Studies on the Resistance of the Pearl Oyster, *Pinctada martensii* (DUNKER), against Changes of Environment

IV. On the Tolerance of the Oyster Considered from the Relation Between the Ciliary Movement and the Heart Beat ※

By

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There are many literatures on the studies of the resistance of the shell against environmental changes, however, in those studies except a few, the quantitative measurement of the activity of that animal has not been done and the definition of the death of it was indistinct, as the determination of the death has been carried out by a whole condition of the shell.

Therefore, some easy measurement for the determination of the death of the shell is required. Recently tolerance of bivalve for the environmental changes has been brought out clearly by the physiological aspects of indicators such as ciliary or shell movements and etc.. In my previous papers, resistance of pearl oyster against changes of environment was described from the ciliary movement of the gill and the pulsation of isolated heart, respectively. But relationships in the two results, and also between the results and those of other investigators were not detailed. This paper is concerned with these relations and considered on the tolerance or suitable condition for culture and transportation of the oyster from these relations.

I wish to express my gratitude to Dr. Professor N. Y. KAWAMOTO, Mie Prefectural University and Dr. Y. MATSUI, Director of the Nippon Institute for Scientific Research on Pearls, for their supporting of my work and for their kindness in revising this manuscript.

I am also greatly indebted to Mr. K. ICHIMURA, Chief of Yamaguchi Prefectural Outer Sea Side Fisheries Research Station, for supplying me with the material for this work.

Material and Methods

Pearl oyster, *Pinctada martensii* (DUNKER), used in this work was picked up from Senzaki Bay in Yamaguchi Prefecture. Shell length of the oyster is from 6

※ Contribution from the Shimonoseki College of Fisheries No. 216.
Report of the Nippon Institute for Scientific Research on Pearls, No. 56.

to 7 cm and the age is from 2 to 3 years old. For measuring the physical activity of the ciliary movement, NOMURA-TOMITA's method was used, and the method for that of the heart beat was suspension method in the sea water in where the isolated heart was kept, and recorded on the Kymograph.

These methods employed were detailed in my previous reports. The most of experimental results in this report are quoted from the values related in those reports. However, more experiments were done again to investigate the effect of temperature on the heart beat, therefore the test of mean value was made by adding the fresh data.

Experiments were carried out in August of 1953.

Experimental Results

On the effect of temperature: All the observed values of ciliary movement and heart beat in relation to temperature are shown in Tables 1, a and b.

Table 1. a. Showing the observed values (mm/min) of speed in ciliary movement of pearl oyster in relation to temperature, and μ values calculated by Arrhenius' formula are also shown in this table.

Temp. \ Exp. No.	1	μ	2	μ	3	μ	4	μ	5	μ
°C.										
13	3.4	μ=33,900 (13–23)	—	μ=33,300 (13–22)	4.6	μ=21,200 (13–22)	—	μ=16,700 (13–21)	3.7	μ=19,900 (13–20)
14	3.5		—		6.2		—		4.3	
15	—		4.0		—		4.1		6.2	
16	4.1		—		8.3		—		6.7	
17	7.8		—		—		6.2		8.0	
18	9.4		6.7		10.6		7.8		8.4	
19	—		—		—		—		10.1	
20	14.1		12.6		13.5		—		10.5	
21	—		—		—		11.2		—	
22	—		15.5		16.7		14.2	μ=7,400 (22–31)	—	
23	19.7		18.6	μ=15,600 (23–26)	—	μ=9,200 (23–30)	—		—	
24	—	μ=8,200 (24–31)	21.0		18.5		13.9		—	
25	19.8		—		20.2		—		—	
26	20.4		24.9		—		16.8		—	
27	—		—		23.		—		—	
28	23.6		—		21.3		16.2		—	
29	—		—		24.7		—		—	
30	23.6		—		25.8		19.2		—	
31	—		—		—		20.3		—	

Each relation between temperature and activities on ciliary movement of the gill and heart beat, was considered with the application of Arrhenius' empirical formula (Fig. 1). Temperature characteristic, μ , and critical temperature resulted from the graph are shown in Tables 2 and 3.

It was tested by means of t-method under 1 % level of significance, whether the value of temperature characteristic calculated on the ciliary movement is the same or not to that calculated on the heart beat. Calculation was done as follows:-

Table 1. b. Showing the observed values (no./min) in heart beat of pearl oyster in relation to temperature, and μ values calculated by Arrhenius' formula are also shown in this table.

Temp. \ Exp. No.	1	2	3	4	5	6	7	8
°C.								
12	*	—	—	—	—	—	—	—
13	3.5	*	*	—	—	—	*	—
14	4.5	4.3	5.4	*	*	*	*	—
16	5.4	5.8	7.8	3.4	7.9	*	*	1.3
18	7.2	8.0	8.7	6.2	10.9	*	7.7	2.8
20	9.0	9.8	12.2	9.0	13.5	1.6	5.1	4.8
22	12.0	11.8	14.7	10.2	16.0	2.5	9.2	6.7
24	14.8	16.8	18.3	15.3	20.1	4.8	11.2	7.4
26	18.8	22.9	23.3	18.5	24.8	8.6	14.1	9.9
28	24.4	27.3	30.0	23.6	*	10.8	*	14.9
30	24.4	29.0	34.1	*	—	*	—	—
32	23.7	27.5	*	—	—	—	—	—
μ	μ=22,000 (13–22); μ=15,700 (24–32)	μ=22,800 (14–24); μ=12,400 (26–32)	μ=18,900 (14–20); μ=18,000 (22–30)	μ=21,600	μ=17,700	μ=43,200	μ=19,800	μ=26,200

Note : Asterisks show that observation was ceased for the irregular pulsation.

Table 2. a. Showing the test on mean of μ values calculated from ciliary movement and heart beat (in range of lower temperature) of pearl oyster.

	Value of μ ($\times 10^3$)	Numbers of individual (N)	Degrees of freedom (n)	Mean
Ciliary movement (x)	33.9, 33.3, 21.2, 16.7, 19.9	5	4	25.0
Heart beat (y)	22.0, 22.8, 18.9, 21.6 43.2, 19.8, 17.7, 26.2	8	7	24.0

From Table 2. a,

$$F = N_x(N_y-1)(S_x)^2/N_y(N_x-1)(S_y)^2 = 1.05$$

And from F-table,

$F_0 = 7.85$ (1% level of significance)

at $n_x=4$, $n_y=7$.

$\therefore F < F_0$

Therefore, hypothesis of $\sigma x = \sigma y$ can not be rejected.

Next, if homogeneity of mean is tested by t-method,

$$t = \frac{\bar{x}-\bar{y}}{S\sqrt{\frac{1}{N_x}+\frac{1}{N_y}}} = 0.215.$$

From t-table,

$t_0 = 3.106$ (1 % level of significance)

at $n=11$.

$\therefore t < t_0$

and thus, hypothesis of $\bar{x}=\bar{y}$ can not be rejected.

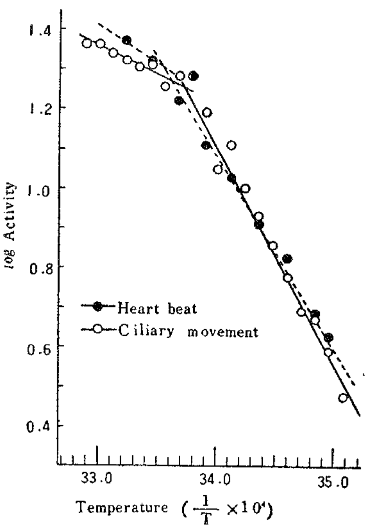

Fig. 1. Showing the application of Arrhenius' formula to the activities of heart beat and ciliary movement in pearl oyster, respectively. The abscissa represents the reciprocal of the absolute temperature.

Table 2. b. Showing the test on mean of value of μ calculated from ciliary movement and heart-beat (in range of higher temperature) of pearl oyster.

	Value of μ ($\times 10^3$)	Numbers of individual (N)	Degrees of freedom (n)	Mean
Ciliary movement (x)	9.2, 8.2, 7.4, 15.6	4	3	10.1
Heart beat (y)	18.0, 15.7, 12.4	3	2	15.4

$F = Nx(Ny-1)(Sx)^2/Ny(Nx-1)(Sy)^2 = 1.56$

And, from F-table $F_o = 99.17$ (1 %) at $nx=3$, $ny=2$.

Because $F < F_o$, hypothesis of $\sigma x = \sigma y$ can not be rejected.

Next, the test of significance of the mean values is treated by t-method.

$$t = \frac{\bar{x} - \bar{y}}{S\sqrt{\frac{1}{Nx} + \frac{1}{Ny}}} = 2.05.$$

From t-table,

$t_o = 4.032$ (1 %) at $n=5$.

$\therefore\ t < t_o$

and thus, hypothesis of $\bar{x} = \bar{y}$ can not be rejected.

According to these results, it may be said safely that no significant difference is discernible between both temperature characteristics. In range of higher temperature, values of μ on ciliary movement were larger than those reported in my previous paper,[6] but these discrepancy would depend on the results calculating from a few measurements in early experiment.

In critical temperature the similar tendency was also observed in each relation as shown in Table 3, but the strict agreement was not obtained.

Table 3. Showing the temperature (°C) of critical point taken from Arrhenius' formula as to activity of ciliary movement (C.M.) and heart beat (H. B.) of pearl oyster.

	Critical temperature (°C)			
C. M.		············ 23—20	············ 11	············ 7
		············ 23	············ 11	············ 6
		············ 20	············ 12	············ 7
		············ 24—23	············ 13	
		············ 23	············ 12	
H. B.	28—30	············ 26	············ 13	
		············ 27	············ 10	
	30	············ 24	············ 12	
		············ 24	············ 13	
	30	············ 26	············ 13	
		············ 22	············ 12	

Influences of lower temperature on the ciliary movement and the heart beat were shown in Table 4. When experiment was treated at the temperature from 6°

to 7°C., the results of ciliary movement affected with cold temperature closely corresponded in 24 hours to those of the heart beat in the same hours.

Table 4. Influence of cold treated on ciliary movement and heart beat of pearl oyster. Relative value is mean of five individuals.

	Relative value				
Time of cold (hr.)	2	5	10	16	24
Ciliary movement (Relative speed %)	103.8	68.2	22.5	19.4	0
Heart beat (Relative work %)	74	94	72	81	0

Temperature ceasing the heart beat did not strictly agree with the temperature ceasing the crawling of the gill pieces by ciliary movement, however, it is interesting that a considerable degree of similarity is found between temperatures (12°—13°C) ceasing the heart beat and the critical point (12°—13°C) in lower temperature range of the ciliary movement,

On the effect of pH : Effects of pH on the ciliary movement and the heart beat give rise to changes in the course of time, therefore those effects were measured for more than 15 hours. By the results shown in Fig. 2, the change of pH of medium gives more remarkable influences in acid side than in alkali. In both subjects there is a inhibition of 50% in acid side at below about pH 6.5, while in alkali side there is rather accelerative action than inhibition. Moreover it is clear that the tolerance of ciliary movement is slightly weaker than that of the heart beat.

On the effect of low osmotic pressure: Experimental medium used in this work was prepared from sea water diluted with distilled water and the measurement was also continued for 15—20 hours as long as in the former experiment. It appeared that the ciliary movement and the heart beat showed still considerable activity in lower osmotic medium when dilution was slowly continued step by step. The tendencies that the activities of them began to affect in dilute sea water with 60—70% and ceased in it with 30—40%, were extremely similar in both subjects (Fig. 3).

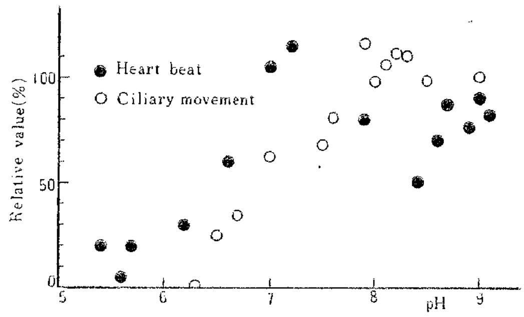

Fig 2. Influence of hydrogen ion concentration on the activities of the ciliary movement and heart beat of pearl oyster. Circles indicate the relative value after 15—20 hours.

If time for keeping in dilute sea water with 30—40%, which ceases the heart beat, is less than 24 hours, the heart beat recoveres within 10—15 hours when it is returned into the normal sea water, and also the relative work recovers to 80—

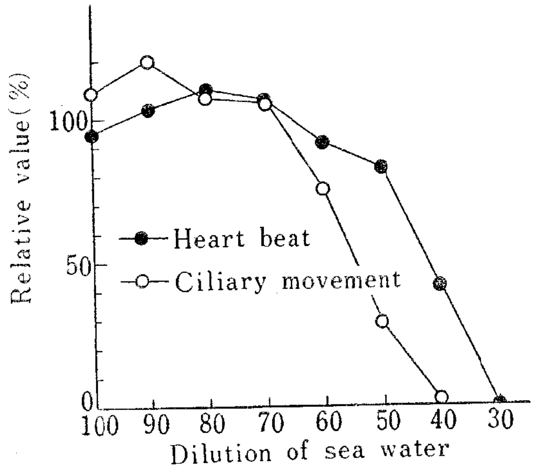

Fig. 3. Effect of dilute sea water on the ciliary movement and the heart beat of pearl oyster. The ordinate represents the relative value after 15—20 hours.

100%. It is also interesting that above mentioned result resembles the fact that gill pieces exposed to diluted sea water with 40% in 20 hours, stop their crawling by ciliary movement but recover the activity of the cilia when it is returned to normal sea water (Table 5).

Discussion

In application of Arrhenius' formula for temperature reaction, no significant differences were discernible in regard to the value of μ, temperature characteristic, between the ciliary movement and the heart beat by means of t-test.

Table 5. Recovery of ciliary movement (C. M.) and heart beat (H. B.) of pearl oyster affected by dilute sea water. (water temp. 16—21°C)

	Activity				
	in n. s. w.	in 40% s. w.	after returned to n. s. w.	Relative value %	Time submerged in 40% s. w.
C. M.	17.6 25.5	0.7 0.0	16.4 23.5	93.2 92.2	18 hrs. 〃
H. B.	77 70	0 0	79.5 56.0	102.9 80.0	5 hrs. 24 hrs.

Notes : Activity shows speed of the gill pieces in C. M. and work done by the heart in H. B., respectively.

According to CROZIER's theories it is said that biological process in temperature reaction is due to the enzyme action in protoplasma. Therefore, from the view point that temperature tolerance of living matter is based on the physical and chemical properties of protoplasma[1]), it is thought that the temperature, which is characterized by Arrhenius' formula, is a fundamental thing for the life of the shell. Because the value of μ in the ciliary movement is recognized to be the same with that in the heart beat though the value is very variable, it might be thought that the temperature reaction is somewhat in common conditioned. By above mentioned reason Arrhenius' formula was applied to consideration of suitable temperature of pearl-oyster.

When these results are compared with the temperature tolerance of pearl oyster inferred by other workers (Table 6), it is interesting that in all cases the range of suitable temperature is nearly similar and the temperature of lower limit is divided in two groups (7°C and 13°C). Because the season of experiment described in Table 6. is not the same, we have to regard the acclimatization for tolerance[7], therefore it will be thought that the temperature of lower limit is 7°C along the facts in below description. Moreover it is inferred that the suitable temperature is 13°—24°C from the following view points; pearl oyster takes hibernation during the temperature below 13°C[4], and the shell movement behaves inactively and irregularly in the temperature above 25°C.[11]

Table 6. Showing the temperature tolerance of pearl oyster inferred by various biological indicator.

	Suitable temperature (°C)	Lower limit (°C)	Observer	
Shell movement	15	13	S. KOBAYASHI,	1949
〃	25…………15		E. SAWANO,	1950
Ciliary movement	23—24…………12—13	7	Author,	1953
Heart beat	26…………12—13	12—13	〃	1955
Whole animal		7	Mie Fisheries Institute,	1927
〃		7.5—8	N. UMEBAYASHI	(Unpublished)

Moreover, in summer experiments, the heart beat ceases at about 12°C, and also the crawling of gill pieces stops at about 7°C, on the contrary in winter experiments the reverse phenomena against above results are observed as shown in the following collected data.

Table 7. Showing the comparison of the activities against lower temperature with regard to the heart beat and the ciliary movement of the gill in pearl oyster.

	Temperature (°C)	6	8	10	12	14	16	18
In summer	Ciliary movement (mm/min)		0	1.0	1.7	4.2	5.9	—
		0	1.3	3.2	5.8	8.2	11.6	13.6
		0	0.5	1.3	3.0	4.3	—	—
	Heart beat (no./min)			0	4.1	5.5	7.9	10.9
			0	*	*	4.3	5.8	8.0
				0	*	4.5	5.4	7.2
					0	5.4	7.8	8.7
In winter	Ciliary movement (mm/min)				0	5.0	8.0	11.3
				0	1.0	2.8	4.9	6.2
				0	1.3	2.6	4.0	6.0
	Heart beat (no./min)	*	4.0	4.8	5.4	6.8	8.5	
		*	3.2	4.5	5.5	7.0	8.6	13.0
		*	*	*	6.8	9.0	10.9	
		0	3.5	4.8	5.8	7.5	10.5	12.4

Note : Asterisks show the irregular pulsation.

From these facts, it may be thought that both subjects are compensated for the activity to each other in the temperature tolerance at least.

Effects of coldness on the ciliary movement and the heart beat were observed to turn inactive within 24 hours of cold treatment (Table 4). These results corresponded with the fact that death-ratio of pearl oyster exposed to the room temperature for 24 hours became to the maximum in winter. [5)]

On the effect of pH or dilute sea water, all experiments were carried out in the course of long time until the activity disappeared or took equilibrium state. On account of reasons that the penetration of hydrogen ion or water will be performed slowly and remarkable time-reduction of the activity occurs in course of experiments (KOBAYASHI and MATSUI, '53, KOBAYASHI, '55), it may be suggested possibly that about pH 7.0 is the lower limit of the oyster life from present investigation. And also from these results it becomes clear that the tendencies suffering the influences and getting the lethal pH value in acid side are very similar to both subjects. And still in case of experiment above pH 7.0, the relative value of activity is larger than that of control experiment, but these physiological significances have not been understood.

Although the relation between the results of ciliary movement and those of the heart beat showed the same tendency against dilution of medium, the heart beat bears slightly stronger tolerance than cilia for lower osmosis (Fig. 3). In both subjects the decrease of activity was observed in degrees below dilution with 70% sea water, therefore, it seems that the dilution in 70% sea water will be lower side of suitable living osmosis. Because, the activities of both subjects rather increase for dilution up to 70% of sea water, so this increasing phenomenon perhaps will be due to the excitation by lower osmosis.

In following, I have tabulated the tolerances for low osmosis investigated by various biological indicators, although the experimental conditons were not the same.

Table 8. Showing the tolerance for low osmosis in pearl oyster, inferred by various biological indicator.

Indicator	Lower limit (salinity %, or sp. gr. σ_{15})	Water temp. (°C)	Observer	
Attaching power by byssus (in young)	13.5	25—27	R. YÛKI,	1951
Ciliary movement	13.2	20—21	〃	〃
〃	13.0	20	Author,	1953
Heart beat	1.0070	26—27	N. Y. KAWAMOTO,	1954
〃	9.5	18—21	Author,	1955
Whole animal	1.010		Mie Fish. Institute,	1927
〃	1.010		N. UMEBAYASHI (Unpublished),	

Results of present experiments do not always mean the result of reaction that shell exhibits as a whole animal. But ciliary movement or heart beat is important as one of physiological factors controlling the vital activity of the shell, therefore these results will offer some fundamental data for the living conditions of pearl oyster and will give a presumption of the tolerance. Moreover these facts are convinced by the interesting comparison with results obtained by the other workers as shown in Tables 6 and 8.

There is also a remarkable agreement in the results between the ciliary movement and the heart beat, so it will be thought that the tolerance may be inferred only by the activity of ciliary movement which is easily measured.

Summary

(1) On the effects of temperature, pH, and osmosis, the relation between the ciliary movement and the heart beat of pearl oyster was investigated.

(2) There existed remarkable agreements in their relations.

(3) Based on these results and on the comparison with the results obtained by other workers, the tolerance of pearl oyster was estimated.

(4) It may be thought that the tolerance of pearl oyster can be indicated by ciliary mevement alone.

References

1) OGAWA, Y., 1952 : Mortality of pearl-oyster as influenced by low temperature in sea water. Medicine and Biology, **24** (5), 174—179, (in Japanese).

2) GALTSOFF, P. S., W. A. CHIPMAN, J. B. ENGLE and H. N. CALDERWOOD, 1947 : Ecological and physiological studies of the effect of sulfate pulp mill wastes on oysters in the York River, Virginia. Fishery Bulletin, **43**, 59—186.

3) KAWAMOTO, N. Y. and E. MOTOKI, 1954 : Influence of Diluted Sea Water on the Pearl-oyster. Report of the Faculty of Fisheries, Prefectural University of Mie, **1** (3), 346—354.

4) KOBAYASHI, S. and M. TOBATA, 1949 : Studies on Culture of Pearl. III. Activity of Pearl Oyster in Winter (1), (2). Bull. Jap. Soc. Fish., **14** (4), 196—202, (in Japanese with English summary).

5) KOBAYASHI, S., 1950 : Death-ratio, with Pearl Oyster were exposed to the Room-temperature in Winter. The Jour. Fuji Pearl Institute, **1** (2), 8—9, (in Japanese).

6) KOBAYASHI, H. and J. MATSUI, 1953 : Studies on the Resisting Power of Pearl Oyster, (Pinctada martensii) against the change of environment. I. On Ciliary Movement of the Gill. Jour. Shimonoseki College of Fish., **3** (2), 123—131, (in Japanese with English summary).

7) KOBAYASHI, H., 1955 : Studies on the Resisting power of the Pearl Oyster, Pteria (Pinctada) martensii (Dunker), against Changes of Environment. (2). On the Heart Beats. Ibid., **4** (1), 95—110, (in Japanese with English summary).

8) KOKUBO, S., T. TAMURA and K. HOJIK, 1934 : The effect of the pH on the heart beat of the oyster as determined by different kinds of acid and alkali. Jour. of Fisheries, No. 37, 1—34.

9) PROSSER, C. L., 1952 : Comparative Animal Physiology, 341—380, W. B. Saunders Co., Phyiladelphia and London.

10) NOMURA, S. and G. TOMITA, 1933 : A simple method of measuring the mechanical activity of cilia. Jour. Shanghai Sci. Inst., sec. iv, **1** (1), 29—39.

11) SAWANO, E., 1950 : Shinzyugai no Chityû-yôshoku-hô no Kenkyû (Studies on pond culture of pearl oyster). Suisankenkyukaiho, **3**, 48—57, (in Japanese).

12) TAKATSUKI, S., 1927 : Physiological studies on the rhythmical heart pulsation in the

oyster, *Ostrea circumpicta* Pils. Sci. Rep. of the Tohoku Imp. Univ. (Biol.), **2** (3), 301—324.

13) YAGI, N., T. GAMÔ, 1942 : Ondo to Seibutsu, (Temperature and Living Matte). Yôkendo, Tokyo. (in Japanese).

14) YÛKI, R. and S. KOBAYASHI, 1951 : Miscellaneous Notes on Youngs of Pearl-oyster, II. The Jour. of the Fuji Pearl Institute, **2** (1—2), 5—19, (in Japanese).

15) YÚKI, R., 1951 : Ciliary Movement of the Gill of the Pearl-oyster. I. Influence of Low Salinities. Ibid, **2** (1—2), 44—55, (in Japanese).