Carapace length for crustaceans - from base of eye to dorso-posterior margin of the carapace.

Gobiomorphus basalis
Galaxias maculatus
Paratya curvirostris
Paranephrops planifrons
Retropinna retropinna

Thus, R. retropinna was the most sensitive and G. affinis the least sensitive to rising temperatures. The CTM values demonstrate a clear response to acclimation temperature, with acclimation to 12°C producing lower CTM's than acclimation to 20°C (Fig.2). The ranking of sensitivity was unaffected by acclimation temperature. Such responses to acclimation are typical of poikilothermic aquatic organisms (Fry 1967). This has important implications for the establishment of thermal discharge criteria, as it indicates that water right restrictions should be flexible enough to account for seasonal changes which influence the physiological tolerance of the animals.

Similar CTM values for the two Gobiomorphus species perhaps emphasises their generic affinity, and thermal tolerance for other members of the genus may be similar.

In using the CTM technique to obtain an indication of the tolerance of these organisms to high temperatures, care must be taken to understand how these results relate to other methods of measuring temperature tolerance and the likely effects of thermal discharges into the animal's habitat. As mentioned earlier, the CTM is determined by steadily increasing the temperature and then recording the temperature at which the test organism is incapacitated. This incapacity can effectively be taken as equivalent to death, as it is assumed that if an animal becomes unable to escape from further stress it will be trapped in a lethal situation. As a means of physiologically analysing the thermal stress and tolerance of an organism, the CTM technique will invariably overestimate the maximum temperature at which an organism can live for an indefinite period of time and retain normal physiological function. The reason for this discrepancy is that the end-point for the CTM is dependent on both time and temperature, and must provide an unambiguous indicator under rapidly rising temperature conditions. Therefore, only the most acute impairment of function will be displayed. Slower breakdowns in physiological processes which lead to death over longer periods and at lower temperatures will be masked, as will subtle temperature-induced modifications in behaviour. This can be demonstrated by comparing various temperature response data for the mosquitofish Gambusia affinis (Table 4). Furthermore, modification of the CTM heating rate can significantly change the CTM values obtained (Table 5). Slower heating rates produce lower CTM values. The advantage of the CTM method is that it can be used to compare individuals and species exposed to a variety of conditions quickly and with good reproducibility.

Continuous observations of the animals during testing revealed two visually distinct responses prior to reaching their CTMs; these were an increase in activity and a loss of equilibrium (Table 6).

Hyperactivity became increasingly apparent at about 26°C for both fish and crustaceans acclimated to 20°C. Information is available from overseas work on the effects of temperature on fish activity. A rise

TABLE 4: COMPARISON OF TEMPERATURE TOLERANCE DATA FOR THE MOSQUITOFISH *GAMBUSIA AFFINIS*.
Figures in parentheses are acclimation temperatures
* - from Coutant (1977).

	Temperature	Source
CTM (20°C)	38.5	Present study
Upper lethal temperature (15°C)	35.4	Brett (1956)
" " " (20°C)	37.3-37.5	Brett (1956), Coffey *et al* (1975)
" " " (25°C)	37.3	Brett (1956)
" " " (30°C)	37.3	"
Upper avoidance temp: adults	29.5	Bacon *et al* (1969) *
" " " 15-19mm	32	" " " *
" " " < 15mm	35	" " " *
Final preferred temp: adults	27	" " " *
" " "	31	Winkler (1973) *

N.B. The lethal temperature is defined as the temperature at which 50% of the population is dead after an 'indefinite' exposure.

TABLE 5: COMPARISON OF CRITICAL THERMAL MAXIMA FOR SHORTFINNED EEL ELVERS (*ANGUILLA AUSTRALIS*) EXPOSED TO DIFFERENT HEATING RATES. ACCLIMATION TEMPERATURE 20± °C.

Heating rate (min/1°C)	CTM value	Source
2	35.6	Present study
5	35.0	WVA 1982
10	31.4	WVA 1982

TABLE 6: SUMMARY OF TEMPERATURES AT WHICH VARIOUS SPECIES SHOWED MODIFICATIONS IN BEHAVIOUR DURING CTM EXPERIMENTS (Ranges are given, the central figure indicates the mode).

Species	Acclimation Temperature(°C)	Temperature (°C) Increased Activity	Equilibrium Loss
FISH			
G. affinis	20	26.0-28.0	34.5-35.0
A. australis	12	19.0-23.0	28.0-30.0
		23.0-26.0	32.5-35.0
Gobiomorphus spp	12	24.0-28.0	26.0-28.5-29.0
	20	27.0-28.0-30.0	30.5-32.5-33.0
G. maculatus	20	26.0-27.0	31.5-32.0
R. retropinna	20	25.5	30.5
CRUSTACEANS			
P. curvirostris	12	22.5-23.0	25.0-26.0
	20	26.0-26.5	29.5-30.0
P. planifrons	12	20.5	27.0
	20	25.5-26.0	29.0-30.0

in activity is associated with increasing water temperature and this can be regarded as a protective response by individual organisms (IAEA 1980). Fish have an acute temperature sense with peripheral temperature receptors detecting external changes as low as 0.03°C (Bull 1936). Transmitted through the central nervous system this information invokes avoidance responses before the internal body temperature is affected, enabling the fish to swim from danger. These responses allow fish to adjust to, or where possible seek refuge from, altered water temperatures during normal seasonal and daily fluctuations, as well as artificial thermal conditions created by waste-heat discharges.

Loss of equilibrium varied slightly between fish, eels and crustaceans. However, the temperature was recorded at the first disruption to an organisms normal body orientation, usually while swimming. For fish, this often involved body rolling to expose the white ventral surface and Paratya were unable to hold the body in a horizontal position and drifted tail down. These symptoms were displayed briefly at first as lapses in normal swimming, but as the temperature continued to rise losses of co-ordination became more evident. These responses were also acclimation dependent (Table 6). Losses in coordination usually began appearing at least 2°C (on average) below the CTM. In this study, disruptions to swimming ability were not examined in detail. However, future work could be directed towards examining this more closely. The temperatures at which these disruptions occur are likely to provide a better indication of potentially hazardous field conditions, such as those created by a thermal plume immediately following discharge, than CTMs or upper lethal temperatures.

On reaching the CTM, animals were placed in water at approximately 20°C and their recovery qualitatively assessed. Individual specimens showed various responses and only overall recovery is described. No account could be made of subtle physiological damage or recuperation. Crustaceans usually recovered immediately when transferred to cooler water and initially did not appear adversely affected by heating. Longer recovery by Paranephrops planifrons was good, but for Paratya curvirostris was highly variable. The effects of direct sublethal thermal shock on subsequent survival should be investigated further. Fish remained lethargic for several minutes following heating and gill opercular beating was deep and rapid. Some fish deaths did occur with moribund specimens being distinguished early in the recovery period by their abnormal body orientation, pale gills, weak ventilation and often dark colouration. Fish either recovered or were dead within 30 to 40 minutes. Those that recovered would take food and appeared healthy in subsequent days.

A general impression obtained at this time was that a very fine balance existed between recovery and death following exposure to extreme temperatures. In this stressful situation exposure of an animal of reduced physiological condition, or slight over-exposure (seconds) before removal, may be sufficient to disrupt physiological processes to a level where death is inevitable.

Summary

This study has provided an indication of the species-specific responses of a number of 'ecologically important' river organisms to

rising temperatures. The CTM determination was chosen to give preliminary and comparative information on which to rank the thermal sensitivity of the animals tested. Along with this, it has also given some clues as to how animals may respond if caught in a thermal plume where they would experience a rapid rise in temperature. This background data can provide the basis on which to plan future experiments specifically designed to look at the impacts of thermal waste disposal. For efficiency, and to provide realistic protective measures, these experiments could be conducted on organisms such as *R. retropinna*, *G. maculatus*, *P. curvirostris* and *P. planifrons*. However, there may well be other species which show even greater thermal sensitivity. Temperature criteria should be set so as to allow all these species to survive, grow, migrate and reproduce.

Accordingly, some likely areas of future investigation could include the following:

- longterm temperature tolerance
- effects of temperature on swimming ability, susceptibility to fatigue, respiration and oxygen consumption, feeding, growth, and gonad development
- acclimation responses, their limits and rates
- effects of temperature shock on survival, swimming coordination and orientation.

ACKNOWLEDGEMENTS

I thank the following people for their assistance with the preparation of this report:

Mrs S. Porter - technical assistance
Mr M. McLea and Ms A. Cree - manuscript
Miss H. Horsley and Mrs I.G. Sizemore - typing

This report was prepared as part of the investigations into the effects of thermal power developments carried out by the Waikato Valley Authority.

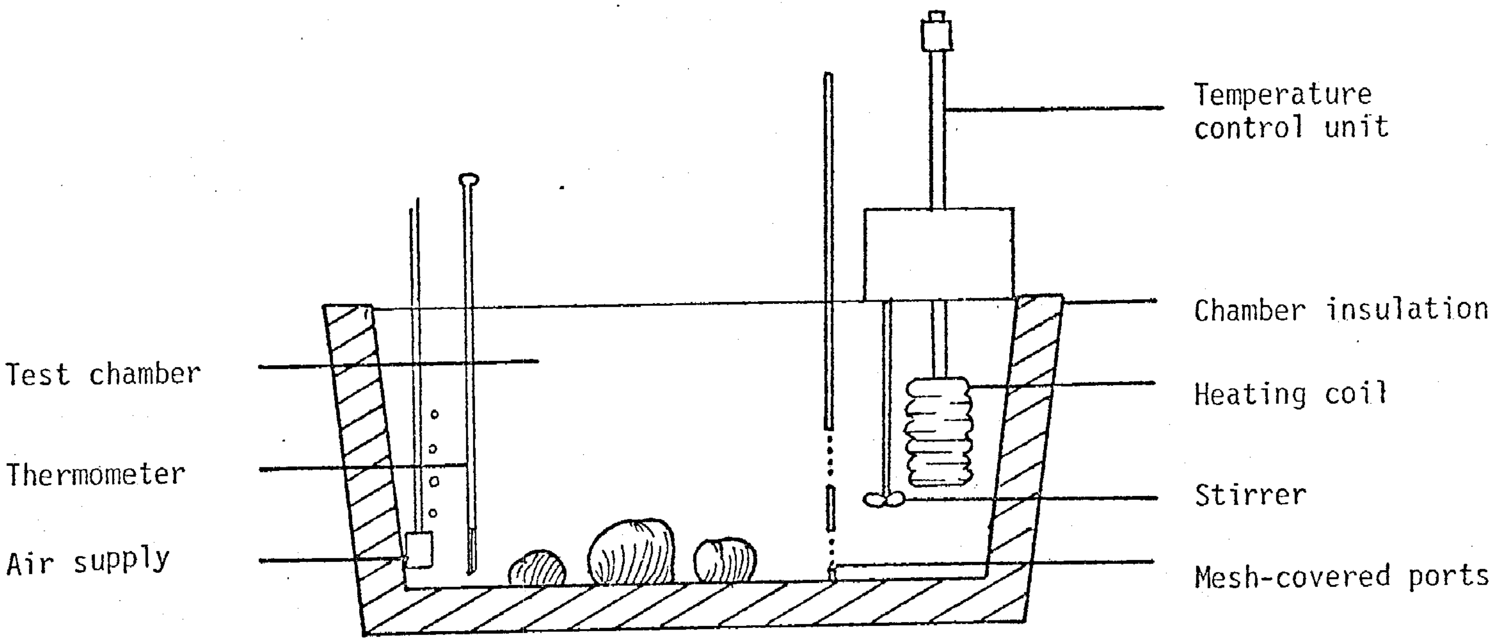

Figure 1 Apparatus used for testing the CTM of fish and crustaceans.

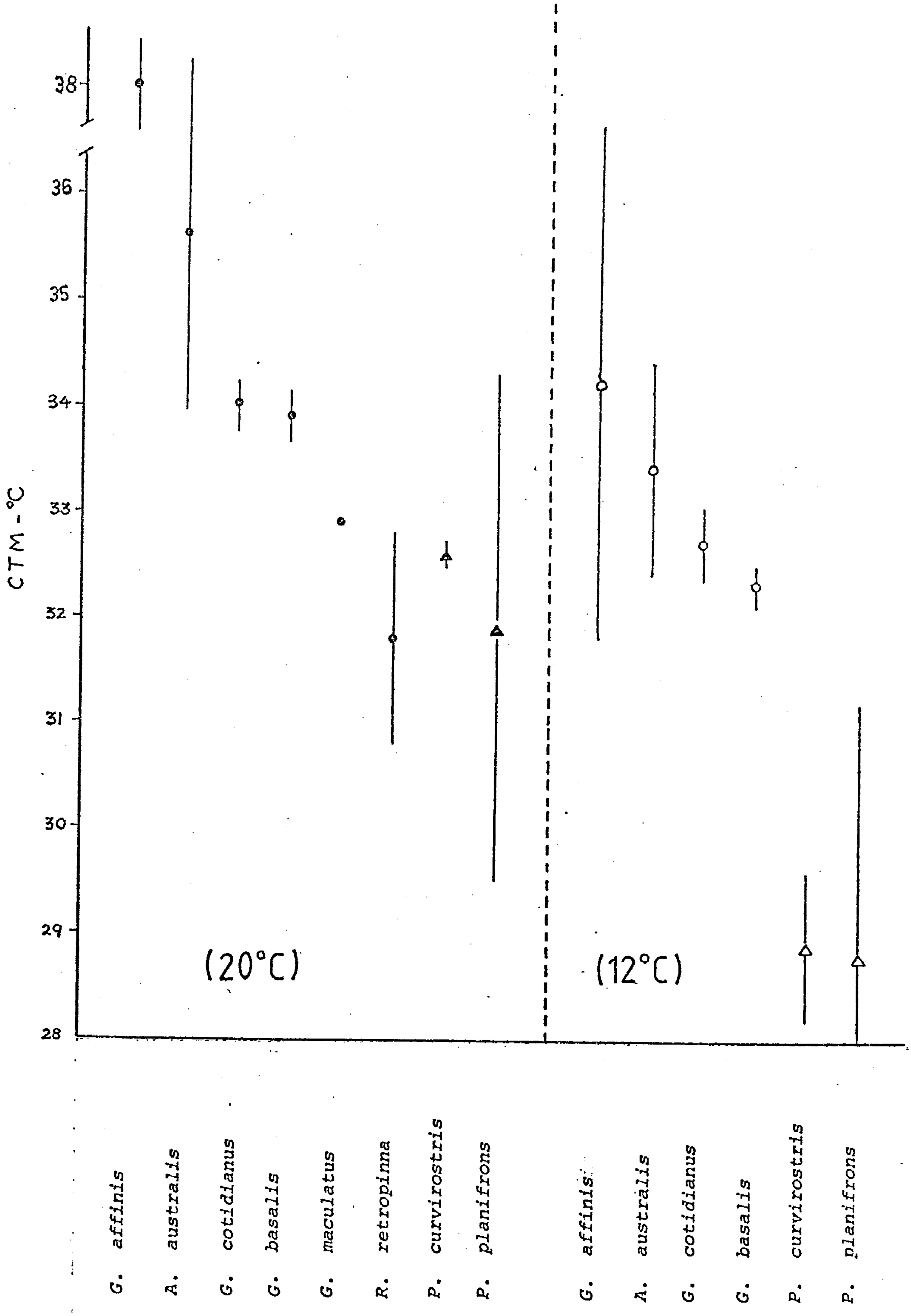

Figure 2 Mean CTM values for fish (circles) and crustaceans (triangles) following acclimation to 12°C and 20°C