HATCHING STATES OF EGGS OF SNOW-MELT *AEDES*[1,2]

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ABSTRACT. Eggs of snow-melt *Aedes* undergo changes in their response to a hatching stimulus. These changes are related to species, prior thermal history, and age of egg. Based on definite hatching responses, eggs may go through 5 hatching states including (1) no response regardless of temperature and stimulation; (2) response to a hatching stimulus which is dependent on hatching temperature; (3) response to a hatching stimulus which is independent of hatching temperature; (4) hatching upon contact with water which is dependent on the hatching temperature and (5) hatching upon contact with water independent of hatching stimulus and temperature.

INTRODUCTION

Hatching of eggs of snow-melt *Aedes* in response to a reduction in the level of dissolved oxygen is under complex controls. Horsfall and Fowler (1961) demonstrated that eggs of *Ae. stimulans* (Walker) must receive exposure first to warm summer temperatures and then to cold winter ones in order to break egg latency and permit hatching. This effectively limits this species to one generation per year. Pinger and Eldridge (1977), working with a laboratory colony of *Ae. canadensis* (Theobald) from the mid-atlantic area, found that a daily light regime of 9 hr light and 15 hr of darkness (9L:15D) during the first 14 days after deposition resulted in egg diapause. Reversal of light regime broke diapause. Shroyer and Craig (1980) found that photoperiod-induced diapause in *Ae. triseriatus* (Say) could be repetitively induced and broken. They further limited the use of the term egg diapause to that which involved photoperiodism. Latency was used to refer to any other seasonally restricted nonresponse of eggs to a hatching stimulus.

Horsfall et al. (1973) divided hatching responses of *Aedes* eggs into 3 types. Type III response was a lack of reaction to a hatching stimulus when submerged. Type II response was hatching when submerged and subjected to a decrease in the dissolved 0_2 level. A Type I response was hatching upon contact with water. This present report will expand on Horsfall et al. (1973) by demonstrating that the eggs of *Aedes* may develop through a series of 5 hatching states. These states are directly related to particular species involved, the age of the egg and current and prior thermal history.

METHODS AND MATERIALS

Four species, *Ae. canadensis, Ae. cinereus* Meigen, *Ae. provocans* (Walker) and *Ae. stimulans,* from Michigan, were studied. Eggs were obtained from field-inseminated females which were collected, transported, and maintained according to Kardatzke (1976). All adults and eggs, prior to exposure to cold, were maintained under a photoregime of 16L:8D at 21°C.

To determine thermal requirements

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[1] Opinions and assertions contained herein are the private views of the author and are not to be construed as official nor as reflecting the views or endorsements of the Department of the Army or the Department of Defense.

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for latency termination and hatching, pooled eggs of the same species were divided into groups of 100. These groups were held in moist chambers according to Horsfall and Fowler (1961). Nine groups of eggs of the 4 species were exposed to a biserial temperature regime of 21°C followed by 4°C. All combinations of exposures of first to 21°C for 40, 70 or 100 days and then to 4°C for 30, 60 or 90 days were made prior to being exposed to the hatching stimulus at 15°C. Five other groups were also held for 100 days at 21°C prior to cold exposure. Two of these 5 were then held at 4°C for 90 days prior to attempted hatching at 4°C. The last 2 were held at 4°C for 400 days prior to hatching at 4° and 15°C. The hatching stimulus was a reduction of the dissolved oxygen level using the techniques described by Kardatzke (1979a).

The multivoltine strain of *Ae. canadensis* was obtained from those eggs which would respond to a hatching stimulus 40 days after deposition. Larvae and adults were maintained using procedures of Kardatzke (1979b). Adults were mated using the technique of McDaniel and Horsfall (1957) as modified by Novak and Liem (1975). The laboratory colony was continuously maintained at 21°C and 16L:8D.

Eggs of the 4 species were also held only at 21°C prior to hatching. Eggs were divided into 3 groups of 100 and held for 40, 70 or 100 days at 21°C prior to hatching at 21°C.

RESULTS

Depending on the species and population involved, eggs of snow-melt *Aedes* may require prior exposure to cold temperatures to be hatched. Eggs of both *Ae. provocans* and *Ae. stimulans* could not be hatched when only exposed to 21°C (Tables 1 and 2). Eggs of *Ae. cinereus* could be readily hatched up to 70 days after deposition (Table 3). However, 100 days after deposition, 63% of *Ae. cinereus* eggs stored under a constant 21°C and 16L:8D photoperiod had become refractory to the hatching stimulus (Table 3). In the field-collected population of *Ae. canadensis*, approximately 80% of the eggs were refractory to the hatching stimulus at 40, 70 and 100 days post-deposition (Table 4). The selected lab-colony of *Ae.*

Table 1. Hatching response of eggs of *Aedes stimulans* in relation to temperature, age, and depression of dissolved oxygen level.

Age of eggs (days)	Storage regime days at 21°C/ days at 4°	Hatching temperature °C	Hatching threshold (% dissolved 0_2)	% eggs hatched
40	40/0	21°	None	0
70	70/0	21°	None	0
100	100/0	21°	None	0
70	40/30	15°	None	0
100	40/60	15°	15	15
130	40/90	15°	15	32
100	70/30	15°	15	5
130	70/60	15°	15	17
160	70/90	15°	15	71
130	100/30	15°	15	45
160	100/60	15°	15	63
190	100/90	15°	15	96
190	100/90	4°	None	0
190	100/90	8°	None	0
280	100/180	4°	15	90
500	100/400	4°	15	100
500	100/400	15°	15	100

Table 2. Hatching response of eggs of *Aedes provocans* in relation to temperature, age and depression of dissolved oxygen level.

Age of eggs (days)	Storage regime days at 21°C/ days at 4°	Hatching temperature °C	Hatching threshold (% dissolved 0_2)	% eggs hatched
40	40/0	21	None	0
70	70/0	21	None	0
100	70/0	21	None	0
70	40/30	15	None	0
100	40/60	15	18	15
130	40/90	15	18	32
100	70/30	15	18	5
130	70/60	15	18	17
160	70/90	15	18	71
130	100/30	15	18	45
160	100/60	4	18	63
190	100/90	4	18	96
280	100/180	4	18	100
500	100/400	4	18	100
500	100/400	15	21	100

canadensis, at 40 days post-deposition, could be readily hatched. After 70 and 100 days at 21°C and 16L:8D photoperiod, this laboratory population of *Ae. canadensis* had also developed a refractory response to the hatching stimulus (Table 5).

Hatching of eggs of *Ae. stimulans* was affected by previous and current thermal conditions. With the thermal sequence of 21°C followed by 4°C (Table 1), the percentage of hatchable eggs increased in direct proportion to duration of storage. While exposure to 4°C is an essential requisite to hatching, the percentage of hatchable eggs increased most markedly with an increase in exposure time to 21°C (Table 1). Thus, warm summer temperatures are an operative factor in egg latency termination.

Eggs of *Ae. stimulans*, whose combined storage at 21° and 4°C was 190 days or less required exposure to a rise in temperature in order to respond to the hatching stimulus (Table 1). After 280 days of storage, these eggs could be hatched at 4°C. Hatching thresholds did not vary with age of the eggs or the hatching temperature (Table 1).

Hatching of eggs of *Ae. provocans* was also affected by previous thermal history. With 21°C followed by 4°C (Table 2), the percentage of hatchable eggs increased in a manner similar to eggs of *Ae. stimulans* (Table 1). After 100 days at 21°C followed by 90 days at 4°C, eggs of *Ae. provocans* would respond to a hatching stimulus at 4°C (Table 2). The hatching threshold remained constant until eggs had been at 4°C for 400 days. These 500 day-old eggs, if they experienced a rise in temperature prior to submergence, would hatch upon contact with water and did not require a reduction in the level of dissolved oxygen (Table 2). A decrease in dissolved oxygen from 21% to 18% was still required if the hatching temperature was 4°C (Table 2).

Hatching of eggs of *Ae. cinereus* after exposure to cold was not directly affected by previous thermal history. At least some eggs were always hatchable regardless of the duration of cold storage (Table 3). With eggs of 190 days, hatching could not be induced at 4°C. A rise in hatching temperature to 8°C was a requisite to responding to the hatching stimulus. Hatching at 4°C would occur with eggs 280 days old (Table 3). With eggs up to 280 days old, the hatching threshold re-

Table 3. Hatching response of eggs of *Aedes cinereus* in relation to temperature, age, and depression of dissolved oxygen level.

Age of eggs (days)	Storage regime days at 21°C/ days at 4°	Hatching temperature °C	Hatching threshold (% dissolved O_2)	% eggs hatched
40	40/0	21	12	93
70	70/0	21	12	87
100	100/0	21	12	37
70	40/30	15	12	88
100	40/60	15	12	64
130	40/90	15	12	70
100	70/30	15	12	14
130	70/60	15	12	23
160	70/90	15	12	64
130	100/30	15	12	100
160	100/60	15	12	85
190	100/90	4	None	0
190	100/90	8	12	20
190	100/90	15	12	100
280	100/180	4	12	100
500	100/400	4	15	100
500	100/400	15	15	100

mained constant at 12% dissolved oxygen regardless of hatching temperature. This threshold was altered to 15% dissolved oxygen by the time the eggs were 500 days old (Table 3). The change in hatching threshold was age dependent but temperature independent.

Aedes canadensis eggs have a variety of hatching responses. Some eggs always were in a hatchable state immediately after embryogeny (Table 4). Other eggs would not hatch without exposure to cold. However, the hatching response after exposure to cold was identical to *Ae. cinereus* (Table 3), a multivoltine species, rather than *Ae. stimulans* (Table 1), a univoltine species. With eggs of 190 days of age, hatching could not be induced at 4°C. A rise in temperature to at least 8°C was a requisite for hatching (Table 4). At a hatching temperature of 8°C, the dissolved oxygen threshold was 12%. At hatching temperature of 15°C, the threshold rose to 15% for 190 day old eggs (Table 4). After storage for 500 days, eggs of *Ae. canadensis* had a hatching threshold of 18% at 4°C. The requirement for a reduction in the level of dissolved oxygen was eliminated if these eggs experienced a rise in temperature to 15°C 24 hours prior to submergence (Table 4).

DISCUSSION

In confirmation of findings of Horsfall and Fowler (1961), eggs of many snowmelt *Aedes* required prior exposure first to warm temperatures and then to cold temperatures to become hatchable. The current study demonstrated that the hatching temperature was an operative factor in the capability of eggs to respond to hatching stimuli. After 90 days at 21°C following 90 days at 4°C, eggs of *Ae. provocans* would respond to a hatching stimulus at 4°C, while eggs of *Ae. stimulans* exposed to the same conditions would not respond to a hatching stimulus unless the hatching temperature was 15°C. Only additional aging of eggs of *Ae. stimulans* at 4°C would result in development of hatchability at 4°C.

A multivoltine species such as *Ae. cinereus* has the inherent capability to respond to a hatching stimulus without exposure to cold. However, prolonged ex-

Table 4. Hatching response of eggs from field-collected *Aedes canadensis* in relation to temperature, age and depression of dissolved oxygen level.

Age of eggs (days)	Storage regime days at 21°C/ days at 4°	Hatching temperature °C	Hatching threshold (% dissolved O_2)	% eggs hatched
40	40/0	21	15	19
70	70/0	21	15	24
100	100/0	21	15	19
70	40/30	15	15	87
100	40/60	15	15	65
130	40/90	15	15	68
100	70/30	15	15	23
130	70/60	15	15	27
160	70/90	15	15	51
130	100/30	15	15	71
160	100/60	15	15	69
190	100/90	4	None	0
190	100/90	8	12	25
190	100/90	15	15	90
280	100/180	4	15	100
500	100/400	4	18	100
500	100/400	15	21	100

posure to a constant 21°C and 16L:8D photoperiod resulted in development, in these eggs, of a latent response to the hatching stimulus. Cold was required to breach this latent condition, but this latent state is not required to survive cold.

Aedes canadensis is a species capable of multivoltinism. It is also a species in which eggs can develop both a latent response to a hatching stimulus and true egg diapause. At least 3 factors can be involved in development of these states. Pinger and Eldridge (1977) found that, for an eastern USA population, exposure of the eggs to a 9L:15D photoperiod during the first 14 days after deposition would induce egg diapause. This present study demonstrated that prolonged storage at 21°C without exposure to a hatching stimulus resulted in development of

Table 5. Hatching response of eggs of a selected multivoltine strain of *Aedes canadensis* when stored at 21°C only.

Days at 21°C	% eggs hatched
40	82
70	28
100	8

egg latency in a lab population regardless of photoperiod. Results from the field-collected populations when compared to the work of Pinger and Eldridge (1977) indicated that source locale of the population may also be a factor in degree of egg latency in a population.

The presence or absence of latency, as measured by a refractory response to the hatching stimulus, indicated that the eggs of snow-melt *Aedes* undergo some yet undetermined physiological changes. Horsfall et al. (1973) referred to these changes as 3 types of hatching behavior. These hatching types really reflected the existing hatching state of the egg as it relates to the environment.

Eggs of snow-melt *Aedes* may be considered to be in one of 5 hatching states at any one time of their life. Hatching state V involves those eggs which, when immersed in water, will not hatch regardless of adequacy of hatching stimulus or temperature. Eggs of all mosquitoes in embryogeny are in state V. In nature, postembryonic eggs of *Ae. provocans* and *Ae. stimulans* are in state V during the summer following deposition and prior to their first winter.

Hatching state IV involves those eggs which, when immersed in water, will not respond to an adequate hatching stimulus unless a critical hatching temperature is reached. Eggs of *Ae. stimulans,* after 100 days at 21 °C followed by 90 days at 4 °C, are examples of eggs in state IV since they require a rise in hatching temperature as a prerequisite to hatching. Horsfall et al. (1973) made similar observations for eggs of *Ae. vexans* (Meigen). In nature, state IV occurs in eggs of *Ae. stimulans* during late winter (January/February). Eggs of *Ae. provocans* did not exhibit state IV in this study.

Hatching state III involves those eggs which, when immersed in water, will respond to an adequate hatching stimulus independent of hatching temperature. Eggs of *Ae. provocans* that are 190 days old and eggs of *Ae. canadensis, Ae. cinereus,* and *Ae. stimulans* that are 280 days old are examples of eggs in state III. For *Ae. provocans,* this state has developed by the first vernal thaw and allows the eggs to hatch during any thaw. For eggs of *Ae. canadensis, Ae. cinereus* and *Ae. stimulans* this state has developed by spring (March/April).

The early appearance of state III in *Ae. provocans* may be a factor which limits the southern distribution of this species. James (1962) observed that early hatching of *Ae. provocans* during an unseasonally early thaw resulted in death of many larvae during a subsequent refreezing. Thus, the early appearance of state III in eggs of *Ae. provocans* may limit this species to more northern areas where cycles of premature thawing and refreezing do not frequently occur. Conversely, the presence of state IV in eggs of *Ae. stimulans* may be a factor in the more southerly distribution of this species.

Hatching state II involves those eggs which, when submerged and having not experienced a previous rise in hatching temperature, require a hatching stimulus; but, having experienced a rise in hatching temperature prior to submergence, hatch upon submergence. State II occurred in eggs of *Ae. canadensis* and *Ae. provocans* after 500 days of storage. In nature, this time would be equivalent to the second spring after deposition. It would not normally occur in nature unless there was an extreme drought the first spring after deposition.

Hatching state I involves those eggs which, regardless of hatching temperature, required only contact with water to hatch. Eggs of *Culex* and 500 day old eggs of *Ae. punctor* (Kardatzke 1979a) are examples of eggs in state I. Like state II, state I in snow-melt *Aedes* does not normally occur in nature since it is the equivalent of at least the second spring after deposition. It does limit the life expectancy of eggs of at least some snow-melt *Aedes* to about 2 years since a heavy wetting of the soil would induce hatching of eggs in state I.

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