

# Thermal Tolerance of *Mytilus edulis* of Eastern Australia

R. L. Wallis

School of Zoology, University of New South Wales; Kensington, N.S.W., Australia

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## Abstract

The tolerance and resistance parameters of *Mytilus edulis* were investigated using probit analysis based on the dosage-mortality response curve. The effect of thermal acclimation on incipient lethal temperatures for a range of 5° to 25°C are described. The ultimate upper incipient lethal temperature is 28.2°C. Changes in the thermal resistance parameters with size, photoperiod and salinity are shown to be statistically significant. The temperature of incapacitation of the mussel is defined in relation to the critical thermal maxima. A correlation was found between latitude and both temperature of incapacitation and temperature of spawning in the laboratory.

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## Introduction

Recent evidence indicates a growing awareness of the influence of thermal discharges on marine life and, in Australia, a growing concern for the lack of literature dealing with thermal tolerance and resistance characteristics for native species (Powles, 1972). This study examines the thermal responses of the blue mussel *Mytilus edulis* with the objectives of ecological correlation and industrial application in mind. *M. edulis* was selected because of the phenomenon of fouling in power-station intake pipes and the increasing commercial importance of this species with the recent introduction of mussel farming. Therefore, the objective of this study was to elucidate the species' tolerance range, thermal resistance characteristics, and factors which modify these responses.

## Materials and Methods

The experimental method employed was a modification of procedures for determining thermal tolerance characteristics of fish (Fry *et al.*, 1942, 1946; Brett, 1944, 1952). As a result, work carried out on *Mytilus edulis* in the Northern Hemisphere is not directly comparable, since other workers (Henderson, 1929;

Pickens, 1965; Read and Cumming, 1967) utilised a variety of methods.

## Source of Mussels

*Mytilus edulis* is common along the southern coast of Australia, reaching northward as far as Port Stephens on the East coast and Perth on the West coast (Hum, 1971). The collection site was restricted to one locality, except when latitudinal influences were being investigated. Specimens were taken as consistently as possible from the 0.1 to 0.2 m level above zero tide at the Cooks River breakwater in Botany Bay. In testing latitudinal effects, mussels were collected from sites throughout their distribution and airfreighted to the laboratory at the University of New South Wales.

## Acclimation

In determining a species tolerance range it has become necessary to stabilise the thermal history of the test animals (Fry *et al.*, 1942, 1946). The maximum deviation allowed from the set acclimation temperature was 0.5°C. The acclimation response was measured according to the method of Doudoroff (1942) by determining a series of 24-h lethal

temperatures over several days. The response approaches a constant as acclimation approaches completion, and this occurred within 14 days. This corresponds to the acclimation rates in *Mytilus edulis* measured by oxygen consumption, filtration rate, and enzymic activity (Newell and Pye, 1970; Widdows and Bayne, 1971). Consequently, all acclimation times were set at 14 days or longer.

Factors which may change the tolerance response were kept constant. These constants were: (1) length of test specimens (3 to 5 cm); (2) tidal level (0.1 to 0.2 m); (3) salinity (29 to 30‰); (4) photoperiod (normal day cycle during acclimation and 8 h light during testing); (5) latitude (same locality); (6) diet (unfed); (7) developmental state (adult); and (8) seasons (late summer to mid winter).

#### *Test Temperatures*

Test temperature tanks were housed in a laboratory set up specifically for tolerance work. Each tank was regulated so that deviation from the set temperature was less than 0.2°C. The number of mussels in each test tank was kept between 10 and 20, with a bias to 20.

#### *Recording Procedure and Analysis*

The response measured was death. Mussels failing to respond by closing on removal from the water were deemed dead, as preliminary experiments showed no sign of recovery of these mussels when returned to ambient conditions. The numbers dead in each test tank were recorded along with their lengths and weights for each observation time. The method is a modification of that suggested by Sprague (1973) for toxicity tests and differs from that of Fry *et al.* (1946), in that the determinations are based on the dosage-mortality response curve yielding the statistic LT<sub>50</sub> (Litchfield and Wilcoxon, 1949) while Fry's method is based on the time-mortality response curve yielding the median resistance time (MRT). The necessity for this change was highlighted by Waugh and Garside (1971) in that "since it is not possible to observe the moment of death of a mussel, as it is for example in fish, median resistance times cannot be computed by the method of Fry *et al.* (1946) and therefore, bioassays would be required from a broad array of fixed exposure times".

When tests for each acclimation temperature were completed and the LT<sub>50</sub>'s calculated from probit analysis of cumulative percent mortality against log test temperature for each observation time, the resistance regression equation was then computed. Each acclimation temperature has a characteristic equation which described the mussel's resistance to lethal temperatures.

#### *Modifying Factors*

Size, photoperiod and salinity were examined. In the case of size, the procedures adopted were the same as those described above. For photoperiod tests, mussels were taken directly from the environment and subjected to a constant lethal temperature (28°C) but at different photoperiods: complete light, complete dark and a normal 12 h dark:12 h light cycle. The rationale for this procedure is based on the assumption that mussels collected at the same time from the same site can be considered acclimatized to the same set of environmental factors. Discrepancies in the response under the same test conditions but with different photoperiods can, therefore, be considered as the result of photoperiodic changes.

Using this same rationale, a similar experiment was conducted using a range of salinities, 10, 15, 20, 30, 35 and 39‰, tested at a constant lethal temperature (28°C) and a constant non-lethal temperature (20°C). Mussels which had acclimated for 10 days at 20°C and at salinities of 39, 35, 30, 20 and 15‰ were tested at 28°C to determine if salinity acclimation changed the response.

#### *Critical Thermal Maxima*

Critical thermal maxima (CTM) determinations have frequently been used as an index for correlating a species maximum temperature tolerance with its distribution. A modification of the method developed by Fry (1967) was used, increasing the temperature of the water bath at a constant rate of 1°C/5 min. The 50% response level was the temperature taken for the CTM. The response recorded was incapacitation, since the moment of death of a mussel is not observable. However, as the water temperature increases, the mussels close up and, as the temperature continues to increase, they open again - the temperature of this latter response was defined as the temperature of incapacitation.

Incapacitated mussels were removed and placed at ambient temperatures and left for 15 h or more, after which the number of recoveries was recorded. This CTM procedure was utilised to detect changes in tolerance with latitude.

## Results and Discussion

### *Tolerance and Resistance*

A summary of the thermal response data obtained for *Mytilus edulis* is given in Table 1. The calculations and original data are voluminous and are presented elsewhere (Wallis, 1973). The degrees of eurythermicity was calculated according to McErlean *et al.* (1969) and was based on Fig. 3. The thermal resistance regression lines (Fig. 1) describe the change from resistance to tolerance as defined by Fry *et al.* (1946); the dashed lines horizontal to the time axis represent the maximum temperature corresponding to each acclimation temperature for which the mussels can withstand an indefinite period of exposure. This demarcates the upper limit of tolerance - the incipient lethal temperatures (Fry *et al.*, 1946). Higher temperatures are regarded as being in the zone of resistance and the mortality response in this zone is described by the resistance (solid) lines. Each line indicates the maximum time of exposure to lethal temperatures that a population of mussels can undergo before 50% die.

The response is different for each acclimation temperature, emphasising the importance of thermal history in this species. The 20°C and 25°C acclimation responses are of special interest, since the 20°C-acclimated mussels are more resistant to lethal temperatures. The decline in ability to resist lethal temperatures when acclimated at 25°C may be related to the condition of the test animals. Observations during the acclimation process revealed that, at 25°C, byssal thread formation was irregular and weaker than in those mussels acclimating at 20°C. Also mortality during the acclimation process was higher at 25°C than at any other acclimation temperature. Van Winkle (1970) found that the primary stressor affecting byssal thread growth in *Mytilus edulis* was temperature. Thus, mussels acclimated at 25°C were weakened by the temperature stress.

The incipient lethal temperatures are plotted against acclimation temperature (Fig. 2) to illustrate the toler-

ance polygon for the species. The tolerance response reveals a "plateau effect" for incipient lethal temperatures corresponding to acclimation temperatures above 20°C. These incipient lethal temperatures are equal and are designated the ultimate upper incipient lethal temperature (Brett, 1944, 1952). For *Mytilus edulis* this temperature is 28.2°C. Ninety-five percent prediction intervals were constructed from the resistance regression equation for these temperatures (see Table 1) to indicate the range of response; they are  $\pm 0.5^\circ\text{C}$  for 20°C, and  $\pm 0.9^\circ\text{C}$  for 25°C.

Resistance times of 30 and 48 h have been added to Fig. 2, to indicate the change in resistance with different acclimation temperatures. The resistance increases at a rate of 1.5°C for every 10°C increase in acclimation temperature (Fig. 2). The resistance zone thus becomes broader as the acclimation temperature increases (Fig. 3).

This type of thermal data is essential for the construction and prediction of allowable thermal limits for heated effluents from industry. Recently much emphasis has been placed on the utility of such data, by Coutant (1972), Sprague (1973) and others.

### *Size*

Size categories were arbitrarily defined as "small", length less than 3 cm; "large", length greater than 5 cm; and the intermediate range, "medium". The effect of size on tolerance was clearly demonstrated in a comparison of large and medium mussels acclimated at 20°C (Fig. 4). In the investigation both size categories were acclimated and tested in the same tanks, thereby eliminating any experimentally generated variations between the size groups.

The 95% confidence limits (obtained from the resistance regression lines) are mutually exclusive, while the resistance lines are parallel. Thus, size makes a significant difference (at the 5% level) in the level of resistance as well as tolerance. Therefore, at any lethal temperature, smaller mussels will succumb more quickly than larger mussels. From Fig. 4 it can be seen that a lethal temperature of 30°C will kill 50% of the "medium" mussels in 36 h and the same proportion of "large" mussels in 70 h. Wallis (1973) has suggested that metabolic factors which vary with age or size are the causative agents and not the structural properties, such as shell size or thickness.

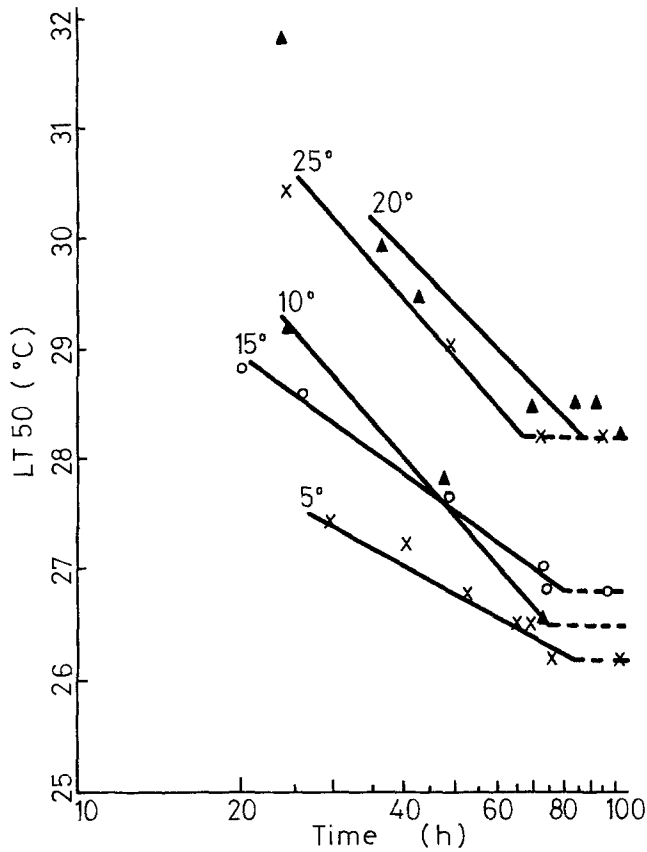


Fig. 1. *Mytilus edulis*. Resistance response (solid lines) for each acclimation temperature after probit and regression analysis. Dashed horizontal lines correspond to incipient lethal temperatures

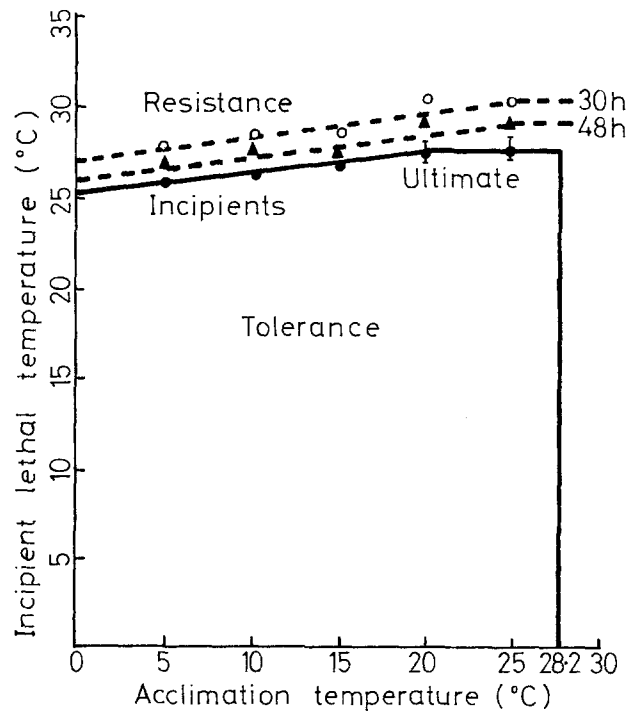


Fig. 2. *Mytilus edulis*. Tolerance polygon with 30 and 48-h resistance lines. Vertical bars represent 95% confidence limits about incipient lethal temperatures which correspond to the ultimate incipient lethal temperature (28.2)

Table 1. *Mytilus edulis*. Thermal resistance data. Sex: mixed; size: 3 cm < length < 5 cm; location: Cooks River, Botany Bay, N.S.W. at 0.1 to 0.2 m intertidal range. Upper tolerance zone: 364 units (degrees of eurythermicity)

Acclimation		Thermal resistance regression parameters <sup>a</sup>				Data limits (°C)		Incipient lethal temperature (°C)
Time (days)	Temperature (°C)	Intercept a	Slope b	N <sup>b</sup>	r <sup>c</sup>	Upper	Lower	
5	28	31.459	-2.748	7	-0.9794	28.0	22.0	26.2
10	18	36.934	-5.551	3	-0.9917	33.0	25.0	26.6
15	21	32.857	-3.128	3	-0.9170	29.6	26.0	26.8
20	20	37.993	-5.029	8	-0.9211	31.8	26.0	28.2
25	21	38.428	-5.587	4	-0.9926	31.7	27.1	28.2 (ultimate)

<sup>a</sup>Parameters based on  $LT_{50} = a + b (\log \text{ time})$ .

<sup>b</sup>Number of times for which temperature to 50% mortality was obtained.

<sup>c</sup>Correlation coefficient.

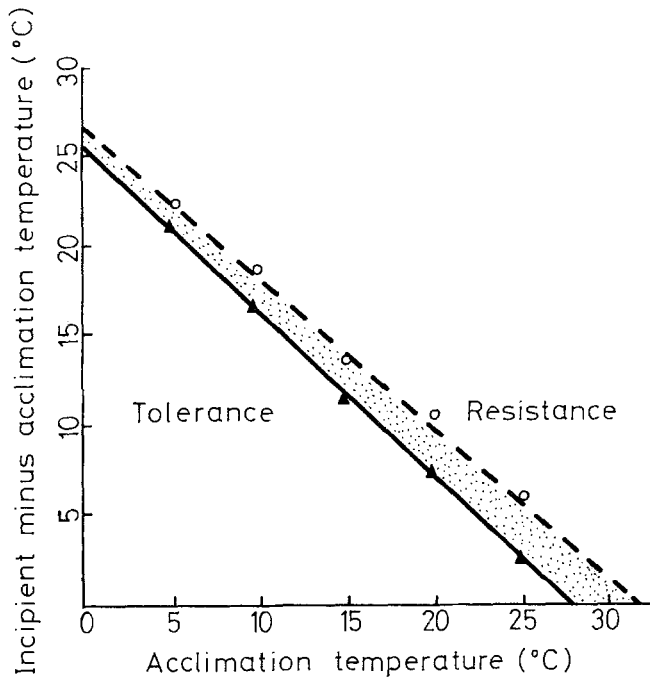


Fig. 3. *Mytilus edulis*. Upper tolerance triangle indicating an increase in resistance zone with acclimation temperature. Eurythermicity calculated from this triangle is 364 units

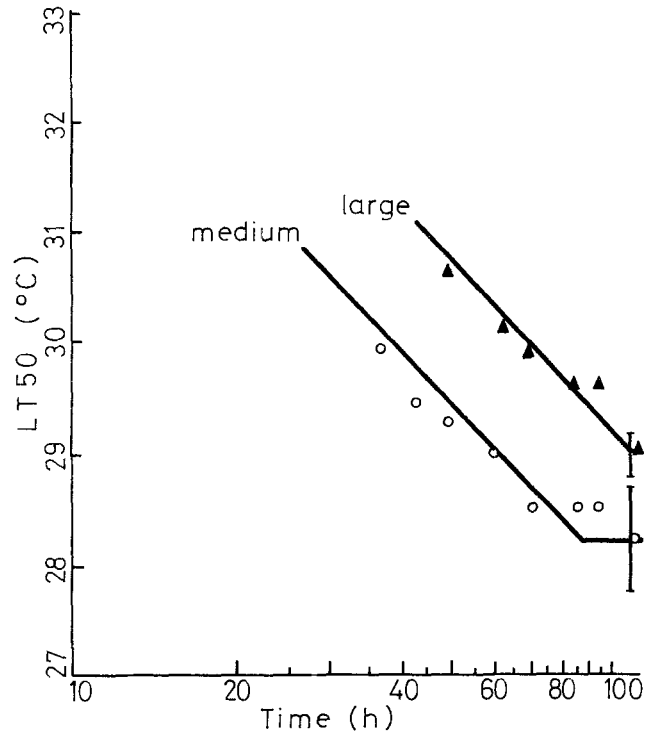


Fig. 4. *Mytilus edulis*. Resistance lines for large and medium, 20°C-acclimated mussels. Vertical bars represent 95% confidence limits about incipient lethal temperature

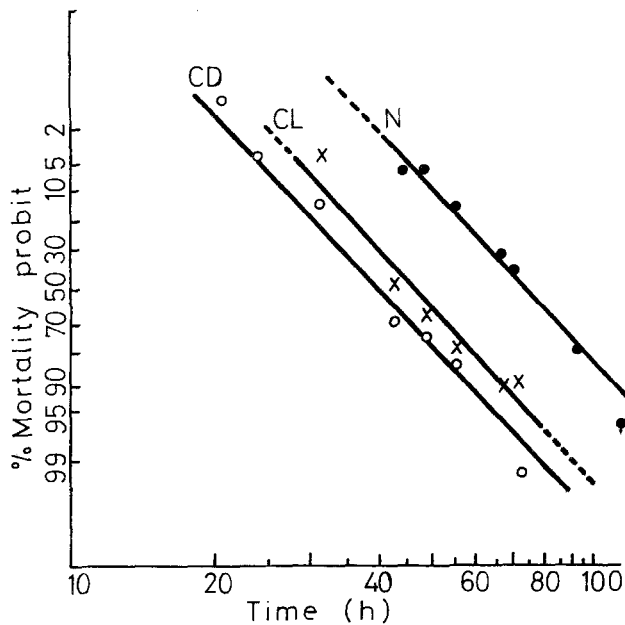


Fig. 5. *Mytilus edulis*. Resistance response of large mussels at different photoperiods to 28°C. N: normal 12 h dark/12 h light cycle; CD: complete dark; CL: complete light

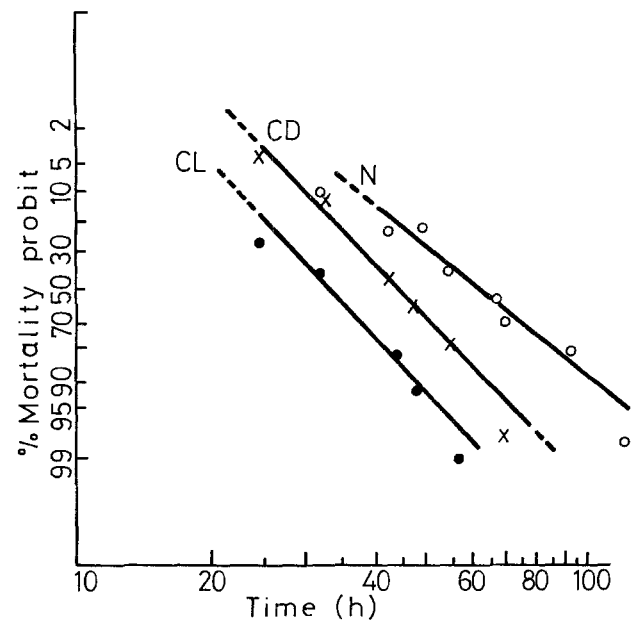


Fig. 6. *Mytilus edulis*. Resistance response of small mussels at different photoperiods to 28°C. abbreviations as in Fig. 5

Table 2. *Mytilus edulis*. Resistance response at different photoperiods including tests for significant shifts in median resistance time (MRT) and population responses of small and large mussels

No. tested	Test condition <sup>a</sup>	MRT (h)	Slope function	$\chi^2$ of line 5% level	Test of significance <sup>b</sup>	
					Population shift (U)	MRT shift (P.R.)
Small						
23	N	65	0.7683	5.07(14.1)	-	-
22	CL	33	parallel <sup>c</sup>	3.19(7.82)	22 (23)**	1.96 (1.6)*
24	CD	47.5	parallel	-	26 (21)	1.368 (1.5)
Large						
19	N	76	0.7997	1.57(12.6)	-	-
19	CL	46	parallel	1.87(11.1)	15.5(15)*	1.652(1.43)*
19	CD	42	parallel	-	17.5(17)*	no test required*

<sup>a</sup>N: normal photoperiod; CL: complete light; CD: complete dark.

<sup>b</sup>U: Test statistic of Mann-Whitney U-test; P.R.: potency ratio of Litchfield-Wilcoxon; parentheses contain values for various statistics at designated significance level.

<sup>c</sup>Response is parallel to large N response by Litchfield-Wilcoxon test for parallelism.

\* P < 5% level.

\*\* P < 2.5% level.

#### Photoperiod

For shellfish, the effects of photoperiod on heat or cold resistance do not appear to have been investigated, or at least reported. The experiments reported here (Table 2) were designed to detect any change in response that may be brought about by the use of abnormal photoperiods in the laboratory, and thereby give some idea of the importance of controlling such factors in thermal tolerance investigations.

Figs. 5 and 6 show the mortality response at 28°C for large and small mussels, respectively, under three different photoperiod regimes.

The most striking phenomenon associated with the response lines is their parallel nature. All the lines may be regarded as parallel according to the Litchfield-Wilcoxon test for parallelism (see Table 2). The parallel nature may be interpreted as evidence for the involvement of the same mechanism of thermal collapse.

The resistance time is also altered from that of the normal photoperiod in that death occurs earlier but proceeds at the same rate. The magnitude of this response was investigated in two ways, basically as a result of problems inherent in the data in relation to assumptions of normality which underlie standard statistical tests. The results

were analysed for significant shifts in the response for each photoperiod as a whole by the Mann-Whitney U-test, and significant shifts in the time to 50% mortality (median resistance times, MRT) by the Potency Ratio test of Litchfield-Wilcoxon. Using the first statistic, all but two responses differed significantly from the normal photoperiod response (N), and with the second statistic all but one response differed significantly from the normal (N) response (see Table 2).

This indicates that loss of some mechanism governed by photoperiodic regulation significantly reduces resistance to lethal temperatures. Consequently the mechanism of thermal resistance in the mussel is in some way connected to the reception of photoperiodic changes and these changes must involve a light and dark response.

The difference in response of the two size groups tested, again illustrates the effect of size on resistance. The MRT of the large mussels is approximately 11 h longer than that for the small mussels under normal light regimes.

#### Salinity

Changes in resistance to a constant lethal temperature due to salinity

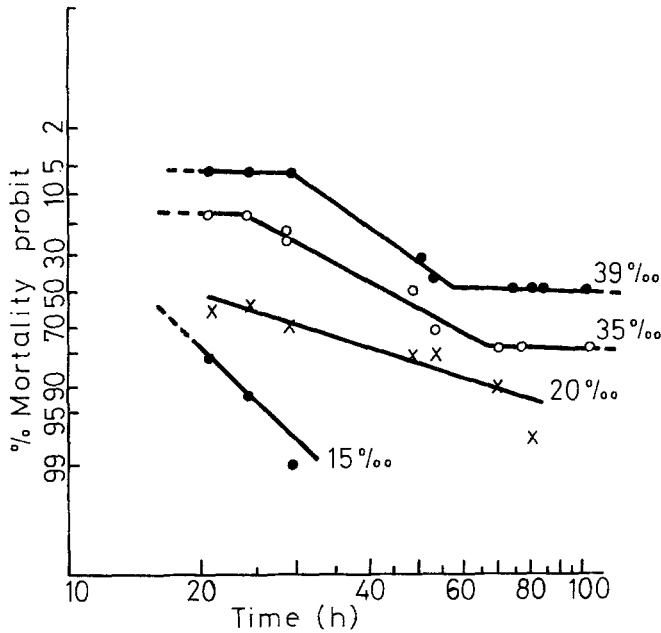


Fig. 7. *Mytilus edulis*. Resistance response at 28°C of mussels salinity-acclimated for 10 days at the indicated test salinities at 20°C

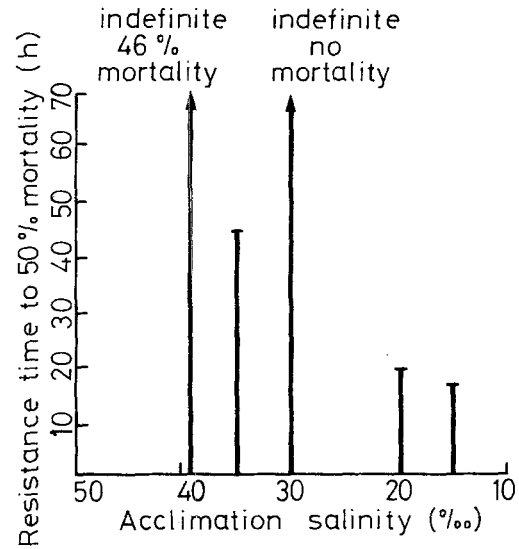


Fig. 8. *Mytilus edulis*. Relation of resistance times at 28°C to acclimation salinity

Table 3. *Mytilus edulis*. Response to a lethal temperature (28°C) at various salinities<sup>a</sup>

Salinity (%)	Acclimated	Number	MRT (h)
39	No	15	40
35	No	15	42.5
30	No	15	39
20	No	15	24
15	No	15	27
10	No	15	31
39	Yes	15	indefinite
35	Yes	13	45
30	Yes	15	indefinite
20	Yes	10	20
15	Yes	12	17

<sup>a</sup>For non-lethal temperature (20°C) no deaths were observed at any salinity by 62 h, but by Day 6, 100% mortality occurred at 10‰S.

differences were investigated (Table 3). The unacclimated mussels tested at 28°C did not have their MRT's altered in any obvious way. High salinities (30, 35 and 39‰) did appear, however, to increase resistance.

Salinity-acclimated mussels, however, gave a general trend of increasing resistance with salinity (Fig. 7). Low salinities, although homogenous in nature, have an increasing slope, while at high salinities heterogeneity is evident. Such heterogeneity is indi-

cative of changes in the mechanisms of thermal resistance (Tyler, 1966), and it appears that the higher salinities aid resistance.

This trend, however, is eclipsed by the lack of mortality in mussels acclimated at 30‰S. Thus, 30‰S may be considered the optimum for thermal resistance. The relation of salinity to resistance is shown in Fig. 8.

Latitude

The relation of latitude to tolerance was obtained by the CTM method (Table 4). The choice of this method for such an investigation results primarily from economic considerations in airfreighting mussels to the laboratory.

Using Spearman's rank correlation analysis, an inverse correlation of  $r_s = -0.463$  was found between temperature of 50% incapacitation and latitude. Therefore, tolerance declines with increase in latitude. A similar relationship for heat resistance using gill pieces of *Mytilus edulis* was found by Schlieper et al. (1967). The temperature range of incapacitation over the geographical area examined is 34.8°C at Hobart to 39.8°C at Cooks River. This represents a 5°C change in tolerance over a 10° latitudinal change and reflects a 7°C difference in the average maximum water temperatures.

The temperature of shell closure may also be significant in delimiting the

Table 4. *Mytilus edulis*. Changes in temperature response with latitudinal position. Mean annual maximum water temperatures for locations near collection sites were: Sydney, 24°C (Hum, 1971); Melbourne, 20°C (King, 1970); Hobart, 17°C (Winstanley, 1972); n.d.: no data

Location	Latitude (°S)	Tidal level (m)	Temperature of shell closure (°C)	Temperature of 50% incapacitation (°C)	Temperature of spawning (°C)
Cooks River	33° 56'	0.3	28-29	39.8	25
Jervis Bay	35° 03'	0.2	28	38.5	24-25
Batemans Bay	35° 42'	0.5	28	38.4	21
Merimbula	36° 54'	1.2	27	38.4	22
Eden	37° 04'	0.5	27	38.8	23
Lakes Entrance	37° 54'	0.2	29	37.8	19
Williamstown	37° 50'	n.d.	26	38.0	no spawning
Hobart	42° 50'	n.d.	25-26	34.8	no spawning
Perth	32°	0.2	28-29	38.4	no spawning

upper range of temperature tolerance for long-term exposures. For example, the Cooks River mussels gave a shell closure range of 28° to 29°C, while the ultimate upper incipient temperature was 28.2°C. The northern samples (Cooks River and Perth) gave 28° to 29°C for the temperature of shell closure, while the southern limit (Hobart) gave 25° to 26°C. This represents a decline of 3°C in the ultimate incipient lethal temperature over the geographic range examined. With respect to temperature of shell closure, Pickens (1965) states, "with few exceptions the temperature limits of a beating heart in an intact mussel correspond to those temperatures at which the shell closes....". Thus, the temperature limits of the beating heart, of shell closure and the ultimate upper incipient lethal temperature are all closely related.

An inverse correlation was found between latitude and the temperature at which spawning occurred during tests for incapacitation in the laboratory. The correlation coefficient was  $r_s = -0.9$  (Spearman's rank correlation analysis). Wilson and Hodgkin (1967) established a correlation between reproductive season and geographical distribution, with temperature as the determining factor. Thus, the relation of spawning temperature to latitude may be biologically important in *Mytilus edulis*.

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Mr. R.L. Wallis  
School of Zoology  
University of New South Wales  
P.O. Box 1  
Kensington, New South Wales 2033  
Australia

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