The Great Lakes Entomologist

Volume 29
Number 2 - Summer 1996 *Number 2 - Summer 1996*
Article 2

June 1996

Laboratory Rearing of *Lycaeides Melissa Samuelis* (Lepidoptera: Lycaenidae), An Endangered Butterfly in Michigan

Catherine Papp Herms
Michigan State University

Deborah G. McCullough
Michigan State University

Deborah L. Miller
North Central Forest Experiment Station

Leah S. Bauer
Michigan State University

Robert A. Haack
Michigan State University

LABORATORY REARING OF *LYCAEIDES MELISSA SAMUELIS* (LEPIDOPTERA: LYCAENIDAE), AN ENDANGERED BUTTERFLY IN MICHIGAN

Catherine Papp Herms[1], Deborah G. McCullough[1,2], Deborah L. Miller[4], Leah S. Bauer[1,3,4] and Robert A. Haack[1,2,3,4]

ABSTRACT

The Karner blue butterfly (*Lycaeides melissa samuelis*) is listed as a federally endangered species in the United States. It occurs in oak savanna and pine barren habitats from eastern Minnesota to New Hampshire. In 1994, we successfully reared Karner blue larvae under controlled laboratory conditions for experimental purposes, and report on those rearing methods here. We collected 20 female Karner blue adults of the spring generation from two areas in Michigan, and housed them in cages in an environmental chamber at 24°–26°C for 5 days. The female butterflies produced 154 eggs, of which 72 hatched in an average of 4.5 days, and 68 first instars survived. Eggs, larvae and pupae were kept in a growth chamber at 24°C. Developmental time from egg to adult averaged 26 days; the average duration of each instar ranged from 3 to 4 days, and the average pupal duration was 8 days. Thirty three laboratory-reared Karner blue larvae successfully completed the 4 instars, and were released as adults into maternal collection sites. Laboratory rearing may be a viable means of providing Karner blue individuals for reintroduction into areas where the species is extirpated, for supplementation of small populations, or for research. Ultimately, such methods may become an integral part in the recovery of this and other rare invertebrate species.

Lycaeides melissa samuelis Nabokov (Lepidoptera: Lycaenidae), commonly referred to as the Karner blue butterfly, is listed as an endangered species by the US Fish & Wildlife Service (USFWS 1992). The species is found in oak savannas and pine barrens, both of which are xeric, sparsely wooded communities (Dirig 1994). Populations of the Karner blue occur discontinuously along a narrow band from eastern Minnesota to New Hampshire (Shapiro 1969, USFWS 1992, Haack 1993). The butterfly overwinters in the egg stage and has two generations per year. Larvae of both the spring and summer generations feed only on wild lupine (*Lupinus perennis*), which grows in the sandy soils of the savanna and barrens habitats, and adults utilize a variety of nectar sources (Schweitzer 1989, Haack 1993, Dirig 1994, Swengel 1995).

The Karner blue was added to the list of United States federal endangered species in December 1992 in response to dramatic rangewide reductions in

[1]Departments of Entomology, Forestry[2], and Pesticide Research Center[3], Michigan State University, East Lansing, MI 48824.

[4]USDA Forest Service, North Central Forest Experiment Station, 1407 South Harrison Road, East Lansing, MI 48823.

butterfly abundance and distribution (USFWS 1992). Population declines are attributed to habitat loss and fragmentation resulting from anthropogenic activities such as agriculture, residential and commercial development, off-road vehicle use and fire suppression (Packer 1987, USFWS 1992, Haack 1993, Dirig 1994). Currently, the species occurs in localized areas in Minnesota, Wisconsin, Indiana, Michigan, New York and New Hampshire, and is extirpated in Massachusetts, Pennsylvania, Ohio, Ontario and most likely Illinois (USFWS 1992, Haack 1993, Baker 1994, Grigore and Windus 1994, Packer 1994). Michigan, New York and Wisconsin harbor the greatest numbers of Karner blue populations (Bleser 1992, Haack 1993, Baker 1994).

Conservation of the Karner blue is mandated by the Endangered Species Act of 1973, which provides federal protection for the butterfly and its designated critical habitat, and requires the development and implementation of plans for species recovery (USFWS 1992). Specific recovery measures to-date include research to elucidate critical habitat needs, habitat restoration and management, and investigation into Karner blue propagation and reintroduction (USFWS 1992, Baker 1994). All components of the butterfly's critical habitat have not yet been identified, limiting habitat restoration efforts (Andow et al. 1994). The potential for propagation of Karner blue through captive rearing is gaining increasing attention, especially in states such as Minnesota and New Hampshire, where only a few, small Karner blue populations occur (Schweitzer 1994). These populations could become extirpated before necessary information regarding Karner blue ecology is acquired, or before the habitat has time to respond to management activities (Packer 1994).

Captive rearing may provide a means to supplement low butterfly populations, reestablish recently extirpated populations (New 1993), or provide individuals for research, with minimal risk to existing populations (Lane and Welch 1994). However, only a few recent attempts have been made to identify methods for collection and captive rearing of the Karner blue (Savignano 1992, VanLuven 1993, 1994; Lane and Welch 1994).

We describe the methods and success of our efforts to rear Karner blue from spring generation butterflies under controlled laboratory conditions in 1994. Larvae acquired from this study were used in a related study to evaluate the susceptibility of Karner blue to *Bacillus thuringiensis* Berliner var. *kurstaki* (*Btk*), a microbial insecticide specific to Lepidoptera, commonly used for gypsy moth (*Lymantria dispar* L.; Lepidoptera: Lymantriidae) suppression in Michigan (Herms 1996).

METHODS AND MATERIALS

Lupine foliage: Wild lupine foliage used for Karner blue rearing activities was obtained from a small field in Ingham County, Michigan, which supports lupine and other remnant prairie plant species but, no Karner blue. To harvest lupine, the stems were cut and placed in a water-filled container, and the stem ends were recut under water. In the laboratory, lupine foliage was covered with a plastic bag to reduce desiccation, and refrigerated at 5° C until needed. New lupine stems were harvested and the old stems discarded every 4–5 days. Leaves with previous insect feeding or other damage were not used for rearing Karner blue larvae.

Field collection of Karner blue adults: We collected a total of 20 female Karner blue adults during the spring flight in June 1994 from five collection sites in the Lower Peninsula of Michigan. Three sites were located in the Allegan State Game Area (Allegan County), and two sites were in the Huron-Manistee National Forest (Montcalm and Newaygo Counties). Sites

https://scholar.valpo.edu/tgle/vol29/iss2/2
DOI: 10.22543/0090-0222.1903

were chosen with the cooperation of officials from the Michigan Natural Features Inventory, the Michigan Field Office of The Nature Conservancy, the Allegan State Game Area, and the Huron-Manistee National Forest. The sites were approved by the US Fish & Wildlife Service. Ten females were collected from the game area and 10 from the national forest. We collected only in sites that had 1993 summer generation adult counts of more than 200 butterflies (based upon actual numbers of butterflies observed; Michigan Natural Features Inventory, unpublished data; Huron-Manistee National Forest, unpublished data). In an attempt to minimize possible impacts on local populations, no more than five females were collected from any one site.

We collected Karner blue females 2 weeks after the first spring generation adults were observed, approximately halfway into the spring flight period (Table 1). Butterflies began flying approximately 5 days sooner in the more southerly sites of Allegan State Game Area than in the Huron-Manistee National Forest; therefore, collections were made on 1 June 1994 in the game area and on 9 June 1994 in the national forest. We attempted to select females with moderate wing wear, rather than extremely fresh-looking females or those with worn wings. We assumed that females with moderate wear would have already mated but still retain much of their egg complement. At the time of collection in the game area, the ratio of males to females in Karner blue populations near the collection sites ranged from 2:1 to 3:1 (no butterfly surveys were conducted in the collection sites) (Herms 1996).

Collections were initiated around 1100 hr and completed by 1300 hr. On both days, the weather was sunny, with temperatures around 22°C. We caught each Karner blue female individually in a butterfly net, and transferred it to a glassine envelope by holding the wings. Envelopes with butterflies were then placed in individual plastic containers to prevent crushing, and kept in a slightly chilled cooler (approximately 20°C) in the shade (Saul-Gershenz et al. 1995). A layer of newspaper was used to prevent direct contact of the containers with ice packs at the bottom of the cooler. Transportation time from each collection site to our laboratory at Michigan State University was ca. 2 hr.

Housing of butterflies: In the laboratory, butterflies were transferred to aluminum frame cages (61 × 61 × 61 cm) with 32 mesh Lumite screen (Bio-Quip Products, Gardena, CA). We opened each envelope inside the cage and allowed the female to walk out onto lupine foliage (described below). Butterflies were caged together by site. Cages were kept on fluorescent-lighted shelves in a walk-in environmental chamber maintained at 24°–26°C, with an 18:6 hr light:dark photoperiod, and relative humidity of 57–68 percent.

We provisioned each cage with a water source, partial shading, nectar

Table 1. Adult flight periods of 1994 spring and summer Karner blue generations in Allegan State Game Area (Allegan Co) and Huron-Manistee National Forest (Oceana Co) in Michigan.

Area	Flight period	First adult seen	Last adult seen
Allegan State Game Area	Spring	19 May	18 June
	Summer	27 June	12 August
Huron-Manistee National Forest	Spring	24 May	*not recorded*
	Summer	5 July	*not recorded.*

source, and ovipositional site. The water source was a wet sponge cut to tightly fit the bottom of a petri dish (100 × 15 mm). One sponge was provided per cage, and was moistened daily. Any standing water or condensation was wiped up immediately, to prevent butterflies from becoming trapped or drowning (Lane and Welch 1994). We provided partial shading by placing layers of paper towels over one corner of the top of the cage.

The nectar source was a 5 percent honey: 95 percent water solution (Lane and Welch 1994). The solution was placed in a sterile 150-ml flask, and then sealed with parafilm. Cotton dental wicking (Accu Bite Dental Supply Inc., East Lansing, MI) was pushed partially into the flask through the parafilm, leaving 3–5 cm of wicking protruding, to provide a suitable place for butterflies to perch and feed. We provided two nectar flasks in each cage, and replaced them every 2 days.

The ovipositional site consisted of a wild lupine stem, 20–30 cm tall, with flowers and leaves, in a water-filled 250-ml flask with a parafilm seal. We placed two flasks with lupine in each cage, and replaced them every 2 days with fresh lupine.

We housed the females for 5 days in the cages, and then returned all survivors to their original collection sites. Female butterflies were transported in a ca. 20°C cooler, in glassine envelopes and plastic containers as previously described, to the appropriate site. At the sites, we released each female by opening the envelope near a lupine plant, and allowing the butterfly to walk onto a leaf.

Egg collection and care: We removed the lupine stems from the cages and inspected them for Karner blue eggs once per day. Eggs were carefully dislodged from the plant using a small blade (Lane and Welch 1994), and placed individually into 30-ml plastic cups (Jet Plastica Industries, Hatfield, PA). When lupine stems were replaced, the old stems were kept with the flasks in the environmental chamber, and examined periodically for any eggs or developing larvae.

Plastic cups containing individual eggs were placed in large, lidded plastic boxes (19 x 10 x 8 cm; Tri-State Plastics, Dixon, KY) lined with moist paper towels, and kept in a fluorescent-lighted growth chamber maintained at 24°C, with an 18:6 hr light:dark photoperiod and ambient relative humidity. Relative humidity inside each box with moist paper towels was ca. 80–85 percent, as measured with a Bionaire instrument (model BT-254F, accuracy ± 5 %; Bionaire Environmental Air Products, Blauvelt, NY). We checked the eggs once per day for hatch. Two days after the eggs were collected, we added a small piece of lupine foliage to each cup in anticipation of hatch. The paper towels in each box were rewetted once at most, but only if there was no condensation on the sides of the box or in the cups. No additional moisture was added to the boxes once the lupine foliage was added to the cups, and the box lids were propped for short periods when necessary to allow excess moisture to dissipate.

Larval rearing: We kept larvae in the same growth chamber as the eggs, and checked them daily for molting, mortality, food supply, and condition of container. Molting was noted via presence of exuvia. Larval length was measured at the beginning of each instar using a dissecting microscope fitted with an ocular micrometer.

First and second instar larvae were reared individually in 30-ml plastic cups, which were kept in the growth chamber in lidded plastic boxes. Larvae were transferred, while on the lupine foliage, to fresh cups every 2 days. If necessary, a #000 paintbrush was first used to place each larva on the lupine foliage. We supplied fresh pieces of lupine every 2 days for first instars, and

https://scholar.valpo.edu/tgle/vol29/iss2/2
DOI: 10.22543/0090-0222.1903

daily for second instars. Old foliage was removed the following day after larvae had moved to the new leaves.

Third and fourth instar larvae were reared individually in petri dishes (100 x 15 mm), which were kept in the growth chamber on trays. We provided an entire lupine leaf to each larva by placing the leaf stem in a water-filled 0.5 dram (2-ml) glass vial stoppered with a cotton plug. In this way, the vials and leaves could be placed in the petri dishes horizontally without water leakage, thus preventing larvae from drowning. Lupine leaves were replaced when more than half of the leaf was eaten, usually every 1–2 days. Third instar larvae were transferred to new petri dishes every 2 days, and fourth instar larvae were transferred to new dishes daily. When replacing old lupine or transferring larvae to new dishes, we cut the leaflets that had the larvae, and then moved the larvae while on the leaflets.

After daily use, paintbrushes, forceps and scissors were sterilized by first soaking in a bleach:water solution (1:4), then washing with soapy water and rinsing in distilled water, and finally autoclaving. To avoid potential disease transmission between individuals, we also cleaned utensils after use with each larva by dipping utensils in the bleach solution, and then rinsing thoroughly with water.

Pupae: We kept pupae in the same growth chamber as the eggs and larvae. Pupae were placed individually in small, lidded plastic boxes (14 x 7 x 4 cm; Tri-State Plastics, Dixon, KY) to allow room for adult emergence. When pupae were attached to a lupine leaf, we cut away excess foliage from around the pupal case to avoid leaf molding. When pupae were attached to the petri dish, we sterilized the dish surface around the pupa with 70 percent ethyl alcohol, and placed the open dish in the box.

Adult butterflies: After emergence, each Karner blue adult with its container was removed from the growth chamber, and kept in a refrigerator at 5°C for 1 or 2 days prior to field release. On the day of release, we transported adults in their boxes in a ca. 20°C cooler to the maternal collection sites. The boxes were then removed from the cooler, and opened in a shady area to allow each butterfly to acclimate and fly away.

Data analysis: Developmental times for male and female Karner blue were compared by ANOVA using SYSTAT (Wilkinson 1990). All statistical analyses were conducted at $p < 0.05$ level of significance.

RESULTS

Collection and housing of female butterflies: All 20 Karner blue adult females were collected and transported without mortality from the collection sites to Michigan State University. The butterflies appeared to adjust quickly to the cages, and began using the nectar and water sources within the first few hours. Females from Allegan State Game Area and Huron-Manistee National Forest began laying eggs 2 and 3 days after collection, respectively.

Ten of the 20 Karner blue females were still alive after 5 days (five each from Allegan State Game Area and Huron-Manistee National Forest), and were returned to the original collection sites. We observed male Karner blue butterflies of the spring generation in the sites when the females were released, so presumably all females could have mated. The ten females that did not survive died after 4–5 days in captivity of apparently natural causes. These specimens are vouchered in the collection of the Department of Entomology, Michigan State University, East Lansing, Michigan.

Egg collection and hatch: We collected a total of 154 eggs from the caged butterflies, of which 61 percent were from Allegan State Game Area fe-

males, and 39 percent were from Huron-Manistee National Forest females (Table 2). Once females began laying eggs, we collected from 0–23 eggs per cage per day. Eggs were most often found on the leaves, petioles and stems of the lupine, and occasionally on flowers. We did not find eggs on the sides of the cages or flasks. Nine eggs laid by the Huron-Manistee National Forest females were overlooked, and were later discovered as second and third instars on the old lupine stems in the environmental chamber. Since females were caged in groups, the exact number of eggs from each female could not be distinguished. Based upon cage averages, the average overall number of eggs per female ranged from 1–16.

Overall egg hatch was 47 percent; however, egg hatch varied by region and site (i.e. cage) (Table 2). Forty-three percent of eggs from Allegan State Game Area, and 53 percent of eggs from the Huron-Manistee National Forest hatched (Table 2). Of the 72 first instars obtained, two died (one was deformed so that it could not feed properly and one became diseased), and two escaped (and presumably died).

A total of 82 Karner blue eggs (53 percent) did not hatch. Of these eggs, we observed six cases where two eggs were stuck together (each was counted as 1 egg, not 2), and two eggs which were oddly shaped as compared to the others. We also observed an unidentified species of mite on five of the unhatched eggs. Mold developed on 47 eggs, even though no excessive moisture was apparent. Twenty of those eggs became moldy 5–6 days after they were collected, and the other 27 eggs developed mold in 8–11 days.

Development of larvae, pupae, adults: We used 59 of the 68 Karner blue larvae in a related study (Herms 1996) to determine the susceptibility of Karner blue to *Btk* used for gypsy moth suppression. The other nine Karner blue that were found as larvae on the old lupine were not used in the *Btk* study, and were reared under normal conditions. Of the larvae used in the *Btk* study, 15 were reared under normal conditions for controls, and the other 44 larvae were placed at varying instars on *Btk* treatments. Information reported here regarding larval and pupal development (Table 3, 4) was taken from the 15 control larvae, and the 44 treatment larvae up to their placement on the treatments.

Table 2. Total numbers of eggs obtained and hatched from caged female Karner blue butterflies collected from Allegan State Game Area (Allegan Co) and Huron-Manistee National Forest (Montcalm Co and Newaygo Co) in Michigan.

Karner blue collection area	Cage no.	No. Karner blue adult females	No. eggs	
			Laid	Hatched
Allegan State Game Area	1	4	64	29
	2	3	13	2
	3	3	17	9
	Subtotal	10	94	40
Huron-Manistee National Forest	4	5	54	28
	5	5	6	4
	Subtotal	10	60	32
	Total	**20**	**154**	**72**

https://scholar.valpo.edu/tgle/vol29/iss2/2
DOI: 10.22543/0090-0222.1903

Table 3. Mean duration ± SE of Karner blue life stages captively reared at 24°C.

		Duration of life stages (days)	
Life stage	Sample size*	Mean ± SE	Range
Egg	62	4.1 ± 0.2	1–6**
1st instar	38	3.2 ± 0.2	2–6
2nd instar	36	3.1 ± 0.1	1–5
3rd instar	31	3.4 ± 0.1	2–5
4th instar	15	4.0 ± 0.2	3–6
Prepupa	15	1.2 ± 0.1	1–2
Pupa	15	7.9 ± 0.2	7–9
1st–4th instar	15	13.1 ± 0.4	11–16
Males	7	12.4 ± 0.5 a	11–14
Females	8	13.8 ± 0.5 a	12–16
Egg–adult	15	26.0 ± 0.4	24–29
Males	7	25.0 ± 0.2 a	24–26
Females	8	26.9 ± 0.5 b	25–29

NOTE: For gender comparisons, means followed by the same letter are not significantly different by ANOVA at $p < 0.05$.

* Some larvae reared in this study were used in related research (Herms 1996). Data reported here represent development of 'treatment' Karner blue larvae before they were assigned to treatments, and 'control' larvae in the related research.

** Only one egg hatched 1 day after collection; however, it was probably overlooked during egg collection and left on the lupine foliage for 1 day.

Table 4. Average body length ± SE of captive-reared Karner blue larvae at the onset of each instar.

		Body length (mm)	
Instar	Sample size#	Mean ± SE	Range
1st	25	1.5 ± 0.04	1.1–1.9
2nd	18	2.5 ± 0.12	1.9–3.5
3rd	31	5.2 ± 0.20	3.3–6.8
4th	28	8.5 ± 0.25	6.2–12.5

Some larvae reared in this study were used in related research (Herms 1996). Data reported here represent development of 'treatment' Karner blue larvae before they were assigned to treatments, and 'control' larvae in the related research.

Total developmental time of Karner blue from egg collection to adulthood at 24°C averaged 26 days overall; however, developmental time for females differed significantly from males by 2 days on average ($F = 11.47$, $df = 1$; $p < 0.005$) (Table 3). Karner blue eggs hatched on average 4 days after egg collection, with several eggs hatching after only 2 days (Table 3). One egg hatched after only 1 day; however, this egg was probably overlooked during egg collec-

tion and left on the lupine foliage for a day. No eggs hatched more than 6 days after collection. Total larval duration (first–fourth instar) averaged 13 days overall; larval duration was ca. 1.5 days longer for females than males on average, but was not significantly different ($F = 4.41$, $df = 1$; $p < 0.056$) (Table 3). The duration of individual instars averaged 3–4 days (Table 3). At the prepupal stage, which lasted ca. 1 day (Table 3), Karner blue larvae stopped feeding and became stationary, attaching themselves to the petri dish or to a lupine leaf with a few silk threads. The pupal stage averaged 8 days (Table 3) for both Karner blue males ($n = 7$, $SE = 0.2$) and females ($n = 8$, $SE = 0.2$). Pupae darkened 1 day before adult emergence.

Larval body length was difficult to measure accurately because larvae were often moving, appearing more elongate than when stationary. Based on the average initial lengths for each instar, larvae increased 1 mm from first to second instar, 2.7 mm from second to third, and 3.3 mm from third to fourth (Table 4).

The 15 control larvae and nine larvae not used in the *Btk* study, plus nine of 44 treatment larvae to survive the *Btk* bioassay, developed successfully to adulthood, producing 33 Karner blue adults for release. Nineteen adults (9 males, 10 females) were released into game area sites; 14 adults (6 males, 8 females) were released into national forest sites. We observed summer generation Karner blue adults from wild populations in the sites at the time of release (Table 1).

DISCUSSION

Laboratory, or captive, rearing, and subsequent reintroduction, have been successful components in the conservation of several butterfly species in the family Lycaenidae, such as the atala hairstreak (*Eumaeus atala* Poey, New 1993) in Florida, and the large blue (*Maculinea arion* L., Clarke 1977, New 1993) and large copper (*Lycaena dispar* Obth.) in England (Duffey 1977, Pyle et al. 1981). Our results are consistent with those of recent Karner blue rearing studies (Savignano 1992, VanLuven 1993, 1994, Lane and Welch 1994) that eggs can be collected from females in the laboratory, and can be reared successfully from larva to adult.

In the present study, we obtained 154 eggs, and subsequently 72 first instars, from 20 spring generation Karner blue females. Survival of larvae, pupae and adults reared under normal conditions was high; only four first instars died. The controlled environments of the walk-in environmental chamber and growth chamber used to maintain butterflies and other lifestages ensured that individuals would not experience detrimental temperature extremes. Although many Karner blue larvae were used in related research (Herms 1996), 33 survived to adulthood and were released into maternal collection sites. Fortunately, summer generation adults of the wild populations were flying at the time of release. The rate at which Karner blue developed in the laboratory at 24°C was similar enough to that of field individuals to allow for overlap. Ultimately, synchronous development of lab and field populations would be a desired outcome for a reintroduction program.

In Wisconsin, Lane and Welch (1994) reported the highest oviposition and hatching rates of any rearing study to date. They obtained 876 eggs from 40 spring generation Karner blue females after a 2-day housing period, and 88 percent of the eggs hatched. Lane and Welch (1994) concluded that captive rearing produced large numbers of larvae with minimal or no impact to local populations, and that survival of larvae to adulthood was higher in the laboratory than in the field.

https://scholar.valpo.edu/tgle/vol29/iss2/2
DOI: 10.22543/0090-0222.1903

Summer generation Karner blue females have been used successfully for captive rearing activities in New Hampshire, although overwintering of the eggs and providing lupine for newly hatched larvae in the spring posed some challenges (VanLuven 1993, 1994). In a 1992 study, VanLuven (1993, 1994) obtained 117 eggs from 11 summer generation females that were housed for 3–5 days, and 110 larvae hatched the following spring.

In this study, we observed lower oviposition rates (eggs per Karner blue female) and hatching success than in previous studies (Savignano 1992, VanLuven 1993, Lane and Welch 1994). These results may have been due to random, uncontrollable variables that impacted egg production and viability, such as field conditions experienced by the females prior to collection. Savignano (1992) reported year-to-year variability in egg hatch among rearing experiments, ranging from 60–90 percent hatch. Lederhouse and Scriber (1987) obtained low oviposition rates and/or egg viability for 10–20 percent of field-collected female tiger swallowtail butterflies (*Papilio glaucus* L.; Lepidoptera: Papilionidae) in each of several trials, which they attributed to random mating failure.

However, oviposition and hatching rates in this study may also have been affected by experimental variables such as age (based on wing wear) of collected females, handling of females (collection, transport), size and type of ovipositional cage, and environmental conditions (temperature, relative humidity, light) used to maintain females and eggs in the laboratory. Although we do not have data to support these possible explanations, we point out several ways our rearing methods differed from those of VanLuven (1993, 1994) and Lane and Welch (1994).

Like VanLuven (1993, 1994), we attempted to collect female Karner blue with moderate wing wear, assuming that these females would have mated (Friedrich 1986) but still retain many eggs. In contrast, Lane and Welch (1994) captured fresh females, many of which were observed ovipositing in the field and were presumed to be gravid. For some Lepidoptera species, the adult females can lay a large proportion of their eggs by the time they appear moderately worn (Friedrich 1986). Age of the Karner blue females may also have impacted egg viability. Lederhouse and Scriber (1987) reported significant declines over time in egg viability of female tiger swallowtail butterflies. Some of the Karner blue females we collected may not have been mated, as proposed by VanLuven (1994) to explain low egg numbers in his 1993 study; any eggs laid by these females would have contributed to the low hatching success we recorded.

The butterfly collection and transportation methods used in this study differed somewhat from the other studies, where butterflies were not directly handled, and had some freedom of movement during transport (VanLuven 1993, 1994, Lane and Welch 1994). Lane and Welch (1994) also provisioned butterflies with water and nectar sources. Transport time from field to laboratory in our study was considerably longer than in the other studies. Keeping the butterflies immobile and cool ensured that they would not experience temperature extremes (Saul-Gershenz et al. 1995), reduced their need for resources during transportation, and did not appear to stress or damage them.

Small butterflies, such as lycaenids, can be induced to oviposit in small containers that restrict movement (Friedrich 1986). VanLuven (1993, 1994) used 240-ml glass jars to house summer generation females for oviposition, however with varying success. We chose to use mesh cages similar to those used by Lane and Welch (1994), with access provided by a cloth sleeve, to facilitate the provisioning of resources, and to minimize the risk of butterflies escaping. However, the oviposition cage used by Lane and Welch (1994) was ca. half the size (30 x 30 x 30cm) of the cage used in this study, which caused the lupine stems to touch the top of the cage. Females were often observed

walking on the cage top and coming into contact with lupine (C. Lane, University of Minnesota, pers. comm.). A smaller cage would increase the likelihood of contact between butterflies and ovipositional sites.

Environmental laboratory conditions, such as temperature, relative humidity and light, used to maintain female butterflies and eggs can affect oviposition rate and egg hatch (Singh and Ashby 1985). Our rearing methods mimicked field conditions less than the other studies cited, because of our use of an environmental walk-in chamber to house caged butterflies and growth chambers to house the other butterfly lifestages.

Temperature is an important variable for determining insect activity and development (Goodenough and Parnell 1985, Singh and Ashby 1985, Saul-Gershenz et al. 1995). We housed female butterflies at 24° to 26°C, temperatures slightly lower than daytime temperatures in the field. VanLuven (1993) observed that female Karner blue butterflies of the summer generation were relatively inactive when housed in the laboratory at temperatures below 27°C. However, guidelines for butterfly rearing suggested 25°C as an acceptable temperature for egg production and oviposition (Friedrich 1986). Lane and Welch (1994) kept caged females at ambient room temperature, which averaged 28°C, but fluctuated widely from 23° to 31°C during the day. We maintained eggs at 24°C, whereas Lane and Welch (1994) kept eggs in ambient room temperature, which averaged 24°C, but ranged daily from 20° to 28°C.

The appropriate level of relative humidity for insect development varies with different lifestages (Saul-Gershenz et al. 1995). Relative humidity can impact egg development (Goodenough and Parnell 1985) by either causing desiccation when humidity is too low or molding when humidity is too high (Singh and Ashby 1985, Friedrich 1986). In our study, molding may have reduced egg hatch; approximately half of the unhatched eggs developed mold, some within 6 days and others within 11 days of collection. The remaining unhatched eggs in our study neither developed mold, nor appeared desiccated. Lane and Welch (1994) similarly reported molding in preliminary rearing attempts with Karner blue. Surface disinfection of eggs would presumably reduce this problem (Singh and Ashby 1985).

The quality of light, both wavelength and intensity, and photoperiod, can impact insect physiology, biochemistry and behavior, including oviposition behavior (Singh and Ashby 1985, Saul-Gershenz et al. 1995). In our study, lighting experienced by caged Karner blue females was provided entirely by fluorescent bulbs, with an 18:6 hr light:dark photoperiod. In the studies by Lane and Welch (1994) and VanLuven (1993, 1994), caged butterflies experienced some indirect natural lighting. However, Lane and Welch (1994) used fluorescent bulbs to provide most of the lighting, with a 16:8 hr light:dark photoperiod. VanLuven (1993, 1994) supplemented natural light with an incandescent lamp during cloudy days.

We did not encounter any problems rearing larvae to adulthood in the laboratory. Karner blue larvae developed successfully without the provision of tending ant species; however, this may be a requirement for other ant-tended lycaenid species (New 1993). Only one larva died from an apparent disease. We emphasized sanitation throughout the rearing process (Singh and Ashby 1985, Saul-Gershenz et al. 1995), especially during larval rearing. Protocols included housing larvae in individual containers which were changed often, keeping larval containers free of frass and moisture build-up, supplying clean foliage regularly, and using sterilized tools.

Karner blue larvae appeared to do well on cut foliage from wild lupine plants. Our initial intention was to rear larvae on wild lupine grown from seed in the greenhouse. A preliminary attempt in 1993 to produce greenhouse lupine was successful; however, in 1994, lupine seedlings became infested

https://scholar.valpo.edu/tgle/vol29/iss2/2
DOI: 10.22543/0090-0222.1903

with western flower thrips (*Frankliniella occidentalis* Pergande; Thysanoptera: Thripidae), a common greenhouse pest, and no plants survived. Savignano (1992) successfully reared Karner blue larvae from eggs of spring generation butterflies on Russell Hybrid (*Lupinus polyphyllus*), a cultivated lupine hybrid that grows more quickly in the greenhouse and produces larger leaves than wild lupine. Greenhouse cultivation of lupine may become a useful way to provide foliage for Karner blue rearing projects, especially when overwintered eggs are used and wild lupine may be difficult to obtain in the spring. Although no attempts have been made, development of an artificial larval diet could also simplify the rearing process of Karner blue.

While we need more information on proper laboratory conditions for Karner blue oviposition and development, captive rearing appears to be a viable means of producing Karner blue individuals to supplement or reestablish wild populations, or for research, with potentially little impact to source populations (Lane and Welch 1994). In considering the use of captive-reared Karner blue for reintroduction, some questions still remain regarding which adult generation (spring or summer) should be used for the egg source, and which life stage should be released in the field (Lane and Welch 1994, Schweitzer 1994). Based upon recommendations from previous butterfly rearing programs, reintroductions should occur only within the species' historic range, and reared individuals used for supplementation or re-establishment should be genetically similar to native individuals in or near the release site (Pyle 1976, New et al. 1995). While captive rearing does not replace the need for conservation of butterfly populations in the natural environment (New 1993, Robinson 1995), it appears to be a viable option in the overall conservation program of the Karner blue.

ACKNOWLEDGMENTS

We thank Mary Rabe, Michigan Natural Features Inventory; John Lerg, Allegan State Game Area; Joe Kelly, Huron-Manistee National Forest; Nancy Sferra, Michigan Field Office of The Nature Conservancy; Susan Walker, Mike DeCapita and Kevin Stubbs, USDI Fish & Wildlife Service; and Cynthia Lane, Department of Entomology, University of Minnesota, for their advice with this study. Many thanks as well to Kevin Ellwood and Katy Albers for their indispensable help in the laboratory, and to Dr. James Miller, Department of Entomology, Michigan State University, for use of the walk-in environmental chamber and laboratory space.

This study was funded by the USDA Forest Service, North Central Forest Experiment Station Cooperative Agreement 23-92-61, and USDA Forest Service, Northeastern Area State & Private Forestry in St. Paul, Minnesota. Funding was also provided through a USDA NAPIAP (National Agricultural Pesticide Impact Assessment Program) grant (NC-26-93), by the Michigan Department of Natural Resources through the 1993 and 1994 Nongame Wildlife Fund and Living Resources Small Grants Programs, the Michigan Agricultural Experiment Station Project No. MICL01710, and The Michigan Nature Conservancy.

Federal and state permits for this study were obtained from the USDI Fish & Wildlife Service and the Michigan Department of Natural Resources, respectively, in 1994.

LITERATURE CITED

Andow, D. A., R. J. Baker and C. P. Lane. 1994. Research needs for management and recovery of Karner blue butterfly. pp. 209–216. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Baker, R. J. 1994. The Karner blue butterfly: 1993 and beyond. pp. 163–169. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Bleser, C. A. 1992. Karner blue butterfly survey, management and monitoring activities in Wisconsin 1990–spring 1992. Report to the Wisconsin Dept. of Natural Resources, Bureau of Endangered Resources, Madison WI. 88 pp. plus appendices.

Clarke, C. A. 1977. Breeding the large blue butterfly *Maculinea arion* and the swallowtail butterfly *Papilio machaon* in captivity. Int'l Zoo Yb. 17: 60–62.

Dirig, R. 1994. Historical notes on wild lupine and the Karner blue butterfly at the Albany Pine Bush, New York. pp. 23–36. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Duffey, E. 1977. The reestablishment of the large copper butterfly *Lycaena dispar batavus* Obth. on Woodwalton Fen National Nature Reserve, Cambridgeshire, England, 1969–73. Biol. Conserv. 12: 143–158.

Friedrich, E. 1986. Breeding butterflies and moths- a practical handbook for British and European species. Harley Books, Essex, England. 176 pp.

Goodenough, J. L. and C. B. Parnell. 1985. Basic engineering design requirements for ventilation, heating, cooling, and humidification of insect rearing facilities. pp. 137–155. *In*: P. Singh and R. F. Moore (eds.). Handbook of insect rearing: Volume I. Elserier, Amsterdam.

Grigore, M. T. and J. L. Windus. 1994. Decline of the Karner blue butterfly in the oak openings of northwest Ohio. pp. 135–142. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Haack, R. A. 1993. The endangered Karner blue butterfly (Lepidoptera: Lycaenidae): biology, management considerations, and data gaps. pp. 83–100. *In*: A. R. Gillespie, G. R. Parker, P. E. Phillip and G. Rink (eds.). Proceedings, 9th Central Hardwood Forest Conference, 1993 March 8–10; West Lafayette, IN. Gen. Tech. Rep. NC-161. St. Paul, MN: USDA Forest Service, North Central Forest Experiment Station.

Herms, C. P. 1996. The endangered Karner blue butterfly (Lepidoptera: Lycaenidae) in Michigan: habitat suitability, potential impacts of gypsy moth (Lepidoptera: Lymantriidae) suppression, and laboratory rearing. Master's Thesis. Michigan State University, East Lansing. 120 pp.

Lane, C. and R. Welch. 1994. Rearing Karner blue butterflies for experimentation, reestablishment and supplementation. Report to USDI Fish & Wildlife Service, St. Paul, Minnesota. 18 pp.

Lederhouse, R. C. and J. M. Scriber. 1987. Ecological significance of a postmating decline in egg viability in the tiger swallowtail. J. Lepidop. Soc. 41: 83–93.

New, T. R. 1993. Introduction to the biology and conservation of the Lycaenidae. pp. 1–21. *In*: T. R. New (ed.). Conservation biology of Lycaenidae (Butterflies). The International Union for Conservation of Nature (IUCN) Species Survival Commission, Occasional Paper No. 8.

New, T. R., R. M. Pyle, J. A. Thomas, C. D. Thomas, and P. C. Hammond. 1995. Butterfly conservation management. Annu. Rev. Entomol. 40: 57–83.

Packer, L. 1987. Status report on the Karner blue butterfly, *Lycaeides samuelis melissa* Nabokov, in Ontario. Report to the World Wildlife Fund and the Ontario Ministry of Natural Resources, Wildlife Branch, Nongame Program. 66 pp.

Packer, L. 1994. The extirpation of the Karner blue butterfly in Ontario. pp. 143–151. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Pyle, R. M. 1976. Conservation of Lepidoptera in the United States. Biol. Conserv. 9: 55–75.

Pyle, R., M. Bentzien and P. Opler. 1981. Insect conservation. Annu. Rev. Entomol. 26: 233–258.

Robinson, M. H. 1995. The conservation of invertebrates: problems and prospects. pp. 5–19. *In*: E. F. Gibbons, Jr., B. S. Durrant, and J. Demarest (eds.). Conservation of endangered species in captivity: an interdisciplinary approach. State University of New York Press, Albany, New York.

Saul-Gershenz, L. S., R. A. Arnold and J. M. Scriber. 1995. Design of captive environments for endangered invertebrates. pp. 51–71. *In*: E. F. Gibbons, Jr., B. S. Durrant, and J. Demarest (eds.). Conservation of endangered species in captivity: an interdisciplinary approach. State University of New York Press, Albany, New York.

Savignano, D. A. 1992. Karner blue butterfly rearing experiments. Report to the New York State Dept. of Environmental Conservation, Endangered Species Unit. 5 pp.

Schweitzer, D. F. 1989. Fact sheet for the Karner blue butterfly with special reference to New York. Report to The Nature Conservancy, New York Field Office. 7 pp.

Schweitzer, D. F. 1994. Recovery goals and methods for Karner blue butterfly populations. pp. 185–193. *In*: D. A. Andow, R. J. Baker and C. P. Lane (eds.). Karner blue butterfly: A symbol of a vanishing landscape. Misc. Publ. 84–1994, Minnesota Agricultural Experiment Station, Univ. of Minnesota, St. Paul.

Shapiro, A. M. 1969. New distributional data on three northeastern United States butterflies. J. Lepidop. Soc. 23: 265–269.

Singh, P. and M. D. Ashby. 1985. Insect rearing management. pp. 185–215. *In*: P. Singh and R. F. Moore (eds.). Handbook of insect rearing: Volume I. Elserier, Amsterdam.

Swengel, A. B. 1995. Observations of spring larvae of *Lycaeides melissa samuelis* (Lepidoptera: Lycaenidae) in central Wisconsin. Great Lakes Entomol. 28: 155–170.

United States Fish & Wildlife Service. 1992. Endangered and threatened wildlife and plants: determination of endangered status for the Karner blue butterfly. Federal Register (December 14) 57(240): 59236–59244.

VanLuven, D. E. 1993. Status report on the Karner blue butterfly and associated management activities. Report to the USDI Fish & Wildlife Service, Concord, New Hampshire. 25 pp.

VanLuven, D. E. 1994. 1994 Karner blue butterfly (*Lycaeides melissa samuelis*) captive-rearing results: Concord Pine Barrens, New Hampshire. Report to The Nature Conservancy, New Hampshire Field Office, Concord, New Hampshire. 12 pp.

Wilkinson, L. 1990. SYSTAT: the system for statistics. Evanston, Illinois: SYSTAT Inc. 677 pp.