1980

The population biology of the giant water bug Belostoma flumineum Say (Hemiptera: Belostomatidae)

Jeffrey Warren Flosi

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Entomology Commons

Recommended Citation


https://lib.dr.iastate.edu/rtd/7326

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in “sectioning” the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.
FLOSİ, JEFFREY WARREN

THE POPULATION BIOLOGY OF THE GIANT WATER BUG BELOSTOMA FLUMINEUM SAY (HEMIPTERA: BELOSTOMATIDAE)

Iowa State University

Ph.D. 1980

University Microfilms International 300 N. Zeb Road, Ann Arbor, MI 48106 18 Bedford Row, London WC1R 4EJ, England
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark ✓.

1. Glossy photographs ✓
2. Colored illustrations
3. Photographs with dark background
4. Illustrations are poor copy
5. Print shows through as there is text on both sides of page
6. Indistinct, broken or small print on several pages throughout
7. Tightly bound copy with print lost in spine
8. Computer printout pages with indistinct print
9. Page(s) lacking when material received, and not available from school or author
10. Page(s) seem to be missing in numbering only as text follows
11. Poor carbon copy
12. Not original copy, several pages with blurred type
13. Appendix pages are poor copy
14. Original copy with light type
15. Curling and wrinkled pages
16. Other

University Microfilms International
The population biology of the giant water bug *Belostoma flumineum* Say (Hemiptera: Belostomatidae)

by

Jeffrey Warren Flosi

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Entomology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Signature was redacted for privacy.

Members of the Committee:

Signature was redacted for privacy.

Signature was redacted for privacy.

Signature was redacted for privacy.

Signature was redacted for privacy.

Iowa State University
Ames, Iowa

1980
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>PART I. THE BIONOMICS OF <strong>BELOSTOMA FLUMINEUM</strong></td>
<td>5</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>6</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>8</td>
</tr>
<tr>
<td>General methods</td>
<td>8</td>
</tr>
<tr>
<td>Habitat</td>
<td>9</td>
</tr>
<tr>
<td>Parasitism</td>
<td>10</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>12</td>
</tr>
<tr>
<td>Description of stages</td>
<td>12</td>
</tr>
<tr>
<td>Seasonal cycle and generations per annum</td>
<td>26</td>
</tr>
<tr>
<td>Reproduction</td>
<td>29</td>
</tr>
<tr>
<td>Respiration</td>
<td>40</td>
</tr>
<tr>
<td>Distribution</td>
<td>46</td>
</tr>
<tr>
<td>Habitat</td>
<td>49</td>
</tr>
<tr>
<td>Feeding</td>
<td>55</td>
</tr>
<tr>
<td>Locomotion</td>
<td>58</td>
</tr>
<tr>
<td>Behavior</td>
<td>63</td>
</tr>
<tr>
<td>Parasitism</td>
<td>64</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>74</td>
</tr>
<tr>
<td>PART II. FLIGHT MUSCLE DEGENERATION</td>
<td>75</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>76</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>81</td>
</tr>
<tr>
<td>RESULTS AND DISCUSSION</td>
<td>83</td>
</tr>
<tr>
<td>Temperature</td>
<td>84</td>
</tr>
<tr>
<td>Photoperiod</td>
<td>86</td>
</tr>
<tr>
<td>Nutrition</td>
<td>86</td>
</tr>
<tr>
<td>Field-collected <strong>B. flumineum</strong></td>
<td>88</td>
</tr>
<tr>
<td>CONCLUSIONS</td>
<td>95</td>
</tr>
</tbody>
</table>
PART III. CIRCADIAN ORGANIZATION OF CUTICLE

INTRODUCTION

General structure of insect cuticle
Supermolecular architecture
Mechanical properties
Daily growth layers in solid endocuticle

MATERIALS AND METHODS

RESULTS

Circadian organization of exoskeleton
Circadian control of daily growth layers
Effects of temperature on cuticle growth

DISCUSSION

CONCLUSIONS

PART IV. AGE RECRUITMENT IN POPULATIONS OF BELOSTOMA FLUMINEUM

INTRODUCTION

MATERIALS AND METHODS

Method of age determination
Validity of method
Field sampling

RESULTS AND DISCUSSION

CONCLUSIONS

LITERATURE CITED

ACKNOWLEDGMENTS
LIST OF TABLES

Table 1. Belostoma flumineum egg incubation periods at different temperatures 15

Table 2. The mean length and width (in mm) of nymphs of the five instars of Belostoma flumineum 15

Table 3. The mean length (in mm) of nymphs of the five instars of Belostoma flumineum, B. malkini (Cullen 1969), Lethocerus maximus (Cullen 1969), L. mazzai (De Carlo 1962) and Hydrocyrius columbae (Miller 1961) 16

Table 4. The mean duration in days of the five nymphal instars of Belostoma flumineum, B. malkini (Cullen 1969), Lethocerus maximus (Cullen 1969), L. mazzai (De Carlo 1962), and Hydrocyrius columbae (Cullen 1969) 17

Table 5. Adult longevity (days) of Belostoma flumineum during successive generations per annum 25

Table 6. Ovipositional records during the successive generations per annum of Belostoma flumineum 33

Table 7. Rate of production of egg-rafts and egg productivity during three successive generations of Belostoma flumineum 34

Table 8. Climate summary for Boone, Iowa; January 1978-December 1978. (Annual Weather Summary; U. S. Department of Commerce) 52
Table 9. Physical and chemical factors of Lost Lake, Ledges State Park, Boone Co., Iowa

Table 10. Prey items eaten by nymphal and adult Belostoma flumineum at Lost Lake, Ledges State Park, Boone Co., Iowa

Table 11. Parasitic associations of larvae in superfamilies of water mites with imagos in orders of aquatic insects

Table 12. The effect of different temperatures upon the indirect flight muscles of adult Belostoma flumineum, 8 days after ecdysis

Table 13. The effect of different photoperiods upon the indirect flight muscles of adult Belostoma flumineum, 8 days after ecdysis

Table 14. The effect of starvation upon the indirect flight muscles of adult Belostoma flumineum, 7 and 14 days after ecdysis

Table 15. The effect of various light-dark (L-D) regimens on cuticular growth ring deposition

Table 16. The number of pairs of rings as a function of photoperiod and temperature

Table 17. The composition of the field samples of Belostoma flumineum
<table>
<thead>
<tr>
<th>Fig.</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dorsal view of <em>Belostoma flumineum</em></td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>Ventral view of <em>Belostoma flumineum</em> (female)</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>Antenna of adult <em>Belostoma flumineum</em></td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>Ventral view of caudal part of male genital organ of <em>Belostoma flumineum</em></td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>Dorsal view of caudal part of male genital organ of <em>Belostoma flumineum</em></td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>Seasonal cycle and generations per annum for <em>Belostoma flumineum</em></td>
<td>28</td>
</tr>
<tr>
<td>7</td>
<td>Dorsal view of the posterior segments of the abdomen of <em>Belostoma flumineum</em></td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>Collection sites of <em>Belostoma flumineum</em> in Iowa</td>
<td>48</td>
</tr>
<tr>
<td>9</td>
<td>Water mite nymphochrysalis, with a portion of the developing water mite showing through a break in the larval integument</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td><em>Belostoma flumineum</em>. Cross-section through metathoracic femur of a bug raised at 12L:12D at 24°C photographed between crossed polaroids set for maximum brightness of birefringence</td>
<td>113</td>
</tr>
<tr>
<td>11</td>
<td><em>Belostoma flumineum</em>. 5 day old adult from a field population</td>
<td>113</td>
</tr>
<tr>
<td>12</td>
<td><em>Belostoma flumineum</em>. Growth layers in adult tibia</td>
<td>113</td>
</tr>
<tr>
<td>13</td>
<td><em>Belostoma flumineum</em>. Growth layers in tibia of last instar nymph</td>
<td>113</td>
</tr>
</tbody>
</table>
Fig. 14. Growth rate, plotted as the reciprocal of the number of paired daily growth rings, as a function of temperature.

Fig. 15. Age frequency histograms of 2 samples (taken 5 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed.

Fig. 16. Age frequency histograms of 2 samples (taken 2 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed.

Fig. 17. Age frequency histograms of 2 samples (taken 5 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed.

Fig. 18. Age frequency histograms of 2 samples (taken 2 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed.
GENERAL INTRODUCTION

Giant water bugs are found throughout the world, except in the arctic regions, the continent of Europe, and northern Asia; and even in Europe fossils indicate that they once occupied this area. The family is now primarily tropical and all genera reach their maximum speciation in tropical regions. The family is rather small, probably numbering less than 150 species belonging to several genera, of which only Lethocerus is cosmopolitan (Lauck and Menke 1961). Hydrocyrius, Limnogeton and Sphaerodema (=Diplonychus) occur in the Eastern Hemisphere, and Belostoma, Abedus and Horvanthinia occur only in the Western Hemisphere. All members of the family are aquatic and predaceous, and some are extremely large. Considering their large size, there is little published work on the Belostomatidae, and the family normally receives scant treatment in more general accounts of the aquatic Hemiptera. Apart from taxonomic works, scattered biological information on belostomatids was given by Torre-Bueno (1906), Hungerford (1919), Leefmans (1923), Hoffman (1924, 1926, 1933), Rankin (1935), Presswalla and George (1936), Rao (1962), De Carlo (1938, 1962), Voelker (1968), Tawfik (1969), Cullen (1969), Smith 1974, 1976a, b) and Tawfik et al. (1978a, b).
The large size of many of the species of *Belostoma* attracted early attention. Under the generic name *Nepa*, Linnaeus included the members of *Belostoma* with the Nepidae. In 1807, Latreille differentiated the two groups and established the genus *Belostoma* for the species, *B. testaceopalidum*, thus establishing this as the type species for the genus (Lauck 1962).

In 1863, Mayr contributed the first monograph on the family Belostomatidae and included 10 species of *Belostoma* under the generic name *Zaitha*. *Zaitha* was established in 1843 by Amyot and Serville for the species *Zaitha stollii*. These authors were apparently unaware of Latreille's description of the genus *Belostoma*. Mayr and subsequent authors continued to perpetuate this error until corrected by Montandon (1900). During the same year that Mayr's monograph appeared, Dufour (1863) independently published a monograph of the family, including 21 species of *Belostoma*. In 1871, Mayr again monographed the family and examined and compared the specimens used by Dufour as well as those studied by Stål, who had subsequently described four species of *Belostoma*. Although Montandon never published a monograph on *Belostoma*, he described 12 new species. The numerous specimens identified by Montandon and scattered throughout European and U. S. museums attest to his thorough knowledge of the group. It is not surprising that his species remain valid.
More recently, De Carlo has made numerous contributions to the taxonomy of the Belostomatidae, climaxed by his "Los Belostomides Americanos" published in 1938. He has also described several new species of Belostoma since his monograph. Even more recently, Menke (1958) published a synopsis of North American species of Belostoma, including an excellent account of the species in this area of the country, and Lauck (1962) published a monograph of the genus Belostoma, with 62 species treated in the monograph. The taxonomic relationship of Belostoma to the other genera of the family is discussed by Lauck and Menke (1961) and keys are furnished to identify the seven genera.

Belostoma occurs throughout the Americas, reaching its maximum diversity in South America. Only a few, closely related species have invaded the colder climates of North America. Belostoma flumineum Say 1831 has the most extensive range of the North American species of the genus. It occurs in the southern half of Canada, throughout the United States, and in northern Mexico. In the United States it becomes rather scarce where the range overlaps with that of B. lutarium (Stål). Although both species occur in Iowa, B. flumineum is most commonly caught.

The present study is based on work carried out in Iowa during 1977-78 on the biology and population dynamics of the giant water bug, B. flumineum. This study was undertaken for
the following purposes: (1) to make comprehensive biological investigations on certain relatively unexplored aspects of the water bug; (2) to examine the incidence of flight muscle degeneration in *B. flumineum* with respect to selected environmental factors; (3) to investigate the circadian organization of growth rings in the solid endocuticle; and (4) to assess the dynamics of age recruitment in field populations of *B. flumineum* utilizing daily growth rings.
PART I. THE BIONOMICS OF *BELOSTOMA FLUMINEUM*
INTRODUCTION

Ecologically, *Belostoma flumineum* has a considerable degree of homogeneity that is apparent in both the nymphal and imaginal stages. Although this insect is a fairly active predator in various aquatic habitats, literature indicates that the species previously has not been subjected to intensive study. *Belostoma flumineum* apparently has a wider range of habitats than any other species of aquatic Hemiptera. The mosaic environment of water bugs includes both highly predictable (permanent water; seasonal cycles) and unpredictable (temporary waters) features. Individuals are found along the grassy margins of streams and rivers, in the wooded overflows of rivers, and in ponds and lakes. There they live in insect communities consisting of aquatic bugs, beetles, anisopteran and zygopteran naiads as well as immature stages of Culicidae, Chironomidae, Ephydridae, and Tabanidae. *Belostoma flumineum* is positively thigmotactic and requires some type of cover such as boards, logs or vegetation. In spite of their moderately large size, these bugs are not conspicuous because they commonly rest at the surface in trash or mats of vegetation and because they are cryptically colored. They rest with the body extending obliquely downward and the tip of the abdomen slightly above the surface film.
Although an intensive study of the species has not been undertaken previously, some studies on the bionomics and male brooding behavior have been completed by a few workers.

Torre-Bueno (1906) provided information concerning the stadia and instars of *B. flumineum* and Hungerford (1919) added observations on its feeding habits, habitat, and tropisms in his treatise on the biology and ecology of aquatic and semiaquatic Hemiptera. Severin and Severin (1911a, b) gave information about overwintering, and experimented with the tropisms of *B. flumineum*. The life history, respiration, feeding, and mating of *B. flumineum* were reviewed by Lauck (1959).

Detailed studies on male egg brooding have been conducted by Smith (1974, 1976a, b) in an attempt to answer several questions about the reversed sex roles found in these insects.
MATERIALS AND METHODS

General methods

The rearing and breeding of giant water bugs in the laboratory is difficult. This is the result of certain biological features characteristic of these bugs, i.e., they are predaceous, requiring an abundant quantity of food, and the timing of their reproductive phase is probably dependent on the timing of their dispersal phase.

Field and laboratory observations and laboratory experiments were employed to accumulate data. Water bugs were observed and collected from the following central Iowa localities: the pond at McFarland Park, Story County; Little Wall Lake, Hamilton County; Lost Lake in Ledges State Park, Boone County; and a number of farm ponds in Story County. All stages of water bugs were easily collected by scooping up floating mats of vegetation with a Needham net or some other type of aquatic net. The mats were carefully sorted to detect the insects because belostomatids feign death.

Rearing of B. flumineum started with a collection of egg-bearing males from Lost Lake at the onset of the active period by early May, 1977 and 1978. In the laboratory, these males were isolated in glass dishes measuring 11.5 cm in diameter by 5.5 cm in depth and containing 3 cm of aged tap water. Each dish was provided with a cork for the insect to
crawl and settle on. Immediately after hatching, nymphs were isolated in small jars where the depth of the water did not exceed 3 cm throughout early nymphal instars. Early nymphal instars were fed on mosquito larvae and small snails, while 4th and 5th nymphal instars were provided with larger snails. Mature insects were later transferred to 10 gallon aquaria in the laboratory. Adult bugs were paired, and each pair was confined to a glass dish for oviposition. Dishes were provided daily with sufficient quantities of snails and mosquito larvae as prey items. The water in all containers was renewed daily.

The specimens upon which the descriptions of instars are based were reared in the laboratory and collected from several farm ponds located in Story County, Iowa. The description of the adults is taken in part from Hungerford (1919). Descriptions were made of specimens preserved in 70% alcohol solution. Measurements were made with the aid of a binocular dissecting microscope equipped with an eye-piece micrometer.

Habitat

Climatological data were obtained from an annual weather summary (U. S. Department of Commerce, 1978) for Boone, Iowa, 6.4 km to the north of the study site.

Water temperatures were recorded from a resistance thermometer (to 0.1°C) and a mercury thermometer (to 1.0°C).
Turbidity was measured with a U. S. Geological Survey turbidity rod (Welch 1948).

Water samples for dissolved oxygen (DO) analysis were collected using standard techniques and precautions. Analysis was titrametric, using the unmodified Winkler technique with an accuracy of ± 0.2 mg/l.

Hydrogen ion concentration was measured using the portable pH meter (Beckman) standardized in the laboratory with a Beckman model "G" and standardized in the field with acetate buffer (pH 4).

The line transect method was used to obtain a relative assessment of the dominant species of macroflora in Lost Lake. In this method, all plants touching or lying beneath a line were counted and identified at 10 m intervals.

Parasitism

The study of water mite life histories and host-parasite interactions was made from field collected parasitized host insects. Larvae, larval sclerites, and associated nymphs were obtained from the hosts. Engorged larvae and nymphochrysalids removed from hosts were generally satisfactory for identification.

The best preservative for adult and nymphal water mites was modified Koenike's solution (GAW) as recommended by Mitchell and Cook (1952). Specimens preserved in alcohol or formalin are more difficult to clear. GAW-preserved specimens of most water mites were flexible, yet firm, and
were easily cleared in acetic corrosive (Andre's fluid) or potassium hydroxide solution (Mitchell and Cook 1952).

The single most satisfactory method for obtaining larval material for study was to rear eggs laid in the laboratory. Females, obtained in the field by the dip-netting technique, were taken to the laboratory as rapidly as possible and isolated in small containers. Two-dram shell vials containing about 1 cm depth (i.e., 1 ml) of water proved a satisfactory habitat for the species reared in the laboratory. Females were then held at room temperature and observed every few days to determine if oviposition had occurred or if water was required to replace loss through evaporation. The original water was from Lost Lake, but the level was maintained with aged tap water. When oviposition occurred, the vial was reassigned to a different shelf to await the hatching of the eggs upon the death of the female. Larvae were harvested as soon as possible after hatching.

For most rearing operations (sorting of field collections, handling of larvae and adults) a low-power, stereoscopic dissecting microscope was used. Initial sorting was carried out under the dissecting microscope without clearing. For critical study and species determination, cleared specimens mounted on slides were examined by transmitted light at 100-400x.
RESULTS AND DISCUSSION

Description of stages

Egg stage  The egg of *B. flumineum* was an elongate cylindrical form, with the opercular end slightly flattened and broader than the basal end which was attached to the back of the male by a secretion of the colleterial gland. When first laid the eggs were a uniform creamy-buff but quickly colored on exposure to air. At the opercular end, the color was brown to one third of the egg-length; the remaining area was an opaque white. The chorion was smooth except for the minute micropylar area that appeared as a light brown crescent to one side of the cephalic end. The polygonal outlines of the follicle cells persisted on the exochorion and, as the pigment was confined discretely to these polygons, a mosaic effect was produced.

Belostomatid eggs increased greatly in volume during incubation. When newly-laid, the egg measured about 1.5 mm long and 0.9 mm wide, but prior to hatching, its length increased to 2.8 mm. Torre-Bueno (1906) noted an increase in one egg from 2.0 mm to 3 mm in *B. flumineum*. Cullen (1969) conducted a more thorough study with the eggs of *B. malkini* Lauck by taking groups of ten from a developing batch every 24 hours and measuring them with a micrometer eyepiece. Immediately after being laid the eggs were 2.68 mm
long; 10 days later, prior to hatching, they were 6.19 mm long. The greatest amount of growth occurred between the third (2.81 mm) and seventh (5.32 mm) days, so that the graph of their growth was a sigmoidal curve. After the nymphs hatched, the eggshells returned to a length of 3.4 mm, demonstrating the elastic properties of the chorion. During the incubation of eggs in this study, slight changes in the color of the eggs were discerned.

The eggs of B. flumineum hatched after 9-12 days. Usually all the eggs hatched during the same night, but the hatching of a few batches was spread over two days, apparently being inhibited during the intervening day. When hatching was imminent, the chorion ruptured in a ring-like course around the micropylar end except for a small area left dorsally which formed the operculum. Examination of several eggs showed that the split was slightly variable in position, but the cap always remained attached at the dorsal side. Hungerford's (1925) description of the hatching of an egg of Lethocerus griseus indicated the cap forced up by a delicate transparent membrane. After the cap was raised by the bubble-like device the head of the bug was nearly out of the shell and it was still "enshrouded by a delicate garment that embraced each limb separately and was shed as the last rite in the hatching process." Newly hatched nymphs of B. flumineum were also completely surrounded by a membrane,
from which they quickly escape. The hatching process was witnessed several times in this species. After the cap was raised the nymph emerged very rapidly, head first, by vigorous bending and peristaltic movements. A "bubble-like device" could not be seen, and it is probable that such aids to hatching are not necessary in B. flumineum. The time required for complete emergence varied from 15 to 30 minutes. The empty egg raft was detached later from the back of the male by the expansion of the hemelytra or when the pad was rubbed against any solid object.

The incubation period of the egg stage under different laboratory temperatures is given in Table 1, which reveals that the incubation period was inversely related to temperature.

**Nymphal stage** There were five nymphal stages (Table 2). The body length of B. flumineum is given in Table 3 along with those of four other species that have been reared in the laboratory by other workers. The average duration of each instar of the same five species of water bugs is given in Table 4.

The newly hatched 1st instar nymph was faint yellow with bright red eyes, but within a few hours this body color changed to a pale brown. The thorax had a few lighter markings and the abdomen was mottled with light areas in 6 transverse rows. The ventral portion of the abdomen was
Table 1. *Belostoma flumineum* egg incubation periods at different temperatures

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean Temp. °C (± 0.5)</th>
<th>Mean Incubation Period and Range (Days)</th>
<th>No. of Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>18</td>
<td>11.5 ± 0.04 (9-12)</td>
<td>274</td>
</tr>
<tr>
<td>July</td>
<td>24</td>
<td>9.6 ± 0.01 (9-11)</td>
<td>422</td>
</tr>
<tr>
<td>August</td>
<td>27</td>
<td>7.2 ± 0.01 (7-9)</td>
<td>186</td>
</tr>
<tr>
<td>September</td>
<td>24</td>
<td>9.1 ± 0.05 (9-10)</td>
<td>140</td>
</tr>
</tbody>
</table>

Table 2. The mean length and width (in mm) of nympha of the five instars of *Belostoma flumineum*<sup>a</sup>

<table>
<thead>
<tr>
<th>Instar</th>
<th>Mean length</th>
<th>Mean width</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instar I</td>
<td>4.6</td>
<td>2.4</td>
</tr>
<tr>
<td>Instar II</td>
<td>6.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Instar III</td>
<td>9.0</td>
<td>3.8</td>
</tr>
<tr>
<td>Instar IV</td>
<td>11.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Instar V</td>
<td>16.5</td>
<td>8.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>N=20 insects per instar.
Table 3. The mean length (in mm) of nymphs of the five instars of Belostoma flumineum, B. malkini (Cullen 1969), Lethocerus maximus (Cullen 1969), L. mazzai (De Carlo 1962), and Hydrocyrius columbiae (Miller 1961)

<table>
<thead>
<tr>
<th>Species</th>
<th>B. flumineum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B. malkini</th>
<th>L. maximus</th>
<th>L. mazzai</th>
<th>H. columbiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instar I</td>
<td>4.6</td>
<td>7.75</td>
<td>15.0</td>
<td>11.0</td>
<td>16</td>
</tr>
<tr>
<td>Instar II</td>
<td>6.2</td>
<td>11.0</td>
<td>22.5</td>
<td>17.0</td>
<td>20-22</td>
</tr>
<tr>
<td>Instar III</td>
<td>9.0</td>
<td>15.5</td>
<td>32.5</td>
<td>26.0</td>
<td>27-30</td>
</tr>
<tr>
<td>Instar IV</td>
<td>11.5</td>
<td>22.25</td>
<td>46.5</td>
<td>37.5</td>
<td>36-39</td>
</tr>
<tr>
<td>Instar V</td>
<td>16.5</td>
<td>32.5</td>
<td>65-70</td>
<td>53.0</td>
<td>49-52</td>
</tr>
</tbody>
</table>

<sup>a</sup>N = 20 insects per instar.
Table 4. The mean duration in days of the five nymphal instars of Belostoma flumineum, B. malkini (Cullen 1969), Lethocerus maximus (Cullen 1969), L. mazzai (De Carlo 1962), and Hydrocyrius columbiae (Cullen 1969)

<table>
<thead>
<tr>
<th>Species</th>
<th>B. flumineum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B. malkini</th>
<th>L. maximus</th>
<th>L. mazzai</th>
<th>H. columbiae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>11.0</td>
<td>10</td>
<td>8</td>
<td>9</td>
<td>14</td>
</tr>
<tr>
<td>Instar I</td>
<td>9.3</td>
<td>7.9</td>
<td>6.5</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Instar II</td>
<td>6.0</td>
<td>11.1</td>
<td>8</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Instar III</td>
<td>6.5</td>
<td>16</td>
<td>13.7</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Instar IV</td>
<td>7.0</td>
<td>21.6</td>
<td>18.5</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Instar V</td>
<td>13.0</td>
<td>21.3</td>
<td>35</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Total (to nearest whole day)</td>
<td>53.0</td>
<td>88</td>
<td>90</td>
<td>72</td>
<td>113</td>
</tr>
</tbody>
</table>

<sup>a</sup>N = 20 insects per instar.
densely clothed with hydrofuge hairs and the spiracles were evident. The tarsi were one-segmented and provided with two claws each. The short, club-shaped antennae were one-segmented, about 1.5x as broad at the base as at the apex, and twice as long as the greatest diameter. The first instar nymphs were able to feed within a few hours after hatching and their first ecdysis occurred in about a week. Ecdysis normally occurred at night but was witnessed in the early morning on several occasions. Each nymph held on to vegetation near the surface with its meso- and methathoracic legs. A split developed along the mid-dorsal line of the thoracic cuticle. The thorax emerged first, accompanied by pumping movements, followed by the head and part of the abdomen. A rapid sweep of the legs drew the rest of the abdomen out of the old cuticle. The nymph floated on the surface with the tip of the abdomen in contact with the surface film. The nymph was at first soft and pale yellow but within 25 minutes had hardened to its dorso-ventrally flattened shape and turned light greenish-brown. The amount of pigmentation developed was variable and related to the darkness of the environment, i.e., darker pigmentation was found in insects reared against a darker background and/or under low light conditions.

In the second instar nymph, the general body color was lighter than that of the first instar. The ventral aspect of the nymph was also much lighter in color.
Spiracles were clearly seen on the venter of the abdomen. The fore-tarsal claws, which were nearly equal in the first instar, were unequal with the inside one greatly reduced in length. The antennae were distinctly two-segmented and a little more slender. The third instar nymph was olivaceous in color. In this instar, the wing-pads were first present, extending back on the metathorax so that the marginal exposed line of this segment was about 0.3 of the entire line of the combined meso- and metathorax. The antennae were distinctly three-segmented, club-shaped, and about twice as long as wide. The fourth instar nymph possessed the same general shape and color as that of the third instar. The wing pads reached about 0.8 of the total marginal length of the meso- plus metathoraces. The antennae had two lateral processes in this instar. The general characteristics of the body of the 5th instar were similar to those of the preceding instar, but the color was a deeper yellowish green and the wing pads reached just beyond the metathorax. The antennae more closely resembled those of the adult. There was still but one segment to each tarsus, which becomes two-segmented in the adult.

**Adult stage**  
The body of an adult *B. flumineum* was moderately large, dorsally flattened, elliptical-ovoid, with the rostrum long and slender, antennae concealed in ventral grooves behind the eyes, front legs raptorial, and the apex
of the abdomen with a pair of short, strap-like, retractile appendages (Fig. 1-5). The general color was light brown to fuscous, suffused with yellow. The pronotum and scrutellum were darker posteriorly; the venter was usually dark with a variable number of darker annulations. The length of the anteocular area was less than the length of the interocular area. The prolongation of the penultimate antennal segment was not constricted at the base. The lateral margins of the pronotum were straight or slightly concave. The hind legs were flattened and ciliated for swimming. The abdominal sternum was longer than the cephalic width. The female genital capsule had the ninth segment forming a V-shaped configuration on the venter. The hairs of the connexivum were of even length.

Both sexes were similar in appearance but females were slightly larger than males. Females measured 18.5-24.5 mm in length and had a maximum body width of 9.2-12.0 mm. The body length of males measured 19.5-23.0 mm and the maximum body width was 9.4-11.5 mm.

Records of adult longevity during successive generations per annum are shown in Table 5 which indicates that males always had longer mean lives than females. In addition, the adults of the first generation had the shortest life span while those of the third generation had the longest, entering diapause during the winter.
Fig. 1. Dorsal view of Belostoma flumineum

Fig. 2. Ventral view of Belostoma flumineum (female)
Fig. 3. Antenna of adult *Belostoma flumineum*

Fig. 4. Ventral view of caudal part of male genital organ of *Belostoma flumineum*

Fig. 5. Dorsal view of caudal part of male genital organ of *Belostoma flumineum*
Table 5. Adult longevity (days) of *Belostoma flumineum* during successive generations per annum

<table>
<thead>
<tr>
<th></th>
<th>1st generation&lt;sup&gt;a&lt;/sup&gt; at 24°C</th>
<th>2nd generation&lt;sup&gt;b&lt;/sup&gt; at 27.4°C</th>
<th>3rd generation&lt;sup&gt;b&lt;/sup&gt; at 24°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females</strong></td>
<td>78.2 ± 5.3 (50-94)</td>
<td>168.6 ± 12.4 (41-288)</td>
<td>231.8 ± 10.0 (125-280)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>165.0 ± 20.1 (60-317)</td>
<td>248.4 ± 3.4 (224-279)</td>
<td>251.5 ± 29.1 (137-309)</td>
</tr>
</tbody>
</table>

<sup>a</sup>15 females and 20 males.

<sup>b</sup>15 females and 15 males.
Seasonal cycle and generations per annum

The active period of *B. flumineum* is from April until November after which only adults in a diapausing state can persist until the following April. In this active period, reproduction results in building 2-3 generations (Fig. 6). The first generation is produced from eggs laid by adults of the overwintering (second or third) generation at the onset of the active period. Encumbered males are commonly observed between mid-May and mid-June. Thus, the nymphs of the first generation exist, in various developmental stages, from late May until late August, giving rise to adults that survive between late June and mid-October. Subsequently, nymphs of the second generation develop to maturity from early July until mid-October; the latest often die before reaching the adult stage. Adults of the second generation survive from late July to the early days of the next June. The latest individuals enter diapause before they deposit their eggs at the onset of the next season's active period. Early adults, on the other hand, produce eggs between late July and the end of October. From these eggs, nymphs of the third generation emerge to exist from mid-July through October. Once again the latest nymphs die before reaching the imaginal stage, while earlier individuals reach this stage to persist during a period extending from early September to the end of next July,
Fig. 6. Seasonal cycle and generations per annum for Belostoma flumineum
H Ovipositional period
N Nymphal period
I Imaginal period

![Bar chart showing the periods of oviposition, nymphal, and imaginal stages for the years 1977 and 1978. The chart indicates the generations (First, Second, Third) and the months of the year.]
ceasing activity throughout the diapause period. The adults of *B. flumineum* overwinter in Iowa, burying themselves in mud of their habitats, and they may sometimes be found in warm days of early spring, perched on an emergent object, in the sun.

**Reproduction**

The eggs of *B. flumineum* are invariably attached to the back of the male in a pad of mucilagenous cement, as is characteristic of the Belostomatinae (Lauck and Menke 1961). Prior to 1899, it was believed that egg-bearers were females carrying their own eggs. Early authors (Dufour 1863; Dimmock 1886; Comstock and Comstock 1899) attributed the depositional process to a long protrusible ovipositor which the female bug was said to extend over her back. Credit for egg-carrying was properly bestowed on the male (Slater 1899) in an account which included the following: "That the male chafes under the burden is unmistakable; in fact, my suspicions as to the sex of the egg-carrier were first aroused by watching one in an aquarium, which was trying to free itself from its load of eggs, an exhibition of a lack of maternal interests not expected in a female carrying her own eggs." Slater went on to say that when the male was attacked by an unidentified agent he "meekly received the blows, seemingly preferring death to the indignity of carrying and caring for the eggs." Torre-Bueno (1906) in
apparent accord with the "humiliated male hypothesis" stated:

"The egg-bearing male...dislikes exceedingly this forced servitude, and does all he can to rid himself of the burden. From time to time he passes his third pair of legs over the dorsum, apparently in an endeavor to accomplish his purpose. If he is not able to get rid of it, as sometimes happens, he carries his burden till in due time all the little ones are emerged, when he at last frees himself from it."

These interpretations of observed behavior are not consistent with modern selection theory. Natural selection could not have favored females programmed to dispose of their ova on the back of a male only to have them discarded in places or conditions that might impede their development. On the contrary, females are always under intense selection to choose oviposition sites that will maximize egg viability. Therefore the back of the male should be the optimal ovipositional substrate for B. flumineum and other species belonging to the belostomatid subfamily characterized by this behavior; the behavior of egg-bearing (encumbered) males should somehow maximize egg viability.

**Copulation-oviposition cycle** Mating preferably takes place at night. The process starts with the female plunging underneath a male clinging to a water plant or some other substratum. After preliminary sparring between the sexes, receptive males perform display pumping in the manner of brood pumping (rocking up and down on the longitudinal body axis) but at a much more vigorous pace.
Females respond by climbing on the male's back as if to oviposit. With one or both hind legs, the male manipulates the female into an inferior copulatory position, sliding backwards until the terminalia of both participants are in contact. Then the male slides his abdomen sideways and bends it ventrally while the female terminalia becomes fully extended; this position enables the aedeagus to be perfectly inserted into the female's gonopore. When this insertion has taken place, the male releases the grasps of his hind legs and the pair floats at the water surface. If disturbed, the bugs separate for about 5 minutes to couple again when perfectly settled. After intromission, the male abruptly scrapes one hind leg on the female's hemelytra. The female repositions herself immediately and begins to lay eggs, starting at the apex of the male's hemelystra. After about 5 minutes, although the female has laid a few eggs, the male's temporary quiescence ends. Forcing the female out of position with his hind leg, the male begins a new bout of vigorous display pumping. This cycle continues until the female's total egg clutch is deposited on the male's back. The male is capable of copulating with several females, but when carrying an egg pad he does not mate again until completing brood care for the current egg mass. Thus, male giant water bugs have developed a whole set of courtship and mating behaviors.
that maximize individual male fitness and preclude altruistic brooding. By "insisting" on copulation prior to oviposition, the male ensures that the first eggs he receives will have been fertilized by him. Limiting the period of oviposition tends to minimize the chance of error, i.e., acceptance of eggs fertilized by another male.

The ovipositional records for the successive generations per annum which were reared in the laboratory at mean temperatures of 24, 28, and 24°C, respectively, are presented in Table 6. The highest number of eggs (420.4 ± 15.1 per female) was produced by first generation females throughout an ovipositional period of 45.6 ± 4.3 days. The number of eggs produced by second generation females was relatively smaller (287.4 ± 9.1 per female), and the ovipositional period was accordingly shorter (34.0 ± 8.1 days). Females of the third generation produced the fewest eggs (104.1 ± 4.6 per female) during the shortest ovipositional period (28.5 ± 8.9 days). Records for the daily number of eggs per female during the first, second, and third generations were insignificantly different, being 34.5, 36.3, and 30.5 eggs, respectively.

The rate of production of egg-rafts during the three successive generations per annum is presented in Table 7. Under laboratory conditions, the number of egg-rafts produced by a single female ranged from 2-4, 1-3, and 1-3 rafts in the first, second and third generations, respectively. Most females (65%) of the first generation deposited 2 rafts,
Table 6. Ovispositional records during the successive generations per annum of *Belostoma flumineum*

<table>
<thead>
<tr>
<th>Ovispositional record</th>
<th>1st generation&lt;sup&gt;a&lt;/sup&gt; (at 24°C)</th>
<th>2nd generation&lt;sup&gt;b&lt;/sup&gt; (at 28°C)</th>
<th>3rd generation&lt;sup&gt;a&lt;/sup&gt; at 24°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-ovipositional</td>
<td>5.6 ± 0.8 (3-7)</td>
<td>5.9 ± 0.5 (4-9)</td>
<td>192.2 ± 6.3 (164-251)</td>
</tr>
<tr>
<td>period (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovipositional</td>
<td>45.6 ± 4.3 (34-61)</td>
<td>34.0 ± 8.1 (24-45)</td>
<td>28.5 ± 8.9 (1-37)</td>
</tr>
<tr>
<td>period (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-ovipositional</td>
<td>11.8 ± 3.8 (6-17)</td>
<td>109.5 ± 6.7 (10-200)</td>
<td>26.5 ± 2.2 (10-45)</td>
</tr>
<tr>
<td>period (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of eggs</td>
<td>420.4 ± 15.1 (341-587)</td>
<td>287.4 ± 9.1 (192-345)</td>
<td>104.1 ± 4.6 (46-168)</td>
</tr>
<tr>
<td>per female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily mean no. of</td>
<td>34.5 (21.0-48.9)</td>
<td>36.3 (27.4-44.0)</td>
<td>30.5 (26.0-64.0)</td>
</tr>
<tr>
<td>eggs per female</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 10 females.  
<sup>b</sup> 12 females.
<table>
<thead>
<tr>
<th>% of females</th>
<th>1st gen.⁠(^a⁠</th>
<th>2nd gen.⁠(^a⁠</th>
<th>3rd gen.⁠(^b⁠</th>
<th>Total number of eggs produced per female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st gen.⁠(^a⁠</td>
<td>2nd gen.⁠(^a⁠</td>
<td>3rd gen.⁠(^b⁠</td>
<td>1st gen.⁠(^a⁠</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>55</td>
<td>75.0</td>
<td>--</td>
</tr>
<tr>
<td>65</td>
<td>30</td>
<td>16.6</td>
<td>--</td>
<td>257.0</td>
</tr>
<tr>
<td>30</td>
<td>15</td>
<td>8.3</td>
<td>--</td>
<td>478.5</td>
</tr>
<tr>
<td>5</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>194.0</td>
</tr>
</tbody>
</table>

⁠\(^a\)20 females.

⁠\(^b\)12 females.
while most of those of the second and third generations laid one raft only. A positive correlation could be seen between the number of rafts and the total number of eggs per female.

**Egg requirements** Eggs that remained attached to the backs of live males in the laboratory enjoyed a high (approximately 97%) hatching success (N=5). To determine if this high egg survival rate depended upon the eggs being carried about on the back of live male *B. flumineum*, four egg-rafts containing immature eggs were removed from the males and placed in separate finger bowls in the laboratory; 2 were covered with tap water and 2 were allowed to dry. None of the eggs survived; those kept in water developed a fungal growth after a few days, and those allowed to dry became desiccated. Next, two egg-bearing males were killed and replaced in their jars with the eggs intact. These eggs also failed to hatch.

These results, similar to those obtained by Voelker (1968), Cullen (1969), and Smith (1976a, 1976b), for eggs of *Limnogeton fiberi* Mayr, *B. malkini*, *B. flumineum*, and *Abedus herberti* Hidalgo, respectively, suggest that successful reproduction for members of the Belostomatidae depends on the attachment of eggs to the backs of healthy males. Male bugs passively prevent desiccation of eggs by always remaining in water, and apparently provide other services to eggs and eclosing nymphs. Among these may be the
facilitation of embryonic gas exchange, as indicated by the total failure of submersed eggs. Stagnant water and static eggs may lethally impede embryonic gas exchange, causing tissues to die from oxygen starvation and from acidification of dissolved metabolic carbon dioxide. Nevertheless, it still appeared that the live brooding male provides further services beyond simply exposing the eggs regularly to atmospheric air.

**Brooding behavior** Emergent vegetation, when available, was the chosen microhabitat for encumbered male *B. flumineum* in the field. Virtually all (approximately 25) egg-bearing males were found associated with emergent vegetation where they seemed to spend a great deal of time positioned in the air acquisition position (i.e., resting with the tip of the abdomen slightly above the surface). This posture exposed the unattached ends of the eggs to the atmosphere. Females and unencumbered males were found on the pond's mud bottom, under mats of algae, and associated with debris. These individuals surfaced periodically, but generally remained at the surface only long enough to renew their sub-hemelytral air supplies. The subsurface behavior of an encumbered male in Lost Lake was observed. This individual vigorously stroked his egg pad with his hind legs. He sometimes stroked alternately with one leg and then the other, but more frequently applied a series of strokes with one leg while the other rested, then without
pause, the roles of both legs were reversed. These bouts of stroking were followed by rest periods of varying length up to 30 seconds. This behavior pattern has been referred to as brood stroking by Smith (1976a).

Further details and variations of this behavior pattern were described by Smith (1976a) as revealed by laboratory investigations on B. flumineum. While brood stroking, the male is supported by his front and middle legs, employing only the hind legs to stroke the eggs. Most strokes are applied smoothly to the full dorsal surface (anterior to posterior) of the egg pad, but occasionally discrete variations in this pattern are common for each individual. At the onset of brood stroking, bugs usually patted their eggs. "Patting" involved short vertical movements of one hind leg against the mid-dorsal surface of the egg pad. Between five and seven "pats" were delivered in rapid succession. Two other distinct variations from the stroking pattern probably led early workers (Slater 1899; Torre-Bueno 1906) to infer that males are reluctant to bear eggs. Smith labelled the two other movements "kicking" and "sawing." Kicking entailed dynamic thrusting of one hind leg at the anterior of the egg pad. Sawing consisted of brisk lateral movements of the hind tibiotarsus pressed into the egg interstices. The leg was sawed back and forth in place for a period not exceeding 5 seconds. Both of these variants were rare, relative to stroking.
Torre-Bueno (1906) noted the "peculiar fact" that copulation takes place in connection with oviposition for B. flumineum. This observation is particularly meaningful in light of Parrer's (1970) conclusion that sperm from the last male to mate in a polyandrous series usually predominate in fertilization. Assuming this is the case for B. flumineum, eggs borne by the individual male will contain predominantly his genes (of the haploid male contribution) even if the female has mated previously. Sperm competition studies on another belostomatid species, A. herberti, have yielded some tentative results that support this idea (Smith 1976b). Slater (1899) assumed that eggs borne by an encumbered male were fathered by him when she conceded that sometimes "paternal instincts predominate." She stated that the third pair of legs were sometimes used to brush eggs free of foreign particles. Cullen (1969) also believed leg movements of B. malkini probably served to keep the eggs clean and parasite-free.

It is not clear, however, that mere cleanliness would enhance egg viability and there are no known parasites of belostomatid eggs besides the microbial growth. Furthermore, the observed rates of brood stroking would seem to suggest another function for this behavior. Long strokes of the hind legs replete with swimming hairs should provide a regularly intermittent flow of water over the eggs concomitantly
renewing dissolved oxygen (even if at low ambient concentrations), and removing embryonic metabolites. Results of detached egg pad experiments suggest that these effects are the function of brood stroking.

Smith (1976a) suggests that patting is a mechanism that allows the bug to determine that he is still carrying eggs. It occurs infrequently (relative to brood stroking) and usually initiates a series of bouts of brood stroking. The functions of kicking and sawing are less obvious, but both patterns may serve to evaluate the adhesion of the egg pad. Kicking tests the margin most vulnerable to failure: the leading edge. It is here that the nidus is exposed to greatest hydrodynamic forces as the bug swims. Sawing may reveal lateral and posterior looseness. Apparently, a loosely attached clutch of eggs is a poor risk. It may be lost entirely, with the consequence that all eggs will fail. For this reason, a male that continues to brood a loose clutch may have a statistically higher probability of wasting his time and energy. Presumably selection has favored males that quickly detect egg pad looseness and discard loose pads. Removal of a firmly attached egg pad and cannibalism of eggs in the face of adverse brooding conditions seem to be other selectively advantageous male options (Smith 1976a).

Smith did not attempt experimentally to assess the direct caloric cost to males incurred by brooding. However,
it seems evident that the brooding male is exposed to extra risks. The additional weight of developing eggs would impair swimming movements and perhaps reduce the ability to escape predators. Smith did measure the swimming speeds of egg-bearing and unencumbered males and found that the latter could swim considerably faster. Additionally, the extra weight of the eggs may impair the feeding efficiency of these insects. The behaviors involved in the male's parental care undoubtedly also increase risk of predation. For example, because water bugs normally rest motionless, protected by their cryptic coloration, the pumping or stroking movements probably make them more vulnerable by betraying their position. It may be partly to balance such risks that water bugs have developed a tremendous bite.

Respiration

Miller (1961) has given a detailed account of the respiratory system of Hydrocyrius columbiae, which differs only in minor anatomical points from that of B. flumineum. Like other aquatic Hemiptera and many Coleoptera, adult belostomatids have a subalar air store. The hind wings, ventral surface of the forewings, and dorsal side of the abdomen are all hydrofuge and the area enclosed forms the air store. Posteriorly from the fifth segment a fringe of long wettable hairs borders the abdomen (Fig. 7). A narrow
Fig. 7. Dorsal view of the posterior segments of the abdomen of *Belostoma flumineum* (parts of the terga of segments 6 and 7 are cut away to expose the retractor muscle of the siphon and its long apodeme)
Siphon retractor muscle

Apodeme

Air gulley

Siphon

Wing margin
naked strip of cuticle separates these wettable hairs from the hydrofuge hair-pile that covers most of the dorsal surface. Anterior to the fifth segment the wings overlap the abdomen and the ventral hair-pile is continuous with the dorsal. This hair-pile is comprised of long and short hairs; the former are about 1 mm long and have no structural modifications at the distal end, whereas the latter are hooked at the tip and are 60-70 microns long. The pile is interrupted 2 mm in from the margin by a slight depression in the cuticle that stretches from the base of the siphon to spiracle 3, and is lined with short, hooked hairs only.

The air in the ventral hair-pile is continuous with that in the dorsal air store. The main function of the ventral air film is to allow the ventrally placed abdominal spiracles to communicate with the dorsal air store and hence with the atmospheric air when the siphons pierce the water surface. In addition the air film serves a subsidiary function as a physical gill, although this is probably of small importance (Miller 1961).

The highly modified ninth segment forms a pair of respiratory siphons that are attached to the external genitalia and anus. Unlike the siphons on the Nepidae those on the Belostomatidae do not join to form a tube but are flattened and lie side by side. Torre-Bueno (1906) describes those of B. flumineum as being strap-like; the two straps fuse at the
basal ends and surround the phallobase. The respiratory siphons are short and can only be extended and withdrawn a very short distance. Long hairs line the outer margin of the siphon and part of the ventral surface, and in addition small hooked hairs are scattered over the ventral surface. Normally on the siphons, the whole ventral surface and the proximal half of the dorsal surface retain an air film. It is frequently seen as a silver bubble lining the edges of the wing covers.

When the bug is submerged, the siphons remain completely retracted under the wings. During the ascent to the surface (which is usually made at about 5 cm/min) the siphons are protracted and retracted in phase with weak ventilatory movements, and with each protraction a column of air is drawn out on the siphon bristles. As soon as the siphons make contact with atmospheric air, strong abdominal ventilatory movements immediately begin. If the air is displaced from the siphon hairs (e.g., rubbing with grease) the bug continues to ascend until the posterior part of the abdomen protrudes above the surface. The abdomen is then flexed ventrally and ventilation takes place through the gap between the wings and the abdomen.

The siphons can perform three types of movement: (1) extension and retraction; (2) lateral movements; and (3) dorsal curving. Extension is achieved by abdominal pressure
and relaxation of the retractor muscles. A pair of muscles, one attached to the base of each siphon and arising on a long apodeme which is fixed to the anterior edge of the seventh segment, retracts the siphone under the wings. It was found in this study that extension of the siphon was not possible in bugs with small parts of the abdominal sclerites removed. Lateral movements of the siphons are sometimes seen when the bug is out of water or when the air film on the siphons has been displaced. They can occur in one siphon alone, but are often seen in both as separating movements. These movements are brought about by small muscles at the base of the siphons, and their relative significance is unknown. The dorsal curving movement is most interesting. The bug is normally less dense than water, and when it surfaces for ventilation and there is nothing for it to grasp, it floats nearly horizontally. In order to pierce the water surface, the extended siphons curve dorsally. This movement is achieved by a series of diagonally situated muscles which are attached to the dorsal and ventral walls inside the siphons. In B. flumineum, as in the other Belostomatidae, the function of the siphons is to place the sub-hemelytral air store, and therefore all but the first pair of spiracles, directly in contact with the atmosphere. There is probably little significance in the location of spiracle 10 at the siphon base, particularly since it is very similar to the other abdominal spiracles in structure.
Nymphal B. flumineum have a large ventral air store trapped by a pile of hairs of several distinct types. These are described by Miller (1961). The nymph does not possess respiratory siphons, and the tenth pair of spiracles lies on the ventral surface of the abdomen. In the last nymphal instar, the outline of the adult siphons is visible, but they remain fused to the rest of the abdomen. Normally the nymph pierces the water surface meniscus with the long hydrofuge hairs on the tip of the abdomen, but any part of the air store may be involved. The dependence of the nymph on the surface for respiration probably means that it does not normally descend deeper than 1-2 m.

Severin and Severin (1911a) reported that B. flumineum could live approximately 12 hours without renewing its air supply. Their experiments were conducted at a water temperature of 20°C.

Distribution

B. flumineum is an abundant species in Iowa. There is little overlapping of range of B. flumineum and B. lutarium in Iowa. Only in Henry and Des Moines counties were B. flumineum and B. lutarium found in the same pond. The collection sites of B. flumineum in Iowa are shown in Fig. 8.
Fig. 8. Collection sites of *Belostoma flumineum* in Iowa
Habitat

Habitat as used in this study is a seral community or physiognomic subdivision thereof, so that all of the area within each habitat is, at zero density of the species, essentially homogenous with respect to the physical and biological features which are relevant to the behavior and survival of the species.

Habitats for *B. flumineum* included both temporary and permanent waters with and without marginal macrophytes and/or emergent vegetation. Water ranged from clear to extremely turbid. Water bugs were found in a few eutrophic ponds when oxygen was less than 1 ppm. The typical habitat was a cattail pond with a foot or more of water. Individuals rest beneath the dead leaves and stems that form a latticed floating mat. They also frequent the grassy margins or overhanging bushes on the margins of ponds. The microhabitat selected by encumbered males in the field seemed correlated with the stage of development of the eggs being carried. Marginal macrophytes were obviously the preferred microhabitat for males carrying eggs in every stage of development.

One excellent habitat yielding an unusual concentration of *B. flumineum* in central Iowa was a large pond, Lost Lake. The organisms found in Lost Lake might well be found in any pond of similar size, location and stage of development; the area of the pond was about 0.75 ha. This habitat
is at least superficially similar to the limnic-wrack beds of Scandinavian shores as described by Dahl (1959). In Iowa, such habitats are only physiognomic subdivisions of the reed-swamp community or occasionally of the floating-vegetation community where the latter extends to the shoreline (Deonier 1961). In general, the limnic-wrack habitat may be considered as an accumulation of decaying plant debris. At Lost Lake the accumulation was of long duration, being repeatedly increased in amount for several seasons.

**Macroflora**  
The dominant macroflora of Lost Lake may be divided into three groups: submerged, floating-leaved, and emergent vegetation. Several species of submerged vascular plants were found throughout the season. Of these the most important was *Potamogeton amplifolius*, a pond weed which thickly covered the pond between depths of 0.5 and 1.5 m. In late summer its submerged and floating leaves and emergent fruiting structures formed a continuous cover.

The pond also supported a fairly large growth of floating-leaved plants including *Nuphar advena* (water lily). *Lemna minor* (lesser duckweed) was a small vascular plant which floated over the surface with the wind in great masses in late summer. *Nuphar* occurred in most of the pond shallow water and its massive tuberous roots were an extremely noticeable component of the substratum. *Lemna* was so abundant
as to effect oxygen reduction locally by limiting light penetration.

*Typha latifolia* (broad-leaved cattail) and *Scirpus validus*, (sedge) two species of rooted emergent aquatic vascular plants, were often uprooted by freezing and thawing and the rafts thus formed became the chief components of limnic wrack when washed ashore. Tangled masses of *Nuphar*, species of *Potamogeton*, *Utricularia vulgaris* (bladderwort), and planktonic filamentous algae constituted the wrack present around shores without a zone of emergent vegetation. *Polygonum amphibium* (amphibious smartweed) and other terrestrial shoreline plants grow on limnic wrack and thus augment its ecotonal characteristics.

**Physical and chemical conditions at Lost Lake**

The monthly temperature and precipitation averages are shown in Table 8. Although these data appear highly regular, extreme variations were common in late fall and early spring. Freezing temperatures occurred on 159 days of the year (November through May). The summer precipitation, though averaging high, was subject to extreme variation during the study season of 1978.

Although water-level variation was pronounced in this period, there was little effect on the floating pond weed and marginal vegetation that shelters *B. flumineum*. The annual cycle of water-level begins with filling in spring, due to snow melting and high precipitation combined with
Table 8. Climate summary for Boone, Iowa; January 1978-December 1978. (Annual Weather Summary: U. S. Department of Commerce) a

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean monthly temperature</th>
<th>Mean monthly precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°F</td>
<td>°C</td>
</tr>
<tr>
<td>January</td>
<td>8.5</td>
<td>-13.1</td>
</tr>
<tr>
<td>February</td>
<td>11.4</td>
<td>-11.4</td>
</tr>
<tr>
<td>March</td>
<td>31.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>April</td>
<td>48.3</td>
<td>9.1</td>
</tr>
<tr>
<td>May</td>
<td>59.7</td>
<td>15.4</td>
</tr>
<tr>
<td>June</td>
<td>68.7</td>
<td>20.4</td>
</tr>
<tr>
<td>July</td>
<td>72.5</td>
<td>22.5</td>
</tr>
<tr>
<td>August</td>
<td>71.1</td>
<td>21.7</td>
</tr>
<tr>
<td>September</td>
<td>67.6</td>
<td>19.8</td>
</tr>
<tr>
<td>October</td>
<td>49.3</td>
<td>9.6</td>
</tr>
<tr>
<td>November</td>
<td>35.5</td>
<td>1.9</td>
</tr>
<tr>
<td>December</td>
<td>19.2</td>
<td>-7.1</td>
</tr>
</tbody>
</table>

Annual mean temperature 45.3 7.4 Total annual precipitation 37.15 943.50

aEnglish units in original publication; metric units provided for comparison.
reduced evaporation. Presumably, in the fall, evaporation is increased as the humidifying influence of the surrounding forest is lost and rainfall is decreased; this causes a drop in water-level. Lost Lake had dried up completely in mid-July of 1977, a drought year in central Iowa.

Since water movement is very slight, great differences in water temperature can occur in short distances. Surface water temperature of the pond varied in the summer as much as 7° in 24 hours; bottom temperature fluctuated only 3° during the same time. Surface water temperature in the summer exceeded 35°C in dense floating-leaved vegetation.

Turbidity was not measured in interstitial and algal mat water since any attempt to do so caused immediate clouding to opacity. Pond turbidity varied seasonally owing to plankton (Table 9); heavy rainfall and decreasing water-level increased turbidity by disturbing the flocculent ooze at the bottom.

The dissolved oxygen content of surface waters of Lost Lake (Table 9) can increase 1.1 mg/l/h and decrease at about the same rate. Seasonally, there was considerable variation in the dissolved oxygen content of Lost Lake; in late winter under heavy ice cover, dissolved oxygen was not detectable. All samples taken in the flocculent "false bottom" lacked dissolved oxygen and the oxygen concentration in any subsurface samples in the pond depend largely on proximity to the bottom. Vascular vegetation of the shore region of
Table 9. Physical and chemical factors of Lost Lake, Ledges State Park, Boone Co., Iowa

<table>
<thead>
<tr>
<th></th>
<th>Temp. (°C)</th>
<th>Dissolved Max (mg/l)</th>
<th>Oxygen Min (mg/l)</th>
<th>Turbidity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 1978</td>
<td>12</td>
<td>7.4</td>
<td>5.5</td>
<td>40</td>
<td>7.1</td>
</tr>
<tr>
<td>May</td>
<td>19</td>
<td>9.3</td>
<td>6.2</td>
<td>10</td>
<td>6.5</td>
</tr>
<tr>
<td>June</td>
<td>26</td>
<td>10.7</td>
<td>6.2</td>
<td>12</td>
<td>7.0</td>
</tr>
<tr>
<td>August</td>
<td>31</td>
<td>11.5</td>
<td>5.1</td>
<td>90</td>
<td>7.1</td>
</tr>
<tr>
<td>September</td>
<td>25</td>
<td>10.4</td>
<td>7.0</td>
<td>--^a</td>
<td>7.1</td>
</tr>
<tr>
<td>October</td>
<td>8</td>
<td>10.2</td>
<td>7.4</td>
<td>60</td>
<td>7.2</td>
</tr>
<tr>
<td>December</td>
<td>1</td>
<td>18.3</td>
<td>12.6</td>
<td>24</td>
<td>7.4</td>
</tr>
<tr>
<td>March 1979</td>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
<td>--^a</td>
<td>5.6</td>
</tr>
</tbody>
</table>

^aWater too disturbed to measure.
the pond probably reduces the oxygen content of the water there since nearly all of these plants have surface leaves which exchange gases with the atmosphere and block light from the water; i.e., duckweed (*Lemna minor*), can at times cover more than one third of the pond surface with compact floating leaves that reduce the oxygen tension under such a mass.

The pH of the pond varied considerably during the course of this study (Table 9). The lowest reading was obtained in late winter under the ice (5.6) and the highest was 7.4 after spring rains had raised the level of the pond. Most readings in the pond were close to 7.0.

**Feeding**

The large appetites of the belostomatids have been well-documented (Dimmock 1887; Rankin 1935). *Belostoma flumineum*, like nepids, captures its prey in the raptorial forelegs. Individuals do not pursue prey, but maintain a relatively stationary position, frequently resting with the respiratory straps breaking the surface film, the middle and hind legs spread, the head slightly forward, and the forelegs spread widely, ready to grasp. Once they seize the prey, they maintain a firm hold. Individuals in captivity occasionally seized prey on the water surface, but preferred prey in the water.

In captivity, the earlier instars did not take food
as readily as later instars and seemed to be less aggressive than the adults. Hungry individuals swam a short distance toward a disturbance in the water. Severin and Severin (1911a) demonstrated that the grasping response to food is caused by vibration in the water rather than by some visual cue.

The prey is usually held with the forelegs while being sucked dry, although the middle and hind legs may be used in holding large or active prey. Such prey as large naiads and snails were often held with all six legs. To attack a snail, for example, the bug will swim quietly around the snail with the beak probing the shell. When pierced by the beak, the snail struggles to retreat into the shell. This type of resistance brings about deeper probing with the beak. Feeding starts with the snail held by the raptorial forelegs of the bug while the middle and hind legs either cling to a submerged plant, or the insect floats at the surface.

Although considerable knowledge has been gained about the action and composition of the venom and saliva of certain Hymenoptera, Diptera, Siphonaptera and other insects that attack man, little work has been carried out on the saliva of predaceous Hemiptera. An analysis of the saliva of the reduviid *Platymeris rhadamanthus* Gaerstecker reveals three trypsin-like proteolytic fractions and weak phospholipase activity (Edwards 1961). He concluded that the protease,
aided by the hyaluronidase present, acts to disrupt the intercellular matrix, abolishing the nerve and muscle excitability and leading to general lysis in the animal. In research on the salivary enzymes of the belostomatid *Lethocerus cordofanus* Mayr, Rees and Offord (1969) characterized the enzymatic activity of the saliva as proteolytic. In addition, hyaluronidase, nuclease, phosphatase and esterase have been identified. Although there is little evidence for an exact comparison of the salivary components of *B. flumineum* to those of *Lethocerus* or *Platymeris*, their actions on humans are known from personal experience to be similar, producing localized pain, vasodilation and slight paralysis. The salivary glands of giant water bugs are quite large, occupying most of the space in the pterothorax not occupied by muscle or tracheae.

Given sufficient prey, nymphal belostomatids will feed virtually continuously. This does not in fact imply a continuous ingestion of food because feeding, as in many true bugs (Hemiptera), is a two-stage process. Saliva, containing a potent mixture of hydrolytic enzymes (Rees and Offord 1969) is first injected into the prey and, after lysis of the tissues, the resulting mixture is sucked back into the bug. With large prey this process can take many hours.

Cannibalism sometimes occurred in belostomatids kept in the laboratory when other food sources were inadequate.
and conditions crowded. The nymphs in particular readily seized nymphs of an earlier instar and, less frequently, those of the same size. Torre-Bueno (1906) writes of *B. flumineum*, "In times of stress it will feed on its own nymphs, which in turn are not averse to preying on each other when hungry, which is always."

**Belostoma flumineum** will feed on a wide range of animals (Table 10). Snails, naiads, and corixids were observed to be the most favored food for adults in both field and laboratory. Young nymphs prefer small corixids and Crustacea, larvae, and young naiads. Adults have been observed feeding on fish three or four times their own length (Todd 1883). The number of animals eaten per day depended upon their availability.

**Locomotion**

**Belostoma flumineum** is a strong swimmer. Individuals glide through the water with alternating strokes of the middle and hind pair of legs which are adapted for swimming. They are flattened and bear hairs so arranged as to be raised during the power stroke of the leg and depressed during the recovery stroke. The swimming legs have the usual two tarsal claws. Swimming and flight reflexes in giant water bugs were investigated by Dingle (1961). On land *B. flumineum* is awkward. Individuals literally slide across a surface,
Table 10. Prey items eaten by nymphal and adult *Belostoma flumineum* at Lost Lake, Ledges State Park, Boone Co., Iowa

<table>
<thead>
<tr>
<th>Mollusca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastropoda</strong></td>
</tr>
<tr>
<td><em>Helisoma trivolvis</em> Say</td>
</tr>
<tr>
<td><em>Gyraulus parvus</em> Say</td>
</tr>
<tr>
<td><em>Physa heterostropha</em> Say</td>
</tr>
<tr>
<td><em>Lymnaea palustris</em> Muller</td>
</tr>
<tr>
<td><strong>Pelecypoda</strong></td>
</tr>
<tr>
<td><em>Pisidium</em> sp.</td>
</tr>
<tr>
<td><strong>Arthropoda</strong></td>
</tr>
<tr>
<td><strong>Insecta</strong></td>
</tr>
<tr>
<td><strong>Odonata: Anisoptera</strong></td>
</tr>
<tr>
<td><em>Libellula luctuosa</em> Burmeister</td>
</tr>
<tr>
<td><em>Libellula pulchella</em> Drury</td>
</tr>
<tr>
<td><em>Platthemis lydia</em> Drury</td>
</tr>
<tr>
<td><em>Sympetrum ribicundulum</em> (Say)</td>
</tr>
<tr>
<td><em>Sympetrum obstrusum</em> Hagen</td>
</tr>
<tr>
<td><em>Anax junius</em> (Drury)</td>
</tr>
<tr>
<td><em>Aeshna</em> spp.</td>
</tr>
<tr>
<td><em>Gomphus amnicola</em> Walsh</td>
</tr>
<tr>
<td><strong>Odonata: Zygoptera</strong></td>
</tr>
<tr>
<td><em>Archilestes grandis</em> (Rambur)</td>
</tr>
<tr>
<td><em>Lestes unguiculatus</em> Hagen</td>
</tr>
<tr>
<td><em>Agrion maculatum</em> Beauvois</td>
</tr>
<tr>
<td><em>Hetaerina americana</em> (Fabricus)</td>
</tr>
<tr>
<td><em>Argia sedula</em> (Hagen)</td>
</tr>
<tr>
<td><em>Enallagma</em> sp.</td>
</tr>
<tr>
<td>Hemiptera</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Hesperocorixa interrupta (Say)</td>
</tr>
<tr>
<td>Hesperocorixa lucida (Abbot)</td>
</tr>
<tr>
<td>Hesperocorixa obliqua (Hungerford)</td>
</tr>
<tr>
<td>Hesperocorixa vulgaris (Hungerford)</td>
</tr>
<tr>
<td>Sigara alternata (Say)</td>
</tr>
<tr>
<td>Gerris buenoii Kirkaldy</td>
</tr>
<tr>
<td>Gerris marginatus Say</td>
</tr>
<tr>
<td>Ranatra fusca Bueno</td>
</tr>
<tr>
<td>Notonecta indica Linnaeus</td>
</tr>
<tr>
<td>Notonecta undulata Kirby</td>
</tr>
<tr>
<td>Buenoa macrotibialis Hungerford</td>
</tr>
<tr>
<td>Buenoa margaritacea Bueno</td>
</tr>
<tr>
<td>Plea striola Fieber</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coleoptera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilus triangularis (Say)</td>
</tr>
<tr>
<td>Hydrobius sp.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diptera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles quadrimaculatus Say</td>
</tr>
<tr>
<td>Anopheles punctipennis (Say)</td>
</tr>
<tr>
<td>Aedes vexans (Meigen)</td>
</tr>
<tr>
<td>Aedes stimulans Walker</td>
</tr>
<tr>
<td>Culex restuans Theobald</td>
</tr>
<tr>
<td>Culex pipiens pipiens Linnaeus</td>
</tr>
<tr>
<td>Culiseta inornata (Williston)</td>
</tr>
<tr>
<td>Chaoborus americanus (Johannsen)</td>
</tr>
<tr>
<td>Chrysops sp.</td>
</tr>
<tr>
<td>Tabanus sp.</td>
</tr>
<tr>
<td>various species of Chironomidae</td>
</tr>
<tr>
<td>various species of Ephydridae</td>
</tr>
</tbody>
</table>
Table 10. (Continued)

Vertebrata

**Amphibia**

*Pseudacris nigrita triseriata* (Wied)

*Rana pipiens pipiens* Schreber
pushing with the middle and hind legs in irregular thrusts.

Although *B. flumineum* does not fly to light as do *B. malkini* (Cullen 1969) and species of *Lethocerus* and *Benacus*, it is apparent that flight does occur. Several times, large numbers of *B. flumineum* present in Lost Lake and Little Wall Lake disappeared in a week's time during July 1977. This reduction in the population coincided with flights of corixids and a reduction in the number of notonectids, nepids, and corixids. Bowden (1964) found a clear lunar periodicity for the flight of *Sphaerodema severinii* Melichar in Ghana. He also found through regression analysis that the catches were to a considerable extent related to an immediately preceding rainfall. Cullen (1969) reported similar evidence for the dispersal flights of *B. malkini*. The findings by Bowden (1964) were the first examples of a lunar rhythm of flight activity in the Hemiptera, but he does not offer any explanation of its adaptive significance. The advantage of flying when there is maximum moonlight is probably that it facilitates the distinguishing of topographical features, particularly expanses of water, which reflect the light.

In addition to the lunar periodicity, there is a seasonal variation in the amount of flight activity. The flight of belostomatids is representative of the normal dispersal movements of most pterygote insects, sometimes resulting in the colonization of new areas. For example,
Cullen (1969) reports that female B. malkini and L. maximus caught during flight have already mated and therefore have the potential for populating new areas. However, the problems of colonization for large predaceous water bugs are certainly different from those of the terrestrial herbivorous insects, which have been used for most studies of insect dispersal (Williams 1957; Johnson 1960; Kennedy 1961; Dingle 1968). Whereas the latter can make use of feeding areas that only exist for part of a year, belostomatids have to colonize permanent habitats because of the nature of their diet.

**Behavior**

Usinger (1956, pp. 203-207) records the death-feigning habit of B. flumineum and species of Abedus. When disturbed by removal from water or by contact with the dorsal and ventral surfaces of the body, the bugs assume a characteristic rigid position and remain in this so-called "death-feint" for varying periods of time. This position was maintained for an average of 16.4 minutes in five successive experiments involving six specimens of B. flumineum (Severin and Severin 1911b). As the bugs never exhibited this posture while in the water, the normal environment, it is uncertain whether this habitat has any adaptive significance.

Another curious habit is the forceful ejection of fluid from the anus of B. flumineum when the bug was held captive out of water. Usinger (1956) suggests that the fluid might be responsible for the odor noted by other observers.
The ejection of fluid seems to be part of the normal process of elimination in $B$. $flumineum$. A bug resting at the surface will occasionally release a jet of fluid, which is usually clear but is black after the insect has been feeding. Dissections of $B$. $flumineum$ showed that the rectum is thin-walled and has a large diverticulum running anteriorly as far as the thorax. Rapid flattening of the abdomen seems to force the fluid under pressure out of the rectum and its reservoir. The advantage of this behavior, projecting fecal material some distance from the bug itself, may be to conceal its presence from potential predators.

Parasitism

Symbiotic relationships between mites and insects involve a variety of associations including commensalism, parasitism, and predation. Perhaps the most common association is phoretic commensalism where ambulatory mites may feed on integumentary exudates or bits of food material clinging to the surface of the insect host. Furthermore, ambulatory mites may feed on the same substratum as the insect host, thus utilizing the insect as a source of transportation from one food supply to another. Only the water mites, Hydracarina (or Hydrachnellae), have become generally adapted to fresh waters. Although Hydracarina are found in almost all types of fresh-water habitats, they are most
abundant and characteristic of streams, rivers, ponds, and the littoral region of lakes, especially where there are quantities of rooted aquatic vegetation. Their bright colors, globular to ovoid shape, and clambering and swimming habits identify them unmistakably.

Water mite larvae typically are ectoparasites on imagos of aquatic insects. This aspect of water mite biology was first documented adequately by Wesenburg-Lund (1919) and subsequently has been reported for all families for which life history data are available. Wesenburg-Lund (1919) favors the theory of some broad preference by larvae for certain insect hosts; thus, he states that the Limnochanidae (Eylaiiodea) usually parasitize hydrometrids, that Eylaiidae (Elyaiodea) mostly parasitize aerial insects, that Hydryphantidae (Hydryphantoidea) usually occur on culicids, and that several other families appear to be almost restricted to insects that do not leave the water. In view of more recent ecological observations, it is possible that his conclusions may be a reflection of restricted mite and insect faunas in the ponds he studied. It would be highly desirable to test this question experimentally in order to find out definitely whether or not a particular larval mite has any specific host preference. The insect host provides the mites not only with the source of nutrients necessary for larval maturation but also with the primary mechanism
for dispersal. A number of studies have documented the association of larvae of species in several genera of water mites with insect hosts in certain groups (e.g., Münchberg 1935; Mitchell 1959, 1964; Lanciani 1969, 1970) and a useful summary of the literature was provided by Jones (1967). However, studies published to date have left the majority of water mite genera unaccounted for, even within limited geographic areas, and consequently have provided little insight regarding the possible significance of host associations in elucidating the ecological background of water mites. This situation has resulted because investigations have been concentrated mainly on insect hosts that are large, conspicuous, and relatively well-known taxonomically (e.g., Odonata, Hemiptera, Coleoptera) and from difficulties in identifying parasitic larval mites taken from hosts. The known associations of larvae in the seven superfamilies of water mites with imagos in six orders of aquatic insects are summarized in Table 11.

**Life history** Water mite species in nearly all genera appear to conform essentially to a characteristic life history pattern, although statements concerning the breeding season vary widely. Fertilized eggs are rarely deposited singly; usually they are extruded in groups of 20 to 400 onto stones, vegetation, and debris. Eggs are usually red. *Hydrachna* oviposits in the tissue of aquatic plants.
Table 11. Parasitic associations of larvae in superfamilies of water mites with imagos in orders of aquatic insects.

<table>
<thead>
<tr>
<th>Superfamilies of water mites</th>
<th>Orders of insects with imaginal hosts of water mites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plecoptera</td>
</tr>
<tr>
<td>Hydrovolzioidea</td>
<td>X</td>
</tr>
<tr>
<td>Hydrachnoidea</td>
<td>X</td>
</tr>
<tr>
<td>Eylaiioidea</td>
<td>X</td>
</tr>
<tr>
<td>Hydryphantoidea</td>
<td>X</td>
</tr>
<tr>
<td>Lebertioidea</td>
<td></td>
</tr>
<tr>
<td>Hygobatoidea</td>
<td>X</td>
</tr>
<tr>
<td>Arrenuroidea</td>
<td>X</td>
</tr>
</tbody>
</table>
Eggs deposited by female adults on the substratum of the aquatic habitat give rise in several days to active hexapod larvae. After a short free-swimming period, the larvae become attached to aquatic insects with their capitulum and assume a parasitic existence. In some species, each larva seeks to locate and attach to a suitable insect host on the surface film (species of Hydrovolziodea, Eylaioidae, and Hydryphantoidea). In others, the larva locates its potential host beneath the surface film, either in the water column or in the substratum (species of Hydrachnoidea, Libertioidea, Hygrobatoidea, and Arrenuroidea) such that at ecdysis of the host, the mite can climb onto the teneral imago and embed its chelicerae at an appropriate attachment site. In both cases the larval mite subsequently engorges on fluids from the host and then remains attached until the host returns to the water. In species that parasitize long-lived hosts such as Hemiptera and Coleoptera (e.g., certain species of Hydrachnoidea and Eylaioidae), the larva transforms to the nymphochrysalis (Fig. 9) and molts to the nymph while attached to the host. The mite then returns to the water as an active, predaceous, octopod nymph. With parasites of relatively short-lived hosts such as imaginal nematocerous Diptera, the engorged larva detaches from its host, re-enters the water and seeks out a suitable substratum in which to embed its chelicerae in preparation for transforming to the
Fig. 9. Water mite nymphochrysalis, with a portion of the developing water mite showing through a break in the larval integument
nymphochrysalis. The mechanisms for detachment from host and for re-entry to water are not understood adequately.

Nevertheless, metamorphosis occurs within the nymphochrysalis, usually in several days, and the mite then emerges as an active nymph. In both cases, the nymph resembles the adult but is sexually undifferentiated. The duration of the nymphal stage is variable and after nymphal maturation the mite again seeks out an appropriate substratum, embeds its chelicerae and transforms to the imagochrysalis in which metamorphosis to the adult occurs in several days. Adults usually mate immediately after emergence and the males die shortly thereafter.

Genus Eylais Species of Eylais are among the most common and conspicuous water mites inhabiting standing waters. The water mites in this group inhabit shallow ponds primarily and can be easily collected in each of their life cycle stages. All known hosts are adult aquatic Coleoptera and Hemiptera, and the air-bathed sites under the wings of these insects - tergites, membranous wings, and undersides of the elytra or hemelytra have been utilized. Larvae of Eylais are known from Hemiptera of the families Belostomatidae (Lanciani 1969; pers. obs.) and Corixidae (Lanciani 1969; Smith and Oliver 1976; pers. obs.). The larvae of Eylais belostomatis Lanciani parasitized only B. flumineum and appear to exploit potential hosts more completely than other
species of Eylais studied. Incidences of parasitism frequently reached 100%, and mites per insect sometimes exceeded 100. Also, larvae succeeded in attaching to hosts in laboratory containers almost every time they were placed together.

**Genus Hydrachna**  The only other water mite found on B. flumineum was *Hydrachna magniscutata* Marshall which attaches to various sites on nymphs and adults, including both the uppersides and (rarely) the undersides of the hemelytra of adults. Often a single specimen of *B. flumineum* harbored as many as 40 to 50 mites. Larval *Hydrachna* attached to the thorax (including the cervix), the legs, and the abdomen. Larvae of *Hydrachna* have been found on Hemiptera and Coleoptera. The Hemiptera include Notonectidae (Lundblad 1927; pers. obs.), Nepidae (Lundblad 1927; Crowell 1960; pers. obs.), Belostomatidae (Lanciani 1969; Smith and Oliver 1976; pers. obs.) and Corixidae (Lundblad 1927; Stout 1953; Davids 1973; Harris and Harrison 1974; pers. obs.).

The larvae of Hydrachnoidea and Elyaiioidea, with the exception of a few species, parasitize hosts that frequent the surface film, particularly Hemiptera and Coleoptera. Larvae of Eylaidae parasitize insects whose imagos are aquatic and regularly penetrate the surface film and expose their abdomens in order to obtain air. The hosts are located and readily infested at these times, and the mite larvae
persist as air breathers, attaching to the host in regions bathed by the air supply (Lanciani 1970). Larvae of these mites, then, are adapted to parasitize essentially aquatic hosts but themselves are not aquatic organisms. Larvae of Hydrachnoidea are truly aquatic and locate their hosts beneath the surface film. Consequently they can parasitize Hemiptera that obtain air through a breathing tube and do not extensively expose their abdomens at the surface (e.g., Nepidae, Belostomatidae), as well as insects parasitized by the Eylaidae. While the Eylaidoidea and Hydrachnoidea have achieved considerable diversity, their adaptive radiations apparently have been constrained relative to those of other water mites because their larvae parasitize large, predaceous insects. Such hosts are long-lived and reproduce relatively slowly.
SUMMARY

The eggs of *Belostoma flumineum* are laid on the hemelytra of the males which then exhibit an altered behavioral pattern. Such brooding behavior includes keeping the eggs wet, frequently exposing them to atmospheric air, and probably maintaining an intermittent flow of water over the eggs by stroking them with the hind legs while below the surface. There are five nymphal instars. The active period of *B. flumineum* is from April until November after which the adults over-winter in a diapausing state until the following spring. A female may deposit as many as four batches of eggs during a season, and there may be two to three generations in a year. Individuals of the third generation had the longest lives. The water bugs are usually found along the vegetated margins of ponds and lakes, although they occur occasionally along vegetated banks of streams. *Belostoma flumineum* is morphologically adapted to feeding on aquatic snails in that the rostral segments are long, slender and curved. This enables *B. flumineum* to reach a snail which has withdrawn deeply into its shell. Additional prey items include aquatic arthropods and amphibia. *Belostoma flumineum* in Iowa is commensalized by two species of Hydracarina, but it does not appear to be injured in any way by the water mites.
PART II. FLIGHT MUSCLE DEGENERATION
INTRODUCTION

In many insects there is a period after the final molt to the adult form during which the flight muscles enlarge and mature. This is followed by a period of intensive flight activity (dispersal phase) and this in turn is followed by a third period (reproductive phase) in which the flight muscles degenerate. This degeneration is a normal part of the life cycle of the insect. The relative importance and duration of these three phases is variable from species to species. Flight muscles are known to histolyze in more than 50 species of 20 families from 8 orders, in some species of which muscles also regenerate later. This suggests that the Pterygota generally have a potential for structural lability in the flight apparatus even after reaching the adult stage. Histolysis, timed in relation to ecological needs, ensures that a habitat or diapause site is not relinquished too soon, though the means seem very drastic.

The earliest record of flight muscle degeneration is that of Janet (1907, as cited from Cullen 1969) who showed that in the ant Lasius niger (L.) the large flight muscles of the females autolyze after the nuptial flight. Degeneration also occurs in the Isoptera (Feytaud 1912), where again it is important for the establishment of the new colony. The phenomenon has since been discovered in
Dermaptera (Mercier 1924; Poisson 1924), some Diptera (Mercier 1924; Roubaud 1932; Hocking 1952, 1954), various Coleoptera (Rüsschkamp 1927; Jackson 1933, 1952, 1956), Lepidoptera (Finlayson 1956), Homoptera (Johnson 1953, 1957, 1959), and Hemiptera (Ferrière 1914; Poisson 1924; Wigglesworth 1956; Cullen 1969; Edwards 1969a, b). Ferrière and Poisson worked with aquatic Hemiptera of the families Nepidae and Naucoridae, while Cullen investigated the tropical water bug Belostoma malkini Lauck. In several insect orders, notably Hemiptera and Coleoptera, the environment influences flightlessness.

As in Isoptera, a similar physiological link between flight muscle breakdown and egg production is seen in the Hymenoptera and Diptera. Thus Hocking (1952), in an examination of the flight muscles of Aedes communis (De Geer), found a good inverse agreement between the degree of egg development and the degree of flight muscle degeneration: specimens in which eggs were almost ready to laid showed no trace of either tergosternal or dorsal flight muscles. In the Coleoptera the function of flight muscle dissolution is not so clear. Rüsschkamp (1927) examined the flight muscles of 250 specimens of Melasoma, Chrysomela, and Chrysochloa and concluded that the flight muscles undergo histolysis in the course of imaginal life. Jackson (1933), working with Sitona hispidula (F.), concluded that in flightless individuals the normal post-metamorphic
development of the flight muscles and the chitinous supports is arrested.

The utilization of flight muscle as a food reserve, or its degeneration to reduce the intake of food by the removal of a large volume of active tissue, carries with it the disadvantage of decreased mobility. This disadvantage has been overcome in the ambrosia and bark beetles (Coleoptera, Scolytidae) in which the flight muscles do not completely degenerate and which later regrow to full size when required for a further period of flight activity (Chapman 1956, 1958; Reid 1958; Atkins and Ferris 1962; Borden and Slater 1969). A similar cycle of degeneration and regeneration occurs in the Colorado potato beetle (Leptinotarsa decemlineata Say), in which the muscles revert to normal at the end of diapause (Stegwee 1964; Stegwee et al. 1963; de Kort 1969). The muscles become greatly reduced during diapause and are partially replaced by fat stores. Before the termination of diapause in the spring, the flight muscles regenerate and the process of regrowth is completed within a few hours after emergence from the soil. Beetles subjected to a short-day photoperiod fail to grow their flight muscles to full size before degeneration takes place (de Kort 1969). During diapause flight muscle metabolism is low and mitochondrial enzyme activity is scarcely detectable. In this instance, as the degeneration is only
temporary, the function cannot be to supply metabolites necessary for the reproductive organs but is more likely to be a mechanism for lowering the overall basal respiratory requirements of the insect during hibernation. Similar considerations may explain the observations made by Jackson (1952) that certain species of water beetles have varying proportions of individuals with degenerate muscles at different times of the year.

In the Homoptera, as in ants, termites and flies, flight muscle degeneration begins when the animals have settled down to a sedentary life, after a dispersal flight or flights, and is associated with the development of eggs. The flight muscles histolyze a day or two after the first flight, although in some insects this can be delayed for more than two weeks when such factors as darkness interrupt flight (Heathcote and Cockbain 1966; Johnson 1957).

In aquatic Hemiptera reduced flight muscles are quite common and degeneration in the adult has often been assumed to be the cause. In their papers on flight muscle degeneration in aquatic Hemiptera, Ferrière (1914) and Poisson (1924) do not form any opinion on its value to the insect. However, Scudder (1971) discusses this interpretation in detail and throws doubt on it by demonstrating that the smaller flight muscles in the flightless Cencorixa bifida (Hungerford) are a consequence of retarded growth, not
degeneration. Whether this explanation is universally applicable to the aquatic Hemiptera remains to be seen. In order to investigate flight muscle degeneration in Belostoma flumineum, a study was undertaken to determine the incidence of histolyzed flight muscles in field-collected specimens and the environmental factors controlling flight muscle histolysis.
MATERIALS AND METHODS

Cultures of B. flumineum were kept in environmental growth chambers at 27 ± 0.5°C, with a 12-hr photoperiod provided by fluorescent lamps. The insects were kept in ten gallon aquaria containing gravel and aged tap water. Each aquarium was also provided with various species of grass stems and pondweed for the insects to crawl and settle on. Snails and mosquito larvae of several species were used for food. Under these conditions the life cycle was completed in about 45 days. For temperature and starvation studies, insects were reared in smaller water-filled jars that were provided with a cork and toothpick for supporting the insect. Constant temperature cabinets were used for temperature experiments. Light-control cabinets were used for photoperiod studies. Insects were tested for flight ability by throwing them into the air; they were further examined by dissection in insect Ringer solution (Knudsen 1966), and the flight muscles were classified as unhistolyzed (A), histolyzing (B), or histolyzed (C) (Edwards 1969a, 1969b). For histological examination, insects were fixed in aqueous Bouin's fluid, embedded in Ester Wax under vacuum, sectioned at 8-10 microns, and stained in iron hematoxylin and orange G.
Two hundred field-collected specimens of *B. flumineum* were dissected between June and November 1978. In each dissection three features were examined: the indirect flight muscles, the reproductive organs and the post-cerebral complex of endocrine organs. Degenerate muscles could be distinguished from normal ones by their glistening white, rather than pink, color, their collapsed appearance and by a tendency to fall away from the meso- and metaphragma.
RESULTS AND DISCUSSION

The main difficulty in obtaining a thorough analysis of the flight muscles of these insects is that they can be induced to fly only rarely and unpredictably (cf. Dingle 1961; Parsons 1960). This difficulty is apparently due to the fact that giant water bugs probably fly in nature only once or twice a year and only at certain seasons of the year. The rest of the time they are completely aquatic. This suggests that the occurrence of flight is controlled by annually recurring cyclic events and that it may not be possible to induce flight in most individuals except for that short period of time each year which corresponds to the naturally occurring time for flight.

In insects of the order Hemiptera, the indirect muscles have a large number (~1800) of small diameter fibers (~7 microns) and the main tracheal trunk runs down the middle of the muscle with branches radiating outwards terminating in blind sacs (Ashhurst 1967). There is little or no penetration of tracheae into the fibers and the individual fibers can therefore be separated without damage. Degenerate fibrillar muscles were readily identified from their normal counterparts because their general shape, position and points of insertion are the same. Otherwise, they present a
characteristic white, slack appearance. The whiteness is due to the fact that the dense tracheal tissue, typical of these muscles normally, is the predominant material remaining on the degenerate muscles. Teased preparations of these muscles showed that some muscle material was still present. The large fibers typical of these muscles were visible but the fibrils within the fibers were apparently much shrunken. This was evident because the nuclei of the fibers were very numerous whereas they are usually obscured in a fiber packed with normal fibrils. Also, although longitudinal striations were evident, cross striations, normally very obvious, were only faintly visible.

Temperature

Third instar nymphs were reared in groups of 10 at different temperatures, i.e., 24°C, 27°C, and 32°C. Two days after the final ecdysis the insects were tested for flight ability. The insects did not ecdyse together; however, 8 days after the ecdysis of the first female, all the insects were dissected. Males generally ecdysed 1 day earlier than females (Table 12). Temperature determined the developmental period but had no apparent effect on flight muscle degeneration. Adult females and the majority of adult males did not fly before flight muscle histolysis, although lowered temperatures lengthened the pre-ovipositional period.
Table 12. The effect of different temperatures upon the indirect flight muscles of adult *Belostoma flumineum*, 8 days after ecdysis (values in parentheses indicate the number of individuals induced to fly)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>24°C</th>
<th>27°C</th>
<th>32°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>State of A</td>
<td>4 (3)</td>
<td>3</td>
<td>3 (1)</td>
</tr>
<tr>
<td>flight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State of B</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>muscles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 13. The effect of different photoperiods upon the indirect flight muscles of adult *Belostoma flumineum*, 8 days after ecdysis (values in parentheses indicate the number of individuals induced to fly)

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>State of A</td>
<td>4 (1)</td>
<td>-</td>
<td>3 (2)</td>
<td>2</td>
</tr>
<tr>
<td>flight</td>
<td></td>
<td>4</td>
<td></td>
<td>5 (2)</td>
</tr>
<tr>
<td>State of B</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>muscles</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>
Photoperiod

After ecdysis, fifth instar nymphs were kept in cabinets with lighting controlled at 0, 6, 12, and 18 hr of light per 24 hr. Following the final ecdysis, the adults (except those in total darkness) were tested for flight ability; all were dissected 8 days after the final ecdysis (Table 13). As in the temperature experiments, females did not fly before the flight muscle histolyzed. After 8 days all had histolyzing flight muscles. Photoperiod, therefore, had no apparent effect upon flight muscle degeneration.

Nutrition

To test for nutritional differences between the various prey items commonly eaten by B. flumineum, some insects were reared on snails and others were given various arthropods (e.g., damselfly naiads, corixids, and mosquito larvae). Dissection 6 to 8 days after ecdysis showed that all adult females had histolyzed flight muscles and developing oocytes. Males were for the most part unaffected, and some were capable of flight. Other groups were given no food between ecdysis and dissection. After 7 days, none of the females could fly and the flight muscles were histolyzed (Table 14). Oocytes had differentiated but remained small and transparent. In adult females starved for 14 days, the fat body was greatly reduced.
Table 14. The effect of starvation upon the indirect flight muscles of adult Belostoma flumineum, 7 and 14 days after ecdysis (values in parentheses indicate the number of individuals induced to fly)

<table>
<thead>
<tr>
<th>Treatment (age)</th>
<th>Fed (7 days)</th>
<th>Starved (7 days)</th>
<th>Starved (14 days)</th>
<th>Starved (7 days) Fed (7 to 13 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>State of flight</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>-</td>
<td>2 (1)</td>
<td>1</td>
<td>4 (2)</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Vitellogenesis</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
Some females were given snails after 7 days of starvation, and were dissected after 14 days. None of the insects was able to fly and only one had normal flight muscles; moreover, dissection showed that the oocytes were underdeveloped, indicating that little feeding had occurred. Thus, the females were apparently starved, and their flight muscles and oocytes were as in the starved females (Table 14). This result suggests that, even with access to food, feeding behavior of individuals can influence muscle breakdown and reproduction.

**Field-collected B. flumineum**

Unlike Ferrière's (1914) and Poisson's (1924) findings, in which the distinction was made only between normal mature flight muscle and what they termed "l' organe trachéo-parenchymateaux", all stages of degeneration could be found, there being a continuous series from normal mature muscle (A) to muscle in a highly advanced state of histolysis (C). The percentage of females containing mature eggs, or which had apparently already oviposited, was 86.4% for those with degenerate muscles but only 38.1% for those with normal muscles. Each female reproductive system was arbitrarily scored from 1 to 5 according to the stage that it had reached in the reproductive cycle. The mean score for females with normal muscles (A) was 1.20, for females with slightly degenerate
muscles (B) 1.73, and for those with muscles in an advanced state of degeneration (C) 2.46.

Although the number of insects involved was small, there clearly seems to be an inverse agreement between the degree of egg development and the degree of flight muscle degeneration. It is probable, therefore, that a value of flight muscle histolysis in *B. flumineum* is in the production of metabolites, perhaps needed in vitellogenesis. It would perhaps be expected from this that the male would show a smaller tendency toward muscle degeneration, as most of the development of the relatively small testes occurs in the last nymphal instar. In fact, muscle degeneration was as frequent in males as in females.

A female *B. flumineum* lays eggs several times during her lifetime. This raises the question of whether muscle degeneration always occurs before the first batch of eggs develops (or possibly at another stage in the reproductive cycle), or whether the onset of histolysis is controlled by a particular set of physiological conditions that might occur at any stage in the reproductive cycle of the insect. The majority of freshly caught female *B. flumineum* with normal flight muscles contained eggs that were at an early stage of development. Several specimens, however, were found with normal flight muscles, yet having their oviducts filled with mature eggs. Clearly, degeneration of the flight muscles is
not always essential for successful development of eggs.

On the premise that any cell differentiation is dependent upon differential activity of genes, any discussion of flight muscle degeneration necessarily presumes complex interrelationships between the environment and genetically based variations.

If muscle degeneration is not obligatory, it must be influenced by a variable external factor, or combination of factors, which affects the internal environment of the insect. Based on laboratory evidence, a likely factor was thought to be the food supply, degeneration being inhibited or delayed while there existed a plentiful supply of food. To try to test for this, ten newly captured individuals of B. flumineum (5 males, 5 females), in which flight capability had been demonstrated, were starved for six weeks, at the end of which they were dissected and their muscles examined. Ten control individuals were fed five snails per week for six weeks and also dissected. In all the bugs except one unstarved female, the flight muscles were histolyzed to some extent. Four of the unstarved females contained mature eggs, but none of the starved ones nor the female with the normal muscles had produced any.

Although the small numbers of insects involved make these results inconclusive, it seems that an adequate food supply can slow down or delay histolysis but that other
factors are involved in its initiation. In addition, although the products of degeneration may contribute to vitellogenesis, these alone are not enough for the production of mature eggs. Johnson (1957) similarly found in aphids that the muscles could only make a very small contribution to the development of the embryo. The 20 bugs were kept in small water-filled jars which were not provided with flight "take-off" positions, and it is possible that lack of muscle use influenced degeneration. It is likely that if the normal dispersal flight results in the location of a suitable habitat, degeneration then occurs, there being no advantage in any future flight. This might explain the occurrence of degeneration in nine of the ten well-fed individuals.

Very little is known about the precise physiological stimuli that initiate histolysis. The control is usually assumed to be hormonal, but little definite evidence for this has been found. If flight muscle degeneration results from a hormone circulating in the hemolymph, then a system to account for its action on only a few muscles of the body has to be postulated. Presumably these muscles could contain a precursor essential for the degeneration-producing hormone to be effective. Alternately, the nervous system could be involved, but Wigglesworth (1956) provided evidence against this for histolysis of the abdominal muscle in Rhodnius.
Stegwee et al. (1963) found that degeneration of the muscles of *Leptinotarsa* could be produced by extirpation of the post-cerebral complex of endocrine glands, i.e., the corpora cardiaca and the corpora allata. Re-implantation of active post-cerebral complexes resulted in a very rapid regeneration of the muscle.

In *B. flumineum*, the paired corpora cardiaca are situated immediately behind the brain, lying dorsolaterally on the esophagus and adjacent to the anterior end of the dorsal aorta. The paired corpora allata also lie on the dorsolateral surface of the esophagus just behind the corpora cardiaca. The corpora cardiaca are single-lobed and egg-shaped. A pair of small nerves, the nervi corporis cardiaca I, run from the brain to the corpora cardiaca and enter the corpora allata. The corpora allata vary in shape from being flattened ovoids to perfect spheres. The corpora allata were qualitatively examined in 100 dissections of *B. flumineum* to determine whether there was any obvious correlation with the state of the flight muscles. The relative diameter of the corpora allata of the males was slightly greater than that of the females.

There is some controversy over whether a neurosecretory organ that shows an increase in size has a corresponding increase in activity, or whether it is storing rather than releasing neurosecretory material. On the basis of the
former alternative, which is more generally accepted (Highnam 1963), the corpora allata would be expected to be smaller in those individuals with histolyzed muscles, if the control of degeneration in B. flumineum is similar to that in Leptinotarsa. A non-quantitative examination of the corpora allata revealed that they appeared slightly smaller in individuals with normal muscles as compared to the corpora allata of individuals with degenerate muscles. A similar situation was reported by Cullen (1969) for B. malkini. Strangeways-Dixon (1962) found that the corpora allata of Calliphora increase in size with an increase in the hemolymph concentration of protein metabolites. If this is generally characteristic of insects, it might help explain the larger size of corpora allata in individuals with degenerate flight muscles, assuming that degeneration results in a higher concentration of metabolites in the hemolymph.

This situation is further complicated by the involvement of the corpora allata in the reproductive cycles of insects. An increase in the size of the corpora allata during maturation of the eggs would probably disguise any changes correlated with muscle degeneration, especially as the eggs tended to be in a more advanced state of development in those individuals with degenerate muscles. The research on Leptinotarsa by Stegwee et al. (1963) appears to be the first experimental attempt to associate flight muscle
degeneration with the insect endocrine system. It is uncertain how far their findings can be applied to the Belostomatidae, as a major effect of the extirpation of the corpora allata in *Leptinotarsa* is the inducement of diapause, and degeneration could be a secondary effect of this.
CONCLUSIONS

It is not known how long the period of flight muscle maturation lasts in adult *B. flumineum* in their natural environment. Laboratory studies of *B. flumineum* indicate that flight muscle degeneration occurs in males and females and in the female is associated, though perhaps not causally, with the development of eggs. Temperature and photoperiodicity do not influence the incidence of flight muscle degeneration in adult *B. flumineum*. Temperature affects the timing of flight muscle degeneration, but it also affects the timing of other events, e.g., copulation and oviposition. All water bugs studied to date have a tendency to degenerate their flight muscles if food is in short supply, though the controlling factors are not fully understood. The effects of nutrition in reproduction are well-known and have been summarized by Wigglesworth (1964). Flight muscle autolysis is associated with feeding and reproduction in such insects as alate aphids (Johnson 1957, 1959). As in *Oncopeltus fasciatus* (Dallas) (Johansson 1958), starved insects utilize fat reserves and the oocytes remain small with no yolk. Starvation accelerates muscle degeneration, but an abundant food supply will not normally prevent it. It is unlikely that the post-cerebral complex of endocrine organs may be involved in muscle degeneration. It might be possible to interfere with this by endocrine surgery on the water bugs.
but this will not be possible until more is known about the normal physiology of the endocrine system in belostomatids. It is concluded that flight muscle histolysis in Belostoma flumineum is determined by the environment, although not in the same way as in polymorphic aquatic Hemiptera (Brinkhurst 1959; Guthrie 1959; Young 1961, 1965). Belostoma flumineum is not polymorphic; at metamorphosis all adults are potentially capable of flight.
PART III. CIRCADIAN ORGANIZATION OF CUTICLE
INTRODUCTION

The cuticle of an insect is the interface between living animal and environment and as such must provide a means of passage for elements essential to its existence. But the cuticle is most notably a skeleton, and an insect skeleton is a formidably complex composite material. The mechanical properties of such composites depend not only on the individual properties of the components but also upon how they interact. Each phase has an important part to play. In materials such as fiberglass the properties of the matrix are of prime importance in crack-blunting, protecting the surface of the fibers, supporting the architecture of the structure and, of course, acting as a transmitter of the load to the strong phase. As a rule the matrix phase of all composites plays all of these roles to some extent although different types of composite materials can be obtained by shifting the emphasis placed on a particular function. Arthropod cuticles can be seen in just such a manner: there is a spectrum of mechanical properties that differ in direction and magnitude. These mechanical properties, in turn, largely depend upon the nature of the matrix.

The cuticles of insects and other arthropods acquire specialized properties which result from structure. Cuticles are polyphasic and their mechanical behavior results both
from the individual properties of the various constituents and, of equal importance, how these constituents are distributed and interact with each other. The whole question of cuticular organization in insects has been extensively reviewed by Neville (1967b, 1975).

**General structure of insect cuticle**

The integument consists of an epidermal cell layer with the overlying cuticle which it has secreted. The basic division of the cuticle is into a thin, outermost layer, the epicuticle, usually about 1 to 2 microns in thickness, which overlies the thicker procuticle (up to a few microns thick). The epicuticle is also the first stratum to be formed in preparation for ecdysis; it appears to be devoid of chitin, and it is usually subdivided into an outer and inner epicuticle of quite different composition (Weis-Fogh 1970). The epicuticle represents a continuous lining of the external surface, including the invaginated ectodermal parts such as the tracheae, the foregut and the hindgut. It is absent only over certain pits in some chemoreceptors (Slifer 1961) where the holes permit entry of stimulant molecules, in the midgut (Bertram and Bird 1961) to permit the uptake of products of digestion, and in the end apparatus of gland cells (e.g., *Periplaneta* oothecal gland, Mercer and Brunet 1959) to provide a route for the secretion of various glandular products. Mechanically, the epicuticle behaves
as a brittle layer (stronger in compression than in tension) that protects the tough and more flexible procuticle from adverse external factors (Hepburn and Ball 1973; Joffe et al. 1975a). In another sense the epicuticle has been considered as the morphogenetically critical determinant of cuticular dimensions at ecdysis (Bennet-Clark 1963; Weis-Fogh 1970).

The succeeding layers between the epicuticle and the epidermal cells are referred to as procuticle. It forms the greater bulk of the skeleton and it consists of protein, chitin, and some lipids. However, the structure, chemistry and mechanical properties of procuticle vary enormously even within the same organism. Procuticle is in fact a collective term for the entire spectrum of structures which range from hard sclerotized plates, through soft flexible membranes, to rubber-like pads of pure resilin. The layers of the procuticle are secreted in order, beginning with the outermost and working inwards.

After ecdysis and expansion of the cuticle in the final shape of the new stage, precisely defined areas of the outer procuticle become sclerotized by means of a tanning process involving phenols. Tanning usually results in some coloration from light amber to deep brown or even black. This is the exocuticle or the hard part of the integument. Before ecdysis the prospective exocuticle is fully formed in a folded state. It has a higher concentration of dry matter
than the inner layer of preecdysial procuticle, the endocuticle. The endocuticle appears to have a lower concentration of protein so that the chitin is more easily visible in the form of lamelle. The endocuticle usually remains colorless and relatively soft, although it has the characteristics of an essentially solid material. After ecdysis, the endocuticle continues to grow and the thickness, mass and strength of the skeletons of locusts increase almost three times after the final molt due to the deposition of endocuticle (Weis-Fogh 1952; Jensen and Weis-Fogh 1962).

Since in most arthropods the external dimensions are fixed by tanning after each ecdysis, the underlying epidermal cells are pushed inwards as they secrete successive layers of cuticle. Where expansion does occur between ecdyses, it is generally confined to parts of the body lacking a tanned exocuticle. For example, the cuticle in larval Galleria is only tanned at the base of the rugosities (Locke 1961), permitting expansion of the cuticle between them even after tanning has occurred.

Supermolecular architecture

It is now well-established that arthropod cuticles are constructed of microfibrils. Rudall (1965) first illustrated microfibrils in electron micrographs of Sirex ovipositors, and subsequently found them in fly larval and puparial cuticle (Rudall 1967). Microfibrillar systems are also
known in the α-keratin of hair (Filshie and Rogers 1961), the 
β-keratin of feathers (Filshie and Rogers 1961), and the oothecal protein of tortoise beetles (Atkins et al. 1966). The microfibrils in most arthropod cuticles have a diameter of about 28 Å (Neville 1975).

Cuticle microfibrils are made of chitin and they are imbedded in a matrix of protein. This was first suspected by Rudall (1967), who found that the surrounding matrix undergoes faster degradation by molting fluid than the chitin during the resorption of the puparial endocuticle in Calliphora. The partly digested inner layers give a 'purified' chitin X-ray diffraction diagram rather than one which is 'obscured' by protein (Fraenkel and Rudall 1947). This presumably permitted better orientation of the specimen under reduced staining in electron micrographs, which led Rudall (1967) to the conclusion that the matrix which stains heavily with electron dense stains is protein, whereas the microfibrils were chitin. Neville (1970) confirmed this by comparing electron micrographs of pure resilin with ones in which resilin and chitin are known to be the only two components present (e.g., the locust prealar arm ligament). The integrated knowledge of cuticle at the microfibril level obtained by X-ray diffraction, enzymatic degradation, infrared spectroscopy and electron microscopy serves as a summary in supermolecular architecture. Quantitative calculations
of volume fraction of microfibrils and matrix multiplied by the respective densities of chitin and resilin have shown that the microfibrils are chitin within a protein matrix and that the microfibrils are pure crystallites of chitin.

The ultrastructure of exocuticle and endocuticle is poorly understood apart from the extensive studies by Neville and his collaborators which have concentrated upon the organization of the chitin component. A two-system model was proposed by Neville and Luke (1969a, 1969b) to explain microfibrillar architecture of arthropod exocuticles and endocuticles. These systems are (i) layers in which the microfibrils are helicoidally arranged in the protein matrix, and (ii) layers in which the microfibrils are all undirectionally oriented to form a preferred layer. Either type of arrangement may occur exclusively, or both may be present in successive layers. In the latter case, the transition is always continuous and smooth, with a undirectional layer many microfibrils in thickness making a similar angle with the first (or last) plane of microfibrils of the helicoid to that angle which the successive planes of microfibrils make between each other. The original model was deduced from electron microscopy of Schistocerca gregaria cuticle (Neville and Luke 1969b), but it has since acquired general significance for understanding any arthropod cuticle ultrastructure. In the case of adult locusts it is known that
the endocuticle contains daily growth layers, with lamellate night zones alternating with non-lamellate day zones (Neville 1963, 1965a). It was known from chemical and polarized light observations that the daily growth layers were formed by changes in the orientation of the cuticle (Neville 1965a). Electron microscopy confirmed that the lamellate night layers were helicoidal, whereas the non-lamellate day layers consisted of unidirectional chitin microfibrils (Neville and Luke 1969b).

Most exocuticles are helicoidal (lamellate) throughout their thickness, with the exception of locusts (Neville 1967b) and the scarab beetle *Plusiotis resplendens* (Caveney 1971a), which all have a single unidirectional layer of microfibrils sandwiched between two helicoidal layers. The distortion of perfectly regular helicoidal exocuticles is attributed to the expansion at the ecdysis.

Insect endocuticles exhibit interesting sequences and relative proportions of helicoidal and unidirectional layers. When both types of orientation are present, one or the other may predominate, so as to give either unidirectional orientation with very thin intervening layers of helicoidal lamellae (e.g., adults of beetles, belostomatid bugs, dragonflies). Alternatively, helicoidal orientation may dominate with very thin intervening layers of unidirectional orientation (e.g., locust nymphs, locust adult ocelli) (Neville and Luke 1969a).
In the light microscope alternately helicoidal and undirectional systems resemble the plywood type of orientation so common in collagen systems, such as vertebrate basement membranes (Weiss and Ferris 1956) or corneas (Jakus 1964). In insect cuticle, however, careful observation revealed that there may be either a single lamella or a small number of lamellae between each unidirectional layer, and this was confirmed by electron microscopy (Neville and Luke 1969b). Hence in Belostoma, Lethocerus, Hydrocyrius (Hemiptera), in Aeshna (Odonata) and in Tenebrio (Coleoptera), two successive layers with unidirectional orientation do not change direction suddenly by a high angle. Instead, the angle change is achieved by a gradual rotation of planes of microfibrils so as to form a partial turn of helicoid in passing from one major direction to another. Neville and Luke (1969b) referred to these systems as 'pseudo-orthogonal.' The timing in pseudo-orthogonal systems may be circadian (Neville 1965b, 1967b; Dingle et al. 1969).

Mechanical properties

The principal significance of chitin in tanned insect cuticle appears to be one of determining the new size of an instar immediately following ecdysis but prior to tanning (Hepburn and Joffe 1976). Of course, the chitin is also responsible for establishing an architecture which must affect the distribution of proteins within the whole
structure. But, as soon as these proteins are tanned the resulting stabilized matrix dominates the mechanical properties of the cuticle, diminishing the relevance of chitin. However, this only applies to tanned cuticles so that in insects such as caterpillars where the matrix remains untanned the cuticle is strongly dominated by the properties of chitin. Unlike the tanned insects, these insects are able to increase their bodily dimensions intra-stadially; the only restraint being that imposed by work-hardening (Hepburn and Chandler 1976).

All chitin architecture known to occur in insects is structurally anisotropic, particularly in the surface planes. Mechanically, however, a perfectly helicoidal structure is isotropically distributed. The occurrence of sclerotization diminishes the importance of chitin and in such areas the mechanical anisotropy may disappear, as it does in helicoidal/preferred cuticles (Hepburn and Roberts 1975; Joffe et al. 1975a, b).

There is a spectrum of cuticular mechanical properties which is related to the properties of the matrix. Matrices are of three kinds: plastically deformable, brittle and a hybrid of these two, all of which are clearly distinguishable by their tensile, mechanical and fractographic properties (Hepburn and Chandler 1976). The first of these, as exemplified by caterpillars, is of very low relative stiffness and
large breaking strains. The second exhibits high values of relative stiffness and low breaking strains. Finally, there is the hybrid kind of matrix which is a mixture of the other two types. The majority of insect cuticles which have been examined to date are the hybrid type, thus representing what is usually understood as "typical solid cuticle." Typical solid cuticle is a remarkable example of case-hardened material, the properties of which derive from the presence of a brittle outer matrix (the exocuticle) and a softer and plastic inner portion (the endocuticle); these can be distinguished mechanically, fractographically, chemically and histologically.

**Daily growth layers in solid endocuticle**

The solid endocuticle of many insects grows by daily increments with a single or a double layer marking each day's progress until cuticle growth ceases (Neville 1963, 1965a, b, 1967a, b; Dingle et al. 1969). The zonation is due to differences in diurnal and nocturnal deposits, one pair of concentric rings being deposited every 24 hr, and is the result of permanent changes in orientation of chitin crystallites occurring at the time of deposition. As revealed in sections examined with the polarizing light microscope, nocturnal layers comprise several lamellae, whereas diurnal layers are not lamellated. The ultrastructural interpretation of these alternating types of
layers forms the basis of the two-system model of cuticle architecture. Neville (1975) lists the 37 species of insects in which the presence of daily growth layers have been established, and to these may be added about 9 species of Diptera (Schlein and Gratz 1972). Daily growth layers are found in both nymphs and adults in Exopterygote insects and in adult Endopterygotes. Moreover, they occur in the tibiae, femora, thoraces, wing veins, ovipositors, ocelli, and compound eyes. So far they have not been found in arthropod classes other than insects.

The discovery of daily growth layers in insect endocuticle has led to experiments establishing the involvement of a circadian clock in chitin orientation control. The organization of cuticular deposition is circadian, will free-run under constant conditions, and seems to reside in the epidermal cells (Neville 1965a). In locusts, the insects most thoroughly studied, the rhythm of endocuticular organization persists with a circadian rhythm for at least 2 weeks in constant darkness. It is, moreover, very nearly temperature compensated with a $Q_{10}$ of 1.04 for the frequency (Neville 1965). The rhythm in the milkweed bug, Oncopeltus fasciatus, shows the same properties and the same precise temperature compensation (Dingle et al. 1969). Only in some Coleoptera are the layers not laid down in a circadian rhythm nor is their rhythm temperature compensated, so that it
appears that true circadian periodicity, or coupling, has been lost by the epidermal cells of beetles (Zelazny and Neville 1972a, b).

In the locust, though not apparently in *Periplaneta*, *Hydrocyrius* (Neville 1965a, b), or *Oncopeltus* (Dingle et al. 1969), constant temperature and constant light will cause uncoupling of the rhythm and result in the deposition of only one type of cuticle; a dermal light sense is apparently involved (Neville 1967a). One of the interesting results from this work has been the discovery that in the locust, epidermal cells in different parts of the insect differ in their response to constant light. Even cells which are spatially close may show quite different responses. For instance in constant low temperatures and high intensity constant light the endocuticle of the thickened proximal region of the hind tibia is laid down continuously in the lamellate form, despite the fact that the endocuticle of the rest of the hind tibia is non-lamellate under these conditions. This suggests that each epidermal cell is controlled by its own biological clock, and the clocks differ in their response to constant light.

Alternatively, (Neville 1967a) suggests that many cells are controlled by a single clock with a different means for the epidermal cells to respond to it or couple with it. The adaptive features of the system, whereby a regime which
induces non-lamellate endocuticle in the major part of the locust but does not prevent lamellate endocuticle production in some specific regions, is of interest. It seems likely that the unaffected parts are those in which lamellae are functionally indispensable, including as they do the ends of the tibia which are involved in the stresses of jumping, the edge of the wing veins, the rubber-like cuticle of the pre-alar arm and wing hinge ligaments, and the cuticle of the median ocellus and compound eye.

In the giant water bug, Belostoma flumineum, paired daily growth layers are also present and seen to consist of lamellate and non-lamellate layers. For insects raised on a day consisting of 16 hr of light and 8 hr of dark (16L:8D) at 24°C, the endocuticle stops growing at about 8 days, resulting in the deposition of about 8 pairs of distinguishable daily growth layers. It was therefore of interest to investigate the effects of photoperiod and temperature on the growth of the endocuticle. At the same time, the underlying organization of the daily growth layers, especially with regard to possible circadian aspects, was also explored. Such information could then be used to validate the method of counting growth layers as an objective estimate of the duration of the teneral period.
MATERIALS AND METHODS

The work was carried out mainly on the solid endo-cuticle of adult B. flumineum. The insects used in these experiments were reared in small water-filled jars placed within self-contained bioclimatic chambers and were fed snails and mosquito larvae regularly. The temperature varied from 18 to 31°C with the photoperiod at either 16 or 12 hr or in conditions of constant light or dark. Most observations of cuticular architecture were made on the middle region of the tibia of the metathoracic leg, because of suitable size, although some sections were also taken from the femora and tibiae of other legs. The growth rings were best observed with a polarizing light with crossed polaroids set for maximum brightness of birefringence, after the method of Neville (1963, 1965a, b) Materials were frozen-sectioned either fresh or after fixation at 4°C for 48 hr in neutral buffered 4% formaldehyde, and then examined in water.
Fig. 10. *Belostoma flumineum*. Cross-section through metathoracic femur of a bug raised at 12L:12D at 24°C photographed between crossed polaroids set for maximum brightness of birefringence (ten pairs of daily growth rings can be counted at the bottom of the photograph).

Fig. 11. *Belostoma flumineum*. 5 day old adult from a field population.

Fig. 12. *Belostoma flumineum*. Growth layers in adult tibia.

Fig. 13. *Belostoma flumineum*. Growth layers in tibia of last instar nymph.
RESULTS

Circadian organization of exoskeleton

Belostoma flumineum cuticle is not nearly so favorable a material for the analysis of daily growth rings as is that of cockroach or grasshopper. Both in Neville's experimentation (Neville 1963, 1965a, b, 1967a) and in this work the concentric layers in the endocuticle of these latter insects stand out with absolute clarity when observed with polariscopy. Difficulties arose in part because B. flumineum is a smaller insect with cuticle and growth rings much thinner, and also because the rings seem generally to be somewhat less defined (cf. Dingle et al. 1969). Adjacent rings, for example, tend to merge in places making it more difficult than in larger insects to determine the exact number. In my work the maximum number of paired rings that could be counted was taken to be the 'true' value for the number present in any given case (fig. 10-13).

In any event, rearing experiments have confirmed the presence of circadian zonation in the solid endocuticle of the legs of nymphs and adults of B. flumineum. When viewed between crossed polaroids the endocuticle was birefringent with one light and one dark layer deposited each day until the completion of growth. Like the locust (Neville 1965), the difference in the layers results from the fact that the brighter (night) ring is lamellate while the darker (day)
ring in non-lamellate as revealed by examination under the oil immersion lens. The appearance of the concentric growth rings was reminiscent of the seasonal growth in trees, especially since endocuticular growth rings were deposited during the first two weeks of adult life. The number of pairs of rings was counted in sections of hind tibia of adult *B. flumineum*, whose age in days from emergence was known. A hind leg was removed from an adult of known age (1 day), and the expected number of endocuticular layers (one pair) confirmed. Four days later, subsequent sectioning of the other hind legs showed 4 extra pairs of growth layers.

**Circadian control of daily growth layers**

The cuticular growth rings of *B. flumineum* are apparently under the control of a circadian rhythm initiated by a light-dark cycle impinging upon the pharate adult. Once started the rhythm cannot be uncoupled from the circadian clock by either constant light or constant dark. The evidence for these conclusions is contained in Table 15. If the bugs are placed in constant conditions any time after the fifth instars have stopped feeding (2-4 days before the end of this instar), there is no effect on the deposition of growth rings. If, on the other hand, constant conditions begin during the fourth instar or first few days of the fifth instar, then no rings are deposited and the endocuticle is
Table 15. The effect of various light-dark (L-D) regimens on cuticular growth ring deposition<sup>a</sup>

<table>
<thead>
<tr>
<th>Condition</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-4 days in L-D cycle following adult eclosion, then constant light or dark</td>
<td>Normal rings</td>
</tr>
<tr>
<td>Constant light from late fifth instar</td>
<td>Normal rings</td>
</tr>
<tr>
<td>Constant light from fourth or early fifth instar</td>
<td>No rings</td>
</tr>
<tr>
<td>Constant dark from late fifth instar</td>
<td>Normal rings</td>
</tr>
<tr>
<td>Constant dark from fourth or early fifth instar</td>
<td>No rings</td>
</tr>
</tbody>
</table>

<sup>a</sup>N=10 insects per regimen.
largely uniform throughout. In the latter, however, some areas of cuticle may show 2 or 3 incomplete rings extending at most 0.25 of the way in these areas. Evidently the circadian rhythm is somehow 'breaking through' in these areas.

In order to get deposition of complete growth rings in *B. flumineum*, the pharate adult, even if only in the earliest stages, had to receive a light-dark cycle.

As with all suspected circadian rhythms, the possibility remained that the daily pattern was triggered by some unknown rhythmical event occurring at the same time on each day. True circadian rhythms run free in constant light (or dark) and constant temperature, but the period for these rhythms differs slightly from a precise 24 hours. If it could be shown that the free-running period of chitin lamellogenesis in constant darkness at a constant temperature differed from 24 hours; this would exclude the possibility of exogenous triggering by an external factor which is phase-fixed in the astronomical 24-hour period.

It was not possible to determine the free-running period of the rhythm because the number of rings deposited at the experimental temperature of 24°C was insufficient for an accurate measurement of any deviation from 24 hours.

**Effects of temperature on cuticle growth**

As in most classical circadian systems, the circadian oscillator controlling cuticle deposition in *B. flumineum*
was independent of temperature, at least over the ranges tested. Over the temperature range 18 to 27°C a single pair of cuticular growth rings was deposited each day, up to a maximum thickness. Thus the $Q_{10}$ of the circadian system closely approximates 1.0.

The total number of rings, however, is markedly affected by temperature as indicated in Table 16. The number of pairs of rings was inversely related to temperature. At 18°C, for example, a mean of 11.2 pairs of rings resulted while at 27°C means for the two samples were 5.6 and 5.5 pairs. At 35°C no individuals showed rings and in fact B. flumineum appears infertile at this temperature suggesting widespread physiological disruption. Growth rate, plotted as the reciprocal of the number of rings, is indicated as a function of temperature in Fig. 14.

The number of paired rings occurring at any given temperature results from the fact that whereas growth rate is a function of temperature the circadian oscillator controlling cuticular deposition and the final thickness of the cuticle is independent of temperature. Cuticular thickness in the mid-region of the metathoracic tibia was unaffected by the number of rings present. Thus, at 18°C, 11 and 12 paired rings are deposited. In contrast, at 27°C the cuticle reached terminal thickness in 3-7 days with the disposition of the appropriate number of rings (mean 5.5-5.6).
Table 16. The number of pairs of rings as a function of photoperiod and temperature

<table>
<thead>
<tr>
<th>Conditions</th>
<th>(hr L:D)</th>
<th>(Temp. °C)</th>
<th>Range</th>
<th>Mean</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12:12</td>
<td>18</td>
<td>11-12</td>
<td>11.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>12:12</td>
<td>24</td>
<td>6-10</td>
<td>7.2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>16:8</td>
<td>24</td>
<td>6-8</td>
<td>6.4</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>12:12</td>
<td>27</td>
<td>5-7</td>
<td>5.6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>16:8</td>
<td>27</td>
<td>3-6</td>
<td>5.5</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig. 14. Growth rate, plotted as the reciprocal of the number of paired daily growth rings, as a function of temperature.
RATE OF GROWTH

TEMPERATURE (°C)

0.08  0.12  0.16  0.20

12L:12D
16L:8D
At higher temperatures, therefore, the rings are fewer but thicker since the accelerated growth permits deposition of greater amounts of cuticle each day.
DISCUSSION

From the foregoing it is apparent that the cuticular deposition in *B. flumineum* is coupled to a clock. The results of experiments in which giant water bugs were reared in temperature- and light-controlled environments showed that chitin lamellae formation is under the timing control of a circadian clock which is normally phased to daily environmental changes. With the almost universal acceptance of the reality of biological clocks, additional indicator processes are being found to be coupled to them (Harker 1964; Bunning 1964). It was therefore not too surprising to find that the daily growth layers in skeletons of *B. flumineum* were also coupled to a circadian clock. The clock must be set by exposure to a light-dark cycle in the pharate adult, but once set cannot be uncoupled from cuticular deposition by constant light or dark. In this way *B. flumineum* was similar to such insects as *P. americana* and *O. fasciatus* and to other belostomatids, but differed from locusts where the situation is further complicated by the fact that uncoupling occurs in various groups of cells under some conditions but not others (Neville 1965a, b, 1967b, 1975). At temperatures higher than 35°C the clock in *B. flumineum* does become uncoupled.

The circadian clock seems virtually independent of temperature, as is generally characteristic of such systems.
The free-running endocuticular rhythm in *B. flumineum* had a $Q_{10}$ of 1.0, which compares well with a value of 1.04 for *Schistocerca* (Neville 1965b) and a value of 1.0 for *O. fasciatus* (Dingle et al. 1969).

Endocuticular thickness in *B. flumineum* increases linearly with temperatures between 18°C and 27°C. Dingle et al. (1969) found similar results in *O. fasciatus* with $Q_{10}$ of 1.76 between 19°C and 31°C. The endocuticle in *B. flumineum* reaches a constant thickness whose value is independent of temperature at the end of the adult teneral period. Thus high temperature results in a total number of fewer and thicker layers, whereas low temperature produces a larger number of thinner layers. Growth is rhythmical in insect skeletons and so is the organization of structure. In view of the involvement of circadian clocks in homeostatic mechanisms (Harker 1964) it might be thought that if chitin morphogenesis is coupled to a temperature-compensated clock, the actual control of rhythmical skeletal structures would then become more independent of the environment. However, consideration of the discrepancy between the temperature coefficients for the timing of chitin lamellogenesis and for the rate of cuticle deposition, shows the homeostatic implication to be paradoxical.

Food availability was a factor which affected growth layer deposition in *B. flumineum*. Cullen (1969) reports
similar findings for experiments carried out in Trinidad with recently ecdysed adults of B. malkini. When prey was scarce for B. flumineum, cuticle deposition ceased so that a minimal age was given by growth layer counts. Deposition of growth rings in the endocuticle of Rhodnius prolixus is similarly food-dependent, in this case upon blood meals (Zwicky and Wigglesworth 1956). Thus only two growth layers may be found in the femora of old females which have fed several times (Caveney 1971). Carnivores and bloodfeeders feed more spasmodically, and this is reflected in their more erratic endocuticle deposition.

In regions of closely opposed cuticle (e.g., swimming legs) in B. flumineum cuticular connections dowel the two sheets of cuticle together across the intervening space. The chitin orientation in these regions was parallel turning to a right angle to cross the intervening space.

The prime function of chitin orientation is for improved strength in certain directions. Ideal ultrastructural reinforcement in the cuticle is provided by the helicoidal system of stacked layers, a design providing multi-ply strengthening. However, once the chitin has been adopted as a reinforcing material and has been crystallized out in this way, the surface of the cuticle can no longer expand and external growth must halt. It is of course true that specialized parts of insect cuticle do expand between molts,
for instance the abdomens of *Rhodnius* nymphs after a blood meal (Bennet-Clark 1963) and the abdomen of the egg-producing queen of termites (Bordereau 1967). These examples, however, merely imply localized regions with preformed foldings or a somewhat disorganized pattern which permits some degree of permanent flow. The general conclusion seems to be that the advantage which chitin offers for the reinforcement of the cuticle also implies that these structures are deprived of possible growth once they have been formed. It is for this reason alone that the insects adopted discontinuous growth as it is known from the molting cycle.
CONCLUSIONS

The endocuticle of adult B. flumineum grows by daily increments until it reaches maximum thickness, when growth ceases. During the night, chitin crystallites are deposited in the endocuticle in organized lamellae; during the day the same amount of chitin is deposited, but in non-lamellate form. Examined in sections under crossed polaroids, the lamellate layers are strongly birefringent, and the daily growth layers thereby detectable as pairs of alternating light and dark bands. These are under the control of a circadian rhythm initiated by a light-dark cycle impinging upon the pharate adult. Cuticular growth is a direct linear function of temperature, and there is therefore an inverse relationship between temperature and the number of rings in fully mature adults. The cessation of cuticular growth ring deposition objectively defines the end of the teneral period in adult B. flumineum.

Belostoma flumineum can thus be added to a list of insects in which circadian control of cuticular deposition has been demonstrated (Neville 1975). Although an advantage in strengthening the cuticle is suspected (Neville 1967b), the precise function of daily growth rings is unknown.
PART IV. AGE RECRUITMENT IN POPULATIONS OF BELOSTOMA FLUMINEUM
INTRODUCTION

In ecology much interest centers upon the population as a unit of study, and the principal variable in any population ecological model is the population density of the organism under investigation within the habitat or geographical area of interest. Accurate age determination is essential to the understanding of population biology. Without reliable data on an animal's age, there is no way to establish the rate of growth, the onset of maturity, the periodicity of reproduction, or the life span. Age determination thus enables a perception of the rate of reproduction and the longevity of specimens. Age determination of animals is indispensable in many studies of comparative anatomy, morphology, and taxonomy, since without this information there can be no determination of the uniformity of comparable data on the character of age variation. Finally, age determination is also very important for all work on the fluctuations of populations. It follows that a precise method for determining the individual age of animals is of considerable theoretical and applied significance, as it can be used to establish the age composition of a population; this in turn makes it possible to estimate its relative size. Age recruitment as a type of population dynamics is an adaptive property which is as characteristic of each
species as are the morphological features precisely determining the relationship with the environment.

No reliably accurate quantitative criteria have existed previously by which the age of an insect could be measured. Although several qualitative methods have been applied to estimating various stages of growth and maturation (e.g., size of nymphs, color groups of adults, and state of ovariole development), Neville's (1963) method of age determination in locusts, using daily cuticular growth layers, constitutes the first experimentally verified example of a direct age index.

The concept of age determination by counting growth layers is well-known from the seasonal growth rings of trees, and from annual rings in scales, otoliths, finspines, opercula, vertebrae, and other bony structures in fish of temperate waters. Among mammals, annual growth rings are found in the wax-like plug which forms in the external ear tube of whales (Purves 1958), and in the horns of rams (Comfort 1961). Dentine laminae in the teeth have been used as a direct age index for some marine mammals, e.g., sea lions (Sheffer 1950), seals (Laws 1952), and whales (Nishiwaki and Yagi 1953; Berzin 1962). In invertebrates, the shells of lamellibranch mollusks show fast-growing summer-growth zones alternating with slow-growing winter zones.
Instead of following annual or seasonal growth changes, the growth layers in insect cuticle follow a daily rhythm. It is not surprising to find an interval of only 0.5 days between consecutive growth layers in an insect, since their life span is considerably shorter (and thus their rates of growth relatively faster) than those of the organisms mentioned above. The principle of using daily growth layers to determine the age of an insect has obvious implications for ecologists and has already been used in a preliminary field application to a population of grasshoppers (Neville 1963).

There has been no study of the population dynamics of Belostoma flumineum. This is in part due to the usual inaccessibility of their habitats, the difficulty of sampling in inshore areas and the very patchy distribution of B. flumineum that prevails in most habitats. The main purpose for this research was to make use of a direct age index consisting of daily pairs of growth layers in the solid cuticle of the exoskeletons of field-collected B. flumineum.
MATERIALS AND METHODS

Method of age determination

The choice of an age-determining method suitable for field application involved two factors. First, a method applicable to determining the age of both nymphs and adults was needed. Daily growth layers had so far been found in the wing veins, legs, and respiratory siphons of *B. flumineum* (pers. obs.). Since wing veins and respiratory siphons were restricted to the adult stage, a method using legs seemed the obvious choice. Second, significantly large samples must be analyzed for studies of population dynamics, so a suitably rapid method was developed. Materials were frozen-sectioned and then examined in water. The daily growth rings were best observed with a polarizing light microscope with crossed polaroids set for maximum brightness of birefringence.

As revealed in sections examined with the polarizing microscope, layers deposited each night are comprised of several lamellae, whereas those grown during the day are not lamellated (Fig. 10-13). A consecutive pair of cuticular layers (appearing as one dark plus one light band between crossed polaroids) represents 24 hours growth.

It was decided to measure the age of adults in terms of days after emergence. A prerequisite for this was to know how many dark and light layers were laid down in the presence
adult while it was developing within the last nymphal integument prior to the final ecdysis. This was ascertained by sectioning an adult killed at emergence when the number of dark and light preimaginal layers proved to be 1 pair. Adult age in days was computed as \( A = \frac{T-n}{2} \), where \( T = \) total number of dark plus light bands visible between crossed polaroids and \( n = \) number of rings in the newly molted adult (i.e., laid down during "nymphal life"). In \( B. \) flumenium, it was found that all of the exocuticle is deposited before emergence, whereas most, and perhaps even all, of the endocuticle is deposited after emergence. Thus, determining the age of an adult is equivalent to counting the number of pairs of dark and light layers in the endocuticle. It was always necessary to count the maximum number of layers visible, since some growth rings are less well-defined.

Validity of method

The following tests gave confirmatory results with the growth layers in the solid cuticle of hind tibiae of the giant water bug, \( B. \) flumineum. The number of dark and light pairs of endocuticular growth layers was counted in sections of legs of adults whose age in days from emergence was known. A hind leg was severed from an adult of known age (1 day), and the expected number of endocuticular layers (one pair) confirmed. The insect was allowed to live for
4 more days, when subsequent sectioning of the other hind leg showed 4 additional pairs of growth rings. Similar results were also obtained from experimentation on fifth instar nymphs. In general terms, the method can be used to ascribe a definite age in days to any adult with proven daily growth layers, for any age up to the time when cuticular growth is complete.

Field sampling

Samples of *B. flumineum* were taken randomly in Lost Lake. Two samples were taken per month in June, August, September and October 1978, with intervals of two or five days separating each sampling date. The samples comprised fifth instar nymphs and adults, and were generally taken during periods of adult emergence (Table 17). The water bugs were fixed in 4° neutral buffered formaldehyde for 48 hours at 4°C.
Table 17. The composition of the field samples of *Belostoma flumineum*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fifth instar nymphs</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (20-21 June 1978)</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>II (26 June 1978)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>III (28 August 1978)</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>IV (30 August 1978)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>V (17 September 1978)</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td>VI (22 September 1978)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>VII (10 October 1978)</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>VIII (12 October 1978)</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

It was possible to use cuticle structure as a method for determining the age of an insect in which daily cuticular growth layers have been established. When observed between crossed polaroid filters, consecutive layers in any chosen region of tibial cuticle appeared alternately dark and light due to birefringence in some oriented component in the cuticle. Depending upon the orientation with respect to the crossed polaroids, any region of a specific band appeared dark or light; if, for example, a chosen band was traced around the whole section, it appeared alternately to extinguish and reappear.

In the analysis of the results, there were two assumptions. The first was that the fifth nymphal instar was represented by only 8 pairs of cuticular growth rings before the adult cuticle started to form. This is at present an unsupported assumption due to the difficulties encountered in obtaining reliable sections of cuticle from the small B. flumineum nymphs. The second assumption was that the pharate adult stage had a duration of 1 day. This is partially supported by laboratory investigations using known sequences of environmental conditions to produce and analyze modified cuticular structure. It is therefore necessary to preface the results with the statement that they represent an exploratory field application of the direct age index.
The results of the age analysis of the samples of \textit{B. flumineum} are expressed as frequency histograms in Fig. 15 through Fig. 18. It can be seen that the nymphs forming the first age peak of sample I later appear to have spent 1 day as pharate adults and 4 days as teneral adults, emerging to a population peak of adults in sample II. Although the other samples exhibited similar age peaks which correspond to subsequent peaks, the relative frequency of these age peaks was quite irregular later in the season. The bimodal and, in some cases, multimodal profiles of frequency distribution do imply the overlap of the successive generations per annum, as well as the significant longevity of \textit{B. flumineum} adults.

Since giant water bugs have no sharply defined breeding season, the nymphs are usually present during the active season. Thus, for these samples made over short periods of time, the specimens fall into recognizable age groups rather than being evenly distributed as to age.

The degree of distinctness of the age peaks probably depends upon several factors. The shorter the incubation period in relation to the life span of the insect, the sharper will be the age peak. Conversely, if there were no definite periods of oviposition and incubation, there would be no age peaks. Obviously if such factors as growth or other changes correlated with age are rapid, the discontinuity between age peaks will be correspondingly greater; and
Fig. 15. Age frequency histograms of 2 samples (taken 5 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed (n, last instar nymph; p, pharate adult; a, adults)
Fig. 16. Age frequency histograms of 2 samples (taken 2 days apart) of a field population of *Belostoma flumineum*. The end of the last nymphal stage and duration of the pharate adult stage are assumed (n, last instar nymph; p, pharate adult; a, adults)
Sample IV (30 Aug. 1978)

Sample III (28 Aug. 1978)

AGE (DAYS)

% FREQUENCY
Fig. 17. Age frequency histograms of 2 samples (taken 5 days apart) of a field population of Belostoma flumineum. The end of the last nymphal stage and duration of the pharate adult stage are assumed (n, last instar nymph; p, pharate adult; a, adults)
Fig. 18. Age frequency histograms of 2 samples (taken 2 days apart) of a field population of *Belostoma flumineum*. The end of the last nymphal stage and duration of the pharate adult stage are assumed (n, last instar nymph; p, pharate adult; a, adults)
if they are slow, the discontinuity will become obscure or disappear. In addition, certain individuals which grow much more rapidly than others may well-overtake slower members of the next age group, and hence the groups will soon seem to merge.

The age peaks can be clear-cut only if the whole sample is taken over a period of time that is short relative to the life span of the insect or, more strictly, relative to the time between the pharate adult stage and the end of the teneral adult stage. If the sample is evenly distributed or taken at random over a long period of time, the concentration of the sample at discrete parts of the life cycle will not be likely to occur, with little determination of age peaks or recruitment.
CONCLUSIONS

A field study of a B. flumineum population shows that the age, in days, of a water bug can be accurately determined. The method, which was experimentally validated for B. flumineum, makes use of a direct age index consisting of daily pairs of growth layers in the solid cuticle of the exoskeleton. The method is applicable to both nymphs and adults. Using the daily growth layers, the expected time for the appearance of adult population peaks can be predicted. The cessation of cuticular growth ring deposition objectively defines the end of the teneral period in B. flumineum. Age recruitment was difficult to study in natural populations because of the great spatial and temporal variability characteristic of natural communities. The great longevity of B. flumineum tends to reduce the significance of temporal variability and increases the degree of predictability of a given age index. The study of age recruitment using daily growth layers has revealed the very detailed interrelation which exists between the dimensions of structure and time.
LITERATURE CITED


Torre-Bueno, J. R. de la. 1906. Life histories of
North American water bugs. I. Belostoma

Data. Annual Summary. Vol. 89. Environmental
Data Service, National Oceanic and Atmospheric
Administration, Washington, D. C.

University of California, Berkeley. 508 pp.

Voelker, J. 1968. Untersuchungen zu Ernährung,
Fortpflanzungsbiologie und Entwicklung von
Limnogeton fieberi Mayr (Belostomatidae:Heimiptera)
as Beitrag zur Kenntnis von natürlichen Feinden
tropischen Süßwasserschnecken. Entomologische

Weis-Fogh, T. 1952. Fat combustion and metabolic rate
of flying desert locusts. Philos. Trans. R. Soc.
London (B) 237:1-36.

Weis-Fogh, T. 1970. Structure and formation of insect

Weiss, P. and W. Ferris. 1956. The basement lamella of
amphibian skin. Its reconstruction after wounding.


Wesenburg-Lund, C. J. 1919. Contributions to the knowl­
edge of the postembryonal development of the Hydra­

Wigglesworth, V. B. 1956. Formation and involution of
striated muscle fibres during the growth and molting
cycles of Rhodnius prolixus (Hemiptera). Q. J.

Wigglesworth, V. B. 1964. The hormonal regulation of
growth and reproduction in insects. Adv. Insect
Physiol. 2:247-336.


ACKNOWLEDGMENTS

I would like to express my appreciation to the members of my committee for their interest and advice. My deepest thanks go to Dr. E. R. Hart for his understanding and supervision throughout the research and preparation of this dissertation. I also wish to express my gratitude to Drs. E. S. Krafsur, W. C. Rowley, and C. J. Ellis for their help and cooperation in allowing me to use equipment and facilities belonging to their laboratories. For additional assistance in collecting specimens and in preparation of the manuscript, I am also indebted to the following: Dennis Green, Scott Ritchie, Tom Chandler, and Kristine Elvin, all of the Entomology Department, Iowa State University; and to Dr. H. Dingle, Department of Zoology, University of Iowa.

To Clare, my wife, who spent long hours helping in laboratory research and manuscript preparation, always maintaining a steady sense of humor, I sincerely appreciate her tireless efforts and encouragement.

And thanks goes to Mrs. Harriet Markel of El Paso, Texas, for typing the manuscript.