Possibility of Establishment of Yellow Fever Mosquito, *Aedes aegypti* (L.) in Japan

2. Cold- and dry-resistance of eggs, ecological zero point of larvae, development of larvae in early spring, and general summary*

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黄熱蚊 *Aedes aegypti* の日本に於ける土着の可能性に関する研究. 2. 卵の耐寒及び耐乾性, 幼虫の発育零点, 幼虫の早春に於ける発育及び総括. 大藤 芳, 長崎大学医学部医動物学教室 (主任：大森南三郎教授)

I Introduction

In the days of the Panama Canal having been excavated the distribution of the Yellow fever mosquito, *Aedes aegypti* was examined all over the Far East. In Japan, the occurrence of the species was confirmed by Yamada (Yamada, 1916) in the Ryukyus and the Bonin Islands (Table 1), while, it was denied even from the southernmost main Island, Kyushu and its adjasent islands.

For many years thereafter there had been no news about the distribution of the species in Japan, while, in 1944 and for eight years thereafter the occurrence of this mosquito had been observed from a small fishing town, Ushibuka of Amakusa Island in Kyushu as will be mentioned later.

Hereupon, the author had a plan to make sure experimentally of the possibility of the establishment of the species in Nagasaki City, the southwestern sea port in Kyushu, through the results of examinations on the cold- and dry-resistance of eggs, on the ecological zero point of the larvae and also on the development of the larvae hatched out in early spring in Nagasaki City.

The species used in the present experiment is originated from Bankok, Thailand in 1957.

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II The Past Occurrence of *Aedes aegypti* in Ushibuka Fishing Town for Eight Years

A sudden epidemy of dengue fever broke out in Ushibuka town in September, 1944. The town (130°00'E, 32°12'N) is located at southern end of Amakusa Island, Kumamoto Prefecture, Kyushu and was a harbor for transports for Southeast Asia during World War II. During the dengue fever survey there on October of the same year, Oguri et al. (1948) had found *Aedes aegypti*

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Table 1 Mean air temperature (°C) at some Meteorological Stations in Japan

District		Station	Longitude & Latitude	Years of observation	The occurrence of *Aedes aegypti* was
Ryukyu	Main Island	Naha	127°39'E, 26°12'N	1891—1944	reported by Yamada in 1916
Bonin Islands		Chichi Is.	142°11'E, 27°50'N		
Kyushu, Japan	Kagoshima Prefecture	Nase	129°30'E, 28°23'N	1921—1950	
		Kagoshima	130°33'E, 31°34'N	1921—1950	
	Kumamoto P.	Ushibuka	130°00'E, 32°12'N	1950—1953	recognized in 1944-1952
	Nagasaki Prefecture	Tomie	128°40'E, 32°37'N	1924—1950	
		Nagasaki	129°53'E, 32°44'N	1921—1950	

Station	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.	Year
Naha	16.1	16.1	17.7	20.6	23.1	26.1	27.9	27.6	26.6	23.9	20.8	17.7	22.0
Chichi													22.5
Nase	14.2	14.4	16.1	19.1	22.1	25.0	27.8	27.6	26.2	22.8	19.4	16.2	20.9
Kagoshima	6.4	7.4	10.2	15.0	19.0	22.5	26.7	27.1	24.2	18.7	13.6	8.7	16.6
Ushibuka	8.1	8.4	10.6	15.4	19.1	21.6	25.6	27.3	24.2	19.9	15.3	10.3	17.2
Tomie	7.0	7.2	10.1	14.2	18.1	21.4	25.8	26.8	23.7	18.9	11.4	9.7	16.4
Nagasaki	5.2	5.8	9.0	13.9	18.0	21.4	25.5	26.5	23.1	17.8	12.8	7.9	15.6

breeding in fire-fighting concreate tanks. They had found the mosquito distributed there for from September, 1944 to May, 1947. Several other authours also had collected the mosquito in the town before October of 1952 when Bekku (unpublished), the then assistant in our Department, had collected the larvae, for the last time, in only 3 tanks out of several tens of containers he had examined. The destruction of the species might be due partly to the general disuse of the fire-fighting tanks after the War and partly to the encouragement of controlling aedine mosquitoes breeding in domestic containers. The air temperatures at the town are fairly higher especially in cold months for Kyushu as seen in Table 1.

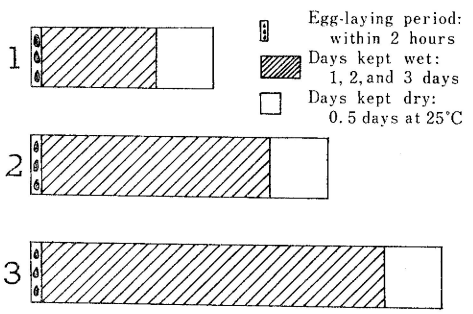
Hereupon the author was interested in investigating whether or not the eggs the most cold- and dry-resistant stage of the species, could survive the winter in Nagasaki where the air temperature is somewhat lower than in Ushibuka town.

III Cold- and Dry-resistance of Eggs of *Aedes aegypti*

(1) Method of Experiments

The eggs were used which were laid within 2 hours by females rearing in large number of cages at 25°C and 60-85% relative humidity room. They were kept wet for one, 2, and 3 days and dried for about 12 hours in the same rearing room as shown in Fig. 1. The plan of experiments to examine the cold- and dry-resistance of eggs was set up as shown in Fig. 2. The mean temperatures during the experiments at laboratory and outdoors in Nagasaki University School of Medicine are illustrated in Fig. 3 together with a line at 12°C which was determined as an ecological zero point of the larvae of *Aedes aegypti* as will be stated later. The mean temperatures and relative humidities during the experiments at laboratory

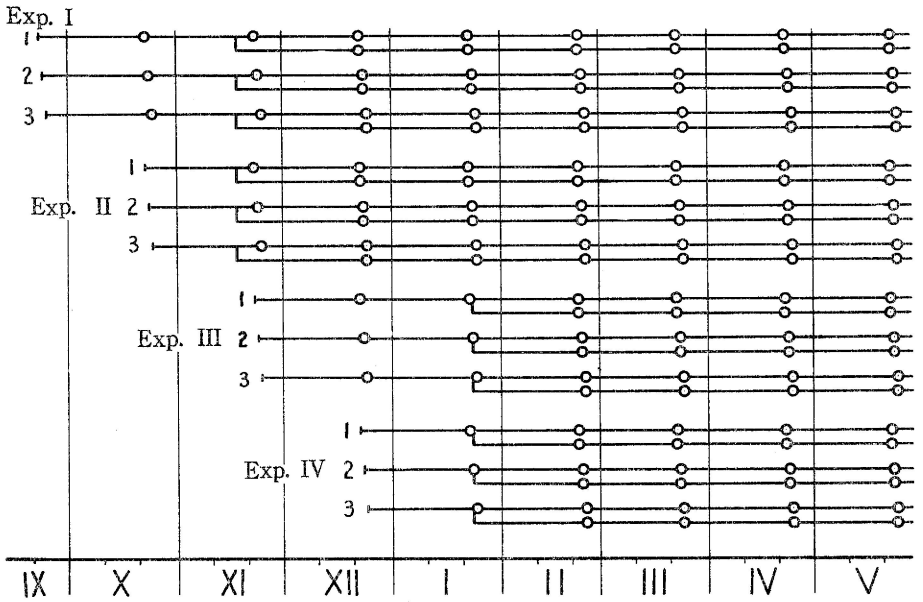
Fig. 1 Preparation of eggs of *Aedes aegypti* at 25°C



dried 12 hours at 25°C and then exposed to laboratory temperatures. On late October a batch of eggs were examined for hatching soaking them in the water at 25°C. On mid-November 6 batches were moved to outdoors. On late November another batch being in the laboratory was examined. Thereafter, each one of the laboratory and outdoor batches were examined for hatching once a month by the same way as above till the end of May of the next year.

The percentage hatchings of eggs which were

Fig. 2 Plan of experiments to examine cold- and dry-resistance of eggs
Exp. I to IV were started at late Sept., Oct., Nov., and Dec., 1960 respectively.
1, 2, and 3 : Days kept wet at 25°C. Upper line: Exposure to laboratory temperature.
Lower line : Exposure to outdoor temperature. Circle : Date on which eggs were examined for hatching at 25°C.



and outdoors are tabulated in Table 2.

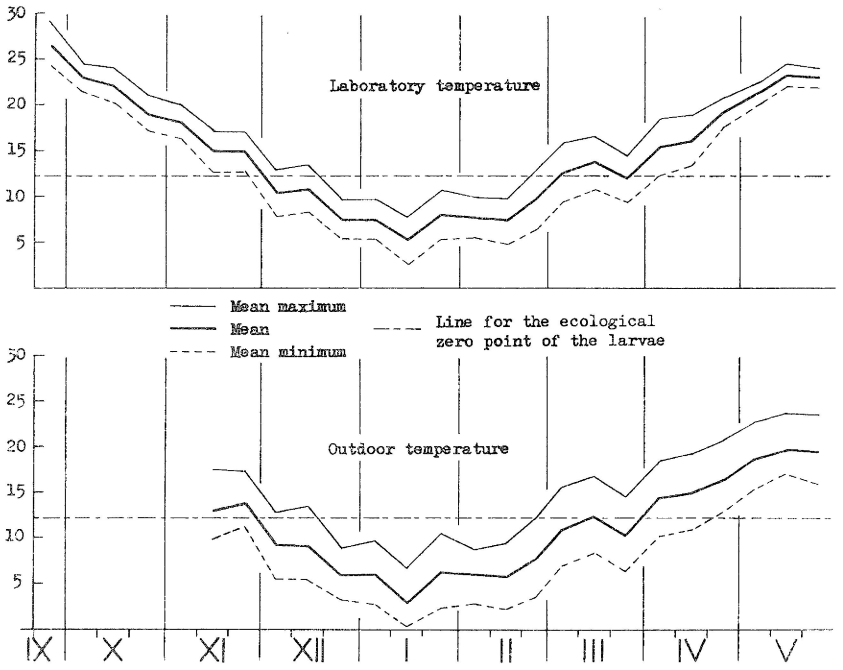
The preparations and the examinations of eggs for hatching were conducted on the end of each month i.e. on the 21st, 22nd, or 23rd of each month, at monthly intervals throughout the experiments.

(2) Results of Experiments

In Experiment I, the first set of about 2100 eggs or 14 batches of each 150 eggs prepared on late September were kept wet for one day and kept wet for one day are given in upper 8 lines in Table 3.1 for the laboratory batches, and in upper 6 lines in Table 3.2 for the outdoor batches, together with the ranges in days from the first to the last hatching. The second and third sets of eggs kept wet at 25°C for 2 and 3 days and exposed to laboratory or outdoor temperatures are given in the middle and lower lines in the same Tables 3.1 or 3.2 respectively.

Experiments II, III, and IV were started respectively on late October, November, and

Fig. 3 Laboratory and outdoor temperatures (°C) at Nagasaki in late Sept., 1960 to May, 1961



December by quite a similar way as in the case of the Experiment I and the results are tabulated in Tables 4.1 and 4.2 ; 5.1 and 5.2 ; and 6.1 and 6.2.

It is well known fact that the eggs of *Aedes aegypti*, when soaked in the water after having been kept dry in the air for some period, hatch out in some cases in masses within a short period and in other cases little by little or irregularly over a very long period. In some cases in the present experiments the periods in days covering from soaking of eggs in the water to the last hatching are very long and in an extreme case the period is as long as 81 days. The periods are subjected to a great fluctuation but in general they appear to be longer in batches of eggs exposed for shorter periods to laboratory or outdoor temperatures and relatively shorter in those exposed for longer periods.

The percentage hatchings of the eggs are also subjected to a great variation. Generally speaking, however, it seems that the longer the period of exposure is the smaller the percentage is, and that the percentage is lower in eggs exposed to lower temperatures.

Before going into consideration about the detailed accounts of the percentage hatchings, those observed under favorable conditions in summer will be referred to. In summer of 1960, percentage hatchings of eggs were examined under natural air conditions of temperatures ranging from 25.2 to 31.8°C and relative humidities from 65 to 80%. The eggs were kept wet for from 48 to 72 hours and dried for only 5 days and then soaked in the water. The mean percentage hatching for 5 batches of each 150 eggs was 82.3% ranging from 73.3 to 88.0%. From the result it may be said that a percentage hatching above 80% will be regarded as a high percentage for the eggs of *Aedes aegypti*.

Tables 3.1 and 3.2 show that the percentage hatchings of the eggs of each set prepared on late September are fairly high at first, decreasing, however, gradually with the prolongation of the period of exposure. The trend is, interesting to

Table 2 Temperature (°C) and relative humidity (%) during the experimental period from late Sept., 1960 to late May, 1961 at laboratory and outdoors in Nagasaki

E: early M: middle L: late

Months and parts		Laboratory				Outdoors			
		M. max.	M. min.	Mean	R.H.	M. max.	M. min.	Mean	R.H.
Sept.	L	28.7	24.2	26.4	72				
Oct.	E	24.4	21.4	22.9	64				
	M	23.9	20.1	22.0	62				
	L	20.8	17.1	18.9	62				
Nov.	E	19.8	16.2	18.0	69				
	M	17.0	12.7	14.9	69	17.5	9.9	13.3	73
	L	17.0	12.7	14.9	74	17.2	11.2	13.7	77
Dec.	E	12.8	7.9	10.3	64	12.7	5.5	9.2	64
	M	13.4	8.3	10.8	67	13.4	5.4	9.0	68
	L	9.5	5.3	7.4	67	8.8	3.2	5.8	66
Jan.	E	9.6	5.3	7.4	70	9.7	2.7	6.0	68
	M	7.7	2.6	5.2	66	6.7	0.3	2.9	65
	L	10.6	5.3	8.0	68	10.5	2.3	6.3	69
Feb.	E	9.8	5.5	7.6	72	8.7	2.7	6.0	73
	M	9.7	4.8	7.3	62	9.3	2.1	5.6	60
	L	12.8	6.4	9.6	58	12.1	3.5	7.6	62
Mar.	E	15.7	9.4	12.6	66	15.5	7.0	10.8	69
	M	16.5	10.7	13.6	70	16.7	8.3	12.3	73
	L	14.3	9.4	11.9	65	14.3	6.4	10.2	67
Apr.	E	18.3	12.3	15.3	65	18.4	10.1	14.3	66
	M	18.6	13.3	16.0	71	19.1	10.8	14.9	76
	L	20.6	17.5	19.1	76	20.9	12.9	16.4	73
May	E	22.5	20.1	21.3	82	22.8	15.3	18.9	78
	M	24.4	22.0	23.2	81	23.8	17.0	19.7	80
	L	23.9	21.8	22.8	76	23.6	15.9	19.5	73

say, nearly similar irrespective of their having been kept wet for 1, 2, or 3 days. This suggests that immature embryos could reach maturity during exposure to such an air conditions as shown in the Sept. L line in Table 2. On the contrary, however, the percentages become in most cases lower in the cases of younger embryos becoming more remarkably so when eggs were exposed to lower temperatures, as seen in Tables 4.1, 4.2, 5.2, 6.1, and 6.2.

Tables 4.1 and 4.2 show that in Experiment II started on late October, the states of hatching are very good not only in point that the percentage hatching of eggs are fairly higher especially in eggs having been kept wet for 2 or 3 days but also in point that the percentages become not markedly lower with the prolongation of the period of exposure than in the cases of the other experiments. This may imply that the eggs with moderately or nearly fully developed embryos are most resistant to cold and dryness when they are subjected to decreasing temperatures of 19°C-

Table 3. 1 Exp. (I) with eggs laid within 2 hours on 21, Sept., 1960 at 25°C

Days kept wet at 25°C	Months exposed to laboratory temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	1	81	150	121	80.7
1	2	43	150	99	66.0
1	3	13	150	77	51.3
1	4	33	150	64	42.7
1	5	12	150	19	12.7
1	6	13	150	6	4.0
1	7	2	150	5	3.3
1	8		150	0	0
2	1	74	150	108	72.0
2	2	42	150	124	82.7
2	3	19	150	101	67.3
2	4	31	150	40	26.7
2	5	11	150	1	0.7
2	6	22	150	9	6.0
2	7	2	150	1	0.7
2	8		150	0	0
3	1	72	150	132	88.0
3	2	41	150	120	80.0
3	3	15	150	78	52.0
3	4	29	150	15	10.0
3	5	3	150	3	2.0
3	6	9	150	2	1.3
3	7		150	0	0
3	8		150	0	0

Table 3. 2 Exp. (I) with eggs laid within 2 hours on 22, Sept., 1960 at 25°C

Days kept wet at 25°C	Months exposed to outdoor temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	3	12	150	47	31.3
1	4	31	150	15	10.0
1	5	6	150	1	0.7
1	6		150	0	0
1	7	20	150	3	2.0
1	8		150	0	0
2	3	26	150	77	51.3
2	4	29	150	27	18.0
2	5	2	150	2	1.3
2	6	5	150	1	0.7
2	7	21	150	1	0.7
2	8		150	0	0
3	3	10	150	54	36.0
3	4	37	150	20	13.3
3	5	5	150	4	2.7
3	6		150	0	0
3	7		150	0	0
3	8		150	0	0

15°C and to further falling ones as in the case of Exp. II which started on late October. In fact, among the eggs exposed to outdoor temperatures, those started on late October is highest in percentage hatching. A fairly good results are also seen in Table 5.1 which were obtained with eggs prepared on late November but exposed to laboratory temperatures. In this case, eggs were exposed at first to about 15°C for about 10 days and to further falling ones.

Table 5.2 indicates that the percentage hatchings of eggs prepared on late November and exposed through laboratory temperatures, to outdoor ones are very low, even though in eggs having fully developed embryos, much less in younger embryos. The similar states of hatching are seen in Tables 6.1 and 6.2. These results show that sudden exposures of eggs having younger embryos to low temperatures are harmful for their living, although the eggs with well developed embryos alone are resistant to the adverse air conditions.

To make comparable more clearly the states of hatchings shown in the tables, the percentages are illustrated in Fig. 4, 5, and 6 by period of having been kept wet.

Fig. 4 indicates that the eggs with one day old embryos are weakest in dry-resistance and especially in cold-resistance excepting those prepared on late September.

Fig. 5 indicates that of the eggs with 2 days old embryos or moderately developed ones. Those prepared on late September and late October are very high in dry- as well as cold-resistance. The eggs of the embryonic age are also dry-resistant even when they were prepared on late November if they were not exposed to outdoor low temperatures. However, when the eggs of the

Table 4. 1 Exp. (II) with eggs laid within 2 hours on 21, Oct., 1960 at 25°C

Days kept wet at 25°C	Months exposed to laboratory temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	1	45	150	73	48.7
1	2	13	150	38	25.3
1	3	39	150	11	7.3
1	4	12	150	5	3.3
1	5	7	150	1	0.7
1	6		150	0	0
1	7		150	0	0
2	1	43	150	117	78.0
2	2	40	150	74	49.3
2	3	31	150	50	33.3
2	4	11	150	26	17.3
2	5	11	150	9	6.0
2	6	20	150	2	1.3
2	7		150	0	0
3	1	41	150	116	77.3
3	2	19	150	99	66.0
3	3	42	150	88	58.7
3	4	11	150	58	38.7
3	5	12	150	21	14.0
3	6	3	150	1	0.7
3	7		150	0	0

same age were exposed suddenly to cold temperature on late December, they scarcely can survive the temperature.

Fig. 6 indicates that the eggs having 3 days old embryos or nearly fully developed embryos are very high in both cold- and dry-resistance even when they were exposed suddenly to severe winter temperature from late December. Eggs prepared on September, however, rather notable in decreasing in the percentage with the prolongation of the period of exposure.

(3) Considerations on the overwintering of the egg

It is a noteworthy fact that the eggs, irrespective of the starting time of exposure, could survive the cold months actually till the end of April. The percentage hatchings of eggs examined at the end of April are compared with each experiment

Table 4. 2 Exp. (II) with eggs laid within 2 hours on 22, Oct., 1960 at 25°C

Days kept wet at 25°C	Months exposed to outdoor temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	2	19	150	40	26.7
1	3	30	150	10	6.7
1	4		150	0	0
1	5		150	0	0
1	6		150	0	0
1	7		150	0	0
2	2	24	150	117	78.0
2	3	30	150	48	32.0
2	4	10	150	12	8.0
2	5	21	150	7	4.7
2	6	9	150	1	0.7
2	7		150	0	0
3	2	10	150	103	68.7
3	3	30	150	84	56.0
3	4	9	150	40	26.7
3	5	11	150	15	10.0
3	6	1	150	2	1.3
3	7		150	0	0

Table 5.1 Exp. (III) with eggs laid within 2 hours on 21, Nov., 1960 at 25°C

Days kept wet at 25°C	Months exposed to laboratory temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	1	15	150	112	74.7
1	2	40	150	52	34.7
1	3	19	150	9	6.0
1	4	7	150	1	0.7
1	5		150	0	0
1	6		150	0	0
2	1	19	150	127	84.7
2	2	30	150	87	58.0
2	3	11	150	50	33.3
2	4	11	150	11	7.3
2	5	20	150	3	2.0
2	6		150	0	0
3	1	39	150	128	85.3
3	2	29	150	69	46.0
3	3	10	150	41	27.3
3	4	19	150	10	6.7
3	5	2	150	1	0.7
3	6		150	0	0

Table 5.2 Exp. (III) with eggs laid within 2 hours on 22, Nov., 1960 at 25°C

Days kept wet at 25°C	Months exposed to outdoor temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	3		150	0	0
1	4		150	0	0
1	5		150	0	0
1	6		150	0	0
2	3		150	0	0
2	4	4	150	1	0.7
2	5		150	0	0
2	6		150	0	0
3	3	10	150	21	14.0
3	4	26	150	9	6.0
3	5		150	0	0
3	6		150	0	0

Table 6.2 Exp. (IV) with eggs laid within 2 hours on 23, Dec., 1960 at 25°C

Days kept wet at 25°C	Months exposed to outdoor temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatche d	% hatching
1	2		150	0	0
1	3		150	0	0
1	4		150	0	0
1	5		150	0	0
2	2		150	0	0
2	3		150	0	0
2	4		150	0	0
2	5		150	0	0
3	2	18	150	61	40.7
3	3	14	150	24	16.0
3	4	1	150	1	0.7
3	5		150	0	0

Table 6.1 Exp. (IV) with eggs laid within 2 hours on 22, Dec., 1960 at 25°C

Days kept wet at 25°C	Months exposed to laboratory temp.	Days covering from soaking to the last hatching	No. and % of eggs		
			used	hatched	% hatching
1	1		150	0	0
1	2		150	0	0
1	3		150	0	0
1	4		150	0	0
1	5		150	0	0
2	1		150	0	0
2	2	11	150	7	4.7
2	3		150	0	0
2	4		150	0	0
2	5		150	0	0
3	1	41	150	100	66.7
3	2	10	150	40	26.7
3	3	14	150	2	1.3
3	4		150	0	0
3	5		150	0	0

started on from late September to December in Fig. 7.

The percentage is highest in eggs prepared on late September and becomes lower with the delay in starting time of exposure.

The percentage hatchings of the eggs examined at the end of March were, however, fairly different in the order: Those of eggs prepared on from late September to late December were 2.0, 5.9, 3.6, and 2.9% respectively showing that the eggs prepared on late October were most resistant.

As above, it proved that the eggs can survive the cold months till the end of April. However, it is a question that whether or not the overwintered eggs can hatch out in nature at outdoor temperatures in March or in April and can develope under the field conditions. The problem will be discussed later.

IV Ecological Zero Point of Immature Stages of *Aedes aegypti*

One hundred larvae, 5 batches of each 20 larvae just hatched out at 25°C were reared by batch in a glass container of 9 cm in diameter and 5 cm in depth, giving Ebios (a sort of brewer's yeast) as bait at a rate of 0.01, 0.02, 0.03, and 0.04g per 8 cc water for 1st, 2nd, 3rd, and 4th instar larvae. The rearing was made at constant temperatures of 18, 22, 25, 27 and 30°C.

The larval period, larval+pupal period of the female, and that of the male are measured at each temperature and the developmental velocity or 100/ developmental period in days is calculated.

Fig. 4 Percentage hatching of eggs kept wet for one day and then exposed for various months to laboratory and outdoor temperatures

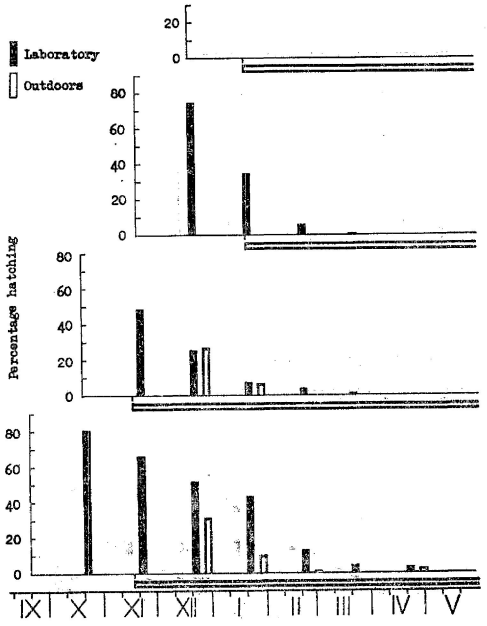


Fig. 5 Percentage hatching of eggs kept wet for 2 days and then exposed for various months to laboratory and outdoor temperatures

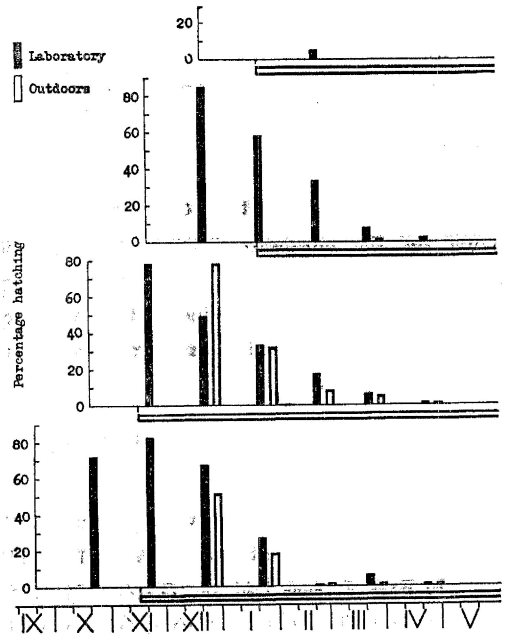


Fig. 6 Percentage hatching of eggs kept wet for 3 days and then exposed for various months to laboratory and outdoor temperatures

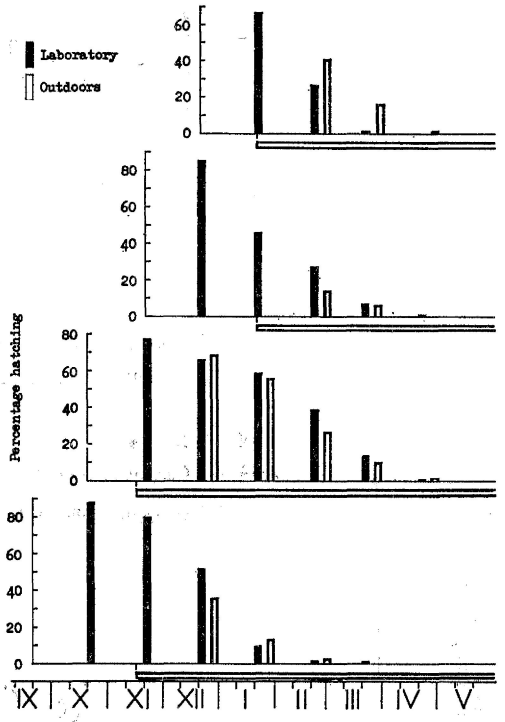


Fig. 7 Percentage hatchings at the end of April of the eggs prepared on the end of each month from September to December, kept wet for 1, 2, and 3 days, and exposed to laboratory and outdoor temperatures

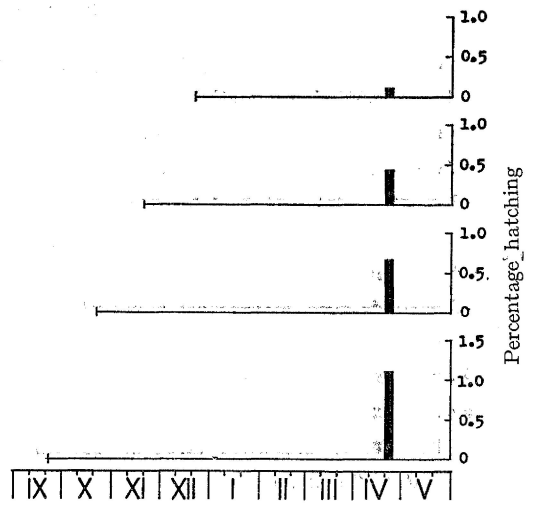
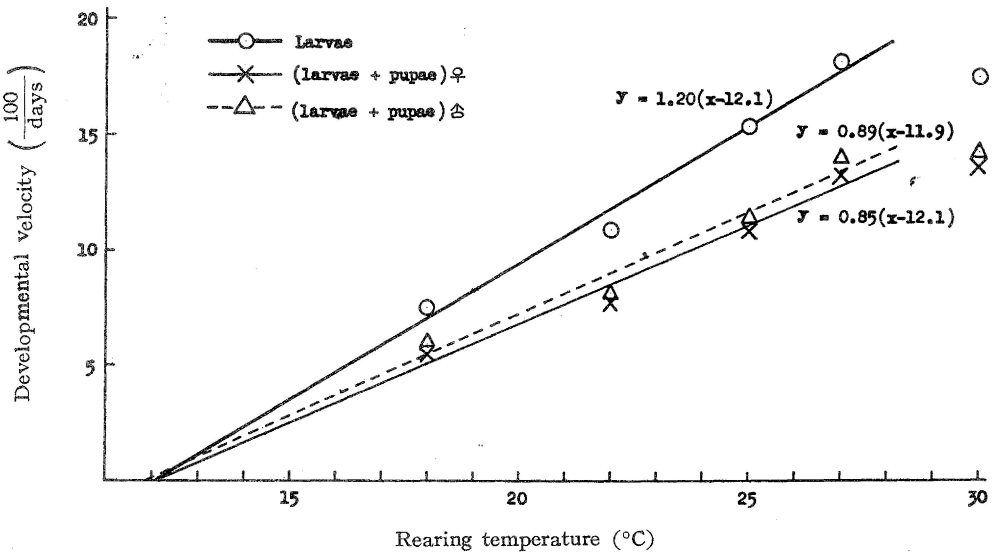


Table 7 Days and velocity in development of immature stages of *Aedes aegypti* at various constant temperatures

Rearing temp. (°C)	Larvae		♀ (larvae+pupae)		♂ (larvae+pupae)	
	Days	$\frac{100}{\text{days}}$	Days	$\frac{100}{\text{days}}$	Days	$\frac{100}{\text{days}}$
18	13.3	7.52	18.2	5.49	16.9	5.92
22	9.2	10.87	12.9	7.75	12.3	8.13
25	6.5	15.38	9.2	10.87	8.7	11.49
27	5.5	18.18	7.6	13.16	7.2	13.89
30	5.7	17.54	7.3	13.69	7.0	14.28

Fig. 8 Regression of velocity in development of immature stages on rearing temperature



for each period. They are tabulated in Table 7.

Then a regression equation of the velocity on the rearing temperature was calculated by the method of least squares as shown in Fig. 8.

Here, the points obtained for 30°C are omitted in calculation of regression lines. The reason is that the developmental period of larvae at 30°C is slightly delayed showing the temperature being not very favorable.

From the equations, the ecological zero points are determined as 12.1, 12.1, and 11.9°C respectively for the larvae, immature stage of the female, and that of the male.

The zero point, about 12°C, is unexpectedly lower for the mosquito distributed mainly in tropics and subtropics. And, this is very important in the case of considering the possibility of establishment in some places in temperate zone such as in Kyushu.

V Development of the Larvae Hatched out in Early Spring

Two kind of experiments were planned with eggs prepared at different time as shown in Table 8. In Exp. I, eggs laid on October 22, 1960 were kept wet for 3 days and dried for 7 days at

25°C and then exposed to laboratory temperatures (cf. Table 2) for 121 days from November 2, 1960 to March 2, 1961. On the last day they were soaked in the water at the outdoor sunny place fixed at the south side of our Laboratory in Nagasaki City. In Exp. II, eggs laid on February 19, 1961 were kept wet about 3 days (0.5+2.5 days) and kept dry for 8 days at 25°C, and were soaked in the water on the same day and at the same outdoor sunny place as the above experiment. That is, in Exp. I eggs were being exposed to the laboratory temperatures during cold months for a long time before their being soaked in the water at the beginning of March, while, in Exp. II, eggs were transferred from a room at 25°C directly to the outdoor place on the same day as in Exp. I.

The states of temperatures, hatching and development of larvae after eggs being soaked in the water at the place are shown in Table 8.

In Exp. I. most larvae died soon after or several days after hatching without going into the 2nd instar stage, and a few of them died in the 2nd and rarely in the 3rd instar stage, and only 5 (1♀, 4♂) or one percent were emerged as adults.

In Exp. II, many larvae died soon or several days after hatching and some died in any larval stages but 33 (12♀, 21♂) or 6.6% were emerged as adults.

The results of these experiments suggest that the dryness of eggs for a long time seems to have some harmful influence upon the living of the larvae hatched out from them, and therefore a little higher temperature conditions than in Nagasaki City, especially in winter, may be needed for the establishment of the mosquito.

VI Considerations on the Possibility of Establishment of the Species in Nagasaki City and in Any Places in Kyushu

Eggs of *Aedes aegypti* can survive the cold months whenever they were started to be exposed to the laboratory or outdoor temperatures from late September to late December in Nagasaki City.

In the city, the mean outdoor temperatures at north side of the laboratory in early, middle, and

Table 8 Hatching and subsequent development of larvae during from March to May at outdoor sunny place in Nagasaki

Exp. I : 500 eggs laid within 2 hours on Oct. 22, 1960 and kept wet for 3 days and then dried for 7 days at 25°C, were kept dry in the laboratory from Nov. 2, 1960 till Mar. 2, 1961 when they were soaked in water and kept at outdoor sunny place.

Exp. II : 500 eggs laid within 12 hours on Feb. 19, 1961 and kept wet for 2.5 days and then dried for 8 days at 25°C, were soaked in water at the same outdoor sunny place from Mar. 2 of the same year.

Temperature (°C) within the period during which larvae hatched and developed							Exp. I			Exp. II		
Month	Date	Max. temp. Range	Max. temp. Mean	Min. temp. Range	Min. temp. Mean	Mean temp.	Date	No. & % of larvae hatched	No. & % of adults emerged	Date	No. & % of larvae hatched	No. & % of adults emerged
March	3—10	18.0—24.0	20.7	4.7—11.3	8.3	14.5	3 ↑	230 or 46% during 49 days		3 ↑ 6	305 or 61% during 34 days	
	11—20	14.3—26.1	20.4	2.7—13.4	9.0	14.7						
	21—31	10.6—18.6	15.6	3.6—11.0	6.9	11.3						
April	1—10	17.6—24.0	20.3	3.5—17.7	10.7	15.5						
	11—20	17.5—23.5	19.4	6.3—15.5	11.2	15.3						
	21—30	18.0—21.8	20.1	14.6—19.5	17.3	18.7	21					
May	1—10	19.8—24.5	22.2	18.1—21.7	20.1	21.1	9 ↕ 15		5 (1.0%)	2 ↕ 17		33 (6.6%)
	11—17	22.2—24.7	23.7	20.6—23.1	21.7	22.7						

late March and April in 1961 were 10.8, 12.3, 10.2, 14.3, 14.9, and 16.4°C, and those at a sunny place at south side were 14.5, 14.7, 11.3, 15.5, 15.3, and 18.7°C respectively as shown in Tables 2 and 8.

At the place with favorable temperatures ranging from 11.3 to 15.5°C, 46% or 230 out of 500 eggs could hatch in nature. However, most larvae died soon or several days after hatching which hatched out from the eggs subjected to dryness under the adverse conditions for a long period (Exp. I in Table 8). And, only 5 individuals or only one percent could reach adults on mid-May. This is contrastive to the high (6.6%) emergence of adults in Exp. II in which eggs were soaked in water at the outdoor place just after the above treatment at 25°C. A supposed overwintering period in Nagasaki for the eggs of the mosquito in nature may exceed 4 months from November to February or more, considering the outdoor temperatures in the city. Then, a high mortality of the larvae will be expected if they hatch out from long exposed eggs in early spring.

Consequently it seems that the possibility of establishment of this species in Nagasaki City may be rather low unless a great many adults are imported during summer months to a very favorable place in the city.

However, there are some places in Kyushu where temperature conditions are more favorable especially in winter than in Nagasaki City. And, if the mosquito are introduced to these favorable places or open ports, high possibility of establishment may be expected.

VII General Summary

In Japan, there had been no record of collecting the yellow fever mosquito, *Aedes aegypti*, excepting those in the Ryukyus and Bonin Islands. However, recently the occurrence of the species was recognized in a fishing town, Ushibuka (130°00'E, 32°12'N), in Amakusa Island, Kumamoto Prefecture, Kyushu for at least eight years from 1944 to 1952. The air temperatures at the town are fairly higher for Kyushu.

The author had a plan to determine the possibility of establishment of this mosquito in southern Kyushu and he made examinations on the overwintering of eggs in Nagasaki City where the air temperature is lower by 1.5°C in annual mean and those in winter months by 2 to 3°C than Ushibuka town, and also made a determination of the ecological zero point of the larvae, and examined the development of larvae hatched out at outdoors in early March and April.

The eggs was proved viable throughout the cold months till the end of April, whenever they were started to be exposed to dryness in the laboratory and outdoors during from late September to late December, though the percentage hatchings decrease with the prolongation of the period of exposure.

Overwintered eggs, when soaked in water at sunny place in early March, can hatch out in some percentage at temperatures ranging from 11.3 to 15.5 °C, but most of the larvae hatched out in this way die soon or several days after hatching if the eggs used were being kept dry for a long period during cold months, and only a few of them can reach adult stage. The early death of the larvae is thought to be mainly due to the long period the eggs having been kept dry, because the then mean temperatures were sufficiently higher than 12°C or the ecological zero point of the larvae.

From the above, it will be concluded that the possibility of establishment of the species in Nagasaki City may be rather low unless a great number of adults are imported during summer months to a very favorable place in the city, while that a high possibility of establishment may be expected if they are introduced to some places or open ports in Kyushu where temperature conditions especially in winter months are slightly more favorable than in Nagasaki City.

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総　　括

黄熱蚊 *Aedes aegypti* (L.) の我が国に於ける分布については, 古く, 山田 (1916) によって, 琉球列島及び小笠原群島に生棲する事が報告されたが, それ以外の土地から採集された報告はない. 所が近年, 本種が熊本県天草の南端の漁港, 牛深町で1944年から1952年迄の少なくとも8年間土着していた事実がある.

著者は本種が西南九州に土着する可能性を実験的に証明するために, 牛深よりも平均気温が約1.5°C低く, 冬期の月平均が2, 3°C低い長崎市に於いて実験室と屋外で卵の耐乾性, 耐寒性について調べ, 又, 別に本種幼虫の発育零点 (ecological zero point) を決定し, 乾燥越年せし

めた卵から3月の自然温度下で孵化した幼虫の発育状況を調べて，次のような実験結果と結論を得た．

25°C温室内で1日，2日，及び3日間湿潤に保った後約半日乾燥した卵群を，9月下旬，10月下旬，11月下旬，及び12月下旬に実験室の自然温度に曝し，後更に夫夫を2分して一部はそのまゝ室内に，他は屋外温度に曝し，以後各月下旬に夫夫一定数ずつの卵を25°C内で水漬して孵化率を調べた結果，何れの月に用意した卵群も室内及び屋外の低温と乾燥に耐えて4月下旬迄は生存し得ることが判明した．4月下旬に於ける孵化率は僅かに1%以下ではあるが，3月下旬には2.0—5.9%と可成りに高い．9月下旬に用意した卵群が7ケ月もの乾燥低温に耐え得ることは極めて重要な事であって，今回の実験で別に決定した本種幼虫の発育零点は約12°Cであるから，3月，4月の屋外の自然温度下で水漬した越年卵がもし孵化し得るならば幼虫は低温に耐えて生存し除々に発育を続け得るものと考えられる．

実験室内の自然温度下で乾燥越年せしめた卵群を，3月初めに，南側の日当たりのよい場所の自然温度下で水漬すると可成りの率に於いて孵化するが，乾燥期間が4ケ月もの長期に及ぶ卵群から孵化した幼虫は，直後又は数日中に死亡するものが多く，僅かに1%が5月中旬に羽化するに過ぎない．

以上の事実は，長崎市では夏期中，極めて好適な環境下へ，極めて多数の成虫が移入されない限り土着の可能性は極めて低い事を暗示する．然し九州には長崎市に於けるよりも平均気温が高く，特に冬期の気温が可成りに高い場所あるいは開港場が少なからずあるから，こゝへ本種が移入されるならば土着する可能性は極めて高いものと推測できる．

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