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TEMPERATURE AND TOLERANCES IN THE ABALONE, *HALIOTIS DIVERSICOLOR SUPERTEXTA* LISCHKE

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Li Lian Liu and Kun-Hsiung Chang (1987) Temperature and tolerances in the abalone, *Haliotis diversicolor supertexta* Lischke. *Bull. Inst. Zool., Academia Sinica* **26**(1): 19-25. Tolerances of the abalone, *Haliotis diversicolor supertexta* Lischke to gradual and sudden temperature changes were studied. When treated with gradual temperature change, i. e. temperature increased or decreased at a rate of 1°C/hr, the upper and lower lethal temperatures for the animals acclimated at 27°C and 21°C in different salinities were 34.8°C-37.5°C, 4.8°C-8.5°C; and 31.6°C-36.5°C 1.7°C-2.7°C respectivly. These lethal temperatures increased with the decreasing of salinity 30‰<35‰<40‰.

The treatment of sudden temperature change for 48 hours shows that the survival rate of abalone under 5°C, 10°C, 35°C with or without aeration were 0%, yet those exposed to 15°C, 20°C, 25°C and 30°C with aeration had 60% survival rate. The upper and lower critical temperature zones for animals acclimated at 27°C were 30°C-35°C and 10°C-15°C, and that for 21°C acclimated groups were 25°C-30°C and 10°C-15°C. The shortest median effective time (ET50) without aeration fell into the test groups of 5°C and 35°C, while that of the longest fell into the 15°C group.

The abalone, *Haliotis diversicolor supertexta*, is an important shellfish resource in the rocky coast of Taiwan. Although the growth oxygen consumption and artificial reproeuction have been studied (Peng *et al.* 1984; Jan *et al.* 1981, 1983; Tzeng 1975, 1977; Yang 1979). But basic physiological and ecological researches are still lacking. About the relationship of environmental factors on small abalone, only the thermal tolerance of its larval stage has been investigated (Yang, 1979). Almost no information is available on environmental tolerances of the abalone.

The present study provides comparative information on the heat and salinity tolerances of the abalone which were tested to determine the median effective time (50 ET), upper and lower lethal temperature. Because the physiological death is difficult to asses in abalone, the tentacle which did not respond to a tactile stimulus was used as the criterion for irreversible damage in these experiments.

MATERIALS AND METHODS

Adult abalone, 4-6 cm in shell length were collected along the coast of Gi-chi in the mid-eastern Taiwan during the period from August 1982 to January 1983. They

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were cultured in the laboratory for at least one week before starting the experiments. To determine the seasonal acclimation effect, the following experiments will repeat with samples acclimated at 27°C as summer temperature or acclimated at 21°C as winter temperature during the cultural period.

Use the methods of gradual and sudden temperature changes (Thompson *et al.*, 1977), the upper and lower lethal temperature were determined by gradual increasing or decreasing the temperature at the rate of 1°C/hr in different salinity treatments of 30‰, 35‰ and 40‰. UV-treated seawater was supplied to the experiments. Test containers were rectangular shaped glass, measuring 17.5×17.5×26.5 cm³. All of the experiments were done in the water bath to control temperature and the oxygen was supplied by using air pump. The temperature and numbers of death were recorded every one hour until all of the abalone were died. The above data were used to compute their upper and lower lethal temperature. The Mann-Whiteney test was used to compare the tolerance of sex difference (Conover, 1971).

Experiments were carried out by sudden temperature change method to determine the survival rate, critical temperature zone, and median effective time (50 ET). Treatments are divided into: oxygen condition (aeration, without aeration), salinity (30‰, 35‰, 40‰), and experimental temperatures (5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C). Each treatment uses one sample and has five duplicates in cylinder acrylic containers (4×4×15.5 cm³).

The numbers of death were recorded every 3-hour from 3 to 48 hours except 5°C and 35°C groups which were recorded every 15-minute. The median effective time (50 ET) for mortality be determined by Probit analysis (Finney, 1962). The unequal sample two-way ANOVA was used to compare the relations of temperature and salinity on abalone's death (Searle, 1971).

RESULTS

The results of upper and lower lethal temperature are shown in Table 1. When treated with gradual temperature change, the upper and lower lethal temperature for the animals revealed the following trends that abalone acclimated at higher temperature had higher lethal limit and which acclimated at lower temperature had lower lethal limit. As to the tolerances of salinity, the sequence is 30‰>35‰>40‰ with respect to the higher to lower lethal limit. The use of Mann-Whiteney test indicated that there was no significant sexual difference.

Use the method of sudden temperature change for 48 hours, the survival rates for each treatment were recorded in Table 2. All of the animals died when treated without aeration or with aeration but under experimental temperatures of 5°C, 10°C, and 35°C. The survival rate of other treatments ranged

TABLE 1

Upper and lower lethal temperature ($\bar{X}\pm SD$) of *Haliotis diversicolor supertexta*, treated with gradual temperature change method under different acclimation temperature and salinities. The figure in the parenthesis denotes the number of abalone under tested

Acclimated temperature (°C)	Temperature change	Lethal temperature (°C) 30%	35%	40%
27°C	Increase	37.5±0.2 (14)	37.0±0.4 (14)	34.8±1.0 (13)
	Decrease	4.8±1.2 (13)	5.3±1.4 (13)	8.5±0 (13)
21°C	Increase	36.5±0.9 (13)	34.7±0.9 (13)	31.6±0.5 (12)
	Decrease	1.7±1.0 (13)	1.8±0.9 (12)	2.7±1.8 (11)

TABLE 2
Survival rate (%) of *Haliotis diversicolor supertexta*, treated with sudden temperature change for 48 hours

Acclimation temperature (°C)	O_2 condition	Salinity (%)	Survival rate (%) 5°C	10°C	15°C	20°C	25°C	30°C	35°C	Control
27	Aeration	30	0	0	100	100	100	100	0	100
		35	0	0	100	100	100	100	0	100
		40	0	0	100	100	100	80	0	100
	Without aeration	30								
		35	0	0	0	0	0	0	0	0
		40								
21	Aeration	30	0	0	100	100	100	0	0	100
		35	0	0	100	100	60	0	0	100
		40	0	0	100	100	80	0	0	100
	Without aeration	30								
		35	0	0	0	0	0	0	0	0
		40								

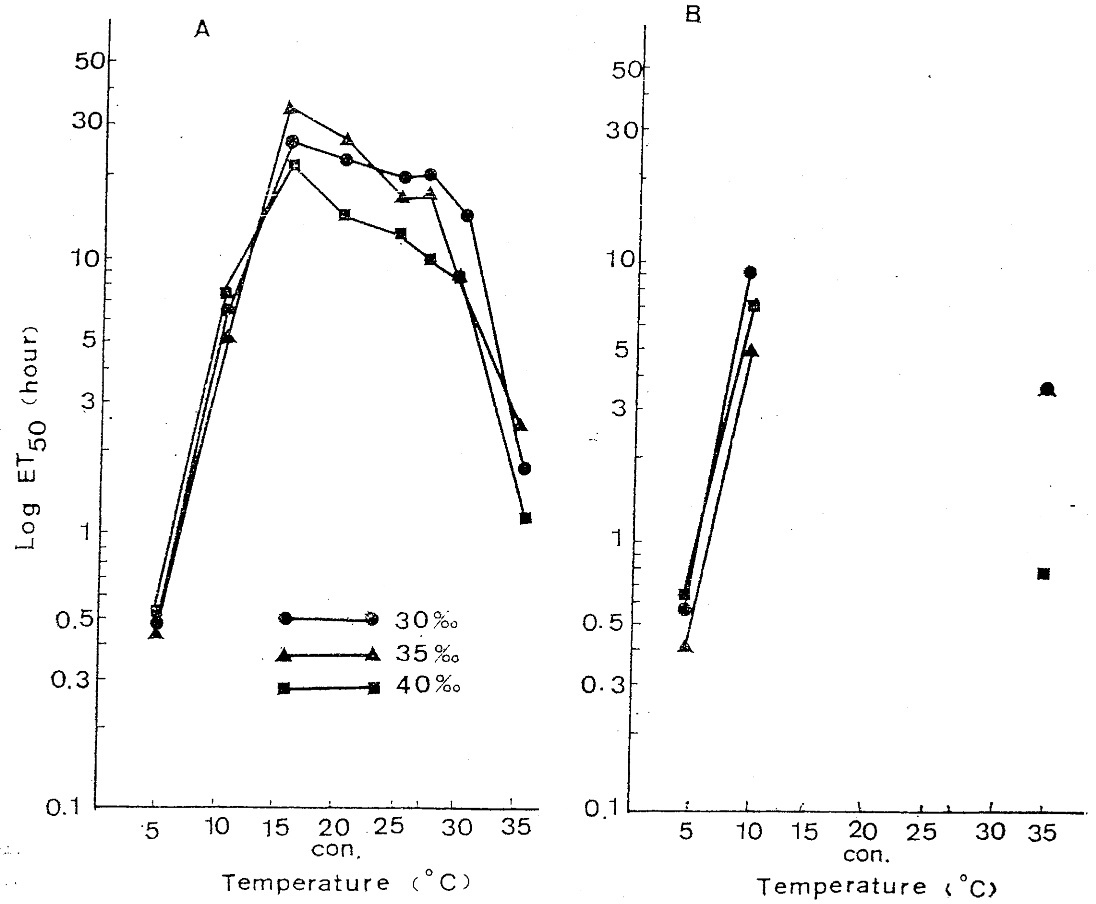

Fig. 1. The median effective time (ET_{50}) curve of abalone acclimated at 27°C in three different treatment of salinity (A) without aeration (B) with aeration.

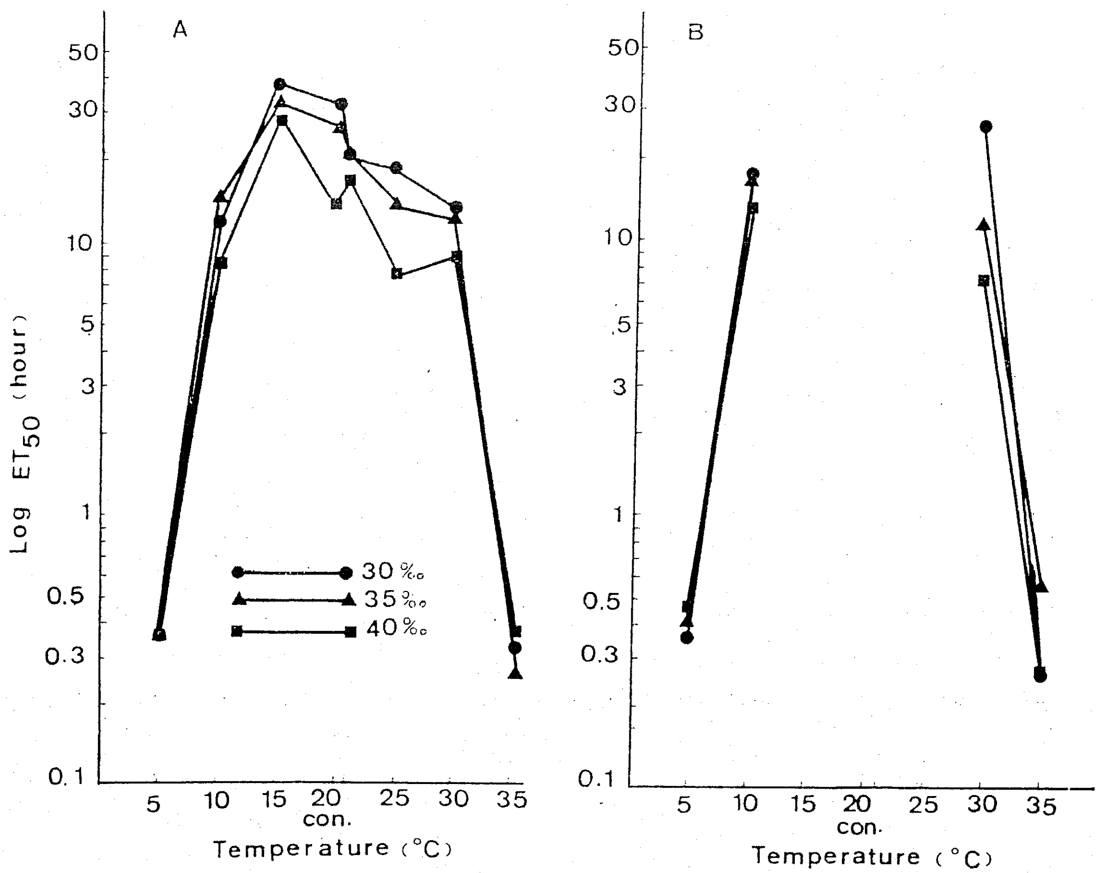

Fig. 2. The median effective time (ET_{50}) curve of abalone acclimated at 21°C in three different treatment of salinity (A) without aeration (B) with aeration.

TABLE 3

Results of two-way ANOVA to determine the effects of salinity and temperature on abalone death which acclimated at 27°C

(A) With aeration

Source	df	SS	MS	F
Temperature	2	831.59	415.80	101.67*
Salinity	2	15.03	7.51	1.84
Interaction	4	20.99	5.25	1.28
Error	34	139.05	4.09	

(B) Without aeration

Source	df	SS	MS	F
Temperature	7	20784.65	2969.23	200.58*
Salinity	2	916.01	458.00	30.94*
Interaction	14	1458.07	104.15	7.03*
Error	192	2842.23	14.80	

*: $p<0.01$

TABLE 4
Results of two-way ANOVA to determine the effects of salinity and temperature on abalone death which acclimated at 21°C

(A) With aeration

Source	df	SS	MS	F
Temperature	3	4580.24	1526.75	331.11*
Salinity	2	338.73	169.36	36.74*
Interaction	6	635.86	105.98	22.98*
Error	48	221.32	4.61	

(B) Without aeration

Source	df	SS	MS	F
Temperature	7	27876.62	3982.37	259.37*
Salinity	2	1847.87	923.93	60.17*
Interaction	12	1234.47	88.18	5.73*
Error	187	2871.22	15.35	

*: $p<0.01$

from 60% to 100%. The upper and lower critical temperatures for those abalones acclimated at 27°C with aeration are 30°C–35°C and 10°C–15°C respectively. But the upper critical temperature decreased to 20°C–25°C when changed the acclimation temperature to 21°C.

The mortality data were plotted on a probability scale versus death time and marked each treatment's ET50 on Figs. 1 and 2. To the thermal tolerances, the shortest and longest ET50 were on groups of 5°C and 15°C under the condition of acclimation at 27°C without aeration. To the groups of acclimation at 21°C without aeration, the shortest and longest 50 ET fell into 35°C and 15°C. When compared with salinity tolerance, it varies in different experimental temperature conditions. For examples, in Fig. 1 the groups of 30‰ can live longer when treated with higher temperature of 25°C or 30°C but change to the group of 35‰ when temperature is lower in 15°C or 20°C. With aeration and in despite of acclimation temperature, there still had survivors after 48 hours in groups of 15°C to 30°C. So the ET50 can't be calculated (Figs. 1, 2).

Two-way ANOVA indicated that when acclimated at 27°C with aeration the experimental temperature would affect the death of abalone significantly ($F=101.67$, $p<0.01$, Table 3). From the F-ratios, the rest conditions, including acclimated at 27°C without aeration and acclimated at 21°C no matter aerated or not, all revealed that experimental temperature also greatly affected the results than salinity, as well as the interaction between temperature and salinity was now significant (Tables 3, 4).

DISCUSSION

When change the temperatures gradually, it seems that the lower limiting temperature is generally higher in warm water forms than that in cold water ones. For the black abalone *H. cracherodii*, the 96 hours 50ET (median effective temperature) value of the 11°C acclimated group was 26.1°C, and that of 16°C acclimated group was 27.4°C (Hines, 1980). For the red abalone *H. rufescens* which treated with 23°C exhibited a lower survival rate of 20% after acclimation in colder water of 10°C and of 100% after acclimation in warmer water of 20°C (Ebert, 1974). Our results showed the similar phenomenon that the lower lethal temperatures were 4.8°C and 1.7°C for abalones acclimated at 27°C or 21°C respectively. Upper lethal temperature demonstrated the same effect (Table 1). Kinne (1963, 1970) had mentioned, that in most cases acclimation to lower temperature tends to shift lower limit downward and acclimation to high temperature tends to shift the upper limits upward.

If increased temperature suddenly, the small abalone will die on the above of 30°C for which acclimated at 27°C; whereas acclimated at 21°C the death of abalone was started on 25°C. But if the temperature was suddenly decreased, the death of both acclimated animals were under 15°C (Table 2). In other words, the small abalone has higher tolerance in the lower temperature rather than in the higher temperature when compared with its normal temperature range of living.

A complex correlation between the biological effects of temperature and salinity, or other environmental factors was also suggested in the present studies as well as previous reports (Aziz 1981, Fry 1958, Kinne 1963). Salinity can modify the effects of temperature, such as enlarge, narrow, or shift the temperature range of an organism. The temperature can modify the effects of salinity accordingly. In some species, increasing salinity has been found to increase resistance to high temperatures, while in others no effect has been found.

The survival rate of the larvae of *H. diversicolor supertexta* (1.3 mm) after rearing in different temperatures for 26 days have different results. Salinity tolerance which rearing in 27°C was 30‰>37‰>27‰>22‰, in 22°C was 37‰>27‰>32‰>22‰, and in 18°C was 22‰>27‰>32‰>37‰ (Yang, 1979). From the *F*-ratios in Table 3 and Table 4, the death of abalone affected by acclimation temperature, experimental temperature, salinity, and aeration conditions were also shown this heterogenous results.

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九孔 (*Haliotis diversicolor supertexta*) 對溫度及鹽度容忍性之研究

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本文係報導本省產之九孔 *Haliotis diversicolor supertexta* 對溫度逐漸與急遽改變之容忍程度。當溫度逐漸改變時，其速率爲每小時 1°C，則分別馴養在 27° 及 21°C 之九孔之高溫及低溫致死溫度範圍分別是 34.8°C～37.5°C、4.8°C～8.5°C，以及 31.6°C～36.5°C 及 1.7°C～2.7°C。此種致死溫度會隨鹽度之降低而增加。

在急遽改變溫度達 48 小時後，在 5°C、10°C 及 35°C 情況下之九孔不論供氧與否均悉數死亡，而在 15°C、20°C、25°C 及 30°C 供氧情況下之九孔有60%到100%的存活率。馴化在 27°C 水溫下之高低臨界溫度爲 30°C～35°C 及 10°C～15°C；馴化在 21°C 水溫下則爲 25°C～30°C 及 10°C～15°C。而未供氧之最短 ET_{50} 落在 5°C 及 35°C 之測試羣中，最長之 ET_{50} 係在 15°C 測試羣內。