LADY BEETLES: 1. LAKE SHORE AGGREGATIONS AND 2. PHYSIOLOGICAL ADAPTATIONS IN RELATION TO HIBERNACULUM

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RICHARD EARL LEE, JR.

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FOREWARD

Two aspects of the biology of lady beetles are reported in this thesis. The first deals with the phenomenon of lady beetle aggregations on the shores of lakes in relation to their natural history. The second part involves a comparative study of specific physiological adaptations of two coccinellids in relation to their respective hibernacula.

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Dr. Robert Gordon identified the reference collection.

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INTRODUCTION

The aggregation of lady beetles on the shores of lakes in the upper midwest is a phenomenon commonly known to local residents, although it has received little attention in the scientific literature. The beetles in these shore aggregations may be so numerous that 5,000 to 10,000 living individuals may be collected in one hour.

As early as 1850, LeConte reported collections of lady beetles on the shore of Lake Superior. Additional observations of coccinellids on lakesshores were made by Wheeler (1887), Snow (1902), Needham (1900, 1904, and 1917), and Park (1930) on Lake Michigan, Schwartz (1890) on Lake Superior, and Smith (1966) on Lake Ontario. In 1975, Simpson and Welborn reported a mixed aggregation of lady beetles and alfalfa weevils, *Hypera postica* (Gyllenhal) along a reservoir in Colorado. Savoiskaya (1965) referred to masses of coccinellids on the shores of lakes, including Lake Ala-kul and Lake Balkhash in Kazakhstan, U.S.S.R. Accumulations of coccinellids have also been reported on ocean shores in England (Marriner, 1939, and Riggall, 1953), the United States (Hagen, 1962), and Egypt (Oliver, 1943). Oliver describes a drift line of dead *Coccinella 11-punctata* L. at least 13 miles long with 70,000 beetles per foot run.

These reports are restricted to species lists and general notes based upon limited observations at single shore sites. In order to determine the significance shore aggregations have in the biology of lady beetles, additional information is required.

The purpose of this study is twofold: 1) to provide a detailed description of these shore aggregations, and 2) to determine the relationship of these aggregations to the life history of coccinellids.

METHODS AND MATERIALS

Periodically during the months of April through November of the years 1975-1978, the shores of lakes were examined for the presence of lady beetle aggregations. A shore site was considered to have an aggregation if it were possible to collect 50 or more beetles during a 15-20 minute search. However, on most occasions when an aggregation was present several hundred and often more than one thousand beetles could be collected during this search period. During the course of this study Park Point, Duluth, Minneosta, on the southwest corner of Lake Superior and Mille Lacs Lake in central Minnesota were sampled intensively during both the fall and spring. Additional samples were collected primarily in the fall at other lakes throughout the upper midwest.

In order to determine the ability of *Hippodamia convergens* Guerin and *H. tredecimpunctata* (Say) to overwinter on Park Point, beetles naturally aggregating on this beach were collected on October 26, 1975, and placed in fine mesh bags. The bags were placed in a protected site beneath the low hanging branches of *Juniperus communis* approximately 40 meters from the water in the lake dune portion of Park Point. In addition, nearly 1,000 beetles were marked with fingernail polish at seven sites 5 to 25 meters from the high water line. Bags and sites were checked on April 3, 1976.

To test the ability of *H. convergens* to survive on the surface of water, single individuals were placed in 500 ml Erlenmeyer flasks partially filled with 200 ml of tap water and held at room temperature. The length of survival was checked daily.

Non-shore collections of coccinellids were made in order to provide comparative information to that obtained from the shore aggregations. Summer collections were made from cornfields on the St. Paul Campus, University of Minnesota, St. Paul, Minnesota. As no overwintering aggregations of *H. convergens* were found in the Minnesota area, samples collected from winter aggregation sites near Grass Valley, Nevada County, California, were purchased from the Bio-Control Co., Auburn, California. Overwintering *H. tredecimpunctata* were collected from a heterospecific aggregation, composed mainly of *Coleomegilla maculata*, located in Winona County in southeastern Minnesota. An additional collection was made on the evening of July 14, 1977, at the Itasca State Park, Clearwater County, Minnesota, when a large number of *H. tredecimpunctata* were attracted to building lights.

Samples were analyzed for the occurrence and relative abundance of each coccinellid species. The sex ratio for *H. convergens* and *H. tredecimpunctata* was determined for most samples. Generally, a portion of the sample was fixed in Kahle's solution for later dissection after the method of Stewart *et al*. (1967). If possible, for each sample twenty males and females of both *H. convergens* and *H. tredecimpunctata* were dissected and examined for fat content and the presence of the braconid parasite, *Perilitus coccinellae*. An individual with high fat content was characterized by a distended abdomen and the presence of a large amount of internal fat globules. Reproductively active females were indicated by the presence of at least two and usually five or more developing eggs of one millimeter or more in length.

Dr. Robert Gordon, Systematic Entomology Laboratory, USDA, determined the reference collection used in this study. A voucher collection has been deposited in the Entomology Museum, Department of Entomology, Fisheries, and Wildlife, University of Minnesota, St. Paul, Minnesota.

RESULTS

General Observations

Shore aggregations were observed primarily during late August, September, October, and May (Figure 1). In addition, nineteen times during this study, I received reports or samples of beetles from persons who had observed shore aggregations. Seventeen of these aggregations occurred in late August or September, while the remainder were observed in May. The largest aggregations took place in September when beetles were sometimes so numerous that 5,000-10,000 individuals could be collected in one hour. Aggregations in other months were often considerably smaller. The probability of finding an aggregation at a particular site was quite variable, even during months were aggregations were most common. As indicated in Figure 1, during the month of September aggregations were observed at only 33% of the site visits.

The aggregations appeared to arrive suddenly on the shore with most of the beetles gradually leaving the area within 2-3 weeks. August and early September aggregations apparently dispersed more rapidly, perhaps due to the warmer temperatures, than those observed in October.

On one occasion, May 5, 1978, large numbers of coccinellids were found dead in the water along the western shore of Mille Lacs Lake. At

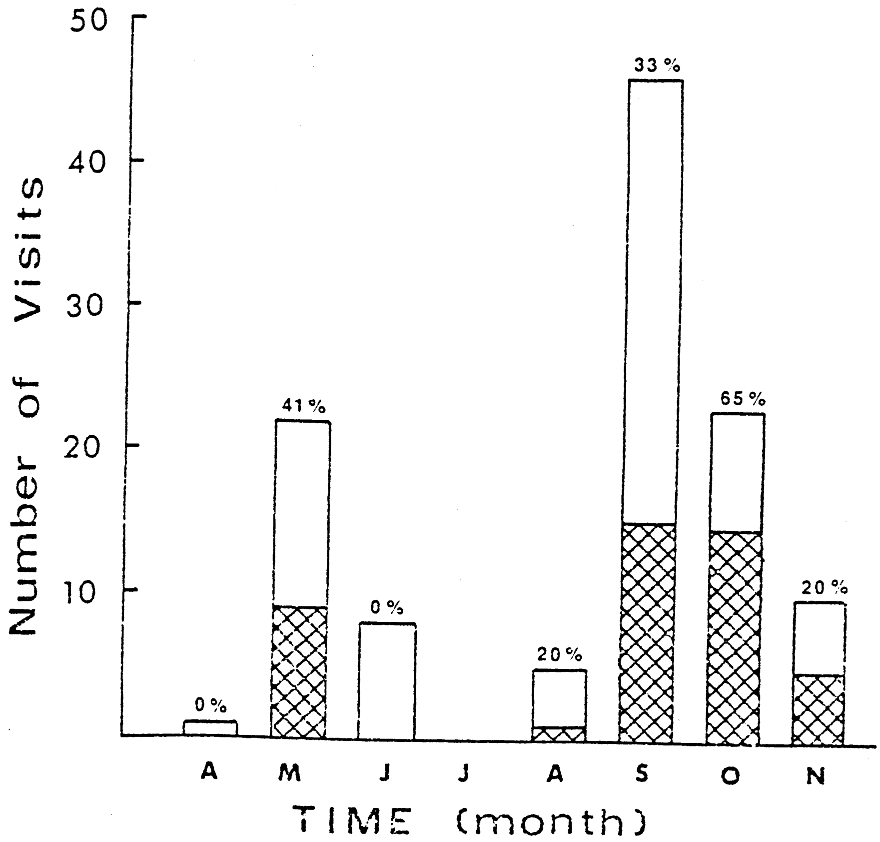

Figure 1. Height of bar corresponds to the number of shore site visits during 1975-1978. Hatched area of bar indicates actual number of sites at which shore aggregations were observed. The number above each column is the percentage of shore aggregations observed in relation to the number of shore site visits for that month.

this time the ice was nearly off the lake; however, at several sites fist-sized and smaller fragments of ice lined the beach, sometimes extending as much as 100 meters out from the shore. The dead beetles were observed floating among this fringe of ice.

On lake shores the greatest concentration of lady beetles was observed within three meters of the high water line, where they were found clinging near the top of drift wood, rocks, vegetation, and any other available object. The distribution of the coccinellids ranged from scattered individuals to clusters numbering in the hundreds. Very few dead beetles were observed in these drift line aggregations. Some individuals exhibited a darkened elytral coloration similar to ones which had been experimentally held in water.

Park Point is a narrow finger of land separating the Duluth Harbor from Lake Superior. Shore aggregations were found only on the lake side of Park Point.

On windy days the beetles moved into protected nooks and crevices. During the day the activity of the beetles appeared to be related to temperature. If disturbed on warm days, the beetles moved about rapidly and sometimes took flight, while on colder days they responded by moving slowly or dropping to the ground.

Copulating pairs of *H. convergens* were found frequently in both fall and spring shore aggregations. No ovipositing females or larval lady beetles were observed on the beaches, although on rare occasions single eggs were encountered on drift wood. Lady beetles were not observed feeding while on the shore, except on two occasions when an adult *H. convergens* was feeding on a conspecific. Furthermore, dissections of more than one thousand individuals of *H. convergens* and *H. tredecimpunctata* collected in both the fall and the spring revealed that the gut was nearly always empty. This fact contrasts with summer dissections of lady beetles collected in cornfields whose digestive tracts were usually distended with parts of aphids.

Geographic Distribution

Shore aggregations were found on lakes throughout the upper midwest. Aggregations were observed at one or more sites on the shores of Lake Superior, L. Michigan, L. Huron, Mille Lacs L., L. Winnipeg, and L. Manitoba. Additional samples of lady beetles were received from fall shore aggregations on Upper Red Lake, Lake of the Woods, and Pelican Lake, Ottertail Co., in west central Minnesota. The shore sites at which these aggregations were located are shown on Figure 2. Spring shore aggregations were observed on Mille Lacs Lake and on Lake Superior.

Species Composition

The most abundant coccinellid in all fall shore aggregations was *H. convergens*. This species usually accounted for more than 99% of the collection sample. *H. tredecimpunctata* was the only other lady beetle collected in relatively large numbers. In one sample from Park Point, this species comprised 11% of the coccinellids in the aggregation. In spring shore aggregations, *H. tredecimpunctata* was generally more common than in fall collections. In 1978, at Mille Lacs Lake in early May, this species constituted more than 98% of the individuals in shore aggregations; however, by May 31, it accounted for only 59% of the sample. At Park Point, in spring collections, *H. convergens* remained the most common species of coccinellid present,

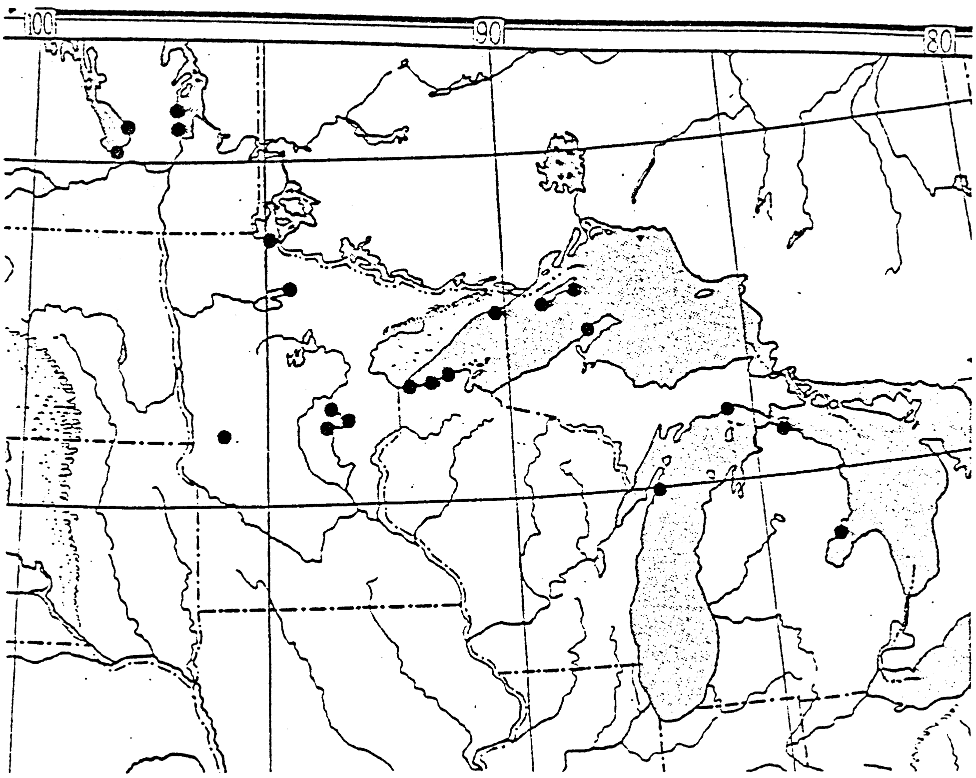

Figure 2. Lake shore sites at which lady beetle aggregations were collected or from which samples were received.

although on one occasion 17% of the sample was *H. tredecimpunctata*.

Other coccinellid species generally accounted for less than 0.5% of the shore aggregation collections. Table 1 summarizes lady beetle species collected at either Mille Lacs Lake or Park Point at any time during the study. The greatest species richness was observed on May 5, 1978, at Mille Lacs Lake when 19 coccinellid species were collected from a one meter-wide strip of shore line thirty meters in length. In even the largest fall shore aggregations, fewer than eight species were typically observed, while spring samples commonly yielded ten or more species. Overall, the species richness at Mille Lacs Lake was greater than that observed at Park Point (Table 1) even though fewer visits were made to Mille Lacs Lake.

Experimental Overwintering on Park Point

When the bags and sites of marked beetles were checked on April 3, 1976, Park Point was clear of snow, but large piles of broken ice still lined the shore. Temperatures were still so low that it is unlikely that beetles would have been able to disperse prior to this time. All beetles in the bags were dead. Extensive movements of sand caused by the action of wind and ice on the beach had buried four of the seven sites where individual beetles had been marked. A two-hour search yielded only four living coccinellids, although over 200 dead individuals were observed. None of the marked beetles were recovered. Searches during November of other years produced very few, if any, living beetles.

	Mille Lacs Lake		Park Point	
	Spring	Fall	Spring	Fall
Adalia bipunctata (L.)	X	X		X
Anatis labiculata (Say)	X		X	X
A. mali (Say)	X		X	X
Anisosticta bitriangularis (Say)	X		X	
Brumoides septentrionis (Weise)	X		X	X
Calvia quatuorodecimguttata (L.)	X			
Chilocorus stigma (Say)	X		X	
Coccidula lepida LeConte	X			
Coccinella novemnotata Herbst				X
C. transversoguttata Brown	X	X		X
Coleomegilla maculata (DeGeer)	X	X	X	X
Cycloneda munda (Say)		X		X
Hippodamia convergens Guerin	X	X	X	X
H. parenthesis (Say)	X	X	X	X
H. tredecimpunctata (Say)	X	X	X	X
Hysperaspis disconotata Muls.	X		X	
H. undulata (Say)	X		X	X
Hysperaspis sp.	X		X	
Macronaemia episcopalis (Kirby)	X		X	
Mulsantina hudsonica Casey	X			
M. picta (Randall)	X			X
Psyllobora vigintimaculata (Say)	X			
Total	20	7	13	13

Table 1. Summary of coccinellids collected from shore aggregations at Mille Lacs Lake and at Park Point on Lake Superior.

Survival in Water

Single individuals of *H. convergens* were able to survive for an average of 10.6 days on the surface of still water (Table 2). Male and female survival times were similar.

Female Reproductive Activity

H. convergens females from spring and fall shore aggregations and overwintering aggregations were reproductively inactive as compared to females collected from cornfields in the summer (Table 3). These results contrast with the data obtained for *H. tredecimpunctata* in which 51.4% of the females from spring shore aggregations were reproductively active, as were those from summer and fall shore samples (Table 3).

Fat Content

In both *Hippodamia* species, the fat content was highest in individuals from overwintering aggregations and lowest for those from spring shore aggregations (Table 4). Fall shore aggregations were composed mainly of individuals with high fat content, although in a small proportion of the population large fat deposits were conspicuously absent. Females in this portion of the population often had well-developed reproductive structures, but lacked developing eggs. This condition suggests that these individuals had reproduced, but that without fat stores would be unable to survive the winter.

Parasitism

Adult coccinellids were commonly observed attached to cocoons of *Perilitus coccinellae* in fall shore aggregations and during the summer

	Mean Survival (days)	S.D. $\overline{X}$	Range	Sample Size (n)
Female	10.7	1.4	2-15	15
Male	10.5	1.4	2-21	15
Total	10.6	0.8	2-21	30

Table 2. Summary data for the survival of Hippodamia convergens in water.

A. Hippodamia convergens

	Summer	Fall Shore Aggregation	Spring Shore Aggregation	Winter Aggregation
Reproductive females (%)	25.0	1.3	0.0	0.0
S.D. $\bar{X}$	4.8	0.5	--	—
Number of individuals (n)	80	464	26	40
Number of samples	4	24	2	2

B. Hippodamia tredecimpunctata

	Spring Shore Aggregation	Fall Shore Aggregation	Summer	Summer at lights	Winter Aggregation
Reproductive females (%)	51.4	34.6	27.5	0.0	0.0
S.D. $\bar{X}$	8.4	9.3	7.1	—	---
Number of individuals (n)	35	26	40	20	24
Number of samples	2	2	2	1	1

Table 3. Proportion of reproductively active females in samples of Hippodamia convergens and H. tredecimpunctata. The solid horizontal bar above the table indicates the lack of a significant difference at the 95% level between all means included by the bar as determined by chi-square tests; all other differences between means are significant at the 95% level.

A. Hippodamia convergens

	Winter Aggregation	Fall Shore Aggregation	Summer	Spring Shore Aggregation
High fat (%)	98.8	86.7	59.4	51.4
S.D. $\overline{X}$	1.2	1.1	3.9	8.6
Number of individuals (n)	80	924	160	34
Number of samples	2	24	4	2

B. Hippodamia tredecimpunctata

	Winter Aggregation	Summer at lights	Fall Shore Aggregation	Summer	Spring Shore Aggregation
High fat (%)	100.0	100.0	63.6	40.0	4.3
S.D. $\overline{X}$	---	---	7.3	5.5	2.4
Number of individuals (n)	24	40	44	80	70
Number of samples	1	1	2	2	2

Table 4. Proportion of individuals with high fat content in samples of Hippodamia convergens and H. tredecimpunctata. Over-scoring is described in Table 3.

in cornfields. Dissection revealed that the incidence of P. coccinellae larvae in H. tredecimpunctata was maintained at relatively constant levels throughout the year (Table 5). In contrast, summer and fall shore collections of H. convergens were parasitized 5-6 times as heavily as those from overwintering or spring shore aggregations (Table 5). H. tredecimpunctata was more heavily infected with P. coccinellae at all times of the year than was H. convergens.

Sex Ratio

In H. convergens, the proportion of females in the fall and spring shore aggregations was comparable to that of winter aggregations, but contained significantly more females than summer samples (Table 6). At several shore sites over 70% of the individuals were female. The data for H. tredecimpunctata revealed no significant differences among the proportion of females from shore aggregations, winter aggregations, or summer samples (Table 6).

DISCUSSION

The most striking behavioral characteristic of coccinellids is their formation of overwintering aggregations. Hagen (1962) describes an aggregation of Hippodamia convergens from which 600 gallons of beetles having 70,000 beetles per gallon were collected. Beetles may remain in these aggregations for extended periods up to ten months. This long dormancy period, described as an estivohibernation for some species, is believed to synchronize the reproductive and feeding stages of the beetles with their aphid food supply (Hodek, 1973). Physiologically, beetles from overwintering aggregations are characterized by the presence

A. *Hippodamia convergens*

	Summer	Fall Shore Aggregation	Winter Aggregation	Spring Shore Aggregation
Infected (%)	18.8	18.3	3.8	2.7
S.D. $\overline{X}$	3.1	1.3	2.1	2.7
Number of individuals (n)	160	924	80	37
Number of samples	4	24	2	2

B. *Hippodamia tredecimpunctata*

	Spring Shore Aggregation	Winter Aggregation	Summer	Fall Shore Aggregation	Summer at lights
Infected (%)	34.3	33.3	31.3	25.0	22.5
S.D. $\overline{X}$	5.7	9.6	5.2	6.5	6.6
Number of individuals (n)	70	24	80	44	40
Number of samples	2	1	2	2	1

Table 5. Incidence of *Perilitus coccinellae* larvae parasitizing *Hippodamia convergens* and *H. tredecimpunctata*. Overscoring is described in Table 3.

A. Hippodamia convergens

	Fall Shore Aggregation	Spring Shore Aggregation	Winter Aggregation	Summer
Female (%)	57.3	56.5	54.3	48.4
S.D. $\overline{X}$	0.6	1.9	1.5	2.2
Number of individuals (n)	6263	701	1176	510
Number of samples	35	5	3	5

B. Hippodamia tredecimpunctata

	Spring Shore Aggregation	Fall Shore Aggregation	Summer	Winter Aggregation	Summer at lights
Female (%)	59.8	51.0	50.3	45.8	46.3
S.D. $\overline{X}$	2.2	3.5	2.8	10.2	3.5
Number of individuals (n)	513	202	324	24	205
Number of samples	5	2	4	1	1

Table 6. Proportion of females in samples of Hippodamia convergens and H. tredecimpunctata. Over-scoring is described in Table 3.

of a well-developed fat body, a reduction in the size of the empty midgut as compared to actively feeding individuals, and a lack of ovigenesis (Hodek, 1973, and McMullen, 1967).

Hippodamia convergens and H. tredecimpunctata from fall shore aggregations share a number of characteristics with ones collected from winter aggregations. The highest levels of fat are found in beetles from these two groups. An empty and reduced gut and a lack of ovigenesis are additional shared traits. Aggregation formation is another prominent behavioral characteristic of both fall shore and overwintering coccinellids.

Similarly, beetles from spring shore aggregations exhibit traits typical of individuals that have recently emerged from winter dormancy. In this study the spring beetles have a lowered fat content, as would be expected for beetles that had recently emerged from overwintering. The extremely low fat content of H. tredecimpunctata in early spring aggregations corresponds to the high reproductive activity of females at this time. Since no aphids were available prior to these early May collections, these data suggest that the fat reserves were used for egg production. High levels of fat for some individuals from summer collections may indicate that a portion of the adult population was produced that summer and was building up fat for overwintering.

Both shore and overwintering aggregations of H. convergens appear to have more females than those from summer breeding and feeding populations. These results suggest no differential mortality between the sexes through the winter (Table 6). Several other workers have reported more females in overwintering aggregations of Coccinella 10-punctata L.

(Marriner, 1939) and *Coleomegilla maculata* (Parker *et al*., 1977, and the author's unpublished data) and in summer samples of six additional coccinellids (Smith, 1966). Smith also reported a sample of *H. convergens* containing only 37.5% females from the shore of Lake Ontario in September. Although these data conflict with the results of this study, his sample consisted of only 35 individuals. A skew in the sex ratio favoring females appears to be another characteristic of overwintering aggregations of *H. convergens* shared by beetles in shore aggregations.

The flight of *H. tredecimpunctata* observed on July 14, 1977, appears to have been individuals en route to a site for estivo-hibernation. These beetles contained high levels of fat and had empty digestive tracts. The lack of reproductive activity was indicated by the absence of developing eggs or the presence of hypertrophied reproductive organs, commonly found in females which had laid eggs earlier that summer. Apparently, these lady beetles had emerged earlier that summer and were preparing to overwinter without reproducing. Since high reproductive activity was observed in spring shore aggregations for *H. tredecimpunctata* (Table 3), it appears that this species begins and ends reproduction earlier than *H. convergens*.

The overwintering habits of *Hippodamia convergens* in the upper midwest are unknown except for the reports of Hodson (1937) and Latta (1928). Both workers observed aggregations of less than 150 individuals beneath loose bark and in the open crevice of a fence post. No overwintering aggregations were located in this study. A summary of Hagen's work (1962) with this species in California is presented in order to provide a basis for a discussion of the significance of his work to the data obtained in this study. In the Central Valley of northern California, Hippodamia convergens is univoltine with feeding and reproduction occurring February through May or June. As aphid numbers dwindle, the emerging adults begin a migration of 50-100 miles to the Sierra Nevada mountains where overwintering aggregations are formed. Migration begins with vertical take-off flights reaching heights of 3500-5400 feet as the beetles are carried along by westerly winds blowing them towards the mountains. These vertical flights are quite unlike the short, low level flights associated with feeding and reproduction. The beetles remain in their mountain aggregations until the first warm days of the year arrive in February or March. At this time a remigration begins as beetles once again take flight, but are now carried on northerly or easterly winds that prevail at this time of the year. The beetles ride these winds until they return to the valleys. Occasionally the returning beetles are carried beyond the Central Valley and are deposited in the Pacific Ocean, as evidenced by their dead bodies washed ashore on the beaches.

A number of characteristics associated with the shore aggregation phenomenon are consistent with the hypothesis that H. convergens undergoes a fall and spring migration similar to the one observed by Hagen (1962) in California. In the upper midwest, H. convergens is commonly found feeding and reproducing in agricultural fields during the months of June, July, and early August, while shore aggregations most commonly occur before or after this period. The high fat content, empty digestive tracts, and reproductive inactivity of beetles from fall shore aggregations are characteristics typical of individuals en route to overwintering sites. The lowered fat levels and increased reproductive activity in spring shore beetles are traits which would be expected for coccinellids emerging from overwintering sites. Even though *H. convergens* is carried by prevailing winds, Hagen (1962) points out that the fall flights result in strongly directional migration, while spring movements are less directed, resembling simple dispersal. Fall shore aggregations of *H. convergens* are much larger than those occurring in the spring. These observations are in accord with mass directional movements in the fall and a less directional spring dispersal.

A number of factors suggest that lady beetles are washed ashore after falling into the water during their fall and spring flights. Park Point extends along a northwest to southeast line bordering the Duluth harbor. The prevailing winds are from the east in May and from the west and northwest in September, October, and November (Anon., 1977). During a study of water movements in western Lake Superior, Ruschmeyer and Olson (1958) found that drift bottles released on a line between Two Harbors, Minnesota, and Port Wing, Wisconsin, were commonly washed ashore on Park Point. Thus, both the wind and water currents tend to move floating beetles onto Park Point.

Several other observations are consistent with this interpretation. First, on several occasions fall shore aggregations were observed on Park Point, but not along the shore north of Duluth at the mouth of the Lester River and at Two Harbors. If lady beetles were actively selecting the shore, one might expect to find them on both sides of Park Point; however, during the course of this study no aggregations were observed on the harbor side of Park Point. The coccinellids that were observed floating among a wide fringe of ice fragments on Mille Lacs Lake had presumably been deposited by the action of wind and waves. Beetles in shore aggregations sometimes had dark elytral markings similar to ones experimentally held in water. Finally, it was determined that *H. convergens* was able to survive for an average of 10.6 days on the surface of water (Table 2).

Summary

Lady beetles from fall shore aggregations and overwintering aggregations are characterized by the presence of large amounts of fat, reproductive inactivity, empty digestive tracts, a skew in the sex ratio favoring females, and the behavioral tendency to form aggregations. During the short period of time they remain on the shore the coccinellids do not feed or reproduce. Most beetles from fall shore aggregations are gone by November. Experimental studies indicated that beetles were unable to overwinter on the shore. *Hippodamia convergens*, the most commonly observed coccinellid in shore aggregations, undergoes long migratory flights to and from overwintering sites in California. It is suggested that similar migratory activity occurs in the upper midwest and that during these flights the beetles may be blown into the water and washed ashore, thus forming aggregations. However, in contrast with overwintering aggregations in California, the shore aggregations reported in this study appear to be temporary shoreline collections, which are neither important nor adaptive to overwintering survival.

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INTRODUCTION

The significance of snow cover in ameliorating environmental stresses, and thereby, enhancing the survival of small mammals (Formozov, 1946) and insects (Mail, 1932) beneath the snow has been known for many years. Pruitt (1957) described the subnivean environment as one having a saturated atmosphere with moderate and relatively stable temperature, while supranivean conditions were characterized by widely fluctuating humidities and temperatures.

Danks (1978) notes that although a number of workers have studied the physiological and biochemical basis of insect overwintering, few investigations have dealt with the significance of particular hibernacula in relation to overwintering physiology. Exceptions to this trend were the comparative studies of Kirchner (1973) with spiders and MacPhee (1964) with insects, in which the level of cold-hardiness was correlated with overwintering site. Arthropods from exposed sites were more cold-hardy as determined by low supercooling points than were those from more protected hibernacula. These studies investigated only one physiological parameter in relation to hibernaculum. Additional investigations which consider a number of factors at one time for a given species are needed.

It is well known that lady beetle adults often form large overwintering aggregations (Hagen, 1962; Hodek, 1973). Beetles may remain in these aggregations for extended periods of up to ten months. In California, Hippodamia convergens Guerin leaves the lowlands in May and June, and migrates to the Sierra Nevada Mountains where aggregations are formed (Hagen, 1962). The beetles remain in the aggregations until February when return migrations to the valleys occur. Hagen reports that most aggregations are not covered by snow through the winter. Although large overwintering aggregations of

H. convergens have not been reported in the upper midwest, Latta (1928) and Hodson (1937) observed aggregations of less than 150 individuals in relatively exposed sites beneath loose bark and in the open crevices of a fencepost.

Coleomegilla maculata (DeGeer) is a common coccinellid of the eastern U.S. that also forms large overwintering aggregations in leaf litter on the forest floor (Hodson, 1937; Hagen, 1962; Parker, Whalon, and Warshaw, 1977). These aggregations are generally covered by snow. Warshaw (personal communication) indicated that aggregations not covered by snow experienced the greatest mortality through the winter. Both species are commonly found in agricultural crops in the midwest during the summer.

Previous workers have demonstrated a reduction in respiration rate during dormancy for H. convergens (Stewart et al., 1967) and for C. maculata (Parker et al., 1977). However, no studies have investigated possible temperature compensation of respiration rate for these species.

Hippodamia convergens and Coleomegilla maculata have similar life histories, but differ in their specific overwintering microhabitat sites. This study provides comparative data on several physiological parameters for individuals collected from winter aggregations. This information is discussed with respect to the significance of the hibernaculum on physiological adaptations related to overwintering.

METHODS AND MATERIALS

Collection - During the fall of 1977 and 1978, Coleomegilla maculata (DeGeer) was collected from overwintering aggregations in Winona County, southeastern Minnesota. Samples of Hippodamia convergens collected from winter aggregation sites near Grass Valley, Nevada County, California, were pruchased from the Bio-Control Company, Auburn, California. Additional collections of both species were made in September, 1978, from corn fields on the St. Paul Campus of the University of Minnesota.

Respiration - The standard techniques, as described by Umbreit et al. (1972) for Warburg manometry were used to determine respiration rates. The center well of the 15 ml flasks was covered with a stainless steel screen to prevent contact between the beetles and the 10% potassium hydroxide solution. Preceding each two-hour respiration trial, a 15-minute period allowed for temperature equilibrium between the flask and water bath. Beetles collected from corn fields were held at room temperature for 2-3 days to allow the gut to empty. Each flask contained 4-10 beetles. The wet weight of the beetles was determined to the nearest 0.1 mg.

Resistance to Dessication - Groups of 50 beetles acclimated to 6° C for a minimum of two months were held at 6° and 20° C with a relative humidity of either 0 or 75% in order to compare the resistance to dessication of *H. convergens* and *C. maculata*. Every three days, the beetles were examined for any movement for 15-20 minutes at room temperature and uncontrolled humidity to determine their length of survival. Probit analyses were used to graphically determine the LD_{50} for each treatment (Finney, 1964).

Supercooling Point Determination - Beetles were held in field cages covered with snow for 2-3 months prior to the supercooling point determinations. The supercooling point (SCP) of individual beetles was measured to the nearest 0.5° C using a temperature probe (YSI #427) and a battery-operated thermometer (YSI Telethermometer). The probe was held against the ventral side of the beetle during cooling. The probe and beetle were enclosed in a test tube and cooled at a rate of 3-4° C/minute in an alcohol bath. Each sample mean is based on SCP determinations for 10-15 beetles.

RESULTS

Respiration

In order to determine the effect of temperature on respiration, both species were acclimated to 6° C for at least two months. An initial set of respiration rates was determined over the 0-20° C range. A second set of determinations was made after acclimation to 20° C and 75% RH for 5-10 days.

After acclimation to 20° C, there was a significant shift in the respiration-temperature (R-T) curve for both species. However, the curves were translated in opposite directions (Table 1, Figures 1 and 2). In *H. convergens*, acclimation resulted in decreased respiration rates, classified as Type I, excess or overshoot compensation by Precht (1958). In contrast, acclimation of *C. maculata* to 20° C resulted in increased respiration rates with a shift of the R-T curve to the left. Precht classed this type of acclimatory response as Type 5, inverse or paradoxical acclimation.

The respiration rates of September field collected beetles for both coccinellid species were more similar to the rates obtained for over-wintering beetles acclimated to 20° C than to 6° C (Table 1). Ambient field temperatures were relatively high at this time. In September, lady beetle populations were low and there were few, if any, aphids available for food. Apparently, many coccinellids had already left the fields to

RESPIRATION ($\mu l\ O_2$ / mg / hr)

Pre-test Condition	Temperature (°C) 0	5	10	15	20
Hippodamia convergens					
6° C	0.096	0.181	0.295	0.480	0.746
	(0.010)	(0.011)	(0.012)	(0.022)	(0.042)
20° C	0.061	0.133	0.209	0.295	0.597
	(0.005)	(0.010)	(0.011)	(0.027)	(0.048)
Cornfield September	0.075	0.161	0.154*	0.307	0.432
	(0.013)	(0.010)	(0.018)	(0.015)	(0.067)
Coleomegilla maculata					
6° C	0.056	0.085	0.127	0.213	0.389
	(0.009)	(0.015)	(0.006)	(0.026)	(0.031)
20° C	0.086	0.187	0.180	0.300	0.587
	(0.006)	(0.015)	(0.015)	(0.034)	(0.045)
Cornfield September	0.068	0.148	0.257*	0.335	0.677
	(0.022)	(0.006)	(0.012)	(0.020)	(0.054)

(   ) = Standard error of the mean.

*Measured at 11° C.

Table 1. Respiration of *Hippodamia convergens* and *Coleomegilla maculata* under varying conditions.

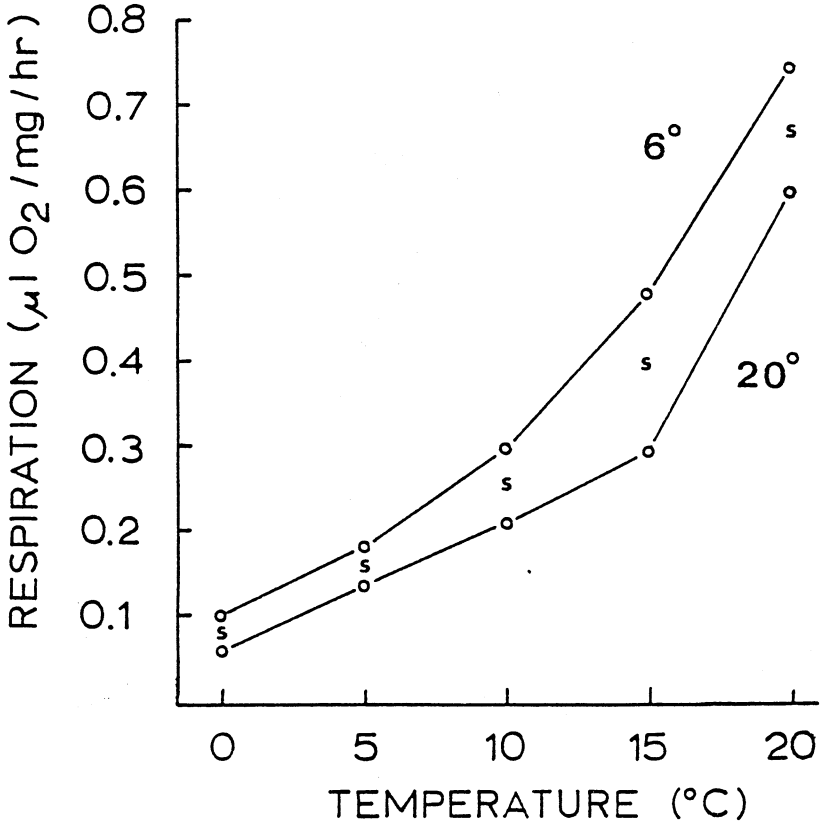

Figure 1. Effect of temperature on respiration rate for Hippodamia convergens acclimated to 6° and 20° C (s indicates $p < 0.05$).

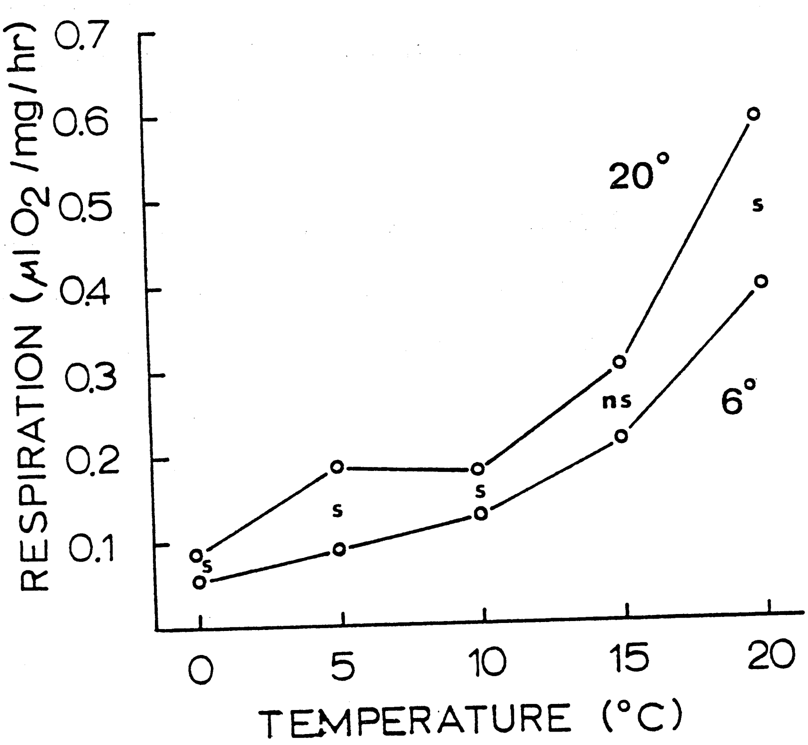

Figure 2. Effect of temperature on respiration rate for <u>Coleomegilla maculata</u> acclimated to 6° and 20° C (s indicates $p < 0.05$, ns indicates $p > 0.05$).

form overwintering aggregations, while the remaining individuals were preparing to do so, as evidenced by their high fat content and their absence from the fields a few weeks later.

Supercooling Point

With respect to biological systems, the supercooling point (SCP) may be defined as the extension of the liquid phase below the colligative freezing point. It is the temperature at which an insect naturally freezes and is a convenient method of quantifying the cold-hardiness of freezing intolerant insects (Salt, 1961). Both H. convergens and C. maculata are freezing intolerant; therefore, the SCP represents the theoretical minimum temperature for survival. However, since the probability of freezing is a function of the length of exposure, the SCP may occur at higher temperatures in the field (Salt, 1966).

H. convergens arriving from California were markedly cold-hardy, having a SCP of -18.2° C $\pm$ 0.7 ($SE_{\bar{x}}$, n = 15) as compared to Minnesota summer determinations of -7.6 ± 0.4. C. maculata undergoes a similar seasonal shift in cold-hardiness with summer SCP values of -5.8 ± 0.3, decreasing to -17.8 ± 1.0 in January.

After 2-3 months in snow-covered field cages, February SCP's reveal similar levels of cold-hardiness for both C. maculata and H. convergens (Figure 3). The response of the two species differed markedly after being held for five days at 25° C and 75% RH (Figure 3). During this period, C. maculata exhibited a rapid increase in SCP (e.g., decrease in cold-hardiness), which was not observed in H. convergens. In fact, H. convergens maintained a constant and low SCP for at least 28 days under these conditions (Figure 4).

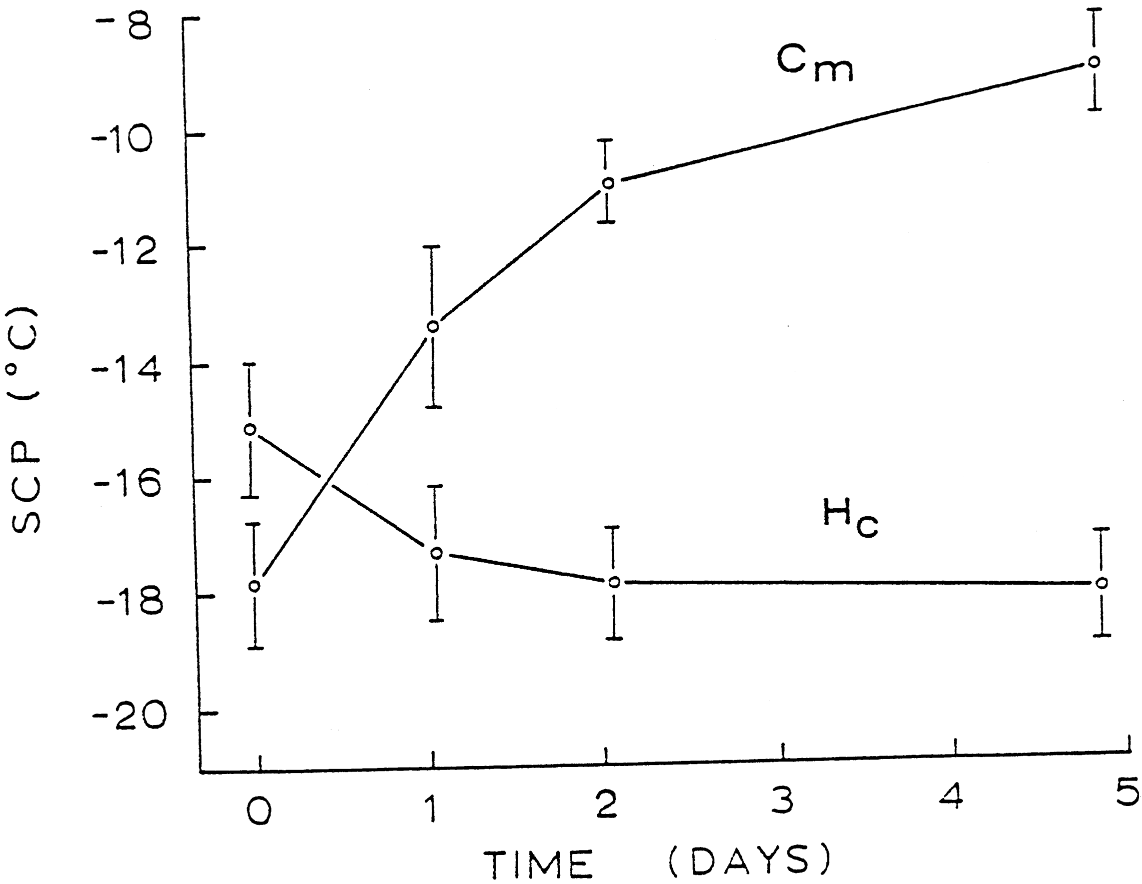

Figure 3. Effect of acclimation to 25° C on SCP ($\overline{X} \pm SE_{\overline{x}}$) of *Hippodamia convergens* and *Coleomegilla maculata*.

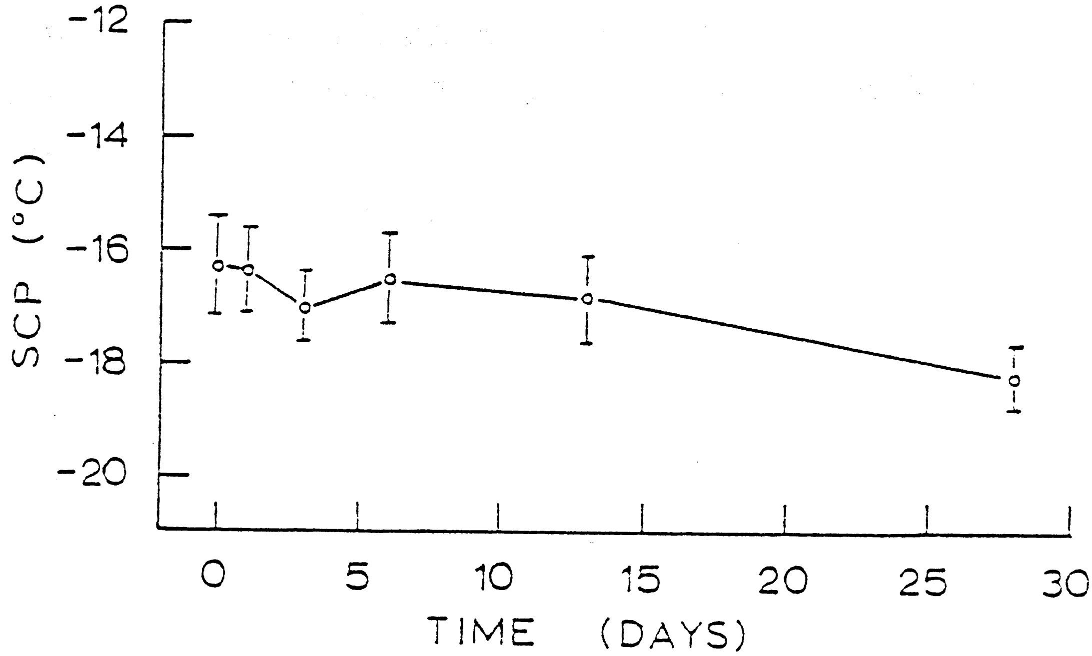

Figure 4. Effect of acclimation to 20° C on SCP ($\overline{X} \pm SE_{\overline{x}}$) of *Hippodamia convergens*.

Resistance to Dessication

H. convergens was more resistant to dessication than C. maculata at 20° C for both 0 and 75% RH and at 6° C and 0% RH (Figure 5 and Table 2). Both species had a high rate of survival at 6° C and 75% RH. Previous workers also found that H. convergens was more resistant than C. maculata using both winter (Hodson, 1937) and summer collected beetles (Ewert and Chiang, 1966).

DISCUSSION

Baust and Morrissey (1975) state that, "Even the most cold tolerant species resident in the Arctic are irreversibly warm acclimated within hours of above freezing exposure." In the Arctic carabid beetle, Pterostichus brevicornis, warm acclimation resulted in a rapid loss of glycerol and an increase in SCP (Baust and Miller, 1970, 1972). A number of other workers have also demonstrated a rapid loss of glycerol, changes in polyol levels and increased SCP (Dubach et al., 1959; Somme, 1964; Asahina, 1969). The rapid increase in SCP following warm acclimation in Coleomegilla maculata was originally reported by Baust and Morrissey (1975), and is confirmed by this study.

However, in contrast, warm acclimation had no effect on cold-hardiness in Hippodamia convergens, as evidence by the retention of low and constant SCP's throughout the warm acclimation period. H. Mantyla of the Bio-Control Co. (personal communication) indicated that the mountain aggregation sites from which the H. convergens samples were collected were not covered by snow and that the ambient temperatures ranged from -7 to 10° C during the winter. An insect which is exposed to widely fluctuating temperatures, perhaps on a daily basis, might be expected to have evolved mechanisms for

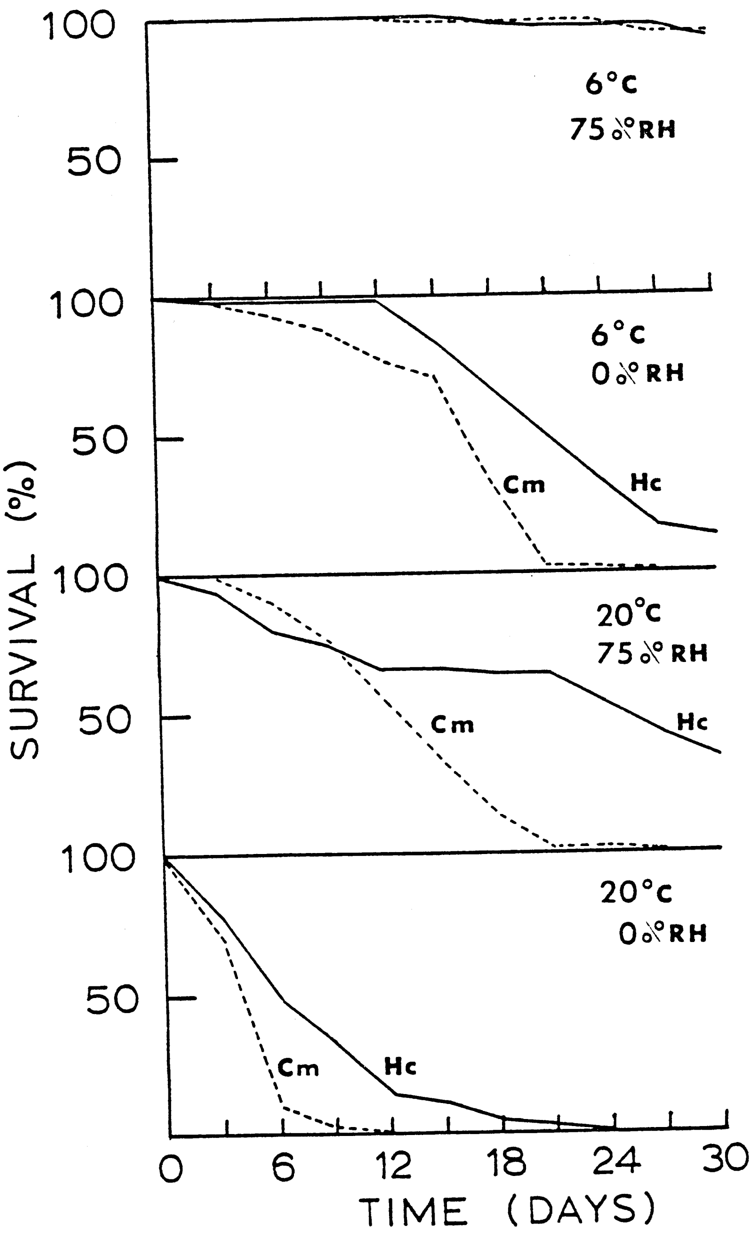

Figure 5. Survival of Hippodamia convergens (solid lines) and Coleomegilla maculata (dashed lines) at varying temperature and humidity.

	LD_{50}		
	20° C		6° C
Relative Humidity	75%	0%	0%
Hippodamia convergens	24.3	5.7	20.6
Coleomegilla maculata	9.2	3.7	16.8

Table 2. LD_{50} (days) for *Hippodamia convergens* and *Coleomegilla maculata* at varying temperature and humidity.

temperature independent retention of cold-hardiness, while insects in subnivean hibernacula such as C. maculata which are not exposed to fluctuating temperatures may lack these adaptations.

It is generally believed that neither compensatory nor non-compensatory acclimation occurs in animals which become dormant or torpid in the cold or warm (Prosser, 1973, 1975; Hazel and Prosser, 1974). However, in this study both non-feeding, reproductively inactive, overwintering lady beetles demonstrated capacity adaptation of respiration rate.

Two contrasting types of acclimatory response were observed. Hippodamia convergens exhibited reduced respiration rates at all temperatures after acclimation to 20° C. This pattern of response was termed Type 1, excess or overshoot compensation by Precht (1958). Overshoot compensation of respiration rate has been commonly observed in a number of insects (Luhmann and Drees, 1952; Marzusch, 1952; Dehnel and Segal, 1956; Nuttall, 1970, and Buffington, 1969). Overshoot compensation of respiration rate results in a reduction of metabolic rate when exposed to increased temperatures. This reduction may result in significant metabolic savings during dormancy when it is not possible to replenish energy reserves.

Conversely, warm acclimation in Coleomegilla maculata resulted in increased respiration as compared to cold acclimated individuals. Precht (1958) termed this pattern of response Type 5, inverse or paradoxical acclimation. Paradoxical acclimation of respiration rate has been rarely reported for insects with the exception of Somme (1968) and Buffington (1969). A number of workers have suggested that paradoxical acclimation may serve as a mechanism to conserve energy in amphibians during the winter (Dunlap, 1971, and Fitzpatrick et al., 1972). In its subnivean hibernaculum, C. maculata experiences only a narrow range of temperatures; the lowered metabolism in response to low temperatures may have evolved as a mechanism for conservation of energy through the winter. The observed increase in respiration rate in response to warm acclimation may correspond to spring warming under natural conditions in which the beetles undergo a metabolic reorganization in preparation for summer feeding and reproduction.

In Wieser's review (1973) of temperature relations in ectothermic animals he describes the development of thought in this area as having passed through two phases. Originally, emphasis centered on the idea that ectotherms were at the mercy of the environment, perhaps best illustrated by Krogh's "normal curve." A second phase centered on the maintenance of metabolic homeostasis in response to environmental perturbations. Wieser suggested that we are now entering a third phase in which at least some ectotherms are best considered as "multistable systems" which respond to environmental temperature changes in ways which are adaptive with respect to the prevailing or "anticipated" conditions. In other words, an ectotherm's response to temperature change at one stage of its life cycle or in a particular habitat may differ greatly from the response observed at other times and/or stages.

The increased respiration rates and the rapid loss of cold-hardiness observed in C. maculata may represent a shift from one stable system associated with energy conservation in the winter to a new system in which the beetles are preparing for summer feeding and reproductive activities. Under natural conditions, C. maculata would normally only experience high temperatures in the spring as the snow melted and the beetles began to disperse from their overwintering aggregations. Further evidence in support of this transition from one stable system to another comes from the work of Solbreck (1974) which details the maturation of post-hibernation flight behavior in C. maculata. He demonstrated that the maturation process was primarily controlled by temperature. Only at temperatures greater than 15^{o} C was there a significant increase in the rate of maturation. Therefore, it appears that the labels of overshoot and paradoxical compensation are misleading and inappropriate terms with which to characterize the observed patterns of acclimatory response of respiration in H. convergens and C. maculata. When viewed in light of Wieser's (1973) suggestion that an ectotherm's response to temperature changes may be adaptive with respect to prevailing or "anticipated" environmental conditions, the observed patterns of acclimatory response appear to be adaptative with respect to the specific hibernaculum site of each species.

SUMMARY

The physiological parameters examined in this study correspond closely to the specific overwintering site of each species (Table 2). The supranivean hibernaculum of Hippodamia convergens exposes this species to fluctuating temperatures and humidities. Correspondingly, H. convergens is resistant to dessication, maintains cold-hardiness independent of temperature, and responds to warm acclimation with a reduced respiration rate. In contrast, the subnivean hibernaculum of Coleomegilla maculata

	Hibernaculum	Effect of Warm Acclimation on Respiration	Effect of Warm Acclimation on SCP	Resistance to Dessication
Hippodamia convergens	Exposed to fluctuating temperature and humidity	Decrease respiration	Retains cold-hardiness	Resistant
Coleomegilla maculata	Snow covered, constant temperatures and high humidity	Increase respiration	Loses cold-hardiness	Not resistant

Table 2. Comparative summary of physiological adaptations of *Hippodamia convergens* and *Coleomegilla maculata* in relation to hibernaculum.

is characterized by constant temperature and humidity. Warm acclimation in this species results in a rapid loss of cold-hardiness and increased respiration rates. This response suggests a transition from an energy conserving metabolic state associated with overwintering to one which would be expected in spring as the beetles prepare for feeding and reproduction.

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