

OVERWINTERING OF *ANOPHELES LINDESAYI JAPONICUS* LARVAE IN THE REPUBLIC OF KOREA

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ABSTRACT. Mosquito larval surveillance for environmental monitoring and pest-control purposes was conducted monthly at dredged soil-dumping areas during the construction of a new harbor in Yongcheon Bay, approximately 5 km SE of Jinhae, on the SW side of Namsan (Mt. Nam) and across the bay from Su-do (Su Island) in Gyeongsangnam Province, Republic of Korea (ROK) from November 2007 through April 2008. During this study, mosquitoes collected as overwintering larvae were *Aedes togoi* in brackish rock pools along the seashore and *Anopheles lindesayi japonicus* along the vegetated margins of a slow-flowing drainage ditch and associated freshwater ground pools containing green algae. Overwintering *An. lindesayi* larvae also were collected along stream margins and stream pools of moderate- to fast-flowing mountain streams near Chungju (Chungcheongbuk Province) (October 2007 and March 2008) and Munsan (Gyeonggi Province) (September 2007 and April 2008). First and second instars were collected and identified in late September 2007 through February 2008. During March and April, collections were primarily 3rd and 4th instars, and by the end of April, pupae were collected. This is the first report of *An. lindesayi japonicus* overwintering as larvae in the ROK.

KEY WORDS Overwintering, *Anopheles lindesayi japonicus*, larvae, Korea, *Aedes togoi*

INTRODUCTION

As early as 1960, mosquito surveillance was conducted throughout the winter months to determine the overwintering life stages of mosquitoes in the Republic of Korea (ROK), and while these surveys focused mostly on the adult resting sites of 2 primary vectors, *Anopheles sinensis* Wiedemann (malaria) and *Culex tritaeniorhynchus* Giles (Japanese encephalitis), several other adult mosquitoes, primarily females, were also collected (Whang 1961). While various types of habitats were surveyed (i.e., building basements, cow stables, air shelters, straw piles, drainage culverts, rock piles, caves, mines, animal shelters, and rat

holes), mountain streams and stream pools were not sampled (Whang 1961, Ree et al. 1976). These studies resulted in the capture of diapausing adults of *Culex pipiens pallens* Coquillett, *An. sinensis* sensu lato, *Anopheles pullus* M. Yamada, *Anopheles sineroides* S. Yamada, *Culex orientalis* Edwards, *Culex hayashii hayashii* S. Yamada, and *Cx. tritaeniorhynchus* (Whang 1961; Ree et al. 1976; Hong et al. 1993, 1994).

Several studies have reported various overwintering habitats and life stages for other ROK mosquito species, including *Aedes albopictus* (Skuse) (egg), *Aedes flavopictus* S. Yamada (egg), *Aedes galloisi* S. Yamada (egg), *Aedes koreicus* (Edwards) (egg), *Aedes alektorovi* Stackelberg (egg), *Aedes chemulpoensis* S. Yamada (egg), *Aedes nipponicus* LaCasse and Yamaguti (egg), *Tripteroides bambusa* (S. Yamada) (egg), *Aedes vexans nipponii* (Theobald) (egg), *Culex pipiens molestus* Forskal (egg, larvae, pupa, and adults), *Aedes dorsalis* (Meigen) (egg), and *Aedes togoi* (Theobald) (egg and larvae) (Hong et al. 1971, Hong 1977, Lee 1986, Shim et al. 1989, Hong et al. 1991, Lee and Lee 1992, Hong and Kim 1995, Sames et al. 2008). Overwintering stages of 19 species of mosquitoes in 5 genera have been reported in the ROK. Survey methods included adult collections made with dry-ice-baited traps and heated tents, egg collections from tree holes or soil samples, and larval collections using dipping or siphoning methods from aquatic habitats.

Recently, Sames et al. (2008) reported the geographical distribution of *Anopheles lindesayi* S. Yamada in the ROK. From their data and previously reported data for this species, they observed that for all *An. lindesayi* specimens

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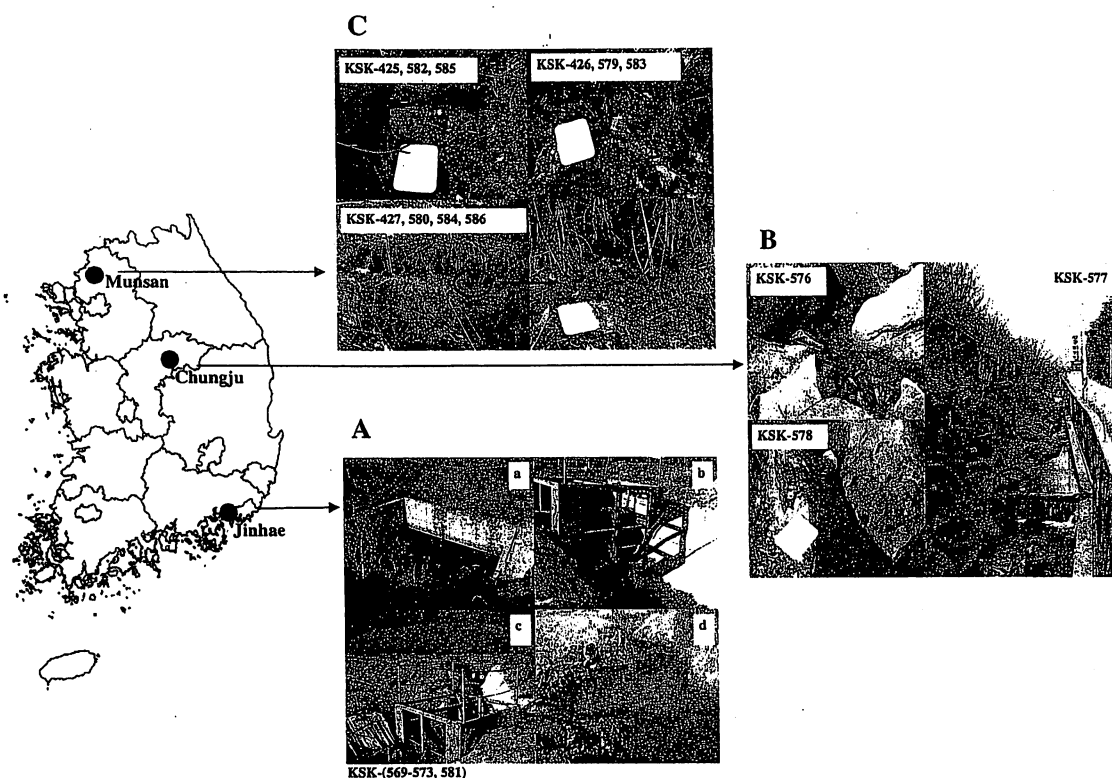


Fig. 1. Map and photographs of collection sites where *Anopheles lindesayi japonicus* immature stages were collected: (A) Jinhae, Gyeongsangnam Province; (B) Chungju (near Mt. Worak National Park), Chungcheongbuk Province; and (C) Munsan, Gyeonggi Province, Republic of Korea, 2007–2008.

reported from the ROK, adults were collected between May and October and larvae were collected between July and October. Based on data from Japan (LaCasse and Yamaguti 1950, US Army 406th Medical Laboratory 1963, Mogi 1996), Sames et al. (2008) suggested that *An. lindesayi* might overwinter as larvae in at least the southern portions of the ROK. Thus, to determine the overwintering status of *An. lindesayi* in the ROK, larval collections were conducted throughout the late fall of 2007 through the spring of 2008 at three geographically dispersed areas.

MATERIALS AND METHODS

Mosquito larval surveillance for environmental monitoring and pest-control purposes was conducted monthly at 3 locations spanning the length of the ROK from September 2007 through April 2008 (Fig. 1). The first location (Fig. 1A) was a dredged soil-dumping area that was being used for the construction of a new harbor (the Busan New Port), in Yongcheon Bay, approximately 5 km SE of Jinhae, on the SW side of Namsan (Mt. Nam) and across the bay from Su-do (Su Island) in Gyeongsangnam Province. Larval

collections were conducted at 4 sites: along slow-flowing drainage ditches, associated ground pools filled with freshwater containing green filamentous algae, near dredged soil-dumping areas, and along a low-volume, narrow, slow-flowing mountain ditch. Bamboo (*Pseudosasa japonica* Makino, 2.0–5.0 m in height) provided partial shade and was present along the ground pool margins.

The second location (Fig. 1B) consisted of 2 collection sites near Mt. Worak National Park, Chungju, Chungcheongbuk Province, approximately 7 km and 40 km east of Chungju along Highway 36, and the third location (Fig. 1C) consisted of several collection sites along a relatively fast-flowing, small mountain stream 7 km west of Munsan (Gyeonggi Province) near Highway 37.

Mosquito larvae were collected using larval dipping and siphoning techniques, and detailed habitat descriptions and photographs were recorded for each collection site. Stream locations and ground pools that were positive for *An. lindesayi* larvae in the fall of 2007 were resampled throughout the study as a means to document changes in development. Larvae of *An. lindesayi* were visually separated from other collected

species based upon their broad palmate setae and characteristic markings and ornamental features and were reared to the adult stage for confirmatory identification.

Adult overwintering collections were made over vegetation in late February and early March near the Jinhae larval collection sites. A 3-m-long, 4-m-deep, and 2-m-high screened tent was placed over vegetation, and a portable propane heater was used to warm the inside tent temperatures to $>20^{\circ}\text{C}$. A collector examined the sides and top of the tent for adult mosquitoes, mouth aspirated them into a screened-pint carton, and provided them with a 10% sucrose solution. All adults were transported to the 5th Medical Detachment, where they were identified morphologically to species or species group. Members of the *Anopheles* Hyrcanus Group were identified to species by polymerase chain reaction (Wilkerson et al. 2003, Li et al. 2005).

RESULTS

From mid-September 2007 through April 2008, 326 *An. lindesayi* larvae were collected at various sites located in Gyeongsangnam, Chungcheongbuk, and Gyeonggi Provinces (Table 1 and Fig. 1).

At the Jinhae site (Fig. 1A), only 1st and 2nd instars of *An. lindesayi* were collected from November 2007 through February 2008 at the 4 dredging soil-dumping sites. During the February survey, 1st and 2nd instars were collected along a slow-flowing drainage ditch where approximately 90% of the surface was covered with ice. In March and April, both 3rd and 4th instars were collected. Water temperatures (November–February) ranged from 5.5°C to 12.1°C with a notable range increase in March and April (15.4 – 18.3°C).

Near Mt. Worak National Park (Fig. 1B), numerous *An. lindesayi* 1st and 2nd instars (>100 observed at some sites) were collected during October 2007 along moderate- to fast-flowing, partially to heavily shaded, rocky mountain stream margins and associated stream pools. Vegetation along the water margin ranged from none to stands of overhanging grasses. Dead leaves and small branches from overhanging trees and the adjacent forest covered the bottom of the stream margins and pools. In March 2008, 31 *An. lindesayi*, 3rd and 4th instars were collected from these habitats (Table 1 and Fig. 1B). Stream and stream pool water temperatures ranged from 7.5°C to 9.0°C .

In September 2007, at Munsan, the northernmost extreme of the ROK near the demilitarized zone that separates the Democratic People's Republic of Korea and the ROK, numerous *An. lindesayi* 1st and 2nd instars were collected along moderate- to fast-flowing, partially to heavily shaded, rocky mountain stream margins and

associated stream pools (Table 1). Abundant dead leaves (e.g., *Quercus* spp. and *Salix* spp.) and branches from the overhanging trees were present and, in some cases, nearly filled the ground pools. In April 2008, 70 *An. lindesayi* 3rd and 4th instars and pupae were collected from this site (Fig. 1C). Water temperatures were only recorded for the month of April, and they ranged from 9.5°C to 12.5°C .

Overall, from September through April, *An. lindesayi* larvae were collected along moderately to heavily shaded stream margins where vegetation or rocks produced an eddy or greatly slowed the movement of the water in moderate- to fast-flowing streams and associated sandy to rocky stream pools, which usually contained an abundance of decaying leaves and small branches. Decaying leaves and tree branches or dense patches of overhanging dead and green grasses were present along the stream margins.

DISCUSSION

Data suggest that *An. lindesayi* eggs are laid in time to hatch in late September through October, and the possibility exists that some might be laid and hatched in early November. Oviposition appears to stop in late November, the 1st and 2nd instars slowly develop, and 3rd and 4th instars become present in March and April of the following year. Pupae are present in late April, and adult emergence occurs in May. These specimens provide the basis for summer populations of *An. lindesayi* and may help further explain why Sames et al. (2008) noted that all reported adult collections in the ROK were made between May and October.

Limited adult overwintering collections conducted at Jinhae, the southernmost area of our study, resulted in the collection of only 4 *An. sinensis* adult females. While there is a possibility that *An. lindesayi* individuals overwinter as adults, based on the numbers of 1st and 2nd instars observed in the river and stream margins and pools, the primary mode of overwintering is through arrested/slow development of the early instars until the temperatures warm in March.

Based on the frequency of collection and numbers of larvae collected in these mountain streams and stream pools, these moderately to heavily shaded environments are the primary habitat for this species (Takata 1950, Mrikubo and Harada 1952, Omori 1952, Sames et al. 2008). At this latitude and the various elevations where these larvae were collected, mean monthly ambient temperatures were similar at Chungju and Munsan and 5 – 7°C colder than temperatures observed for Jinhae (Fig. 2). During periods of very cold weather, the stream-margin surfaces were frozen, while the stream pools were often completely frozen. Therefore, these larvae may

Table 1. Collection records for *Anopheles lindesayi japonicus* larvae collected in the Republic of Korea, 2007–2008.

Site	Serial no. ¹	Date collected	Grid coordinates	Water temp (°C)	Habitats ²	Altitude (m)	Total no. collected ³	Female ³	Male ³
Jinhae	KSK-569(a)	November 17, 2007	35°05'37"N, 128°44'56"E	11.1	D	12	5	3	2
	KSK-569(b)	November 17, 2007	35°05'37"N, 128°44'53"E	12.1	D	12	3	1	2
	KSK-569(d)	November 17, 2007	35°05'30"N, 128°45'28"E	10.0	GP	14	1	0	1
	KSK-570(a)	December 8, 2007	35°05'37"N, 128°44'56"E	6.2	D	12	13	4	9
	KSK-570(b)	December 8, 2007	35°05'37"N, 128°44'53"E	8.1	D	12	1	0	1
	KSK-570(c)	December 8, 2007	35°05'30"N, 128°45'27"E	9.9	D	12	4	2	2
	KSK-571(a)	January 19, 2008	35°05'37"N, 128°44'56"E	6.8	D	12	8	5	3
	KSK-571(c)	January 19, 2008	35°05'30"N, 128°45'27"E	6.9	D	12	23	12	11
	KSK-572(a)	February 16, 2008	35°05'37"N, 128°44'56"E	5.5	D	12	1	1	0
	KSK-572(c)	February 16, 2008	35°05'30"N, 128°45'27"E	6.8	D	12	7	3	4
	KSK-573(a)	March 15, 2008	35°05'37"N, 128°44'56"E	18.3	D	12	1	1	0
	KSK-573(c)	March 15, 2008	35°05'30"N, 128°45'27"E	15.4	D	12	1	0	1
	KSK-581(d)	April 12, 2008	35°05'30"N, 128°45'28"E	18.1	GP	14	1	0	1
Chungju	KSK-554	October 16, 2007	36°55'16"N, 128°13'26"E	–	SM	172	30	17	13
	KSK-556	October 16, 2007	36°55'07"N, 127°56'38"E	–	SM	87	21	10	11
	KSK-558	October 16, 2007	36°55'07"N, 127°56'38"E	–	SM	87	24	13	11
	KSK-576	March 31, 2008	36°55'16"N, 128°13'26"E	7.5	SP	172	2	1	1
	KSK-577	March 31, 2008	36°55'07"N, 127°56'38"E	9.0	SM	87	2	1	1
	KSK-578	March 31, 2008	36°55'07"N, 127°56'38"E	9.0	SM	87	27	9	18
Munsan	KSK-425	September 10, 2007	37°53'28"N, 126°49'40"E	–	SP	49	29	14	15
	KSK-426	September 10, 2007	37°53'27"N, 126°49'41"E	–	SP	56	33	17	16
	KSK-427	September 10, 2007	37°53'25"N, 126°49'44"E	–	SP	67	19	11	8
	KSK-579	April 04, 2008	37°53'27"N, 126°49'41"E	9.5	SP	56	9	3	6
	KSK-580	April 04, 2008	37°53'25"N, 126°49'44"E	10.2	SP	67	8	6	2
	KSK-582	April 15, 2008	37°53'28"N, 126°49'40"E	10.0	SP	49	20	10	10
	KSK-583	April 15, 2008	37°53'27"N, 126°49'41"E	12.5	SP	56	5	1	4
	KSK-584	April 15, 2008	37°53'25"N, 126°49'44"E	11.5	SP	67	2	0	2
TOTAL	KSK-585	April 28, 2008	37°53'28"N, 126°49'40"E	10.0	SP	49	22	6	16
	KSK-586	April 28, 2008	37°53'25"N, 126°49'44"E	11.5	SP	67	4	3	1
							326	154	172

¹ Collection numbers used by authors with (a, b, c, d) representing a specific subsite.² D, drainage ditch; GP, ground pool; SP, stream pool; SM, stream margin.³ Number of adults that emerged from field-collected larvae.

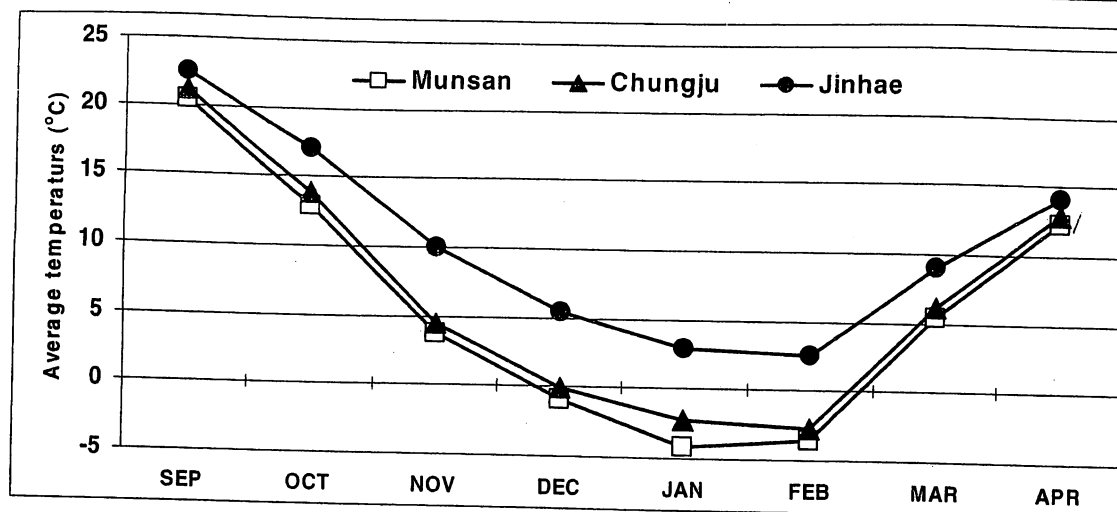


Fig. 2. Average ambient temperatures (°C) for 3 locations where *Anopheles lindesayi japonicus* immature stages were collected from September 2007 through April 2008.

have a mechanism to survive freezing temperatures, whether they are contained within the ice or in the cold water below the ice surface. Some of the smaller pools where larvae were collected later during warmer winter and early spring periods were observed to be completely frozen. It is most likely that they survived the frozen stream pool habitats rather than being washed into these pools after the ice thawed.

Even though Sames et al. (2008) suggested that during heavy rains, stream pools may flood, and some *An. lindesayi* larvae may be washed downstream to rice-growing areas, these rice paddies are not the primary habitats for *An. lindesayi*. Similar to Omori (1952), in nearly all immature collections in the ROK, larvae were collected from sites that were moderately to heavily shaded and were rarely collected in fully sunlit areas, e.g., rice paddies, pools, and ponds. For species collected during this study, *Ae. togoi* had been reported previously as overwintering in the ROK, but this is the first report of *An. lindesayi* overwintering as larvae, and it is the only reported anopheline species that is known to overwinter as larvae in the ROK. Additional surveillance is needed to further define the distribution, adult behaviors, overwintering habitats, and the method of cold environment survival for *An. lindesayi*.

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