CAUSES OF DEATH OF BAIT FISHES

SPECIAL SCIENTIFIC REPORT: FISHERIES No. 62

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Explanatory Note

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Special Scientific Report - Fisheries No. 62

(54) A STUDY OF THE CAUSES OF DEATH OF BAIT FISHES

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Translated from the Japanese language by

W. G. Van Campen Pacific Oceanic Fishery Investigations

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1/ Associated with the Imperial Fisheries Experimental Station; from Journal of the Imperial Fisheries Experimental Station, No. 7 (Papers No. 52-60), pp. 51-90.

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Introduction

To promote efficiency in transporting the bait fish used in skipjack fishing, the type of bait tank used hitherto, that is one in which the water is circulated only by the motion of the boat, is unsuitable. For this reason in July, 1931, this Station, in cooperation with the Shizuoka Prefecture Fisheries Experiment Station, planned some improvements, rebuilt the bait tank of the Shizuoka Station's guide vessel, the Fuji Maru, and installed new equipment to circulate the water thoroughly by means of a pump. Since that time basic experiments have been carried on along with practical tests and real results have been steadily obtained until finally the point has been reached where the efficiency of holding bait fish has advanced remarkably. As is made clear in No. 3 of the Journal of the Imperial Fisheries Experimental Station, 1) both the useful volume and the density of contents of the new bait tank are about 40% greater than those of the old-style tank so the total amount held is about twice as much. Furthermore the death rate has been reduced greatly to less than 10%. Bait fish which have just been captured, hitherto thought impossible to transport because of their high death rate, that is to say, what are commonly called "new bait", have been transported in tests, but as was expected the mortality was heavy and several trials all ended in failure. This must, after all, be ascribed to a number of defects in the methods of handling the bait fish due to the fact that insufficient research had been done on the causes of bait mortality. In order to take the most appropriate measures against mortality, it is necessary to make a detailed study of all of the causes of death in the bait fish such as deficiency of free oxygen, sudden changes in the water temperature, wounds, and so forth.

With such views in mind, the author, during the approximately four years from 1932 to 1935, carried on the investigations indicated in the title of this paper, using as material sardines, <u>Sardinia melanosticta</u>, and anchovies, <u>Engraulis japonicus</u>, of a size suitable for skipjack bait, at Enoura and Mito in Shizuoka Prefecture, aboard the Fuji Maru at anchor in Mito Wan, at the Kanagawa Prefecture Misaki Marine Laboratory, and at Katsuura and Tateyama-Hojo in Chiba Prefecture.

This study was undertaken on the orders of the Chief of the Station and of Technician Itaro Takayama. The study owes not a little to the assistance of the personnel of this Station, the personnel of the Shizuoka Prefecture Fisheries Experiment Station, to the Masudas, father and son, of Shishihama in Shizuoka, Tadanori Aiso and Haruo Yamamoto of Mitsu in the same prefecture; Dr. Ikusaku Amemiya, professor in the Faculty of Agriculture of the Tokyo Imperial University, and Dr. Sadanori Mita, professor in the Faculty of Medicine, have constantly given extremely kind guidance. In the preparation of the report Technicians Saburo Hoshino, Toru Sato, and Kinosuke Kimura supplied profitable advice. I wish here to express my heartfelt thanks to all of these persons.

Death From Lack of Free Oxygen^I

Since the sardine belongs to the class of comparatively active swimmers, the amount of oxygen it uses must be great, as is clear from the study of Hall²). For this reason when the bait fish are being transported, and a great number of them are confined in a small bait tank aboard the boat, in a tank like the ones used heretofore, where the only water circulation is that resulting from the movements of the hull, and even in the new bait tank described above, where the water is circulated by a pump, the danger that the bait fish may use up the limited free oxygen in the sea-water, breathe abnormally, and then finally die of suffocation is an item that should naturally be taken into consideration. In actual fact, it not infrequently happens that while the bait fish are being transported, an oxygen deficiency develops in the bait tank and almost all of the precious bait dies off in a brief space of time leaving the fishermen dumbfounded.

Accordingly, the basis for counter-measures must be formed by experimenting to determine how much free oxygen the bait fish consume under any particular conditions and what is the lethal level of free oxygen.

a) Abnormal Respiration of Bait Fish^{II}

Abnormal respiration is the respiration which takes place in seawater that does not circulate, or in sea-water in which, because of insufficient circulation, the supply of oxygen is inadequate. A suitable example of this is the respiration of bait fish confined in a bait well.

The author experimented on this point early in September, 1932, at the Kanagawa Prefecture Misaki Marine Laboratory, using a tank for physiological experiments on fish, described below, of his own design.III

This tank was made of wood and was 4.95 shaku long and 1.65 shaku in both width and height (internal dimensions) / 1 shaku = 0.994 foot /. It was divided into three equal compartments by partitions which could be freely opened or closed from the outside, according to the object of the experiment. Each compartment had a glass observation window with a door, a tap for sampling the water. a thermometer. and other instruments.

I. Various valuable advice was received from Mr. Nobunari Kawamoto.

- II. There is an interesting study by Kawamoto on abnormal respiration of fishes when being transported alive.
- III. In planning this tank I received valuable advice from Dr. Morisaburo Tauchi. In the experiments I was assisted by Mr. Hidematsu Tokunaga, a member of the staff of the Station. The details of the construction of the tank and the method of operating it will be reported at some future time.

The left and right chambers were equipped with electric heating devices, cooling devices, and agitators. The tank was filled with sea-water, the cover was tightly closed so as to prevent contact with the air, the sardines which were to be tested -- Sardinia melanosticta that had been in captivity for two days were used -- were placed in the tank, and as the sardines consumed the limited free oxygen in the water and until they began to respire with difficulty, water samples were drawn off from time to time (from the sampling tap using a force pump), and the degree of diminution of free oxygen was determined by Winkler's method.

In the following Table 1, Table 2, and Table 3 the average number of fish is 10, and the average total weight is 263 gm. In the experiments recorded in Table 4, Table 5, and Table 6 these amounts were about doubled, that is, the average number of fish was 20 and their average total weight was 556 gm. The duration of the experiment was until all of the fish being tested showed marked difficulty in breathing. In the case of the first experiment it was three hours, and in the later experiment it was one hour and thirty minutes. The amount of free oxygen is shown as the number of cubic centimeters contained in 1 liter of sea-water.

The diminution of free oxygen which gave rise to abnormal respiration, as shown in each table, is shown graphically in the following figures. In the first three, that is, in Figures 1, 2, and 3 corresponding to Tables 1, 2, and 3, the slope of the curves is gentle. In the latter three, that is, in Figures 4, 5, and 6, corresponding to Tables 4, 5, and 6, they are steep. It can be seen that, regardless of the steepness of the curves, the values for the concentrations of free oxygen that produced difficult respiration are all roughly equal. It can be seen clearly that in the case of the latter three, which involved nearly double the number of fish (total weight) of the first three experiments, the oxygen was consumed and the difficult breathing appeared with nearly twice the speed shown in the first three. The author has observed and reported on the same relationship in the case of mixed groups of sardines and anchovies.⁴)

Accordingly, if the number (total weight) of bait fish kept in a bait tank is increased, either the amount of water circulated must be amplified, or other appropriate means must be employed to supply enough free oxygen.

b) Lethal level of free cxygen

In the preceding section it has been described how the sardines begin to breathe abnormally as they consume the limited free oxygen in sea-water by their respiration, and how the concentration of oxygen finally gets so thin that it will not support life. This section reports the results of experiments on the concentration of free oxygen insufficient to support life.

The experiments were carried out in the latter part of August, 1932, and in late December, 1933, at the Kanagawa Prefecture Misaki Marine

			LAULE I EXPERIMENT ON ADDORMAL RESpiration of Sardines	spiration of Sardines	
Time	Water Temp.	Oxygen Cont.	Notes	Materials	
nin	ខ្លុ	<u>cc/1</u>			
0	24.7	5°03	Before putting sardines in.	Number of fish tested	10
20	24.7	3.87		Total body weight Average body weight	264.8gm 26.5
40	24.8	3.27	One fish died.	Average body length	13.9cm
60	24.8	3,03	Breathing became innearlan	Survived	Died
80	24°9	2°59		Number 6	4
100	24.9	2.34		, 16 2	+ 4 0 0 98.9 24 7
120	25.0	2°12	l fish died. Fish ran wild from		23°4 13°4
140	25.0	1.91	l fish died.		
160	25.1	1.74	Fish ran completely wild.		
180	25.1	1.54	<pre>l died. Others showed conspicu- ously difficult respiration.</pre>		

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Table 1 Experiment on Abnormal Respiration of Sardines

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Sardines
of
Respiration
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Table

		10	296.3 gm 26.9	13.8 cm	Died	e 5 8 2	200.9	14.4		<u></u>		
Materials		Number of fish tested	Total body weight Average body weight	Average body length	Survived	Number 4	ର ଋ					
Notes		Before putting sardines in.		l fish died.	Fish showed sions of measiness				l fish died. Fish in a confused state.	l fish died.	<pre>5 died. Fish showed difficulty in breathing.</pre>	
Oxygen Cont.	<u>cc/1</u>	5.03	4.09	3.59	3.23	2.95	2,58	2.46	2.24	2.08	1.84	
Water Oxygen Temp. Cont.	ပ္ပ၊	24.7	24°8	24.8	25.0	25.0	25.0	25.0	25.1	25.2	25.2	
Time	uin	0	20	40	60	80	100	120	140	160	180	

Table 3 Experiment on Abnormal Respiration of Sardines

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		DI	227.7 gm 22.8	13.4 cm	Died	6 8 8 6 6 7 8 6 8 8 8 8 8 8 8 8 8 8 8 8	141.4	14.0				
Materials		Number of fish tested	Total body weight Average body weight	Average body length	Survived	6 23	Total weight 86.3 Av. weight 21.6					
Notes		Before putting sardines in.							l fish died.	3 died. Fish somewhat wild.	3 died. Fish running wild.	
Oxygen Cont.	<u>cc/1</u>	5.03	4.22	3.94	5.4 8	3.18	3,05	2.85	2.64	2.54	2.42	
Water Temp.	01	24°7	24.7	24.8	24.8	24. 8	24°9	24.9	24.9	24.9	25.0	
Time	nim	0	20	40	60	80	100	120	140	160	180	

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Table 4 Experiment on Abnormal Respiration of Sardines

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		21	58%.0 gm 27°7 14.2 cm	Died	18 210 2 8	27.4 27.4	14.1
Materials		Number of fish tested	Average body weight Average body length	Survived	5 5	Total Weight 89.0	
Notes		Before putting sardines in.	l fish died. Fish showed signs of uneasiness.	3 died. Fish ran wild from time to time.	4 died.	3 died. Fish ran wild.	7 died. Other breathing with marked difficulty.
Oxygen Cont.	<u>cc/1</u>	4.15	2.50	1 . 90	1.76	1. 56	1.57
Water Temp.	р,	24.5	24.7	24.8	24.8	24.9	24.9
Time	nin	0	50	40	60	80	8

Table 5 Experiment on Abnormal Respiration of Sardines

1_

		19	29.0 8m 29.0	14°L CM	Died	13	0+	29°0	·····
Materials		Number of fish tested	totar body weight Average body weight	age body lengun	Survived	er 6	Potal weight 173.5		Av. length 14.1
		Quan N	Aver	AVEL		Number	Tota		
Notes		Before putting sardines in.	l fish died.	l fish died.		L dled. Flsh showed signs of uneasiness.	Fish somewhat wild.		3 died. Breathing conspicuously difficult.
Oxygen Cont.	<u>cc/1</u>	4.15	2.91	2.42	6 (60°%	1,88	s	1,80
Water Temp.	50	24.5	24.7	24.7		64° /	24.7		24°7
Time	nin	0	20	40	() C	00	80		100

Table 6 Experiment on Abnormal Respiration of Sardines

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		19	535.0 gm 28.2	L4.1 Cm	Died	16	96 510	455.5	28.5	14.2	
Materials		tested	gnt eight	engtn	Survived	8	\$ \$ \$	79.5	26.5	14.0	
X		Number of fish tested	Total body weight Average body weight	AVETAGE DOGY 1	Su	Number	6 1	Total weight	Av. weight	Av. length	
Notes		Before putting sardines in.		4 fish died. Fish somewhat wild.		L ULEU. DWIMMING MOVEMENUS WEak.	3 died. School in a disordered	condition.		Fish completely wild. Breathing conspicuously difficult.	
Oxygen Cont.	<u>cc/1</u>	4.15	2°96	2.39	90 F	1.00	1.83			1.81	
Water Temp.	р <mark>і</mark>	24.7	24.9	24.9	2	, , , ,	25°0		1	22°1	Ţ
Time	uin U	0	20	40	C S	8	80		ě	06	



Consumption of oxygen by abnormal respiration of sardines

Laboratory and on the coast at Mitsu, Tagata-gun, Shizuoka Prefecture. Materials used were sardines and anchovies. It would have been all right to have used the same method as that employed in the experiments described in the preceding section, placing the fish in a perfectly tightly closed container of sea-water of a definite volume, leaving them until they died of a lack of free oxygen, and then determining the oxygen content at the time the fish died, however, the point to be particularly noted here is that there is considerable variation in the resistance to oxygen deficiency as between individuals and between different sizes of fish (see Table 7). Therefore when a large number of bait fish are suffocated at one time and an attempt is made to determine the lethal level of oxygen, IV in the end the fish with the highest resistance are left alive. Consequently the value for the lethal oxygen level obtained at this time is thought to be inaccurate for these fish. Therefore the author made several score experiments on individual fish using a bowl-shaped glass vessel of known capacity as shown in Figure 7. This glass was placed in the water in a vessel of large size in which a number of bait fish had already been released. Then, taking care not to excite the fish, one was caught inside the vessel and it was quickly sealed by a cover ground to fit perfectly. The experiment was then kept under close observation until the fish suffocated. When it was completely suffocated, the vessel was removed, shaking it during the removal, the cover was taken off. and the water was immediately sampled. The water temperature and the free oxygen content were determined, and by repeating such experiments several score times and finding the average values the lethal level of free oxygen for bait fish was obtained.

At this time, in order to judge accurately the instant of suffocation of the fish, care must be paid to the following items. When the fish is first shut up in the glass bowl, it immediately becomes more or less agitated, but with the passage of time it settles down and as it does so its breathing rate returns to normal and becomes about 60 per minute (in the case of the sardine). However, as the concentration of free oxygen becomes thinner and approaches the lethal point the fish again becomes excited and shows marked uneasiness and restlessness. The rate of respiration exceeds 100 per minute, showing the so-called hyperphoe. In a bait tank at this time the fish stick their snouts above the surface of the water in the so-called hanaage [nose-raising 7. When the oxygen is gone and the lethal level is finally reached, the fish are almost still, the depth of respiration is great, and the breathing rate diminishes greatly to 2 - 3 per minute. Then the fish appears to be unconscious, lies on its side, and the only movement that it makes is to bend its body to the left and right from time to time, in many cases with the mouth wide open. When this condition is reached respiration by means of the gills has already completely ceased, and consequently the sardine no longer consumes the free oxygen in the water. Thereafter the fish carries on so-called airless breathing, and according to the studies of

IV. Technician Kinosuke Kimura is carrying on experiments by this method.⁵⁾



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(<u>Sardinia</u> melanosticta)
(Sardinia
for Sardines
for
Oxygen
of
Density
Lethal
Table 7

						•				•	
No。	Body	Body	Sex	(1)	(11)	$(II)^{-}(I)$	Before	Exnt.	After	Expt.	Lethal
	Weight	Length		Vessel's	Displace-		Water			0	Period
				Water Content	ment of Sardine		Temp。	Cont.	Temp.	Cont。	
	5	ទី		8	ទ	8	SI	<u>cc/1</u>	SI	<u>cc/1</u>	nin
Ч	30°5	14.3	0+	800	34	766	24.2	4 . 84	24 °5	1.80	22
ଋ	29 ° 0	14.4	60	#	30	770	24.5		24.9	1.98	25
ю	29°0	14.4	¢	F	30	770	24.5	4.84	24.6	2.42	24
4	34 °0	15.0	0+		30	770	24.4	3.08	24 . 6	2°83	19
Ŋ	36.0	15.1	01-	2	32	768	24°4	5.08	24°7	2°55	20
9	39.0	14.4	(0)	#	36	764	24°2	5.12	24°4	1.35	27
2	29•0	14.7	0+ (E	30	770	23°7	4.02	23.7	1.33	31
00	30.0	14.2	•	F	30	770	23°7	4.02	23.7	1.33	30
6	33.0	15.1	0 +	E	33	767	23 。 7	4.15	22°8	2.89	20
10	33°0	15.0	40	E	32	768	23°9	4.15	23°9	1.90	25
IJ	31.0	14.7	0+	E	36	764	24.2	4. 84	25°0	2°32	35
12	29°0	13.9	€	t	28	772	24°4	4°84	25°0	1.71	44
13	31.0	14.3	01	2	34	766	24.4	4.84	24.8	1°97	40
14	34.0	14.6	0+ (ŧ	32	768	24°3	4.84	24°7	2 . 06	38
15	30° 5	14.2	0	8	28	772	24°3	4 . 84	24.6	2°34	28
16	34.5	14.8	6	t	32	768	24.2	5.12	24.5	2°73	26
17	31.0	15.0	40	T	32	768	23.7	4°02	23.9	1. 34	36
18	25.0	14.1	40	T	27	773	23°7	4.02	23 . 8	1.20	31
19	23.0	13.3	0+	¥	27	773	23°8	4.15	24°0	2.26	27
20	24•5	14.1	0+	=	26	774	23 . 8	4.15	24.1	1.83	23
			4 4 4								
average	31°3	14.5	11:9	800	31	769	24.1	4.59	24°4	2°05	24
21	31.0	14.2	(0	356	25	331	24.4	4.84	25. 0	1.74	20
22	28.5	14.0	01-	2	25	231	24.4	4.84	24°9	1.50	19
23	32.0	13,9	01-	=	29	327	•	5.12	24.8	2.46	17
24	26.0	14.4	€0	\$	26	330	•	4.02	23.9	2.70	18
25	29 • 0	14.9	64	E	28	328	23 。 7	4.15	23.8	1.94	26
			\$:5	1	[C C	
average	29•3	14.3		356	27	529	24°T	4.59	2 4 •5	ו01	202

Ta	Table 8 L	Lethal Density of	nsity	of Oxygen for Anchovies	for And	hovies (1	(Engraulis	is Japonicus	(sno
No.,	Body	Body	Sex	Amount of	Before	e Expt.	After	Expt.	Lethal
	Weight	Length		water	Water	Oxygen	Water	Oxygen	Period
	1				Temp。	Cont。	Temp。	Cont。	
	ES	5		ပ္ပ	20	<u>cc/1</u>	ົວ	<u>cc/1</u>	nim
~-1	2.55	6°9	40	160	12°3	4 。98	0°6	1.61	58
R	2°93	6.7	••	8	12.3	4.98	0° 6	1.34	63
ю	1.95	6 ° 3	69	æ	12.3	4.98	9°1	1.98	84
4	2,85	7.0	01	58	13°5	4°85	10.5	2.77	30
പ	4,75	8°5	•	88	13 . 5	4 。85	9°2	1.24	40
9	2,90	7.4	0+	38	13.5	4°85	0°0	1.47	70
2	2,85	6°9	0 †	tt	13 ,5	4 。85	0°6	1.90	72
Ø	2°35	6°8	69	86	13°6	4 . 85	9°2	2.27	45
თ	1.35	5°6	60	8	13°6	4°84	ദ്ദ	2°73	55
10	3.20	7.2	(0)	8	13,6	4 。84	9°4	1.39	47
11	3.40	7.5	0+ (¢a ðr	14°0	4°84	10.5	1,89	51
ମ	3°35	7.4	0+1	B 6	14°0	4。81	10.2	1.26	59
13	3,15	7°3	60 (W.	13,5	4°81	ອູ	1.67 I	58
14	1.70	6°0	6	88	13°5	4°84	10.5	1.70	50
15	3.25	7.5	0+	8	13.5	4°84	10.0	2°09	48
			00						
average	2.84	7 ° 0	7 ° 8	160	13.3	4 。87	6°6	1.82	55
16	12.5	11.1	40	200	22°0	4.44	23.0	1.89	26
17 1	0,11	11.0	01-	æ	22°0	4°44	23°1	1.58	32
18	9 5	10,2	0 †	8	22°3	4.40	22.9	2.02	25
19	10.0	10.5	6	88	22°0	4°44	22°9	1.50	40
20	9,5	10.5	6	8	22.1	4.40	22.7	1.76	27
	(4° 4				(1	
average	10,1	10.7	2:3	200	22°1	4.42	22.9	1.95	30

Toriu⁶⁾ at this time probably the oxygen within the swimbladder (which in the sardine is connected to the cecum of the stomach by a ductus pneumaticus) or the oxygen produced by the breakdown of the tissues is consumed. Soon there are several spasms of the eyeballs, pectoral fins, and ventral fins in that order, proceeding from the head toward the tail, and the fish is finally completely dead. A peculiarity of the rigor mortis of the dead fish is that ordinarily those which have their mouths open are somewhat more numerous than those which have them closed, however, there is no such interesting relationship as that seen in Hypomesus olidus during the spawning season, when the males die with their mouths open and the females die with theirs shut.⁷)

The lethal levels of free oxygen determined by this method for sardines and anchovies are shown in the following tables. As can be seen from these tables, the values for the lethal level of free oxygen differ more markedly as between individuals than in relation to the water temperature at the time of the experiment. For <u>Sardinia melanosticta</u> the lowest value per liter was 1.20 cc, and the highest was 2.92 cc. For <u>Engraulis japonicus</u> the lowest was 1.24 cc and the highest was 2.77 cc. However, if these are averaged the lethal quantity for the sardine is about 2 cc per liter, which can be seen to be somewhat lower in the case of the anchovy. This means that if water circulation in the bait well is carried on so that there will be at all times a free oxygen content of 3 cc or more per liter, the lives of the bait fish can be absolutely guaranteed as regards oxygen deficiency.

When we come to a discussion of the consumption of free oxygen in respiration, we naturally have to take into consideration the increase in carbonic acid gas, but in brief experiments like those described above the accumulation of carbonic acid gas has far less ill effects on the organism than has the rapid diminution of oxygen. Even supposing that the operation took place over a long period of time, in a bait tank with a plentiful circulation of water it would be absolutely impossible for the accumulation of carbonic acid to cause the death of bait fish. For this reason it was decided to leave for another time the performing of experiments on the effect of the accumulation of carbonic acid gas on bait fish.

c) Comparative studies of the capabilities of the new and old types of bait tanks in regard to free oxygen deficiency

The amount of water is greater in the new-style bait tank, in which the water is circulated positively by a pump, than in the type used hitherto, where the only water circulation is that supplied by the movements of the hull. It can be affirmed on the basis of the reports of this Station that in the future the number of bait fish that can be held will be doubled with little danger of a deficiency of free oxygen, however, the author wishes, in this section, to report the results of further experiments to show how superior the capabilities of the newstyle tank, with pump circulation, are over the old type, particularly from the point of view of the lack of free oxygen.

The figures shown in Table 9 are the results of experiments in the new bait tanks of the Fuji Maru. Because this was the very beginning of the tests and the operators were unfamiliar with the equipment, a great quantity of bait was too quickly placed in the tank at one time, and the spray pump was not used, so the excited fish congregated densely near the surface, and the increased circulation was almost without effect. Barely 30 minutes after the fish were placed in the tank the free oxygen concentration had fallen far below the lethal level for sardines and anchoyies, and all of the bait quickly suffocated and perished.

This indicates that when placing bait fish in the tanks one should avoid putting a great number in at one time, and the spray pump should be utilized fully to prevent the fish, immediately after they have been placed in the tank, from congregating densely near the surface and creating a localized lack of free oxygen.

The experiments shown in Table 9 utilized new "wild" bait fish while the results of previous experiments using tame bait were as shown in Table 10. Because the fish had become accustomed to confinement in a net live-pound and the number put into the tank was comparatively few, not many of the sardines crowded together near the surface when they were placed in the tank, and so, even though the spray pump was not used, and the free oxygen content 30 minutes after the fish had been put in the tank was only a thin 2.70 cc per liter, as the fish settled down the effectiveness of the pump circulation was apparent, the free oxygen increased quickly, and it was possible to complete the experiment without mishap.

From the two examples cited above it will be seen that when bait fish are held in a new pump-type bait tank, the oxygen supply must be made ample during the first hour or so when the fish are excited and liable to crowd together near the surface and that thereafter there is absolutely no need to worry about a lack of free oxygen. On the other hand, in the old-fashioned bait tanks used hitherto, which depend wholly on natural circulation, even though it is possible to get the bait into the tank safely in the first place, if the motion of the vessel becomes slight, it often happens that because of insufficient oirculation a lack of free oxygen develops and the fish begin to stick their noses out of the water. In Tables 12 and 13 are shown the results of actual tests in which the new and old style tanks of the Fuji Maru were compared by putting into them an approximately equal number, in relation to their respective water capacities, of wild bait fish caught 5 hours before and then cruising for a long period of time in the calm waters of Suruga Wan.

As can be seen from these two tables, in both cases good success was attained in reducing to a very great extent the diminution of free oxygen in the critical first hour after the fish were placed in the bait

Table	9
-------	---

Date of Experiment	Oct. 7, 1931	Time elapsed after fish were placed in tank	Min O	25	30
Species	Mixed sardines and anchovies	Free oxygen concentration	cc/1 5.21	1.49	0.96
Quantity of fish	599,000 gm.	Water temperature	°C 22.0	22.1	22.1
Time elapsed since capture	9 hours 30 min	Notes			all suffocated

Table 10

Date of Experiment	Oct. 5, 1931	Time elapsed after fish were placed in tank	Min O	30	Hour 1	5
Species	Mixed sardines and anchovies	Free oxygen concentration	cc/1 5.24	2.70	3,20	5.89
Quantity of fish	392,700 gm.	Water temperature	о _с 22.0	22.0	22.0	22.0
Time elapsed since capture	2 days 18 hours	Notes				

.

Tank
Bait
New-style
with
Experiment
75
Table

Date of ExperimentTime elapsed after fish were placedMinHourHour914ExperimentMar. 25, 1934fish were placed0 $ô0$ 1 2 4 9 14SpeciessardinesFree oxygen 4.52 4.33 4.33 4.79 5.01 4.31 Ouantity(60 scoops)Water temperature $ -$ Time elapsed5 hoursNotesNotesNotesNotesNotes 1.66 1.00 1.00 1.00		ATO 8 1	TAULE IS EXPERIMENT WILD NEW-SUYLE BAIT "ANK	New-S1	ATE P	ur Tan	×			
sardines Free oxygen 4.52 4.21 4.39 sardines concentration 4.52 4.21 4.39 (60 scoops) Water temperature	Date of Experiment		Time elapsed after fish were placed in tank	Min O	0.0	Hour	N	4	G	14
(60 scoops) Water temperature	Species	sardines	Free oxygen concentration	4.52	4.21		4°82	4 °79	5.01	4.31
5 hours	Quantity of fish	(60 scoops) 935,520 gm。	Water temperature				ļ		Į	
	Time elapsed since capture	5 hours	Notes						no ill effects	

Tank
Bait
Old-style
with
Experiment
13 13
Table

		TANK TO TWATCHTINGIN MICH OTO-SCATE DAIL JANK	S-DTO T	nyte b	ALU LANK			
Date of Experiment	Mar. 25, 1934	25° 1934 fish were placed 0 in tank	Min 0	40	hr. min. 1 - 40	3 - 40	8 = 40	3 - 4 0 8 - 40 14 - 30
Species	sardines	Free oxygen concentration	4°14	4.14 2.85	2°08	2.12	1,17	3,93
Quantity of fish	(40 scoops) 623,680 gm.	Water temperature			1			
Time elapsed since capture	5 hours	Notes					many nose- out	

tank. This is, after all, worthy of attention as it tells realistically of the improvement in handling methods obtained from the experience of a few experiments. After the bait was thus safely got into the tanks, the vessel cruised around Suruga Wan for a few hours, but about 8 hours after the fish had been placed in the tanks the surface of the sea became as calm as an oil slick and the boat's motion almost ceased. Circulation in the old-style tank was insufficient and the free oxygen content fell below the lethal level indicated by the basic experiments to as little as 1.17 cc per liter with the result that many of the fish lifted their snouts out of the water. The new-style bait tank, on the other hand, in readings taken at about the same time showed the extremely superior value of 5.01 cc of free oxygen per liter.

The facts recorded above tell how much superior the new bait tank with pump-type circulation is simply from the point of view of free oxygen.

Death from Sudden Changes in Temperature

Because fishes are poikilothermic animals, their body temperature is easily affected by the temperature of the surrounding water, and is raised or lowered by the conduction, radiation, and convection of heat, but there are, of course, limits of high and low temperature beyond which they cannot exist. These limiting values appear to vary quite a bit with the species of fish, but at any rate if these limits are passed, the fish cannot survive. For this reason there are quite a few cases in which, when bait fish have been loaded into the bait tanks and the vessel has headed for the skipjack grounds of the South Seas, the vessel has unwittingly entered a zone of water temperatures higher than the fish can withstand and the precious bait has been killed off, or where fish held in captivity in sheltered waters have been damaged by a sudden drop in water temperatures in the winter.^{VII}

In the case of the former, the plan of temporarily cooling the sea-water in the bait-tanks and trying to get out of the danger zone has been proposed by this Station already, and at the Taiwan Fisheries Experiment Station they are already planning to put this into effect, but the problem which naturally arises here is to find out how high and how low the lethal temperatures for bait fish are and how they are affected by sudden changes in water temperature.

The resistance of fishes to high temperatures has been studied by Day,⁹) Knauthe¹⁰), and Hathawayll) while their resistance to low temperatures has been reported on by Dutrochetl²) Heathl⁵) and Carterl⁴). On the sardine, however, there is nothing aside from some extremely simple notes in Dr. Kishinouye's study¹⁵) on the ecology of the sardine.

Therefore the author, in the early part of November, 1934, and the latter part of June, 1935, at Mitsu Beach in Tagata-gun, Shizuoka

VII. According to the testimony of Yuzo Masuda, Haruo Yamamoto, and other operators.

Prefecture using sardines and anchovies, and experimenting in the natural order, attempted to find first whether or not the sardine is completely lacking in the ability to regulate its body temperature, and then to what extremes of high and low temperature the sardine can maintain life.

a) Chemical evidence of the fact that the sardine does not have the faculty of regulating its body temperature VIII

In the homiothermic animals, from the birds on up, the temperature center is supposed to be in and around the tuber cinereum of the hypothalamus behind the hypophysis. The fact that when this area is subjected to cold and heat stimuli it brings about a raising or lowering of the body temperature has been gradually revealed on the basis of the cooperative studies of Isenschmid and Krehl¹⁶). Furthermore, according to Meyer¹⁷, animals which have a body temperature regulating center develop fever when injected with sympathetic nerve poisons and when parasympathetic nerve poisons are injected, their body temperature goes down -- an interesting fact. Because of this the author decided to leave the anatomical study of the temperature center to some future time and to determine by Meyer's method through the reactions to injections of nerve poisons whether after all the sardine lacks the capacity to regulate its body temperature, or in other words, whether the sardine does not after all nave a temperature center.

First of all subcutaneous injections were made in sardines using very small amounts (0.2 - 1.0 cc) of a 5% solution of the hydrochlorate of ac-tetrahydro-B-naphtylamin, a chemical which markedly excites the temperature center in the diencephalon and other sympathetic nerve ganglia and brings about centrally the production of high fever. of very small amounts (0.2 - 0.5 cc) of a quadruple solution of the hydrochlorate of adrenalin, which operates centrally and peripherally to cause fever, and of very small quantities (average 0.5 cc) of pituitrin, which works centrally to lower the body temperature. After injection the body temperature was determined by inserting a fish body temperature thermometer into the esophagus (this is a mercury thermometer with a fine, more or less curved tube and a scale which can be read to an accuracy of 0,1°C, manufactured by the Physical and Chemical Instrument Manufacturing Corp.). As a result of repeating these experiments several score times it became clear that the body temperature of sardines changes with the temperature of the surrounding water and aside from that such drugs cause absolutely no rise or fall in the body temperature, that is, no pharmacological changes in the body temperature can be detected. However, the author would like to note here his misgivings on the score that even supposing that a slight fever had been produced by the action of the above-mentioned drugs, it would probably have been impossible to detect it because it would have been immediately dissipated by the conduction and radiation of the heat. Nevertheless, if there had been any fever produced by the drugs, such fever would ordinarily have continued over a rather long period of time, and even though the degree of fever might be slight, its accumulation should reach an amount which could be measured.

VIII. The kind assistance of Technician Migita and Attaché Komoda was obtained in the preparation of chemicals and in other work. More accurate conclusions will be reached after the above-described experiments have been repeated at another suitable opportunity using a thermocouple. -- Accordingly it can be presumed with a fair degree of certainty that fishes, or at least sardines, do not have a heat or cold center. In other words, we can affirm that it is actually impossible for sardines to adjust their body temperature to a level which differs from the temperature of the environment.

b) Resistance to high and low water temperatures

The experiments described in the preceding section have shown how the surrounding water temperature controls the body temperature of the sardine, but the sardine is a living creature and therefore has its own particular limits of water temperature within which it can exist. The author performed the following experiments to find these limits and the resistance of bait fish to sudden changes in water temperature.

The experimental tanks, as shown in Figure 8, were two wooden boxes 100 cm long by 60 cm wide by 60 cm deep. One was used for the experiment and the other for the control. Both had an opening for the water supply pipe in the middle lower portion, and a drain 10 cm from the upper edge. Mercury thermometers were inserted at two opposite corners. One of these tanks was connected by a siphon to a reservoir through a temperature regulator which could cool or heat the sea water to the proper temperature. The control tank was connected directly with the reservoir without passing through the regulator.

With such equipment a suitable supply of sea water was constantly drawn from the reservoir and supplied to both tanks. At the same time 20 each of sardines and anchovies were transferred as speedily as possible from the net live-pound. The resistance to high and low temperatures was tested three times at each temperature with the following results.

1) Resistance to high temperatures

March & Same

To put it in different words, this experiment was to find out how much of a sudden rise in water temperature is required to kill bait fish which are living at a normal temperature. According to Table 14, when sardines which at the time of the experiment were living in sea water of about 22°C were transferred directly into an experimental tank having a water temperature of 28°C there was only 10 - 15% mortality in the course of three hours. Table 15, however, shows that when sardines living under almost the same conditions were transferred directly to an experimental tank having a water temperature of 30° C, there was within 1 hour and 30 minutes a mortality of 65 / sic. Should be 95. 7 - 100%, a result almost equivalent to total destruction.

With anchovies which were taken at the same time as the above-mentioned sardines and which after capture were kept in the same net live-pound



Fig. 8

- A experimental aquarium
- B control aquarium
- C temperature controller
- D water tank
- E coiled tube
- F temperature adjustment
- G thermometer
- H drain

14	
Table	

(20 sardines)	Lenzth cm	0		• · •••			1			ļ		no deaths	11.8 - 13 . 3	80
Experiment (t i	Dead	0	0	0	0	0	0	0	0	0	0	0	Death Rate
Control E		Temp.	22	£	E	*	=	22.1	E	52	E	#	E	
	Trial	Length cm		ł				I			1	12.4-13.0	dead 13.2 alive 12.0- 12.8	15%
each trial)	Third	Number Dead	0	0	0	0	0	0	0	0	0	പ		Death Rate
sardines ea	Trial	Length cm	Į		ļ				13.4	}	12.4, 12.9	1	alive 11.9- 13.0 ´	15%
ment (20	Second	Number Dead	0	0	0	0	0	0	r-1	0	્ય	0	0	Death Rate
Main Experiment (20	First Trial	Length cm	P		1		l		-		13.1	12.2	alive 12.1- 13.5	10%
	First	Number Dead	0	0	0	0	0	0	0	0	Ч	1	0	Death Rate
	Water	Temp. oC	28	6	£	=	£	F	Ŧ	£	#	8	H	
Elapsed			0	10	20	30	40	50	60	90	120	150	180	

200,650

	Leneth cm			1	()			Î				-	I	Į	no deaths	11,9-13 .4	8
Evnowi mont		Dead	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Death Rate
Contuc] B	1.	Temp. oc	22	8	8	Ŧ	\$	8	æ	#	2	Ŧ	8	3	22°2	22°3	
	d Trial	Length cm			13.1			12,1	12°1, 14.7	12,1-13,1	12.5-13.2		ŝ	S.	Ì	alive 12.6	95%
each trial)	Third	Number Dead	0	0	r-1	0	0	m	ຸ	12	ю	0	0	0	0	0	Death Rate
(20 sardines eac)		Length cm				-	12.9, 13.1	11.4-13.4	12.1=13.7	11.9-12.8	12.7	12.6	1	12.5, 13.2		alive 12.5	95%
+		Number Dead	0	0	0	0	ର୍ୟ	ю	g	4	r-1	Ч	0	୍ୟ	0	0	Death Rate
Main Exnerimen	. Trial	Length cm				13. 8	12.5	11.2-13.8	12.2, 12.4	12.0	12.5	12.4, 12.5	12.4, 12.7	14.7	}	12.3	100%
	First	Number Dead	0	0	0	н	Ч	ω	ଋ	-4	Ч	ત્ય	ର୍ୟ	Ч	0	Ч	Death Rate
	Water	Temp. oc	30	E	æ	\$	8	8	· 85	· 8	\$ F	\$	\$	*	*	¥	
Elapsed	Time		0	ß	10	15	80	25	30	35	40	45	50	55	60	06	

24

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there was, as is shown in Table 16, only 10 - 30% mortality in an experiment which continued for 3 hours at 30° C. As Table 17 shows, a mortality rate approaching total destruction was obtained only as the result of an experiment of an hour and a half's duration at the high temperature of 32° C.

Looking at these results it can be seen that anchovies have greater resistance to high water temperatures than have sardines.

2) Resistance to low temperatures

According to Table 18, when sardines which at the time of the experiment were living in sea water with a temperature of about 22° C were transferred directly into an experimental tank with sea water at the low temperature of 8° C, there were no deaths even after three hours and a half, and, as Table 19 shows, even when they were placed in a tank with sea water at 6° C, there was only 5 - 10% mortality after three and a half hours. However, the anchovies which were taken at the same time as these sardines and which were kept after capture in the same holdingnet, had a mortality, as shown in Table 20 and Table 21, of 25 - 30% at a water temperature of 8° C (which had caused no mortality at all among the sardines).

Looking at these results it can be seen that sardines have greater resistance to low water temperatures than have anchovies.

According to these experiments, the anchovy can resist high temperatures (sudden rises in water temperature) better than the sardine, and the sardine can resist low water temperatures (sudden drops in water temperature) better than the anchovy. The range of livable water temperatures is about $7^{\circ} = 29^{\circ}$ for the sardine and $11^{\circ} - 31^{\circ}$ for the anchovy.

These are the results of experiments with bait fish from the Pacific coast of central Honshū made during early summer when the sea water temperature was approximately 22°C, and it may not be possible to apply them generally. At a different season, with fish from a different locality, or with fish which had been adapted to gradual changes in temperature, it is thought that, if we follow Loeb's theory of immunization to temperature changes, the fish would be able to survive somewhat higher and somewhat lower (the possibility is greater in the case of the latter) temperatures than the results of these experiments indicate. At any rate these results are well supported by the fact that the most experienced operators say that sardines are weak at high temperatures and strong at low ones while anchovies are just the opposite, and the fact that generally sardines are distributed in the cold northern regions while anchovies are found in the warm south.

Next I shall attempt to say a word concerning the condition in which bait fish die of such sudden changes in water temperature and the reasons for this mortality.

r ansed			Main Experiment (20	ment (20	anchovies	each trial)			Experiment	(20 anchovies)
Time	Water	First		Secon	Second Trial	Third	Third Trial	Water	Number	Length cm
	Temp.	2 F		Number Dead	Length cm	Number Dead	Length cm	Temp. oc	Dead	
0	30	0		0	1	0		22	ο	1
IO	*	0	1	0	l	0	ł	F	0	1
20	*	0	ļ	0		н	11.5	F	0	1
30	2	0		0	I	0	1	£	0	1
40	\$	0		0	1	ч	10.6	22°3	ч	10.4
60	8	0	1	0	1	ч	10.0	Ŧ	0	
60	*	0		0		ŝ	10,0-12,1	ŧ	0	ļ
06	3		10.0	Ч	6°6	Ъ	10.9	Ŧ	0	
120	*	Ъ	10.7	Ч	10.4	0		Ŧ	0	1
150	#	0	1	~	9°9, 11.1	0		Ŧ	0	
180	E	0	alive 10.1- 11.1	Ч	dead 10,2 alive 8,9- 12,0	0	alive 10.0- 10.5	22.1	0	alive 9.9- 11.1
		Death Rate	10%	Death Rate	25\$	Death Rate	30%		Death Rate	5%

			Experiment (20	anchovies	each trial	(1		Experiment	(20 anchovies)
	First	First Trial	Secol	Second Trial	Thir	Third Trial	Water	Number	Length cm
	Number Dead	Length cm	Number Dead	Length cm	Number Dead	Length cm	Temp. oC	Dead	
L	0	1	0	.	0	ł	22	0	
	0		ο	l	0	I	2	0	
	0	1	Ч	11,5	0]	ŧ	0	1
	0	1	Ч	10,6	-1	10.4	E	0	
	0	l	ю	10.0-12.1	0		Σ	0	
		10.7	~	10.0, 11.0	2	10,2-10,9	Ŧ	0	
	0		ц	9.9 . 11.1	თ	9 .9-11. 4	2	0	
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	10.1, 10.6	н	10.0	Ч	10,1	#	0	1
	2	9 <b>.</b> 8-11.0	0		0		<b>.</b>	0	
<u></u>	ю	10.2-10.9	Ч	10°9	0	1	22.2	0	no deaths
	0	alive 10.0- 11.1	0	1	l	dead 10.1 alive 9.8	2	0	9.7-10.8
<u> </u>	Death Rate	65%	Death	100%	Death	95\$		Death	80

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| (20 sardines) | Length cm | | | | | 1 | ł | ł | | 1 | 1 | | no deaths
11.9-13.9 | 80 |
|---------------|-------------|----------------|------|------|----------|----|----|----|------|------|-----|-----|------------------------|---------------|
| Experiment | | Dead | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Death
Rate |
| Control | · · | Temp。
oC | | 22°2 | æ | 3 | £ | * | 22°7 | 22°8 | F | £ | 23°0 | |
| | Third Trial | Length am | | | 1 | ļ | | l | 1 | 1 | | 1 | no deaths
11.4-13.4 | 80 |
| each trial) | Third | Number
Dead | PC44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Death
Rate |
| sardines | Trial | Length cm | | ł | | ļ | | 1 | 1 | 1 | ł | 1 | no deaths
12.0-13.7 | 8 |
| iment (20 | | Number | neau | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Death
Rate |
| Main Experim | First Trial | Length cm | | ļ | 1 | ļ | | 1 | 1 | | 1 | 1 | no deaths
11.9-14.0 | 8 |
| | First | mn C | nean | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Death
Rate |
| | Water | Temp. | 2 | œ | 8 | ų | E | * | 5 | ¥ | * | 8 | \$ | |
| Rlansed | Time | | | 0 | OT | 20 | 30 | 60 | 06 | 120 | 150 | 180 | 210 | |

| Flanced | | | Main Exnerim | ent (20 | anchovies ea | each trial | | Control Experiment | | (20 anchovies) |
|---------|----------|---------------|------------------------|---------------|----------------------------------|---------------|---------------------|--------------------|---------------|---------------------------|
| Time | Water | First | First Trial | Second | | Thir | Third Trial | Water | Number | Length cm |
| | Temp. | Number | ch cm | Number | Length cm | Number | Length cm | Temp。 | Dead | |
| | c)
N | Dead | | Dead | | חבמת | | > | | |
| 0 | 9 | 0 | 1 | 0 | ļ | 0 | | 22.2 | 0 | - |
| JO | F | 0 | 1 | 0 | ļ | 0 | ļ | # | 0 | an a <u>a b</u> ian da se |
| 20 | ۲ | 0 | | 0 | 1 | 0 | 1 | £ | 0 | |
| 30 | 5 | 0 | 1 | 0 | | 0 | I | F | 0 | |
| 60 | t | 0 | | 0 | 1 | 0 | 1 | E | 0 | • |
| 6 | = | 0 | 1 | 0 | ł | 0 | 1 | £ | 0 | |
| 120 | ₽ | 0 | | Ч | 13.5 | 0 | Į | 22.5 | 0 | |
| 150 | 7 | 0 | | 0 | 1 | -1 | 12.4 | * | 0 | 1 |
| 190 | E | 0 | | 0 | 1 | 0 | 1 | E | 0 | |
| 210 | ŧ | 0 | no deaths
11.8-12.8 | | dead 12.9
alive 12.0-
13.7 | 0 | alive 12.6-
13.5 | 22.7 | 0 | no deaths
12.0-13.2 |
| | | Death
Rate | \$0 | Death
Rate | 10% | Death
Rate | 5% | | Death
Rate | 50 |

| 80 | |
|------|--|
| able | |
| q | |

| Elapsed | | | Main Experiment | ment (20 | sardines | each trial) | | Control | Control Experiment | (20 sardines) |
|----------|-------|---------------|---------------------|----------------|-----------------------|----------------|-----------------------|-------------|--------------------|---------------------------------|
| Time | Water | First | First Trial | Secon | l Trial | Third | Trial | Water | Number | Length cm |
| | | Numb
Dea | 5 | Number
Dead | Length cm | Number
Dead | Length cm | Temp.
oC | Dead | |
| 0 | DI | 0 | | 0 | | 0 | 1 | 23 | 0 | |
| 10 | * | 0 | | 0 | 1 | 0 | 1 | 2 | 0 | ļ |
| 20 | \$ | 0 | ļ | 0 | l | 0 | 1 | 7 | 0 | 1 |
| 30 | * | 0 | 1 | 0 | 1 | 0 | 1 | Ŧ | 0 | 1 |
| 60 | 2 | 0 | | 0 | | 0 | 1 | * | 0 | 1 |
| 06 | # | 0 | -internet | 0 | | 0 | 1 | £ | 0 | ļ |
| 5
120 | = | Ч | 10.7 | 0 | 1 | 0 | 1 | 2 | 0 | 1 |
| 150 | # | 0 | | 0 | 1 | 0 | 1 | 22.8 | 0 | ł |
| 180 | \$ | 0 | | 0 | | 0 | 1 | 2 | 0 | 1 |
| 210 | \$ | 0 | ļ | 0 | | 0 | | 23 | 0 | ļ |
| 240 | * | 0 | alive 10.0-
11.0 | 0 | no deaths
9.9-10.9 | 0 | no deaths
9.9=11.1 | E | 0 | no deaths
10 . 1~11.2 |
| | | Death
Rate | 5% | Death
Rate | 30 | Death
Rate | 80 | | Death
Rate | % |

| Elapsed | | | Main Exper | iment (2 | Experiment (20 anchovies e | each trial | 1)
1 | Control I | Control Experiment | (20 anchovies) |
|---------|-------|----------------|---------------------------------|----------------|----------------------------|----------------|---------------------|-------------|--------------------|------------------------|
| Time | Water | First | rrial | Seco | Second Trial | Thir | Third Trial | Water | Number | Length cm |
| | Tempo | Number
Dead | Length cm | Number
Dead | Length cm | Number
Dead | Length cm | Temp.
oC | Dead | |
| 0 | ω | 0 | | 0 | | 0 | | 22 | 0 | ſ |
| 10 | * | 0 | | 0 | | 0 | | # | 0 | ł |
| 20 | 7 | 0 | | 0 | | 0 | | * | 0 | 1 |
| 30 | 3 | 0 | ł | 0 | | -1 | 10.5 | E | 0 | |
| 60 | 7 | 0 | | 0 | | 0 | | E | 0 | |
| 06 | 3 | Ч | l | Ч | 10°7 | 0 | | E | 0 | |
| 120 | Ŧ | 0 | | 0 | | -1 | 10.2 | 22°1 | 0 | ł |
| 150 | \$ | 22 | 11.0 | ю | 9°9=11°0 | Ч | 10°1 | 23°0 | 0 | |
| 180 | F | 5 | 10.2, 10.3 | -1 | 10°4 | ્ય | 10,1, 11.0 | Ŧ | 0 | |
| 210 | ۶, | н | dead 9.9
alive 10.0-
11.0 | 0 | alive 10.1-
11.0 | 0 | alive l0.l=
11.4 | £ | 0 | no deaths
10.0-10.9 |
| | | Death
Rate | 30% | Death
Rate | 25% | Death
Rate | 25\$ | | Death
Rate | \$0 |

When the temperature first rises sharply, the movement of the fish generally becomes more rapid and the respiration increases (as the water temperature rises the amount of saturation of free oxygen decreases, and the situation therefore gradually becomes disadvantageous to the bait. When bait fish which were carried in the old-fashioned bait tank with its poor water circulation encountered a zone of high water temperatures, they could hardly escape the bad effects of oxygen deficiency). As the temperature rises and the amount of respiration increases, the amount of combustion within the body also increases. At this time, if the water temperature goes to a lethal level, the bait fish immediately begin to run about wildly, and some bend the middle portion of their bodies strongly from time to time, while others are seen to project their noses above the water and others leap clear out of the water. Finally they begin to die off, the least resistant ones first. This type of death from high water temperatures is probably, as Mayer19) says, the result of the accumulation of H2CO3 in the tissues. There have not yet been any cases of heat rigor at the experimental temperatures mentioned above.

If the water temperature drops sharply, the bait generally begins to swim slowly and the amount of respiration decreases. If the temperature falls to a lethal level at this time, the fish gradually become quieter, some begin to lie on their sides from time to time, and finally they begin to die, the least resistant ones first. However, the cause of death in this case is not as clearly apparent as with the high temperatures, and it is probably paralysis of the sinus venosus of the heart.<sup>20</sup>)

Death from Wounds

In the foregoing sections I have dealt with mortality due to lack of free oxygen and mortality resulting from sudden rises and falls in the water temperature, both of which become lethal in an extremely short space of time. In this section I will deal with mortality due to wounds, that is, the type of mortality that results from injuries received at the time of capture, at the time the fish were placed in the bait tank, or thereafter, which in many cases causes death and becomes apparent only after the passage of a long period of time, a type of mortality which can occur most easily and which is rather difficult to diagnose.

When, as has been done hitherto, bait fish which have been kept for some time in a holding net in sheltered waters were loaded into the bait tank, this type of mortality had already been liquidated in the holding net and so it never became much of a problem, but when, as at present, bait that has just been captured, that is, wild bait, is immediately transported in the boats, a good deal of economic loss can result if sufficient attention is not paid to this type of mortality. There are, however, absolutely no reports of studies designed to deal with this problem, and therefore the author has attempted the following investigations concerning the mortality resulting from wounds to bait fish, copying the method of classification used for wounds to human beings.<sup>IX</sup>

VIII. [sic] According to the teachings and writings (21,22) of Professor Sadanori Mita.

L
a) Major wounds

These are, as shown in Plate I, such large injuries as the loss of eyes, lower jaw, opercle, or head, the breaking of the trunk, and the exposure of the viscera, and almost all of the injuries of this type are caused when the fish, at the time of their capture, in struggling to escape crowd together at the lead line and are pinched in the net. Many of these fish die immediately, but in point of numbers these are not more than 1 - 2% of the fish that are captured. When these dead fish are placed in the bait well along with the other healthy bait, they putrefy and pollute the water<sup>X</sup> and for this reason they should be got rid of as soon as possible.

The author gathered up from a bunch of anchovies taken for bait purposes in a lift net off the Kamogawa in Chiba Prefecture on July 3, 1933, all which had received major wounds in the net, and made a study of the location of their wounds. Out of 45 fish selected at random, 30 had the viscera exposed by pressure on the belly, 19 had lost eyes, and 18 had lost their opercles. Fish with the heads missing and the trunk broken were found to be comparatively few.

No special observations were made on sardines, but in general their injuries were about the same as those of anchovies except that it appeared that a good deal fewer of them lost eyes. (See Plate I)

b) Superficial wounds

When bait fish are carefully examined, especially those which have died, the first thing that is noticed is the loss of scales, not one of them having all of its scales. Furthermore, the majority of the fish show more or less bleeding at the eyes, along the belly and sides, or in other places. This bleeding is liable to be wrongly considered all due to simple lacerations received on the surface of the body either at the time the fish were netted or when they were placed in the bait tanks. Even the fishermen appear to be of this opinion, however, if these wounds are carefully examined the number of simple lacerations is unexpectedly small, and most of the injuries are seen to be either subcutaneous bleeding caused by external damage or hemorrhage caused by the rupture of viscera.

1) Loss of scales<sup>21)</sup>

The loss of scales can never directly cause the death of bait fish, but there is no doubt that it lowers their vitality somewhat and makes them more liable to receive secondary wounds. Because of this the fishermen even estimate the vitality of bait fish by the degree of scale loss among them. The author carried out an extremely practical experiment in order to find out to just what degree bait fish lose scales under any given circumstances. On March 25, 1934, twenty sardines which had almost all of their scales were carefully selected from a school being

X. Poisonous matter like ptomaine appearing.

held at Mito Wan in Shizucka Prefecture. These fish were placed one at a time in a partly filled basin of water and allowed to jump about for 30 seconds. Then the number of scales which had fallen off during that time were accurately counted. As a result it was found that sardines averaging 13.3 cm in length lost an average of 62 scales in 30 seconds. Scales were lost most easily from the lower sides of the fish and very similar values were obtained regardless of the size of the specimen. However, the rate of scale loss appears to be fastest at first and to gradually diminish so that it is not possible to calculate the number of scales lost for units of time from 1 second to 1 minute on the basis of these results.

Looking at the results shown in Table 22 it can be gathered that sardines lose their scales very easily and that in a fairly short time with the operation of a certain amount of external force almost all of the scales would be lost. As a matter of fact, quite a few individuals are found among bait fish which have lost almost all of their scales. This means that an extremely large proportion of the fish are liable to receive other mortal injuries because of the rubbing off of their scales.

2) Lacerations

These are injuries to the surface of the body received from the time the fish are taken in the net to after they are placed in the bait tanks, especially in the case of fish which have lost scales. The external force applied is not great enough to cause the subcutaneous wounds described in the next section, and most of the injuries consist of the rubbing off of the epidermis with more or less bleeding, the wounds not being directly related to death.

3) Bites

Among bait kept in live-pounds there are occasionally discovered individuals which have lost vitality through rather large wounds on the tail and other parts of the body, as is shown in Figure 9. Many wounds of this character are bites resulting from the attacks of mackerel which are mixed in with the bait fish in captivity. It should be ascertained whether or not any mackerel are mixed in with the bait, and if there are, they should be speedily fished out. Even where such injuries are fortunately not fatal, there is bound to be some danger of the fish finally dying of bacterial infection when the water temperature is high.

c) Subcutaneous wounds

These are divided into subcutaneous hemorrhages, broken bones, brain contusions, and ruptured viscera. In many cases subcutaneous hemorrhage is associated with brain contusions or ruptured viscera, but sometimes they appear alone. In either case this type of injury consists

| Body Length
of Sardine | Number of Scales
Lost in 30 sec | Body Length
of Sardine | Number of Scales
Lost in 30 sec |
|---------------------------|------------------------------------|---------------------------|------------------------------------|
| CM | | CM | |
| 11.0 | 70 | 13.3 | 71 |
| 11.2 | 60 | 13.3 | 83 |
| 11.5 | 64 | 13.4 | 53 |
| 12.4 | 55 | 13.7 | 62 |
| 12.5 | 69 | 13.9 | 62 |
| 12.6 | 47 | 14.1 | 66 |
| 12.6 | 60 | 15.2 | 58 |
| 12.7 | 41 | 15.2 | 77 |
| 12.8 | 54 | 15.4 | 64 |
| 12,9 | 62 | 16.3 | · 62 |
| Average | values | 13.3 | 62 |

Table 22 Experiment on the Loss of Scales by Sardines

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only of the breaking of the subcutaneous capillaries and the blood quickly coagulates, leaving only gray scars which persist for some time, without any relation to the mortality of the fish. In fishes, broken bones do not occur as frequently as they do in the case of injuries to human beings, and where they prove fatal it is usually the result of internal bleeding caused by contusions. Therefore, in this study the two firstnamed types of inuries will be omitted, and the mortality resulting from the latter two will be discussed.

The result of brain contusions or the rupture of viscera is that the large portions of the deep arteries and veins are broken. The blood running out of these vessels effuses into the tissues and, in fishes, it finally reaches a condition where it can be seen from the outside. If these are observed inattentively, they are liable to be confused with the minor lacerations and simple subcutaneous hemorrhages described above. Consequently it is understandable that hitherto all evidences of bleeding appearing on the outside of the fish have been mistakenly, and without deep consideration, believed to be simple external wounds. The author has fortunately been able to distinguish them in this study, and has been able to discover that brain contusions, ruptured viscera, and other types of subcutaneous wounds are the main causes of the deaths which hitherto have occurred several days after the fish have been captured.

In the following paragraphs the motive for this discovery and the results of the study will be explained.

1) On the peak of mortality which appears several days after the bait fish have been placed in captivity

Fishermen who handle bait fish know very well from experience that when fish are caught and held in captivity the mortality is extremely small at first and that the peak of mortality appears after 1 or 2 days have passed. They are so familiar with this phenomenon that when a day or more has passed after the capture of the fish and the mortality is gradually increasing, they indirectly forecast the imminent appearance of the peak mortality by such sayings as "Well, it looks as if the sardines' heads have begun to ache."XI Kiumra<sup>24</sup>), who made a statistical study of the mortality of bait fish, reported from actual cases that the maximum values of the death rate appeared after the passage of 1 - 2days from the capture of the fish, and he gives a clear death rate curve, as shown in Figure 10.

The author, at Mito in Tagata-gun, Shizuoka Prefecture, in December, 1933, using as material sardines (average length 14.2 cm, average weight 25.1 gm) which had just been captured and which appeared to be comparatively uninjured, pierced the brain, liver, and spleen with a cannula to

XI. Expression used by the Mito bait-dealer Mr. Yamamoto, as well as many other commercial operators.

Figure 10 Death Rate Curves for Bait Fish



- ... approximately 7,500 sardines
- o approximately 15,000 anchovies
- n ... number dead
- k ... mortality coefficient (original figure by Kimura)

induce bleeding. The sardines were then immediately released in a live pound and their subsequent progress was observed. As the following table shows, those in which cerebral hemorrhages had been produced reached their maximum death rate about a day later while those in which hemorrhages had been induced in the liver or spleen showed the peak of their mortality somewhat later.

Again in the latter part of March, 1934, in the bait fish experiment aboard the Fuji Maru, Assistant Technician Mishio<sup>23</sup>) observed a large number of dead sardines and, although this was merely judging from external appearances, he reported that the hemorrhages in the head appeared first and those of the belly later.

When we collate the accounts of the actual experience of fishermen, as given above, the examples cited by Messrs. Kimura and Mishio, and the author's experiment in artificially causing injuries to sardines, it seems most correct to consider that the main cause of the abrupt increase in mortality of sardines 1 - 2 days after they have been placed in captivity is not the lack of free oxygen already referred to, nor is it based on sudden changes in the water temperature, but it is rather the result of hemorrhages from subcutaneous wounds.

2) Brain contusions

When, because of a collision or from some other cause, a rather large force of inertia is applied to the brain, brain contusions are caused, hemorrhages occur locally (mostly in the cerebrum) in the brain tissues, finally a large amount of blood is shed in the brain case, and within a day after the injury death ensues.

The presence of brain contusions can generally be detected indirectly by the bleeding in the snout of the fish resulting from the impact. The occurrence of brain hemorrhage can be judged through noting the extravasation of blood into the cornea or into the T-shaped region at the top of the skull corresponding to the fonticuli. (See Plate I.)

3) Rupture of internal organs and accompanying injury to blood vessels

When a large force is applied to the belly of the fish, in most cases such soft parts as the liver and spleen or the deep arteries and veins associated with these organs are injured so that large quantities of blood are hemorrhaged into the body-cavity. (See Plate I.) The time involved varies considerably depending on the extent of the injury, but usually most of the fish die several days after being hurt. Hofmann<sup>26</sup> in his work on forensic medicine gives a detailed account of how roughly the same results appear in the case of ruptures of the internal organs in humans.

The foregoing deals with brain contusions and ruptures of the liver and spleen which are sufficient to indicate clearly the cause of death,

| Wounds |
|-----------|
| Simulated |
| with |
| Sardines |
| of |
| Rates |
| Death |
| 23 |
| Table |

| Elapsed Time (hours) | 4 | 8 12 | | 16 | 20 | 24 | 28 | 32 | 36 | 40 44 | | 48 | 52 | 56 | 60 |
|--|--------|------|---|----|----|-------|----|----|----|-------|----|------|------|----|----|
| Deaths from cerebral hemorrhage (fish) | , TT (| 2 | 5 | 9 | 23 | 48 | 50 | 44 | 46 | 17 | 6 | 9 10 | 4 15 | 15 | 21 |
| Deaths from hemorrhages of the liver
and/or spleen (fish) | 15 | 8 | 4 | 5 | 16 | 12 14 | 14 | 16 | 29 | 58 | 37 | 39 | 33 | 25 | 21 |

but actually when bait fish which have died after several days in captivity are subjected to a detailed examination, they do not all show such clearly apparent injuries. From time to time these dead fish are discovered to individuals which show only slight bleeding caused by the breaking of subcutaneous tissues. The cause of these deaths, according to the teachings of Professor Mita, is poisoning by the intermediate products of the decomposition of protein, a subject which is under study at present in forensic medicine. The protein that, when the animal is in a healthy state, would already be changed into a nontoxic final product of metabolism before being transported through the arteries invades the circulation from the injured tissues while it is still in the toxic stage of an intermediate metabolic product or visceral poison. It is wondered whether these deaths should not be interpreted as being due to poisoning from this source.

4) On the magnitude of the lethal external forceXII

The author constructed the device shown in the following sketch and carried out experiments to establish a general standard for the magnitude of the external force that is lethal to bait fish.

As the figure shows, the device consisted of a board made of zelkovawood, with a vertical post. To the post a short tube was fastened by one end in such a way that it could move up and down. This tube served to make the fish fall vertically, and experiments were made with it fastened at heights of 20 cm, 40 cm, and 80 cm. The fish dropped onto the board by means of this tube were quickly marked and immediately released in the live-pound. (Such an experiment required only from 30 seconds to 1 minute.) The numbers of deaths that occurred during two days were complied and compared.

| First Exp | periment | Average | Weight | 22 . 5 | gm | Aver age | Length | 13.4 | cm |
|-----------|------------|---------|--------|--------|----|-----------------|--------|------|----|
| Second | 19 | 91 | 49 | 20.7 | 11 | 9 9 | 11 | 12.8 | 11 |
| Third | 1 9 | 88 | ** | 23.0 | 99 | 11 | 11 | 13.5 | Ħ |

Of each of these groups of fish, 50 were dropped from each height with the results shown in the following table / Table 24 7. From these data it can be inferred that when bait fish like those used in these tests are dropped vertically from heights of 40 cm or more, the external force which they receive from the board on which they land is lethal.

Death from Pathological Damage to the Blood Vessels

When several days have passed after a school of fish is placed in a live-pound, it begins to swim in an orderly fashion and in a definite direction<sup>27</sup>) (mostly clockwise) around the circumference of a hypothetical vertical axis at the center of the live-pound. Bait fish which have

XII. Instructions were received from Technician Toru Sato and Attache Susumu Kurita.





| Table 2 | 4 |
|---------|---|
|---------|---|

| | Height | 20 cm | 40 cm | 80 cm |
|--------|-------------------|-------|-------|-------|
| Number | First Experiment | 3 | 36 | 37 |
| of | Second Experiment | 1 1 | 24 | 30 |
| Deaths | Third Experiment | 4 | 37 | 39 |

attained this condition form a well-regulated school and consequently there is very little opportunity for them to be injured by collision or rubbing together. Furthermore, as has already been stated, the internal injuries which are received at the time of capture or when the fish are placed in the bait-tanks will after a few days either result in death or will be naturally cured, and therefore the usual thing is for bleeding fish to gradually disappear and for the death rate to abruptly decline when a certain amount of time has passed after the fish have been placed in captivity.

However, when several weeks have passed after the fish have been placed in the live-pound, red spots due to bleeding, called by the fishermen "red rust", appear under their skin (Figure 12). If fish on which these red spots have appeared are carefully examined, it is clear that in most of them it is a matter of simple subcutaneous bleeding and that the internal organs are completely unharmed, but the peculiarity of this condition is that most of the fish die of just this subcutaneous bleeding. From this fact it can easily be gathered that this bleeding is of a different sort from that described earlier as due to force. Since I have not yet had an opportunity to study this phenomenon, I cannot speak with conviction, but in view of the fact that these symptoms present certain points of resemblance to the Morbus maculosus Werlhofi<sup>28</sup>) that occurs in humans and other animals, it is thought that it cannot be far wrong to consider these red spots as a type of pathological damage to the vascular system under certain external conditions.

Death from Excessive Production of Air Bubbles

On the occasion of an experiment in holding bait sardines in the bait tanks of the Fuji Maru during a three-day period beginning July 24, 1931, an imperfection in the intake of the water pump caused the production of a great number of bubbles, and at the same time a large number of dead fish appeared. 1) When these dead fish were carefully examined. items that deserve mention as impressions obtained directly after the fish died are the fact that almost all of the dead fish had a great many of the above-mentioned small air bubbles on the surface of their bodies and on their gill lamellae and gill-rakers, and that rather conspicuous bleeding could be seen at their snouts, eyeballs, and under their skins. Particularly worthy of note is the fact that the free oxygen did not reach the saturation point (rather there was somewhat of a deficiency)1). and the fact that there were no air bubbles produced in the eyeballs or under the skin, which facts testified eloquently that the cause of death was not bubble disease. In other words, the cause of these deaths must be sought elsewhere than in bubble disease. Kimura<sup>5</sup>) recently has kept sardines in a bait tank in which great quantities of bubbles were produced experimentally and has observed the great number of deaths which resulted. He concluded that the cause of these deaths was the injuries resulting from the disruption of the swimming of the sardines as the bubbles rose in the tank. Ishii<sup>30</sup>), using goldfish as material, conducted a roughly similar experiment and found that air bubbles of a radius of 0.03 cm or

more would remain attached to the surface of the body of the fish for as long as 10 minutes or more without dissipating.

If we collate and examine these facts and the experiment described above, we see that even though the presence of so many small air bubbles may have the advantage of increasing to some extent the free oxygen in the water, physically the bad effect on the bodies of the fish outweighs this. When the air bubbles rise in the water, they not only cause injuries indirectly by disrupting the swimming of the fish, but if they stick to the gill lamellae, which are the breathing apparatus of the fish, they make respiration difficult, and in either case there is plenty of danger of their finally leading the fish to its death.

Death from Unnatural Feeding

The natural food of sardines is phytoplankton such as diatoms and zooplankton such as copepods, so even in a net live-pound or in the baitwells of a boat as long as there is a certain amount of circulation of water the fish will not go completely without food even though they are not fed. However, if the fish are kept for a long time without feeding, they become as shown in Figure 13, with the body lean and only the head large, and not only is their quality as bait lowered but their resistance to all sorts of damage is weakened. Accordingly, when bait fish are to be kept for a long time or transported a long distance they should be properly fed<sup>31</sup>, <sup>32</sup>, but if errors are made in the time and amount of feeding the results are sometimes worse than no feeding at all.

If bait fish are held in captivity and a few days are allowed to pass, then, as described earlier, the school will begin an orderly circling inside the live-pound, and somewhat later they begin to show a condition that the fishermen call "puffing out their cheeks". This term describes the movement in which the opercles are spread as wide as possible, fully opening the gills, the fish swimming for a while in this condition and then closing the opercles. This is the food-seeking action peculiar to these fish, and it is carried on simultaneously with respiration.XIII Since bait fish which show this action are in a good state of health, the fishermen use its presence or absence as a criterion by which to judge the quality of bait. Fish which act in this way may be fed without any trouble, but fish which do not show this type of activity will never take the food that is given to them, and feeding will only result in fouling the water so this is a matter which requires care. Ordinarily it happens only rarely that deaths are caused by mistakes in feeding. but sometimes when bait fish which have been kept for a long time without feeding are being transported and are mistakenly fed the wrong amount. very regrettable results ensue. On July 5, 1931, in the course of an experiment in holding bait aboard the Fuji Maru, the author suddenly

XIII. The author has experimental proof, but will leave its publication to another time.

gave 4,000 grams of groundup sardines to some live bait (50,677 fish) that had been kept for 47 days since their capture without feeding. In 16 - 18 hours after the feeding many dead fish appeared, all of which were gorged with food<sup>1</sup>).

In healthy fish swimming in the broad sea, a sardine 15 cm long ordinarily has 1 gram or less of material in its digestive tract15) 33), but for fish inside a small bait tank, with a restricted scope for movement, this appears to be a little bit too much. The author thinks that the right amount is such that there is no food in the cardiac portion or the blind sac of the stomach, a little in the pyloric part, and a moderate quantity in the intestine, as shown in Figure 14, that is, so that the food does not remain for long in the stomach. When food is suddenly to be given to bait fish that have not been fed for a long time, care should be taken to give them a very small amount at first and then to gradually increase the quantity.

On Vomiting in Bait Fish

If bait fish are placed in bait tanks immediately after capture and transported, the water inside the bait tanks becomes turbid with their excreta, at times to such a degree that the water shows a yellow color. The fishermen explain that this phenomenon is the result of vomiting by the fish, but the author could not help having considerable doubt as the presence of a vomiting center in lower vertebrates such as fish, and therefore it was decided to try the following experiment. Small amounts (0.1 - C.3 cc of a C.5% solution) of apomorphinhydrochloride, the only drug that can be injected to stimulate the vomiting center and cause forced vomiting when human beings have drunk poison with the object of committing suicide, were injected into the body cavities of bait fish shortly after feeding and the reaction was observed. As the following table shows, it was found that the fish regurgitate conspicuously. (The same reaction is found in mackerel-scad, Chelidonichthys kumu, mackerel, Sebastichthys elegans, and Sebastodes inermis.)54)

Thus it was found to be possible that a vomiting center exists in sardines (fish in general). Since a vomiting center is easily excited by spiritual agitation<sup>35</sup>), there is always a possibility that the bait fish, which receive mental shocks during their capture, may regurgitate immediately after being placed in the bait tanks. Not only is the fish itself considerably weakened by vomiting, but the material regurgitated immediately causes a change for the worse in the character of the water, and for this reason, even though the vomiting itself may not be related to the death of the bait, every care is required in handling these fish.

Death of Bait Fish Exposed to the Air<sup>36</sup>)

In placing bait in bait tanks at present dip-nets or buckets are used, and several dozen separate transfers are made. At this time quite



Fig. 14 Suitable feeding of captive bait fish

- Digestive tract of sardine (grey portion-contents of the digestive tract)
 Digestive tract of anchovy (grey portion-contents of the digestive tract)
 - P.C. Pars cardiaca
 - B.S. Blind sac
 - D.P. Ductus pneumaticus
 - P.P. Pars pylorica

| | (Sardines were u | used in the experiment | ;) |
|----------------|--------------------|----------------------------------|--------------------------------|
| Body Length cm | Amount Injected cc | Reactions | Material Vomited |
| 13.0 | 0.3 | immediate reactions | food masses and mucus |
| 12.6 | 0 . 3 | 11 | n |
| 11,5 | 0.2 | n | n |
| 12.0 | 0.1 | immediate obscure | only a little mucus |
| 12.7 | 0.1 | reactions
immediate reactions | small amount of food
masses |

Table 25

a few fish fall onto the deck, and even those which do not are exposed to the air during the time that they are being carried in the scoop-net. Being fish that are weak even in the water, it need hardly be said that the life of the bait is very precarious when it is exposed to the air. Therefore, in order to test how long bait fish can live when left out of the water, the author carried out an experiment on the subject indicated by the heading of this section on March 23, 1934, (clear, atmospheric temperature 15°C) aboard the Fuji Maru using 50 fish that had been caught several days earlier. The fish were taken from the bait tank one by one, their bodies were confined so that they had no freedom of movement, and they were left in the air, the time that elapsed until they were completely dead of suffocation being determined in each case. The lethal time was recorded as the period from the time the fish were taken out of the water and left in the air until the complete cessation of respiration, accompanied by two or three convulsions passing from the anterior to the posterior part of the body. In this way it was possible to make the graph shown in Figure 15, with the lethal time on the vertical axis and the body weight of the fish on the horizontal.

The first things that one perceives in looking at this graph is that as the body weight of the fish increases the lethal time is extended to a greater or lesser degree, and that for a difference in body-weight of about 50 grams there is a difference of almost 30 minutes in the lethal time. Also, the hypothetical curve has a sigmoid shape with reference to the horizontal axis from which it can be inferred that under conditions similar to those of this experiment, no matter how small the fish may be it will not die in less than about 10 minutes, and no matter how large a fish may be, it will not be able to maintain life longer than about 40 minutes. (If the fish is not restrained and is allowed to jump about, it will die in a shorter period of time.) However, it is self-evident that fish should not be left for such long periods of time in the air if it is intended to return them safely to the water, since the experiments described above record the period of time until the fish were completely dead.

Measures to be Taken to Prevent Deaths

These involve eliminating insofar as possible the external factors described above as being the causes of death of bait fishes, so I will attempt to avoid repetition here and will only summarize the items which should be given the most careful attention when transferring newly-caught fish to the bait tanks of a vessel.

First of all, in catching bait fish the fishing should not, of course, be carried on when the weather is stormy. Minute care should be given to the method of operating the fishing gear so as to avoid, as far as possible, causing brain contusions, ruptures of internal organs, and other similar dangerous subcutaneous injuries that give rise to the breakdown of the tissues. In purchasing bait fish from bait fishermen, attention must be paid to the loss of scales and bleeding around the



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eyes and the belly. This is because if these external evidences are conspicuous, the subcutaneous injury will accordingly be great and many of the fish are bound to die later on. Captured bait should not be immediately placed in the bait tanks, but if circumstances permit, a live-pound should be prepared in advance and the fish should be kept in it temporarily to permit them to get over their excitement, to weed out those which are badly hurt and about to die, and to permit the fouling material that the fish vomit because of spiritual perturbation to be naturally washed away. For transferring the fish to the bait tanks. it is ideal if a strong, water-tight port can be constructed in the vessel's side, in accordance with Section 15, Article 9 of the inspection regulations for wooden vessels, through which the bait fish can be poured into the tanks, but where this sort of installation cannot be made they should be transferred using several buckets (more successful than dip-nets) and moving a few fish at a time without haste. When the fish are placed in the tank it must be lighted and the fish must be prevented from bunching together in one place. (If the fish do crowd together in one place they may easily be injured and at the same time they may cause a localized deficiency of free oxygen.) Care must be taken that the free oxygen content does not fall below 3 cc per liter. For about an hour after the fish are put in the tank every effort should be made to give a thorough circulation of water and at the same time the spray pump should be used freely to supplement the supply of oxygen. When more than an hour has passed after the fish have entered the bait tank, with the new pump circulation system the fear of an oxygen deficiency is completely swept away. If, however, for some such reason as a defect in the operation of the pump a great many air bubbles should be produced in the seawater in the tank, there will be deaths from this cause so caution is required. Since fishes in general have the habit of swimming against the current, provision should be made so that the water will gently circulate in a direction opposite to that in which the bait fish swim (clockwise in most cases). When bait fish are transported to the South Seas fishing grounds, deaths due to sudden rises in water temperature must be avoided by immediately cooling the water in the bait tanks somewhat $(2^{\circ} - 3^{\circ})$ is enough) if the vessel encounters warm water zones with temperatures above 27°C in the case of S. melanosticta (the lethal temperature is over 29°C. but a margin of 2°C is left because the saturation point of oxygen is lowered as the water temperature rises) or above 29°C in the case of E. japonicus (lethal temperature over 31°C).

In addition, constant attention should be paid to the causes of death treated in this study, and care should be taken to apply timely and appropriate measures.

Summary

1. This study is chiefly the results of a biological investigation of the causes of death in the various types of mortality that occur when bait fish that have been recently captured, commonly called "new" bait, are placed in bait tanks for transportation.



Plate I Figures of Bleeding Accompanying Subcutaneous Injuries

- 1. From the conspicuous bleeding at the snout there can be inferred, not only the damage to the snout, but also more or less injury to the brain.
- 2. The large hemorrhages in the snout, eyeballs, cephalic canals, and gill openings are sufficient evidence of brain contusions.

C.c ... Cephalic canals

- 3. The hemorrhages in the fonticuli and elsewhere indicate brain contusions. Fo ... Fonticuli C.n ... Cavum nasi
- 4,5. Fish with such hemorrhages in the eyeball, particularly in the cornea, in most cases have suffered injuries to the brain.

Pa ... Palpebra

6. Ruptured cerebral blood vessels.

O.l ...Olfactory lobe Ce ... Cereberum M.s ... Medulla spinalis

7. Contusions of the cerebrum.

8. Rupture of blood vessels of the viscera.

He ... Hepar Ve ... Ventriculus A.p .. Appendix pylorica Li ... Lien I ... Intestinum

9. Contusions of the liver.

2. This study was made from 1932 on aboard the Fuji Maru, guide vessel of the Shizuoka Prefecture Fisheries Experiment Station, at Mito Beach in Shizuoka Prefecture, at the Kanagawa Prefecture Misaki Marine Laboratory, and at several other places.

3. The fish used in the experiments were Sardinia melanosticta and Engraulis japonicus of sizes suitable for use as bait.

4. By keeping a school of bait fish in a tightly closed vessel and studying the rate of speed at which they consumed oxygen by abnormal breathing it was demonstrated that since the average lethal concentration of oxygen for bait fish is roughly fixed, the more fish (total body weight) there are, the faster the oxygen will be used up and the quicker the fish will die.

5. By shutting up a single sardine or anchovy in a small tightly closed vessel and measuring the lethal concentration of oxygen it was found that sardines die when the free oxygen falls below about 2 cc per liter and that the figure for anchovies varies only slightly from this.

5. With the old-fashioned type of bait tank with natural water circulation the danger was always present that the fish would begin to show signs of oxygen deficiency, not only right after they had been placed in the tanks and in their excitement were using up large quantities of free oxygen but also whenever the vessel was not rolling very much, however, with the present style of tanks with pumped water circulation, if only a little care is taken for a short while after the fish are put into the tank, there is thereafter no danger at all of oxygen deficiency. This has been shown by citing actual experiences.

7. Minute quantities of ac-tetrahydro A naphthylamin hydrochloride 5% solution, which causes fever centrally by exciting the temperature center and other sympathetic nerve ganglia, a xl000 solution of adrenalin, which causes fever by central and peripheral action, and pituitrin, which causes the body temperature to drop by acting centrally, were injected subcutaneously and were found to cause no reaction. Thus it was learned that sardines appear to have no center for regulating body temperature and that their body temperature changes with the temperature of the surrounding water.

8. A controlled-temperature tank was used to study the resistance of bait fish to sudden rises and drops in the water temperature. With the sea-water at approximately $22^{\circ} - 23^{\circ}$ C at the time of the experiment, it was found that sardines could endure a temperature range of $7^{\circ} - 29^{\circ}$ C while anchovies could live in a range of $11^{\circ} - 51^{\circ}$ C. This demonstrated that sardines have resistance to low temperatures (sudden drops in temperatures) while anchovies are more resistant to high temperatures (sudden rises in temperature).

9. Injuries received in capture or in transfer to the bait tank were divided into three classifications for study as large wounds immediately fatal, nonfatal superficial wounds, and subcutaneous wounds causing death after the passage of a considerable period of time.

10. It was possible to prove experimentally that the occurrence of the peak of mortality of bait fishes several days after capture was due to internal bleeding from the subcutaneous wounds described above.

11. By dropping bait fish headfirst from various heights the magnitude of the lethal force was measured, and it was found that this force correspond to dropping a fish of about 22 grams weight from a height of 40 cm or more.

12. When several weeks have passed after the fish have been placed in captivity, the blood spots called "red rust" by the fishermen appear on the surface of their bodies. This appears to be bleeding due to pathological damage to the blood vessels.

13. If, because of failure of the intake of the pump or for any other reason, a great many small air bubbles are produced in the bait tanks, the fish sometimes find respiration difficult or receive injuries indirectly and die.

14. When captive fish that have been completely without food for a long time are suddenly fed a large amount, it sometimes brings about their death.

15. There was a marked reaction when 0.1 = 0.3 cc of a 5% solution of the emetic apomorphin hydrochloride was injected beneath the skin of sardines. Consequently it is thought that there may be a vomiting center in sardines. If this is the case, it is possible that sardines vomit freely because of spiritual perturbation immediately after capture. If they begin vomiting, not only will the sardines be somewhat weakened, but the water within the bait tanks will be polluted.

16. Sardines taken out into the air can stay alive longer than was expected (10 - 40 minutes).

17. The best plan in order to prevent the death of bait fishes is thought to be to eliminate insofar as possible the external conditions which cause death as described above.

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Fig. 9 - Photograph showing condition of sardine attacked by mackerel.

Fig. 12 - Photograph showing "red rust" on sardine.

Fig. 13 - Photograph showing emaciated sardine.

Plate II - Photographs showing major wounds of bait fish.