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ON THE MASS REACTIONS OF GOLD FISH AND CARP AGAINST THE TOXIC SUBSTANCE

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Against the similar intensity of stimulation, the mass reaction of animal aggregation varies in accordance with its density. Here, the present authors worked out the mass reaction of gold fish and carp against the toxic effect of sublimate solution.

Material and method. Gold fish used, ranging from 0.8 to 7.8 g in body weight, averaged 3 to 4 g. Carp, ranging from 1.2 to 11.9 g, averaged 4 to 5 g. They were previously trained for a life in a small aquarium before the experiment. As a stimulating substance, the sublimate solutions of various concentrations from 0.1×10^{-4} to 10×10^{-4} normal were employed. The cylindrical glass bath, 30 cm in diameter and 15 cm in height, is filled up with 5 liters of the sublimate solution. For killing, gold fish or carp is put into this solution either singly or together each 2, 5, 10, 20, 30 and 100 fish respectively. The toxic effect is expressed with the mean death time (T measured in minute) of N fish used singly or together. The fish is considered as dead when it lies down on the bottom and shows no reaction against the stimulation upon the eye with the pointed end of a glass rod. Death time (T_n) is the duration of time in which the fish can live after it is put into the toxic solution. Mean death time (T) is calculated from $\Sigma T_n/N$, the lag period (Tmin) is the minimum death time expended and the death interval is Tmax$-T$min, where Tmax is the maximum death time. Death process of N fish group as a whole is characterized with T, Tmin and Tmax$-T$min.

In independent experiment, fresh sublimate solution of 5 liters is used in every experiment, while in repeated experiments the sublimate solution is used repeatedly till the toxic effect becomes unrecognizable. Temperature (θ°C), pH-value, O_2-content (%) and consumed amount of $KMnO_4$ (mg/l) are measured at every experiment. The sublimate concentration is measured colorimetrically with the diphenylcarbazone method.

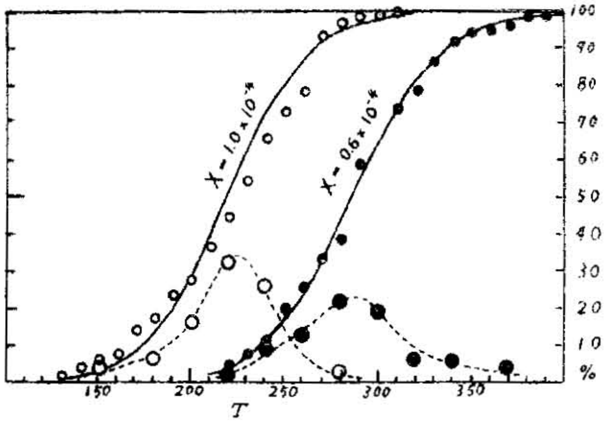

Fig. 1. Death process of a group of 30 gold fish (small circles) and the change in death velocity (large circles, see Appendix 5).

Result. The death process of N fish group follows generally to Robertson's law (Fig. 1 and Table 1) The mean death time is nearly similar to the time expended from the time that they were put into the toxic solution until half of the number of fish used ($N/2$) have died. The mean death time differs not only among the groups of different individual numbers but also in the sublimate solutions of different concentrations. In addition, death time seems dependent upon the body weight of fish and the temperature of solution. The greater the weight of the fish is, the more the death time is prolonged (Fig. 2) Nevertheless, the individual variation in body weight is considered as negligible, so far as we are coneerned. On the other hand, the temperature of the solution is a decisive factor in determining the death time. The relation of the temperature (θ°C) to the death time (T) can be fit to the Arrhenius' formula (Fig. 3). Critical temperature is 10°C or thereabouts both for gold fish and for carp. The coefficient of this formula above 10°C differs from that under 10°C as below

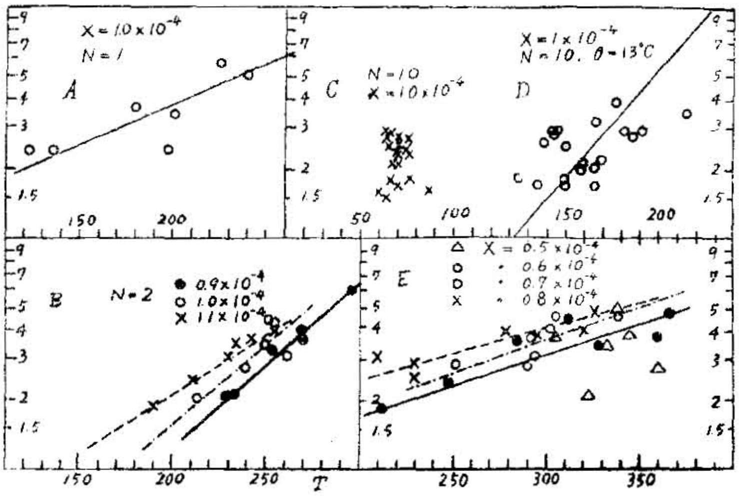

Fig. 2. Relation of body weight (g) to the death time (T in minute) in gold fish (see Appendix I–V).

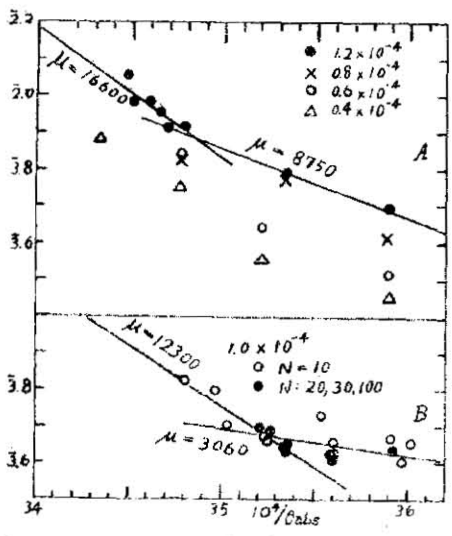

Fig. 3. The relation between the absolute temperature (θ abs.) and the mean death time (T). A: Carp, B: Gold fish.

	sublimate concentration	temperature of solution $<10°C$	$>10°C$
Gold fish	1.0×10^{-4}	$\mu=12300$	$\mu=3060$
Carp	$0.4\times10^{-4}-12\times10^{-4}$	$\mu=16600$	$\mu=8750$

Table 1. Death process of groups of N gold fish.

Temperature (θ°C)	>10°C				<10°C				
X (10^{-4} norm.)	N=1	10	30	100	30	10	30	100	100
T(in minute)	%	%	%	%	%	%	%	%	%
100	5.0	1.3		3					
120	7.5	11.2		4		2.9	0.8		
140	7.5	20.0		—		7.1	3.3	10	
160	7.5	13.7	10.0	13		14.3	3.3	8	
180	20.0	18.8	6.7	24		11.4	10.8	6	
200	20.0	17.5	16.7	27		12.8	15.9	14	2.4
220	17.5	10.0	33.3	26		10.0	25.0	9	5.9
240	7.5	7.5	26.7	3	3.3	11.4	17.5	25	17.6
260	5.0		3.3		10.0	8.6	20.0	23	11.8
280	2.5		3.3		13.3	5.7	2.6	4	30.4
300					23.3	2.9	0.8	1	7.1
320					20.0	2.9			16.4
340					6.7	4.3			2.4
360					6.7	—			2.4
380					—	5.7			1.2
400					10.0				2.4
420					6.7				

Hence the death times must be compared with each other only either among those obtained from the experiments carried out above 10°C or among those under 10°C respectively. As is seen in Table 1, so far as the number of fish used remains constant, the mean death time is smaller and the death interval (Tmax $-$ Tmin) is shorter under the high temperature than under the low. In addition, the mean death time seems to be prolonged proportionally to the increase in the number of fish used and this tendency is clearly intensified more under the low temperature than under the high. When the temperature is sufficiently high, the death time is not much prolonged according to the increase in the number of fish used together. Therefore, the fish seem very feeble against the toxic effect under high temperature.

When the number of individuals used together (N) remains unchanged, the mean death time is in inverse ratio to the sublimate concentration (X) and the death process progresses more slowly in accordance with the decrease in the sublimate concentration (see Fig. 1) The relation between the mean death time (T) and the sublimate concentration (X) is shown with Ostwald's formula as below (Fig. 4)

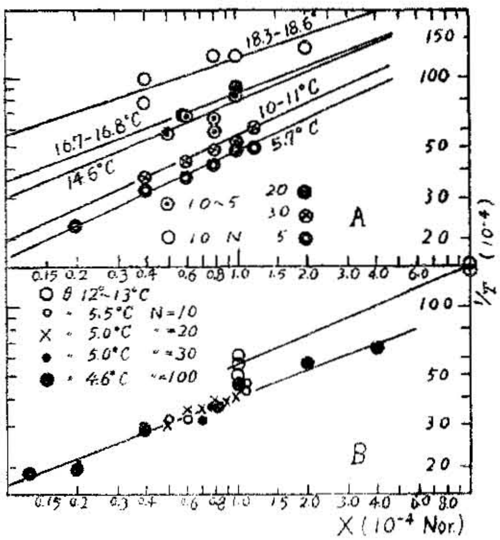

Fig. 4. Relation between the sublimate concentration (X) and the death time (T) A: Carp, B: Gold fish.

From the results obtained from all independent experiments (Table 2), it is known that when the solution is very high in concentration the mean death time remains nearly unchanged disregarding the number of fish used (N). However, it is usually prolonged in proportion to the increase in the number (N) and this tendency is more intensified in the weak solution of the sublimate than in the strong one. This is assumed to be due to the mass reaction which acts usefully on animal aggregation.

On the other hand, it is also noticed that the mean death time is rather shortened, when the number of fish used together increases beyond 30 in gold fish and also 10 in carp in 5 liters of the solution. In other words, a group of 30 gold fish and a group of 10 carp show the maximum protective power for toxic effect of sublimate solution and can live longest in it. There is the optimum density for fish in 5 liters of 1.0×10^{-4} normal solution of sublimate. The optimum density must vary not only because of the concentration of the sublimate but also because of the volume of the solution, into which they are put. Until the number of fish individuals used together increases to the optimum density, the mass reaction is useful for animal aggregation, while it begins to be harmful for it beyond the optimum density. It is considered by W. C. Allee that the mass reaction is due to "biological conditioning" of medium.

Gold fish	$N = 2,\ \theta : 4° - 7°C$	$1/T = 42\ X^{0.47}$
	$= 10,\ \theta < 10°C$	$1/T = 44\ X^{0.59}$
	$= 30,\ \theta < 10°C$	$1/T = 43.5\ X^{0.60}$
Carp	$N = 5,\ \theta < 10°C$	$1/T = 48\ X^{0.44}$
	$= 5,\ \theta > 10°C$	$1/T = 75\ X^{0.28}$
	$= 10,\ \theta < 10°C$	$1/T = 50.2\ X^{0.43}$
	$= 10,\ \theta = 6.1°C$	$1/T = 61\ X^{0.68}$
	$= 20,\ \theta > 10°C$	$1/T = 77.6\ X^{0.02}$
	$= 30,\ \theta > 10°C$	$1/T = 61.9\ X^{0.42}$

Table 2. Change in the mean death time (T) according to the number of fish used together (N), temperature of solution (θ°C) and the sublimate concentration ($X \times 10^{-4}$)—single experiment.

θ°C	<10°C				>10°C				
$X \times 10^{-4}$ Nor.	1.0	0.8	0.6		10.0	1.0			
$N =$ 1	225.0				63.4	182.6			
2	245.7	260.5	290.8		69.3	—			
10	221.0	265.0	311.3			188.2			
30	225.1	270.1	349.7			224.0			
100	228.1					200.4			
$X \times 10^{-4}$ Nor.	1.2	0.8	0.6	0.4	1.2	1.0	0.8	0.6	0.4
$N =$ 1					79.7	120.3	142.5	143.3	1
2					101.5	120.5	77.5	—	164.8
5	202.3	241.4	274.4	310.3	—	133.6	148.4	144.0	175.0
10					112.7	188.5	207.3	228.0	275.0
20					108.6		120.2		203.0
30					104.1		141.8		

In the repeated experiments (Table 3), 5 liters of sublimate solution of 1.2×10^{-4} normal solution are at first used, into which individual groups of 10, 20 and 30 carp are put in several times. Due to the repeated use of same solution, the mean death times are gradually more prolonged and solution becomes nontoxic more rapidly in the large number of fish than in the small number. This fact suggests also that the mass reaction acts more usefully in a large aggregation than in the small. In every case, sublimate concentration (X_n) is measured after experiment and its decreasing amount (ΔX_n) is then calculable. When the total weight of fish used is W(g), then the relation between them is formulated as below

a group of 10 carp $\Delta X/W = 76\ X^{1.21}$
,, 20 ,, $\Delta X/W = 90\ X^{0.76}$
,, 30 ,, $\Delta X/W = 94\ X^{0.52}$

Hence the decrease of sublimate concentration seems due to adsorption of sublimate to fish body and also to the organic matter excreted from fish. This may be called here as "biological conditioning". As the adsorbed amount of sublimate increases in proportion to the increase in the number of fish used, then the toxic effect is necessarily relaxed.

Table 3. Repeated experiments with carp.

Exper. no.		$X \times 10^{-4}$	Temperature	pH-value	ΔX_n	W (g)	T (min.)	O_2-content(%)		$KMnO_4$(mg/l) consumed
								before	after	
I	1	1.2	15.2—16.0	7.0	0.45	46	121.7	90.4	87.0	193
	2	0.75	16.2—17.0	7.0	0.06	44	121.3	--	83.3	—
	3	0.69	18.3—29.0	7.0	0.22	45	132.5	99.3	78.3	261
	4	0.47	—	—	0.10	41	111.6	95.5	65.3	296
	5	0.37	16.7	7.0	0.12	40	192.1	101.0	70.8	—
	6	0.25	17.0—17.6	7.0	0.12	38	177.8	97.7	62.5	493
	7	0.13	13.7—15.5	7.2	—	42	404.3	113.0	—	522
	8	?	15.6	—	—	39	863.3	—	47.8	—
II	1	1.2	15.5—15.8	7.0	0.53	102	111.8		70	435
	2	0.67		6.9	0.27	98	121.6		63	522
	3	0.40		6.9	0.35	76	131.0		53	754
	4	0.15		7.1	—	86	427.9		47	754
	5	?		—	--	100	1038.1		—	842

	1	1.2	0.55	162	104.1		48	493
III	2	0.65	0.45	174	141.8		—	608
	3	0.20	—	120	421.3		42	842

Note: exper. I: 10 fish group, II: 20 fish group, III: 30 fish group. Sublimate concentration after the second experiment measured colorimetrically.

Considered from the increase in the consumed amount of $KMnO_4$ (mg/l) and the decrease in O_2-content (%) in sublimate solution, the more is the number of fish used, the more the organic matters are excreted, and, accordingly, the more remarkably the toxic effect of solution is relaxed. Nevertheless, the mean death time is shortened, when the number of individuals used together increases beyond the optimum density. This fact suggests that the solution will become intolerable again for fish, because it is contaminated excessively with organic excrements. This is the reason why the mass reaction is useful in the aggregation under the optimum density for a given space and in turn becomes harmful above the optimum density.

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APPENDIX

(1) Killing experiments of gold fish and carp with the sublimate solutions were carried out from November 24, 1943 to April 18, 1944 and again from October 27 to November 15, 1944 by Tomoo Hayashi and Nobuhiko Hanamura.

(2) In the following Appendix tables, the abbreviations are
$X(10^{-4})$: sublimate concentration, θ°C: temperature of sublimate solution pH: pH-value of sublimate solution, W: body weight in gram, T: death time in minute.

Appendix 1. Groups of 1 gold fish (independent experiment).

$X(10^{-4})$	10		1													
θ°C	12−13		13		12.5−13		10−12		8.5−9.0		11		7.8−8.5		10−12	
pH	7.2		7.1		7.4		7.4		7.4		7.1		7.0		7.0	
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	1.7	60	1.6	143	2.0	222	2.6	200	1.5	205	1.8	208	3.7	205	9.7	230
	1.8	67	2.3	190	2.1	232	3.0	217	1.5	135	1.9	225	4.2	308	3.7	226
	2.2	65	2.5	152	2.4	178	3.4	194	2.3	192	2.5	237	6.0	200	6.2	192
	2.1	60	2.6	123	4.2	198	2.5	223	4.1	180	2.7	165	3.0	280	6.4	250
	2.9	65	2.8	100	4.7	195	2.6	140	3.9	215	3.8	183	3.4	295		
Mean	2.1	63	2.4	124	3.7	179	2.4	200	3.4	201	2.4	136	2.5	249	5.1	241

Appendix 2. Groups of 2 gold fish (independent experiment).

$X(10^{-4})$	1.1		1.0		0.9		0.8		0.7		0.6		0.5	
θ°C			(A) 5.5−7.0		(B) 5.5−6.2		(C) 4.0−6.5							
pH			(A) 7.2		(B) 7.0		(C) 7.0							
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
(A)	1.9	190	2.1	214	2.2	230	3.1	210	2.8	290	1.8	212	2.2	322
	2.5	210	2.8	240	2.3	232	2.9	230	3.1	295	2.4	248	2.8	360
(B)	3.5	234	4.5	252	3.3	254	3.5	230	2.9	252	3.6	285	3.5	332
	3.7	242	3.1	263	3.7	270	3.8	295	4.6	305	3.8	360	3.9	345
(C)	3.0	231	3.5	250	4.0	270	3.9	278	3.7	293	4.5	312	3.8	305
	4.0	255	4.4	255	6.0	295	4.0	320	4.1	303	3.4	328	4.8	338
Mean	3.2	227	3.4	246	3.6	259	3.4	261	3.5	290	3.3	291	3.5	334

Appendix 3. Groups of 10 gold fish (independent experiment).

$X(10^{-4})$	10				4.0		2.0		1.1		1.0					
θ°C	12−13				4.6−6.0		4.5−5.5		5.5−8.5		12−13				12.5−13.0	
pH	—				—		—		—		7.2−7.1				7.1−7.0	
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	1.5	63	1.6	60	2.4	113	3.0	150	1.9	202	1.8	125	1.7	134	2.5	197
	1.6	87	1.7	70	2.5	138	2.5	154	2.0	210	2.0	165	1.7	150	2.9	220
	1.8	78	1.8	65	3.4	138	4.0	154	2.8	210	2.1	129	1.7	165	2.9	190
	1.7	65	2.1	70	2.0	145	3.7	170	3.1	195	2.6	138	1.8	150	3.2	220
	2.3	75	2.1	67	2.9	147	2.2	175	3.2	190	2.8	143	2.2	169	3.4	184
	2.5	70	2.2	70	3.3	147	4.4	180	3.5	257	2.9	144	2.9	150	3.5	215
	2.5	65	2.3	70	4.0	160	4.6	185	3.6	218	2.9	181	2.0	158	3.6	120
	2.7	65	2.4	73	3.2	165	5.2	190	3.8	225	3.1	165	2.7	185	3.7	180
	2.7	70	2.4	70	3.3	170	5.0	195	5.2	265	3.2	214	2.8	190	4.2	199
	2.8	65	2.9	63	5.2	197	3.3	205	5.5	223	3.9	176	2.8	144	4.2	225
Mean	2.3	71	2.2	68	3.2	152	3.8	176	3.5	219	2.7	161	2.2	160	3.4	199

$X(10^{-4})$	1															
θ°C	12.5—13		10—12				8.5—9.0				8.5—		14.5—14.0			
pH	7.1—7.0		7.4—7.6—7.2				7.4—7.2				7.1		—			
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	2.8	215	2.5	210	2.7	240	2.1	155	1.6	155	1.5	222	5.5	127	4.1	127
	3.0	105	2.6	175	2.8	145	2.6	160	1.7	162	1.6	205	1.9	212	1.7	265
	3.0	190	2.7	185	2.8	150	2.6	214	1.9	157	1.7	233	5.7	142	4.0	130
	3.5	180	2.8	190	2.8	250	2.7	217	2.0	155	1.9	235	5.8	155	5.4	145
	3.5	187	3.0	247	2.9	235	2.8	192	2.0	185	2.2	133	4.9	170	4.5	147
	3.5	208	3.0	207	2.9	240	3.0	173	2.1	130	2.2	272	3.9	176	3.3	150
	3.6	215	3.1	205	3.2	202	3.1	140	2.1	162	2.7	350	4.0	180	5.5	157
	3.7	203	3.1	240	2.0	206	3.1	238	2.5	175	2.8	236	5.0	190	4.9	170
	4.2	225	3.2	225	2.5	228	3.5	175	2.8	165	3.6	275	6.6	205	3.9	172
	4.2	225	3.2	245	2.9	213	3.9	193	2.8	172	3.6	182	5.2	208	6.6	175
Mean	3.5	195	2.9	213	2.8	211	2.9	186	2.2	161	2.4	234	5.3	169	4.8	150

$X(10^{-4})$	1								0.8		0.6		0.5	
θ°C	5.5—7.5				10.7—12.0				5.5—8.5		5.5—8.5		5.5—8.5	
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	1.7	203	1.5	255	4.0	180	4.2	185	2.3	210	2.4	265	2.5	325
	1.9	212	1.7	265	5.0	180	4.2	198	2.6	260	2.8	287	2.9	280
	2.3	195	1.9	268	5.2	197	4.4	199	2.7	280	2.9	340	2.9	306
	2.5	170	2.4	290	3.2	197	4.3	202	2.8	276	3.0	385	3.1	268
	2.5	235	3.1	380	3.5	199	5.2	215	3.4	233	3.2	290	3.2	300
	3.1	185	3.3	300	4.6	206	3.7	220	3.6	267	3.5	298	3.3	390
	3.2	216	3.3	320	4.9	212	3.5	230	3.7	262	3.6	278	3.9	288
	3.2	245	3.6	340	6.1	227	5.5	234	3.7	282	4.2	360	4.0	340
	3.5	240	3.9	290	4.2	247	3.5	245	3.9	265	5.8	300	4.0	372
	3.8	250	4.3	380	6.0	255	6.4	—	4.9	315	6.2	310	4.1	315
Mean	2.8	215	2.9	309	5.3	210	4.5	214	3.4	263	3.8	311	3.4	318

Appendix 4. Groups of 20 gold fish (independent experiment).

$X(10^{-4})$	1.0											
θ°C	10.7—12.0				10—12							
pH	7.0				7.0							
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	7.5	165	3.7	200	4.5	160	3.2	207	3.7	183	5.6	230
	3.6	180	6.9	203	4.6	180	5.5	208	3.8	196	5.5	234
	3.7	185	5.6	207	3.5	182	4.7	210	5.8	203	6.6	235
	3.7	185	5.7	230	3.6	185	4.2	212	5.4	210	6.0	238
	4.9	187	5.0	230	2.7	185	6.5	220	3.8	212	3.8	238
	4.8	192	8.1	235	4.2	187	6.7	225	5.3	215	5.1	240
	6.7	193	4.9	235	2.7	192	3.7	227	5.2	221	5.6	240
	6.4	195	5.2	238	2.8	195	7.0	230	6.8	223	3.8	250
	5.1	197	4.3	240	3.7	196	4.3	237	6.3	223	6.0	258
	4.9	200	5.7	250	4.6	200	6.4	238	5.0	228	7.2	275
Mean			5.3	208			4.5	204			5.3	227

Appendix 5. Groups of 30 gold fish (independent experiment).

$X(10^{-4})$	1.0															
θ°C	8.5−9.0						7.8−8.5						7.8−8.5			
pH	7.4−7.1						—						—			
	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T
	2.3	140	2.9	180	3.2	220	1.3	270	2.1	226	2.6	244	1.0	235	2.2	245
	2.4	190	2.9	215	3.2	235	1.6	260	2.1	230	2.8	214	1.6	183	2.2	260
	2.4	215	2.9	240	3.3	230	1.6	175	2.2	144	2.8	220	1.6	250	2.3	157
	2.5	195	3.0	183	3.4	230	1.9	227	2.2	212	2.9	180	1.6	265	2.4	215
	2.5	205	3.0	220	3.4	230	1.9	230	2.2	215	2.9	237	1.7	245	2.4	238
	2.6	220	3.1	195	3.5	225	1.9	240	2.2	235	3.2	270	1.7	250	2.4	266
	2.7	200	3.1	235	3.8	195	2.0	155	2.5	175	2.5	195	1.8	235	2.5	243
	2.8	208	3.1	250	4.0	220	2.0	205	2.5	238	3.7	270	1.8	258	2.5	240
	2.9	105	3.2	210	3.0	230	2.0	238	2.5	266	3.7	234	1.9	220	2.5	257
	2.9	172	3.2	215	3.0	245	2.1	130	2.6	206	4.7	260	2.2	225	2.6	266
Mean					3.3	210					2.5	221				

$X(10^{-4})$	1.0														0.8	
θ°C			8.0−9.0						10−14						5.0−7.5	
	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T
	2.7	250	1.6	180	2.0	248	2.7	233	4.7	163	6.2	220	5.7	242	3.2	245
	2.9	260	1.6	219	2.1	205	2.9	280	5.0	173	7.5	220	5.6	243	2.2	250
	3.0	300	1.7	211	2.1	262	3.0	269	2.8	182	4.5	222	5.0	248	2.3	255
	3.1	263	1.7	243	2.2	225	3.1	234	4.0	183	5.3	222	4.0	250	2.4	278
	3.3	248	1.8	210	2.3	183	3.6	275	5.8	200	5.7	224	5.0	250	2.4	280
	3.3	262	1.9	180	2.4	235	4.0	275	4.3	206	4.9	231	5.2	252	2.5	283
	3.3	266	1.9	205	2.5	250	4.0	280	5.8	208	4.8	234	5.5	245	2.6	290
	3.7	288	1.9	260	2.6	236	4.6	263	5.6	209	6.5	238	4.2	267	2.8	212
	4.0	277	2.0	215	2.6	249	4.7	270	3.1	214	5.2	239	3.4	284	2.9	250
Mean	2.44	248					2.5	221					5.1	224		

$X(10^{-4})$	0.8				0.6					
θ°C	5.0−7.5				5.0−7.5					
	W	T	W	T	W	T	W	T	W	T
	3.1	245	3.9	255	2.3	268	3.2	268	3.8	340
	3.1	255	4.0	260	2.4	277	3.2	330	4.0	313
	3.1	273	4.1	260	2.5	298	3.4	255	4.0	313
	3.1	300	4.1	275	2.6	335	3.4	325	4.1	310
	3.2	270	4.6	273	2.8	318	3.5	313	4.3	292
	3.2	310	4.7	293	2.9	323	3.5	330	4.5	330
	3.3	288	4.9	295	2.9	362	3.6	315	4.6	420
	3.5	290	5.1	263	2.9	365	3.6	340	5.6	437
	3.5	296	5.1	302	3.0	283	3.6	412	6.7	400
	3.6	280	5.3	306	3.0	298	3.7	305	6.9	416
Mean			3.4	270					3.7	250

Appendix 6. Groups of 100 gold fish (independent experiment).

$X(10^{-4})$	11.0				1.0				0.6			
θ°C	11				5.5−7.5				5.5−8.0			
pH	7.4−7.0				7.1−6.8				7.0−6.9			
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	1.0	171	2.0	176	0.8	140	2.5	258	1.8	243	3.2	287
	1.1	107	2.0	179	0.9	140	2.5	262	1.9	220	3.2	315
	1.2	132	2.0	206	0.9	145	2.6	231	2.0	215	3.3	258
	1.2	167	2.0	211	1.0	153	2.7	260	2.0	265	3.3	320
	1.3	117	2.0	224	1.0	155	2.7	202	2.0	287	3.3	240
	1.3	167	2.0	252	1.2	140	2.7	218	2.1	210	3.4	258
	1.3	182	2.0	229	1.2	146	2.7	234	2.1	243	3.4	280
	1.4	122	2.0	223	1.3	142	2.7	240	2.2	330	3.4	303
	1.4	127	2.1	193	1.3	148	2.7	258	2.3	230	3.5	267
	1.4	162	2.1	199	1.3	165	2.8	220	2.3	247	3.5	287
	1.4	162	2.1	201	1.4	158	2.8	228	2.3	252	3.5	295
	1.4	168	2.1	208	1.4	166	2.8	258	2.4	265	3.5	324
	1.4	188	2.1	220	1.4	176	2.8	270	2.5	230	3.5	328
	1.4	200	2.2	188	1.4	190	2.9	257	2.5	230	3.5	336
	1.4	209	2.2	201	1.4	199	2.9	263	2.5	250	3.6	287
	1.4	218	2.2	212	1.5	163	2.9	265	2.5	290	3.6	297
	1.5	165	2.2	213	1.5	181	3.0	204	2.7	220	3.6	297
	1.5	174	2.2	217	1.5	204	3.0	239	2.7	243	3.7	285
	1.5	175	2.2	222	1.7	168	3.0	250	2.7	285	3.7	300
	1.5	186	2.2	227	1.7	194	3.1	259	2.7	287	3.7	373
	1.5	187	2.2	230	1.7	203	3.2	259	2.7	322	3.8	285
	1.5	191	2.3	187	1.7	233	3.2	260	2.8	240	3.8	293
	1.5	192	2.4	204	1.8	210	3.2	264	2.8	278	3.8	295
	1.5	207	2.4	229	1.8	167	3.2	274	2.8	285	3.8	320
	1.6	125	2.5	207	1.8	179	3.3	261	2.8	285	3.8	355
	1.6	187	3.5	212	1.8	193	3.3	263	2.8	288	3.9	273
	1.6	189	2.5	213	1.8	214	3.3	264	2.8	290	3.9	335
	1.6	189	2.5	219	1.8	227	3.3	270	3.0	267	3.9	336
	1.6	195	2.5	221	1.9	169	3.3	282	3.0	290	4.0	310
	1.6	200	2.6	129	1.9	212	3.4	255	3.0	293	4.0	315
	1.6	202	2.7	178	1.9	264	3.4	270	3.0	298	4.0	336
	1.6	223	2.7	212	2.1	187	3.4	280	3.0	322	4.1	258
	1.7	187	2.9	184	2.1	210	3.5	216	3.0	324	4.2	368
	1.7	198	2.9	226	2.2	251	3.5	237	3.1	245	4.3	345
	1.7	200	3.0	228	2.2	252	3.5	260	3.1	247	4.4	273
	1.7	211	3.0	226	2.3	214	3.5	263	3.1	256	4.4	283
	1.8	164	3.1	233	2.3	245	3.5	270	3.1	260	4.4	330
	1.8	185	3.1	234	2.3	249	3.6	358	3.1	285	4.8	300
	1.8	190	3.1	234	2.3	256	3.6	261	3.2	240	5.0	265
	1.8	200	3.2	222	2.3	262	3.6	270	3.2	287	5.0	338
	1.8	219	3.2	252	2.4	230	3.6	280			5.0	405
	1.9	187	3.3	221	2.4	236	3.7	292			5.4	297
	1.9	194	3.5	230	2.4	245	3.8	263			5.6	380
	1.9	197	3.5	231	2.5	215	4.0	240			5.7	305
	1.9	205	3.6	228	2.5	241	4.1	261			6.7	270
	1.9	206	3.6	242	2.5	248	4.2	262				
	1.9	219	4.0	228	2.5	250	4.5	258				
	1.9	225	4.1	228	2.5	250	4.7	259				
	2.0	172	4.1	242	2.5	258	4.7	265				
	2.0	183	4.3	245	2.5	258	7.8	<305				
Mean			2.8	200			3.1	228			3.5	286

Appendix 7. Groups of 10 carp (independent experiment).

$X(10^{-4})$	1.2						1.0		0.8		0.63		0.6		0.4		11.5	
θ°C	18.3		17.1		14.5		10.4		10.5		10.8		11.6		18.3		11.5	
	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T
	1.5	64	2.5	84	1.2	99	4.5	115	7.9	160	1.2	161	1.4	170	4.4	91	2.9	214
	4.3	84	8.8	89	4.1	140	2.7	135	1.9	161	2.0	175	4.8	192	6.8	97	3.5	230
	4.8	97	2.4	111	2.2	147	2.5	160	2.0	161	3.6	175	4.2	205	6.2	101	3.9	234
	5.8	101	3.5	114	4.7	156	5.9	163	4.5	170	2.7	195	3.7	213	4.3	138	4.0	240
	2.8	101	2.7	122	4.5	170	4.2	165	3.2	172	4.0	207	5.2	227	9.5	139	1.7	249
	3.2	108	6.2	123	8.3	171	3.4	172	2.8	173	2.7	215	5.5	249	3.5	144	3.7	253
	6.0	109	8.8	132	5.1	178	4.4	172	4.5	185	4.9	225	6.7	254	3.9	147	4.8	290
	4.3	115	6.5	135	7.4	183	5.3	175	5.2	213	2.4	225	6.2	255	8.4	150	8.3	300
	5.0	115	8.7	135	3.8	205	6.6	177	9.1	225	3.7	230	7.8	258	8.8	151	8.9	350
	6.0	118	8.4	135	8.0	207	9.2	180	7.7	265	3.7	265	4.3	262	6.2	151	4.4	390
			3.9	67	2.0	84	2.2	89										
			1.6	80	4.1	140												
			6.8	95	2.5	90												
			4.5	108	1.9	105												
			7.0	109	1.8	108												
			3.2	121	2.2	113												
			3.5	125	6.2	138												
			3.9	127	3.0	155												
			6.9	127	8.0	158												
			9.8	139	4.0	175												
Mean	4.4	101	5.5	114	4.2	143	4.9	161	4.9	189	3.1	207	5.0	228	6.2	131	4.6	275

Appendix 8. Groups of 10 carp (repeated experiments).

$X(10^{-4})$	1.2		0.75		0.69		0.47		0.37		0.25		0.13		?	
θ°C	15.6		16.6		18.7		18.7		16.7		17.3		17.3		14.6	
pH	7.0		—		7.0		7.1		7.0		7.0		7.2		—	
no.	I		II		III		IV		V		VI		VII		VIII	
	W	T	W	T	W	T	W	T	W	T	W	T	W	T	W	T
	2.8	146	3.5	82	2.2	93	2.0	91	6.2	178	3.0	132	2.6	385	2.0	
	3.0	86	5.7	86	1.8	110	5.8	101	6.4	179	6.5	170	5.8	396	3.9	700
	3.8	113	5.0	98	3.4	125	4.4	103	4.5	187	3.2	174	5.1	398	5.5	
	3.8	116	8.2	100	4.2	127	5.7	104	3.9	197	2.5	177	3.1	431	2.4	720
	4.2	99	2.8	101	5.8	130	3.6	103	6.0	198	2.7	179	5.4	460	2.9	763
	4.5	100	7.7	105	3.7	148	5.3	108	2.5	201	2.3	190	3.5	471	6.5	857
	5.5	142	3.3	139	6.5	148	5.5	110	6.5	237	3.7	187	6.0	510	4.5	1075
	6.2	148	4.4	156	10.1	180	4.7	115	3.7	241	3.0	210	2.8	512	4.2	1120
	7.3	122	5.0	157	3.4	182	6.8	125	2.8	264	4.1	233	5.7	590	4.2	6830
	7.6	128	6.3	176	7.5	197	5.0	130	(3.2)	(63)	7.3	255	4.7	652	—	—
Mean	4.9	120	5.0	120	4.9	144	4.9	110	4.7	195	3.8	192	4.5	481	3.8	1396

Appendix 9. Groups of 20 carp (repeated experiments).

$X(10^{-4})$	1.2				0.67				0.40				0.15		?	
θ°C	15.5–15.8				15.1–16.4				1.65–16.2				12.6			
pH	7.0				6.9											
no.	I				II				III				IV		V	
	A		B		A		B		A		B		A		A	
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	1.8	67	2.1	92	3.7	102	1.8	93	1.2	108	2.1	220	1.8	380	5.6	480
	3.2	87	7.8	93	4.4	105	3.0	96	1.9	115	3.9	245	3.2	390	5.3	1545
	3.7	92	2.7	96	8.5	109	2.2	97	1.5	116	4.9	255	3.2	390	9.5	1545
	5.0	100	4.7	96	4.1	110	3.7	106	2.1	117	5.5	256	5.3	394	2.7	1570
	4.3	104	3.3	100	4.7	111	2.9	108	2.9	119	2.9	258	5.5	396	3.0	1660
	5.7	105	3.4	100	3.9	112	4.2	113	2.2	125	5.6	264	11.5	400		
	3.9	108	6.0	102	4.2	115	5.1	115	2.0	125	3.4	265	3.9	411		560
	9.1	110	7.5	103	4.9	116	4.3	119	9.0	126	6.8	267	1.9	413	4.1	
	3.8	110	4.9	104	4.6	116	3.9	121	5.0	127	2.8	272	2.1	420		1510
	4.2	111	4.9	106	4.2	117	4.0	122	2.7	129	2.6	272	3.1	430	(13	
	6.6	111	4.6	107	6.4	120	7.9	123	3.0	132	6.5	275	2.3	430		
	7.8	112	5.7	110	6.0	126	3.4	125	3.2	134	6.9	276	2.6	448		
	3.4	114	3.9	112	5.3	129	5.5	128	5.6	136	4.4	279	5.4	478		
	4.6	115	6.1	114	6.2	130	5.4	128	6.3	137	3.9	283	1.3			
	6.1	115	5.2	115	3.1	132	6.4	129	3.4	138	4.9	288	5.6			
	5.9	128	3.7	118	2.9	133	5.0	130	5.0	139	5.8	295	6.3			
	9.9	129	4.7	119	5.2	134	8.6	131	3.1	139	6.3	300	2.3	515		
	3.4	130	5.7	125	3.5	136	3.6	134	5.9	150	5.9	311	8.5			
	3.5	136	3.8	126	5.7	137	6.5	138	5.1	153	3.5	362	5.7			
	6.2	151	5.8	134	4.6	143	—	—	5.1	158	—	—	—	—		
Mean	5.1	112	4.9	109	4.8	122	4.6	119	3.8	131	4.7	276	4.3	428	5.0	1038

Appendix 10. 30 individual group of carp (repeated experiments).

$X(10^{-4})$	1.2				0.65				0.20			
θ°C	16.0–17.1				13.8–13.4				10.0–13.7			
pH	—				—				6.8			
no.	I				II				III			
	W	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*	*W*	*T*
	3.1	50	4.7	105	3.3	90	6.3	146	3.0	195	2.4	420
	2.7	70	8.7	105	2.2	90	5.5	158	5.0	197	8.2	420
	2.5	74	6.0	106	4.4	90	5.4	158	1.3	245	4.4	420
	2.2	85	5.5	86	4.9	90	5.2	90	1.3	285	3.2	335
	4.5	88	2.8	90	3.3	90	10.0	90	4.3	380	3.7	390
	2.9	93	5.8	95	4.5	120	7.6	123	5.2	390	1.5	394
	6.0	98	7.6	98	7.2	130	4.3	130	2.7	405	3.6	405
	8.0	101	7.5	101	3.6	133	2.4	135	1.4	410	5.0	410
	3.8	102	2.5	103	7.8	138	3.9	145	4.6	410	3.3	420
	7.7	106	4.1	110	3.8	158	5.0	162	4.5	440	2.7	440
	2.6	111	3.7	114	7.1	162	5.3	162	5.2	470	9.7	470
	6.5	118	7.7	118	2.8	165	6.6	175	5.8	470	4.3	470
	9.0	118	5.6	123	6.9	178	8.3	178	4.1	480	7.4	480
	5.7	125	8.3	132	6.7	182	11.9	182	3.4	480	2.0	480
	9.2	137	4.0	152	8.9	198	9.2	205	2.4	480	—	—
Mean			5.4	104			5.8	142			4.0	422