Response of the Heartbeat of ***Daphnia pulex*** to Variations in Physical and Chemical Surroundings

Mo-chao Liu Yu

INTRODUCTION

Daphnia is a very useful laboratory subject because it is transparent, and its internal organs can be readily observed through the transparent carapace. That is why the rate of its heartbeat is chosen for this study.

The purpose of this study is to determine the responses of :

(1) the room temperature (25°C) and low temperature (4°C).

(2) Atropine and Adrenaline,

(3) Pilocarpine and physostigmine sulphate,

(4) Potassium chloride and sodium chloride, on the heartbeat of *Daphnia pulex*.

REVIEW OF LITERATURE

The heartbeat of *Daphnia* was studied by MacArthur and Baittie in 1929. There were many factors effect upon heartbeat of *Daphnia* such as density of population, age, sex and temperature. The heartbeat was studied over a range of temperatures between 0° and 40°C. Maximal rates were recorded around 29° to 35°C; in this range some individual continued to speed up, while the beats of others slowed down, became irregular, or stopped altogether. The rate of heartbeat was comparatively slow at birth, rose rapidly for the first hours up to the seventh day, then dropped, at first rather abruptly and later very gradually until the animal was moribund. The average heartbeats per second were 1.69 at 8°, and increased to 4.26 at 18°, and 6.84 at 28°C. (MacArthur and Baittie 1929).

A very definite general correlation between temperature and rate of heartbeat prevails in Simocephalus exspinosus; the higher the temperature, the faster the heartbeat is an almost universal rule. The diversity among different individuals in rate of heartbeat at identical temperatures is much wider at high temperatures (Seiwell 1930).

The heart rate usually increases with rising temperatures within the normal environmental range. The temperature coefficient is greatest below 10°C. Values of 3 to 5 are common between 5° and 10°; between 10° and 25° the coefficient is relatively constant with an average value of about 2; above 20-25° it declines to values between 1 and 2. At some optimum temperature, often between 20° and 30° for temperate Decapoda, the heart rate becomes maximal and there after declines or becomes irregular before passing into diastolic heat arrest between 36° and 52°C. At higher temperatures the relaxed myocardium passes into heat contracture (Waterman 1960).

Atropine is the poisonous alkaloid of the deadly nightshade, makes the heartbeat faster because if neutralizes the action of acetylcholine, generated at the endings of the parasympathetic fibers in the heart. A heart asserted by muscarin, acetylcholine or pilocarpine can be revived by atropine. Physostigmine inhibits the destructive action of cholinesterase and thereby prolongs the cardiac inhibition brought about by vagal stimulation or by acetylcholine.

A heart of the type where the rhythm originates in the muscular tissue of the heart is termed myogenic such as mammal's heart. Most Crustacea have a heart of a different type, in which the beat originales in nerve cells on the wall of the heart. This is the neurogenic type. The two types of heart differ in their reactions to certain drugs. The mammalian heart is accelerated by adrenaline, which is produced at the ends of the sympathetic nerves; while it is slowed by acetylcholine, which is produced by the parasympathetic fibers.

The hearts of most Crustacea are accelerated by acetylcholine. Surprisingly they are also accelerated by adrenaline, so that here the two substances are not antagonistic. Exceptions to the general rule among the Crustacea are found in the Cladocera. Acetycholine slows the heart of *Daphnia*, so that it appears to be myogenic, but the situation is not identical with that in the mammals, for no clear effect of adrenaline has been found. (Green 1961).

In crustaceans ionic regulation is a universal phenomenon, depending on selective output of ions in excretory fluids and controlled uptake of ions by the more permeable surfaces. The body fluids of crustaceans have the same osmotic pressure as their environment. (Waterman 1960)

MATERIALS AND METHODS

(1) With a dropper, select one *Daphnia pulex* from a culture and place it on a slide, withdraw as much water as possible from the slide, leaving just enough to keep the organism alive.

(2) Examine the slide under the low power of the microscope.

(3) Observe the heartbeat very rapidly in the transparent animal and count the number of heartbeat per minute under the normal conditions (25°C) as control.

(4) Transfer it into refrigerator room (4°C). After thirty minutes count the heartbeats per minute again.

(5) Examine ten animals as before and record their heartbeats, then take the average of them.

(6) Connt the heartbeat per minute as before under the normal conditions then place a drop of Atropine solution (1M) on the *Daphnia*. Notice the reaction of *Daphnia* and count the heartbeat per minute.

(7) Examine ten animals and record the heartbeats, then take the average of the heartbeats.

(8) Calculate the standard deviation and standard error with following formula:

$$\sigma = \sqrt{\frac{\Sigma d^2}{N}}$$

$$e = \sqrt{\sigma \text{ control} + \sigma \text{ treated}}$$

(9) Use Adrenaline (0.1 and 1 M), Physostigmine sulphate (1 M), Pilocarpine (1 M), Potassium chloride (0.01, 0.1 and 1 M) and Sodium chloride (0.01 0.1 and 1 M) to treat ten *Daphnia* separately as before and record their heartbeats.

RESULT AND DISCUSSION

The result of the experiment (Table I) indicated that the temperature influences the heartbeat of *Daphnia pulex* obviously. The average rate is 269 beats per minute at 25°C; only 95 per minute at 4°C. It is much slow down when it is in low temperature, the heartbeat range from 256-282 at 25°C and from 88 to 104 at 4°C. It proves the early study (Seiwell 1930)that the higher the temperature;

the faster the heartbeat is an almost universal rule. Since the temperature increases the chemical processes of the body speed up, the metabolic rate is increased and more oxygen is required to satisfy animals' need.

Many chemicals are known to increase or decrease the rate of heartbeat. From the result of this study using Atropine to treat *Daphnia* we found Atropine accelerates the heartbeat of *Daphnia* in low concentration (0.1 M), but no effects when using 1 M Atropine. The heart of *Daphnia* is the type of neurogenic. It may be different from myogenic type.

Adrenaline is a stimulant on the myogenic heart but the result of this experiment (Table III) indicates that adrenaline (0.1 M and 1 M) has no effects on the heartbeat of *Daphnia.* According to Green's statement (1961), exceptions to the general rule among the Crustacea are found in the Cladocera. Acetycholine slows the heartbeat of *Daphnia* so that it appears to be myogenic, but the situation is not identical with that in the mammals, for no clear effect of adrenaline has been found.

Physostigmine sulphate and pilocarpine are strong postganglionic stimulation which also produces minor stimulation at other chalinergic endings. From the result of this study, (Table VI and VII) it shows decrease in the rate of heartbeat of *Daphnia* The control animal heartbeat is 270 per minute, but after treated it slows down to 176 per minute.

The effects of potassium chloride and sodium chloride are various by the different concentrations on the heartbeat of *Daphnia*. The result of this study shows (Table VIII, IX, X, XI, XII, and XIII) the low concentration of potassium chloride or sodium chloride has no effects on the heartbeat of *Daphnia* but both 1 M potassium chloride and sodium chloride slow down the rate of heartbeat rapidly and all animals died after treated. *Daphnia*, like other fresh water animals, always keep their fluids hyperosmotic to the medium. They face with a continuous osmotic inflow of water which they have to excrete, and with the problem of active absorption of iron from a very dilute medium to replace those lost by outward diffusion and excretion. The high concentration of potassium chloride and sodium chloride unbalance the osmotic uptake of water, the *Daphnia* hardly exist in this situation.

Table I. Effect of Temperature(25°C and 4°C) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (25°C)	d	d^2	Heartbeats per minute (4°C)	d	d^2
1	272	-3	9	94	1	1
2	256	13	169	96	-1	1
3	262	7	49	98	-3	9
4	280	-11	121	99	-4	16
5	276	-7	49	104	-9	81
6	268	1	1	95	0	0
7	282	-13	169	93	2	4
8	274	-5	25	91	4	16
9	257	12	144	88	7	49
10	263	6	36	92	3	9
			772			186

$X = 269$ $\qquad X = 9.5$

$$6_{25°C} = \sqrt{\frac{772}{10}} = \sqrt{77.2} = 8.7$$

$$6_{4°C} = \sqrt{\frac{186}{10}} = \sqrt{18.6} = 4.3$$

$$e = \sqrt{8.7+4.3} = \sqrt{13} = 3.6$$

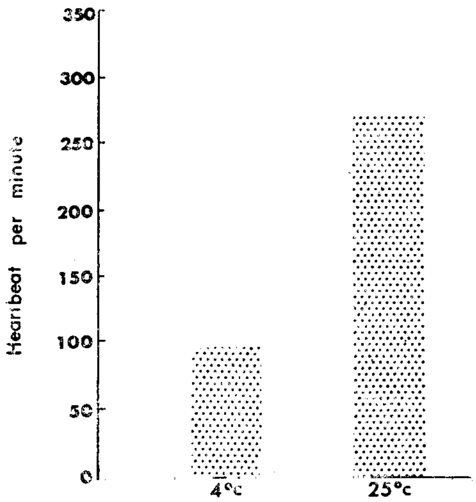

Fig. 1. Effect of Temperature on Heartbeat in Daphnia

Table II. Effect of Atropine (0.1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.1M)	d	d^2
1	264	-8	64	312	13	169
2	252	4	16	320	5	25
3	244	12	144	328	-3	9
4	260	-4	16	334	-9	81
5	260	-4	16	316	9	81
6	250	6	36	322	3	9
7	240	16	256	324	1	1
8	258	-2	4	336	-11	121
9	275	-19	361	330	-5	25
10	257	-1	1	330	-5	25
			914			546

$\bar{X} = 256$ $\quad\quad$ $\bar{X} = 325$

$$\sigma_c = \sqrt{\frac{914}{10}} = \sqrt{91.4} = 9.5$$

$$\sigma_{0.1M} = \sqrt{\frac{546}{10}} = \sqrt{54.6} = 7.4$$

$$e = \sqrt{9.5+7.4} = \sqrt{16.9} = 4.1$$

Table III. Effect of Atropine (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d²	Heartbeats per minute (1M)	d	d²
1	198	4	16	212	-6	36
2	214	-12	144	222	-16	256
3	190	12	144	200	6	36
4	194	8	64	195	9	81
5	202	0	0	198	8	64
6	210	-8	64	212	-6	36
7	199	3	9	214	-8	64
8	198	4	16	197	9	81
9	206	-4	16	198	8	64
10	210	-8	64	212	-6	36
			537			754

$\bar{X} = 202$ $\quad\quad$ $\bar{X} = 206$

$$\sigma_c = \sqrt{\frac{537}{10}} = \sqrt{53.7} = 7.3$$

$$\sigma_{1M} = \sqrt{\frac{754}{10}} = \sqrt{75.4} = 8.7$$

$$e = \sqrt{7.3+8.7} = \sqrt{16} = 4$$

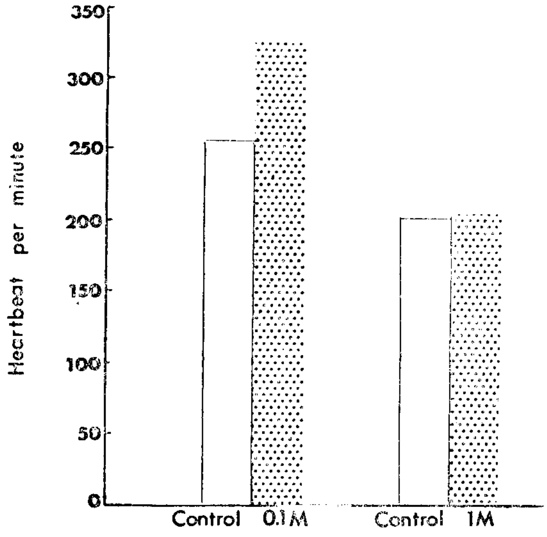

Fig. 2. Effect of Atropine (0.1M and 1M) on Heartbeat in Daphnia

Table IV. Effect of Adrenaline (0.1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.1M)	d	d^2
1	268	-10	100	258	-1	1
2	249	9	81	252	5	25
3	255	3	9	249	8	64
4	270	-12	144	270	-13	169
5	250	8	64	259	-2	4
6	264	-6	36	263	-6	36
7	271	-13	169	261	-4	16
8	254	4	16	254	3	9
9	251	7	49	249	8	64
10	249	9	81	252	5	25
			749			413

X=258 X=257

$$\sigma_c = \sqrt{\frac{749}{10}} = \sqrt{74.9} = 8.6$$

$$\sigma_{0.1M} = \sqrt{\frac{413}{10}} = \sqrt{41.3} = 6.4$$

$$e = \sqrt{8.6+6.4} = \sqrt{15} = 3.8$$

Table V. Effect of Adrenaline (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (1M)	d	d^2
1	258	-4	16	260	-6	36
2	252	2	4	242	12	144
3	248	6	36	250	4	16
4	268	-14	196	265	-11	121
5	254	0	0	248	6	36
6	258	-4	16	270	-16	256
7	254	0	0	256	-2	4
8	242	12	144	248	6	36
9	253	1	1	251	3	9
10	244	10	100	250	4	16
			513			674

X=254 X=254

$$\sigma_c = \sqrt{\frac{513}{10}} = \sqrt{51.3} = 7.1$$

$$\sigma_{1M} = \sqrt{\frac{674}{10}} = \sqrt{67.4} = 8.2$$

$$e = \sqrt{7.1+8.2} = \sqrt{15.3} = 3.9$$

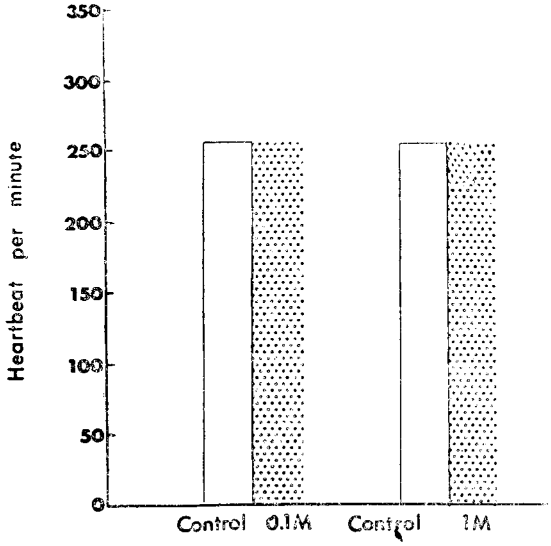

Fig.3. Effect of Adrenaline (0.1 and 1M) on Heartbeat in Daphnia

Table VI. Effect of Physostigmine (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (1M)	d	d^2
1	254	-12	144	184	-9	81
2	248	-6	36	188	-13	169
3	242	0	0	168	7	49
4	240	2	4	180	-5	25
5	229	13	169	159	16	256
6	238	4	16	158	17	289
7	242	0	0	181	-6	36
8	250	-8	64	175	0	0
9	251	-9	81	180	-5	25
10	230	12	144	173	2	4
			658			934

$X = 242$ $X = 175$

$$6_c = \sqrt{\frac{658}{10}} = \sqrt{65.8} = 8.1$$

$$6_{1M} = \sqrt{\frac{934}{10}} = \sqrt{93.4} = 9.6$$

$$e = \sqrt{8.1 + 9.6} = \sqrt{17.7} = 4.2$$

Table VII. Effect of Pilocarpine (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (1M)	d	d^2
1	271	-1	1	170	6	36
2	280	-10	100	190	-14	196
3	259	11	121	178	-2	4
4	258	12	144	174	2	4
5	281	-11	121	184	-8	64
6	282	-12	144	172	4	16
7	271	-1	1	170	6	36
8	262	8	64	I58	18	324
9	275	-5	25	188	-12	144
10	261	9	81	176	0	0
			802			824

$$X = 270 \qquad X = 176$$

$$6_c = \sqrt{\frac{802}{10}} = \sqrt{80.2} = 8.9$$

$$6_{1M} = \sqrt{\frac{824}{10}} = \sqrt{82.4} = 9.06$$

$$e = \sqrt{8.9+9.6} = \sqrt{17.96} = 4.2$$

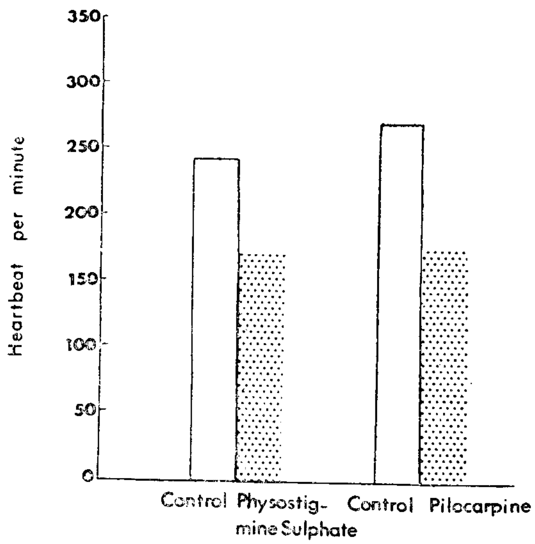

Fig. 4. Effect of Physostigmine Sulphate and Pilocarpine on Heartbeat in Daphnia

Table VIII. Effect of Potassium Chloride (0.01M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.01M)	d	d^2
1	278	15	225	280	16	256
2	282	11	121	289	7	49
3	282	11	121	288	8	64
4	302	-9	81	299	-3	9
5	304	-11	121	309	-13	169
6	300	-7	49	301	-5	25
7	286	7	49	298	-2	4
8	302	-9	81	302	-6	36
9	298	-5	25	300	-4	16
10	296	-3	9	294	2	4
			882			632

X = 293 X = 296

$$\sigma_c = \sqrt{\frac{882}{10}} = \sqrt{88.2} = 9.4$$

$$\sigma_{0.01M} = \sqrt{\frac{632}{10}} = \sqrt{63.2} = 7.9$$

$$e = \sqrt{9.4+7.9} = \sqrt{17.3} = 4.1$$

Table IX. Effect of Sodium Chloride (0.01M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.01M)	d	d^2
1	320	-11	121	304	-2	4
2	300	9	81	303	-1	1
3	302	7	49	316	-14	196
4	308	1	1	298	4	16
5	302	7	49	299	3	9
6	311	-2	4	300	2	4
7	304	5	25	298	4	16
8	327	-18	324	309	-7	49
9	305	4	16	306	-4	16
10	311	-2	4	288	4	16
			674			327

X = 309 X = 302

$$\sigma_c = \sqrt{\frac{674}{10}} = \sqrt{67.4} = 8.2$$

$$\sigma_{0.01M} = \sqrt{\frac{327}{10}} = \sqrt{32.7} = 5.7$$

$$e = \sqrt{8.2+5.7} = \sqrt{13.7} = 3.7$$

Table X. Effect of Potassium Chloride (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d²	Heartbeats per minute (1M)	d	d²
1	293	6	36	124	-15	225
2	298	1	1	96	15	225
3	300	-1	1	101	8	64
4	308	-9	81	102	7	49
5	320	-21	441	110	-1	1
6	309	-10	100	120	-11	121
7	283	16	256	108	1	1
8	296	3	9	101	8	64
9	294	5	25	112	-3	9
10	289	10	100	118	-9	81
			1050			840

X=299 X=105

$$6_c = \sqrt{\frac{1050}{10}} = \sqrt{105} = 10.2$$

$$6_{1M} = \sqrt{\frac{840}{10}} = \sqrt{84} = 9.1$$

$$e = \sqrt{10.2+9.1} = \sqrt{19.3} = 4.4$$

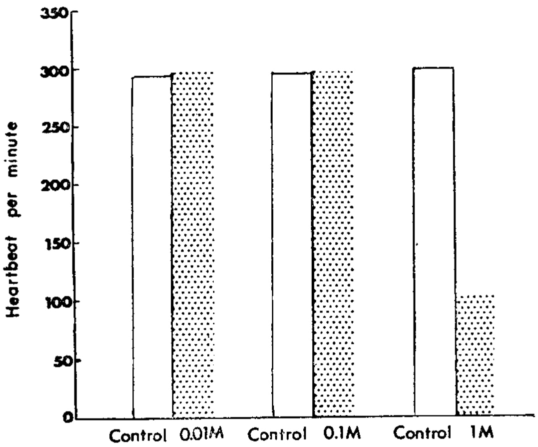

Fig. 5. Effect of Potassium Chloride (0.01M, 0.1M and 1M) on Heartbeat in Daphnia

Table XI. Effect of Potassium Chloride (0.1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.1M)	d	
1	280	14	196	279	16	256
2	304	-10	100	292	3	9
3	284	10	100	292	3	9
4	302	-8	64	300	-5	25
5	288	6	36	298	-3	9
6	288	6	36	296	-1	1
7	296	-2	4	289	6	36
8	301	-7	49	302	-7	49
9	300	-6	36	303	-8	64
10	296	-2	4	300	-5	25
			625			483

X=294 X=295

$$\sigma_c = \sqrt{\frac{625}{10}} = \sqrt{62.5} = 7.8$$

$$\sigma_{0.01M} = \sqrt{\frac{483}{10}} = \sqrt{48.3} = 6.9$$

$$e = \sqrt{7.8+6.9} = \sqrt{14.7} = 3.8$$

Table XII. Effect of Sodium Chloride (0.1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (0.1M)	d	d^2
1	319	-10	100	304	-1	1
2	310	-1	1	303	0	0
3	302	7	49	316	-13	169
4	308	1	1	298	5	25
5	302	7	49	299	4	16
6	312	-3	9	300	3	9
7	304	5	25	298	5	25
8	317	-8	64	309	-6	36
9	305	4	16	306	-3	9
10	311	2	4	299	4	16
			318			306

X=309 X=303

$$\sigma_c = \sqrt{\frac{318}{10}} = \sqrt{31.8} = 5.6$$

$$\sigma_{0.1M} = \sqrt{\frac{306}{10}} = \sqrt{30.6} = 5.5$$

$$e = \sqrt{5.6+5.5} = \sqrt{11.1} = 3.3$$

Table XIII. Effect of Sodium Chloride (1M) on Heartbeat in *Daphnia*

Animal No.	Heartbeats per minute (control)	d	d^2	Heartbeats per minute (1M)	d	d^2
1	318	-11	121	136	-5	25
2	303	4	16	126	5	25
3	315	-8	64	137	-6	36
4	299	8	64	139	-6	36
5	300	7	49	138	-7	49
6	298	9	81	133	-2	4
7	305	2	4	127	4	16
8	309	-2	4	117	14	196
9	311	-4	16	123	8	64
10	312	-5	25	136	-5	25
			444			476

$\bar{X}=307$ $\bar{X}=131$

$$6_c = \sqrt{\frac{444}{10}} = \sqrt{44.4} = 6.7$$

$$6_{1M} = \sqrt{\frac{476}{10}} = \sqrt{47.6} = 6.9$$

$$e = \sqrt{6.7+6.9} = \sqrt{13.6} = 3.7$$

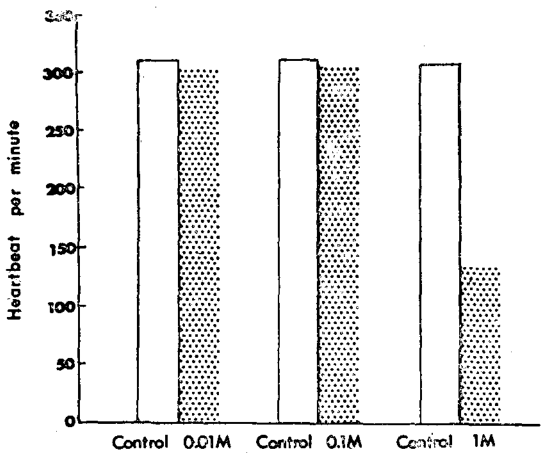

Fig. 6. Effect of Sodium Chloride (0.01M, 0.1M and 1M) on Heartbeat in Daphnia

CONCLUSION

It may be concluded that the heartbeat of *Daphnia* is influenced by physical surrounding as well as chemical surrounding.

The significant different results occured in each of my experiments:

1. The heartbeat of *Daphnia* varies in the different temperatures, the higher the temperature, the faster the heartbeat. The range is from 88 (4°C) to 282 (25°C) per minute.
2. Treated with 1M Atropine, there were no apparent effects, but treated with 0.1M Atropine, the heartbeat of *Daphnia* increased from 256 to 325 per minute.
3. When 0.1M and 1M Adrenaline were treated on *Daphnia*, there are no effects on the heartbeat at all.
4. When 1M Physostigmine sulphate and Pilocarpine were applied to *Daphnia*, there was an obvious decrease in the heartbeat.
5. Using the different concentrations of potassium chloride (0.01, 0.1 and 1M) treated *Daphnia*, there were no effects on the heartbeat, but 1M potassium chloride and 1M sodium chloride decreased the heartbeat rapidly and all animals died immediately.

SUMMARY

The heartbeat of *Daphnia* varies in physical and chemical surroundings. The higher the temperature, the faster the heartbeat is an almost universal rule.

There are no apparent effects on the heartbeat of *Daphnia* when it is treated by the following solutions:

Atropine (1M)
Adrenaline (o.1 and 1M)
Potassium chloride (0.01 and 0.1M)
Sodium chloride (0.01 and 0.1M)

Treated with 0.1M Atropine on *Daphnia*, the heartbeat increased from 256 to 325 per minute.

Applying 1 M Physostigmine sulphate and Pilocarpine to *Daphnia*, the heartheat decreased obviously.

ACKNOWLEDGEMENTS

I wish to express my gratitude especially to Dr. A.M. Winchester for his invaluable advise and for his excellent photography. Gratitude must likewise be extened to Mr. Paul Richard for supplying me the cultured *Daphnia pulex*.

LITERATURE CITED

BSCS group, 1963.
Laboratory Investigations for Biological Science: Molecule to Man. Boston: Houghton Mifflin, American Institute Biological Science, L.93.

Cutting, Windsor C, 1967.
Handbook of Pharmocology: The Actions and Uses of Drugs. Appleton.

Green. J., 1961.
A Biology of Crustacea. Chicago: Quadrangle Books, p. 48.

Ivie, J.N., 1967.
The Effects of Sodium Fluoride and Temperature on Diapause in *Daphnia pulex*. (Unpublished thesis)

MacArthur, J.W. and W.H.T. Baittie 1929,
Metabolic Rates and their relation to longevity in *Daphnia magna*. J. Exptl. Zool, 53:243-268.

Prosser, C. L. ete el. 1952.
Comparative animal physiology, Philadelphia & London: W.B. Saunders.

Seiwell, H.,R., 1930.
Influence of Temperature on the Rate of Beating of the Heart of a *Cladoceran*. J. Exptl. Zool., 57: 331-346.

Thimann. K.V., 1952.
The Action of Hormones in plants and Invertebrates, New York: Academic Press Inc. P. 205.

Waterman, T.H. 1961

The Physiology of Crustaces: Metabolism and Growth. New Bork, Academic Press, pp. 196-197.

Wilmoth, J.H. 1967.
Biology of Invertebrata. New Jersey: Prentice-Hall, Inc.

中 文 摘 要

物理因素及化學因素對水蚤心跳的影響

劉 慕 昭

本研究以水蚤 *Daphnia pulex* 爲材料，試驗溫度及各種化學物質對於心跳的影響。用室溫25°C 處理則每分鐘心跳269次，如以低溫 4°C 處理則降爲95次。用 Atropine (1M)， Adrenaline (0.1及1M) Potassium chloride (0.01及0.1M)， Sodium chloride (0.01 及0.1M) 分別處理無顯著反應，但用 0.1M Atropine 處理則能促進心跳，用 1M Physostigmine sulphate & Pilocarpine分別處理，則會減少每分鐘之次數。