



Biological systematics of *Zaitzevia thermae* (Hatch)
by Mark Mitchell Hooten

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Entomology
Montana State University
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Abstract:

Zaitzevia thermae (Hatch) is a riffle beetle restricted to a small warm spring in the Bozeman, Montana, area. It is of interest to systematists and ecologists due to its limited range and thermophilic habits. Additionally, *Z. thermae* is found literally next to its sister species, *Zaitzevia parvula* (Horn), a cold water inhabitant found commonly in foothill streams. The basic problem in understanding the two species lay in their cryptic nature with respect to each other.

A systematic analysis of *Z. thermae* was undertaken to determine its taxonomic identity. Analysis of *Z. thermae*'s morphology, ecology, and genetic identity was pursued.

Results revealed morphological and isozyme markers that clearly identify *thermae* and suggest that it is reproductively isolated from regional populations of *Z. parvula*. Investigations into the thermal ecology of the two species indicates that they have different preferences.

It is concluded that *thermae* is a distinct taxonomic unit at the species level.

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MONTANA STATE UNIVERSITY
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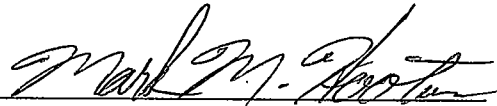
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TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	viii
NOMENCLATURE	ix
ABSTRACT	x
INTRODUCTION	1
TAXONOMIC HISTORY	6
Introduction	6
Synonymy of <u>Zaitzevia thermae</u> (Hatch)	6
MORPHOLOGICAL ANALYSIS	8
Introduction	8
Methods and Materials	9
Standard Analysis	9
Morphometric Analysis	11
Larval Instar Analysis and Morphology	12
Results	13
Standard Analysis	13
Pronotum	13
Wings	15
Metendosternite	16
Genitalia	16
Morphometric analysis	21
Larval Instar Analysis	22
Larval Morphology	23
ECOLOGY	25
Introduction	25
Respiration	26
Avoidance	27
Dispersal	30
Development and Thermal Ecology	31
Methods and Materials	33
Observational	33
Experimental	33

TABLE OF CONTENTS - Continued

	Page
ECOLOGY - <u>Continued</u>	
Results	36
Observational	36
Experimental	37
ELECTROPHORETIC ANALYSIS	41
Introduction	41
Methods and Materials	43
Results	45
CONCLUSION	53
Summary	64
REFERENCES CITED	66
APPENDICES	75
Appendix A - Conservation Management of <u>Zaitzevia</u> <u>thermae</u> (Hatch)	76
Appendix B - Materials Studied of <u>Zaitzevia</u> Champion	81

LIST OF TABLES

Table	Page
1. Summary of ANOVA on size (volume) of <u>Zaitzevia</u> spp. ...	21
2. Summary of LSD on size (volume) of <u>Zaitzevia</u> spp.	22
3. Summary of ANOVA on pronotal proportional size	22
4. Summary of LSD on pronotal proportional size	23
5. Summary of ANOVA on thermal gradient analysis	39
6. Summary of LSD on thermal gradient analysis	40
7. Tray and gel buffers used in electrophoresis with corresponding loci examined	44
8. Allelic frequencies at all observed loci in electrophoresis	46
9. Genetic similarity and distance values for populations examined	48
10. Mean heterozygosity values for populations examined ..	49

LIST OF FIGURES

Figure	Page
1. Map of localities from which <u>Zaitzevia</u> spp. were collected for this study	10
2. Habitus photograph of <u>Zaitzevia thermae</u> (Hatch), BCWS, and <u>Zaitzevia parvula</u> (Horn), NS	14
3. Wing of <u>Zaitzevia thermae</u> , BCWS	15
4. Wing of <u>Zaitzevia parvula</u> , BC	17
5. Wing of <u>Zaitzevia parvula</u> , NS	18
6. Wing of <u>Zaitzevia parvula</u> , SFS	19
7. Wing of <u>Zaitzevia</u> sp., BRWS	19
8. Metendosternites of <u>Zaitzevia</u> spp.	20
9. Histogram of larval head widths of <u>Zaitzevia thermae</u> , BCWS	24
10. Photograph of thermal gradient bar	34
11. Histograms of thermal gradient trials	38
12. WPGMA tree of <u>Zaitzevia</u> spp.	51
13. Distance Wagner tree of <u>Zaitzevia</u> spp.	52

NOMENCLATURE

List of Textual Acronyms *

1. ANOVA - Analysis of Variance
2. BC - Bridger Creek
3. BCWS - Bridger Canyon Warm Spring
4. BFCDC - Bozeman Fish Cultural Development Center
5. BRWS - Brooks Warm Spring
6. LPPC - Little Prickly Pear Creek
7. LSD - Least Significant Difference
8. MR - Musselshell River
9. MSU - Montana State University
10. MTEC - Montana State University Entomology Collection
11. NS - Nimrod Spring
12. OTC - Otter Creek
13. PWS - Potosi Warm Spring
14. SFS - Snowflake Spring
15. UPGMA - Unweighted Pair-group Method with Arithmetic Averaging
16. USFWS - United States Fish and Wildlife Service
17. WFS - West Fork Spring
18. WPGMA - Weighted Pair-group Method with Arithmetic Averaging
19. WSC - Warm Spring Creek

* Acronyms used as citations are found in REFERENCES CITED.

ABSTRACT

Zaitzevia thermae (Hatch) is a riffle beetle restricted to a small warm spring in the Bozeman, Montana, area. It is of interest to systematists and ecologists due to its limited range and thermophilic habits. Additionally, Z. thermae is found literally next to its sister species, Zaitzevia parvula (Horn), a cold water inhabitant found commonly in foothill streams. The basic problem in understanding the two species lay in their cryptic nature with respect to each other.

A systematic analysis of Z. thermae was undertaken to determine its taxonomic identity. Analysis of Z. thermae's morphology, ecology, and genetic identity was pursued.

Results revealed morphological and isozyme markers that clearly identify Z. thermae and suggest that it is reproductively isolated from regional populations of Z. parvula. Investigations into the thermal ecology of the two species indicates that they have different preferences.

It is concluded that Z. thermae is a distinct taxonomic unit at the species level.

INTRODUCTION

Zaitzevia thermae (Hatch), (Elmidae Coleoptera), is restricted to Bridger Canyon Warm Spring (BCWS), a warm spring of 21°C average temperature, in Gallatin County, Montana (MBMG 1981). It is unusual, though not unique, among elmids because of its restricted range and thermophilic habits. The other North American member of the genus is the sister-species, Z. parvula (Horn), which is widespread and common throughout Western North America. This makes Z. thermae a unique organism for systematic investigation. A brief summary of our recent understanding of, and interaction with Z. thermae, will elucidate the current importance of such an investigation.

Zaitzevia thermae is a microphytophage (Brown 1987, Chapman and Demory 1990), found mostly on the lithic substrate of Bridger Canyon Warm Spring (BCWS). Like other elmids, however, Z. thermae is commonly found amid aquatic vegetation, and may derive some of its oxygen supply from aquatic plants (Thorpe and Crisp 1949, Brown 1987). The species' members are flightless, and their life cycle is restricted entirely to the warm spring environment.

Bridger Canyon Warm Spring is located on United States Fish and Wildlife Service (USFWS) land emptying directly into Bridger Creek (BC), a typical foothill stream, at the

base of Drinking Horse Mountain (MBMG 1981). The elevation of BCWS is approximately 1518 m. and the universal transverse mercator measurement is 502.10 east by 5061.33 north (USGS 1987). The spring's source is in the Madison limestone group (MBMG 1981) and the topographic surface of the spring occupies an area of approximately 35 square meters.

Bridger Canyon Warm Spring is one of several naturally occurring warm springs in Montana whose waters originate in the Madison limestone geologic group (MBMG 1981). These springs are of approximately $\geq 10,000$ years of age, many of which are of Pleistocene age or older (Dr. John Montagne, personal communication, Mitchell 1974). Most of these springs have similar water chemistry and average temperatures (16°C to 30°C). Flow rates may vary dramatically from spring to spring (300 to 273,000 liters/minute). In comparison, BCWS exhibits relatively low flow (300 to 1000 l/min.). (See MBMG (1981) for details on water chemistry, temperature and flow).

Less than five square meters of BCWS is habitable by Z. thermae due to anthropogenic alterations in the spring environment. The Bozeman Fish Cultural Development Center (BFCDC) is located approximately one-quarter of a mile south of BCWS and utilizes water from the spring in hatchery operations. Since its construction in 1890, the BFCDC has utilized water from BCWS for fish culture.

In the early years (approximately 1890 to 1920) water was piped directly from the spring to the BFCDC with little modification to the spring. Though the exact date is not known, an open-topped spring house was constructed in this early period, raising the level of the spring. However, shortly after Emma M. Braton reportedly committed suicide in the spring house on March 22, 1918, a cover was constructed to avoid subsequent incidents (Gallatin County Hall of Records, BFCDC records).

The first cover(s) on the spring house were made of wood. A wooden cover probably allowed light to penetrate the joints between boards, thus allowing algal and planktonic growth inside the spring house (BFCDC records). Thus, the population of beetles was likely able to persist inside the spring box as long as light entered. Evidence of this is revealed in a collection of Z. thermae by D.J. Pletsch, 6 March, 1938, "from impounded water" (Montana Entomology Collection (MTEC), Montana State University).

The history of the spring box becomes obscured in BFCDC records after 1938. Local residents recall a metal cover being installed in the early 1950's (Ina Denton and Melvin Osborn, personal communication). The structure was reroofed with corrugated iron in the early 1970's (Carlie Smith, BFCDC Director, personal communication). This structure persists to the present time. These metal covers completely exclude light from the spring box, thus eliminating it as

suitable long-term habitat for Z. thermae.

The USFWS lists Z. thermae as a Category 2 candidate species (USDI 1989). A Category 2 candidate is a species under consideration for protection under the Federal Endangered Species Act (Category 1 Species). Category 2 species are given Category 1 status or Category 3 status (species of special concern) only after a clear understanding of their biological and population status is established. An understanding of the taxonomic, biological, and ecological status of Z. thermae is therefore critical to making sound management decisions designed for preserving the species as well as perpetuating historically important human activities in the area (i.e. BFCDC operations).

However, the taxonomic status of Z. thermae has been uncertain due to the morphological similarities it shares with Z. parvula. In addition, the two species occur literally side-by-side (Z. parvula is abundant in BC). Thus, uncertainties of the taxonomic status of Z. thermae have demanded an in-depth approach to the biological systematics of the species.

Since its description, Z. thermae (Hatch 1938) has received little attention in the taxonomic and ecological literature. Various authors have dealt haphazardly with its taxonomy, speculating over the validity of the species without systematic study (Hinton 1939a; Sanderson 1954; Hatch 1965; Brown 1972). In the latest review of North

American Elmidae, Brown (1972) treats Z. thermae as a possible ecological variant of the sister-species Z. parvula.

In testing the hypothesis that Z. thermae is a valid species, I will also examine the null hypothesis that Z. thermae is not a biological species. This will be undertaken with a multifaceted systematic approach including: 1) the taxonomic history of Z. thermae; 2) a morphological comparison of the skeletal morphology of Z. thermae and Z. parvula adults and larvae; 3) a descriptive and experimental examination of the ecology of Z. thermae, with particular reference to thermal ecology; and 4) an isozyme electrophoretic analyses for comparison of identified allelic frequencies in Z. thermae and Z. parvula populations.

TAXONOMIC HISTORY

Introduction

The first records of Z. thermae were collected by C.J.D. Brown of Montana State University from Bridger Canyon Warm Spring on 6 December, 1936 (Hatch 1938). Holotype and paratype specimens were housed in the Hatch collection, and are now in the collections at the National Museum of Natural History, Washington, D.C. and Oregon State University, respectively.

Subsequent records are widely separated, chronologically. Since its description, the species was collected by Pletsch, 6 March 1938 (MTEC), and not again until 16 January, 1987, by M.A. Ivie and D.L. Gustafson. Since that time, Z. thermae has been observed in situ repeatedly by D.L. Gustafson, M.A. Ivie, and M.M. Hooten (also see Appendix B).

Despite the lack of specimens of Z. thermae, various authors have synonymized Z. thermae with or split it from its sister-species Z. parvula, without apparent justification. The taxonomic history of Z. thermae can best be summarized as follows.

Zaitzevia thermae (Hatch)

Macronychus thermae Hatch 1938 : 18.

Zaitzevia thermae: Sanderson 1954 : 11. Brown 1972 : 21.

Zaitzevia parvulus (not Horn) : Hinton 1939 : 181 (in part).

Zaitzevia parvula (not Horn): Hatch 1965 : 11 (in part).

Hinton (1936) moved Macronychus parvulus Horn (type locality, California) to Zaitzevia following Champion's (1923) generic description. Hatch (1938) described Z. thermae from Bridger Canyon Warm Springs, near Bozeman, Montana, in Macronychus, apparently missing Hinton's work.

Hinton (1939a) synonymized M. thermae Hatch with Z. parvulus, but Sanderson (1954) resurrected it under the new combination Z. thermae. Hatch (1965) missed or chose not to follow Sanderson's view and listed Z. thermae as a synonym of Z. parvula.

Brown (1972) discussed Z. thermae as a possible ecological variant of Z. parvula, but recognized it as a valid species.

MORPHOLOGICAL ANALYSIS

Introduction

Identification of sympatric sister-species is often difficult on morphological grounds due to lack of divergence (Wiley 1981) and an inexactness of ecological scale involved in capture techniques (Allen and Star 1982, Wiens 1989). This may lead to errors in the recognition of sister-species (Howard 1983, Crego et. al. 1990).

Hinton (1939a) synonymized Z. thermae with Z. parvula on the basis of gross morphological similarities. However, even a cursory comparison showed a subtle, but constant difference in habitus. Therefore, a comparative and detailed investigation of skeletal morphology of both species was undertaken in order to test Hinton's hypothesis.

In addition to adult morphology, larval morphology was pursued in the same vein. Separation of congeneric larvae at the species level has proven to be a difficult task in the Elmidae and has met with little or no success (Brown 1972, Brown and White 1978). In order to make direct comparisons between instars, a larval instar analysis of Z. thermae preceded the morphological comparisons of sister-taxa larvae.

Methods and Materials

Standard Analysis

Methods similar to those of Hinton (1939b), Doyen (1966), and Brown (1981) were employed in a sclerite-by-sclerite comparison of Z. thermae and Z. parvula. Fresh specimens were soaked in lactic acid for at least 24 hours prior to dissection in order to clear soft tissues. Sclerites were then removed, put in glycerine mounts and examined under dissecting and compound microscopes.

Dorsal, ventral, and lateral views were compared and a series of working drawings were made using a drawing tube on Wild dissecting and compound microscopes. Drawings, as well as side-by-side comparisons of sclerites served to identify structural differences between the species.

Specimens dissected included approximately fifty Z. thermae from BCWS, and seventy Z. parvula from West Fork Spring (WFS), Snowflake Spring (SFS), and Bridger Creek (BC). In addition, wing and metendosternite morphology were examined in Z. parvula from Nimrod Spring (NS), Potosi Warm Spring (PWS), and Brooks Warm Spring (BRWS). Specimens from each locality have been deposited as vouchers in the MTEC (Appendix B). (See Figure 1 for a map of localities of materials utilized in this and all subsequent analyses).

Since it was not the purpose of this study to redescribe the sister-taxa but to elucidate potential morphological differences, discussion and illustrations are

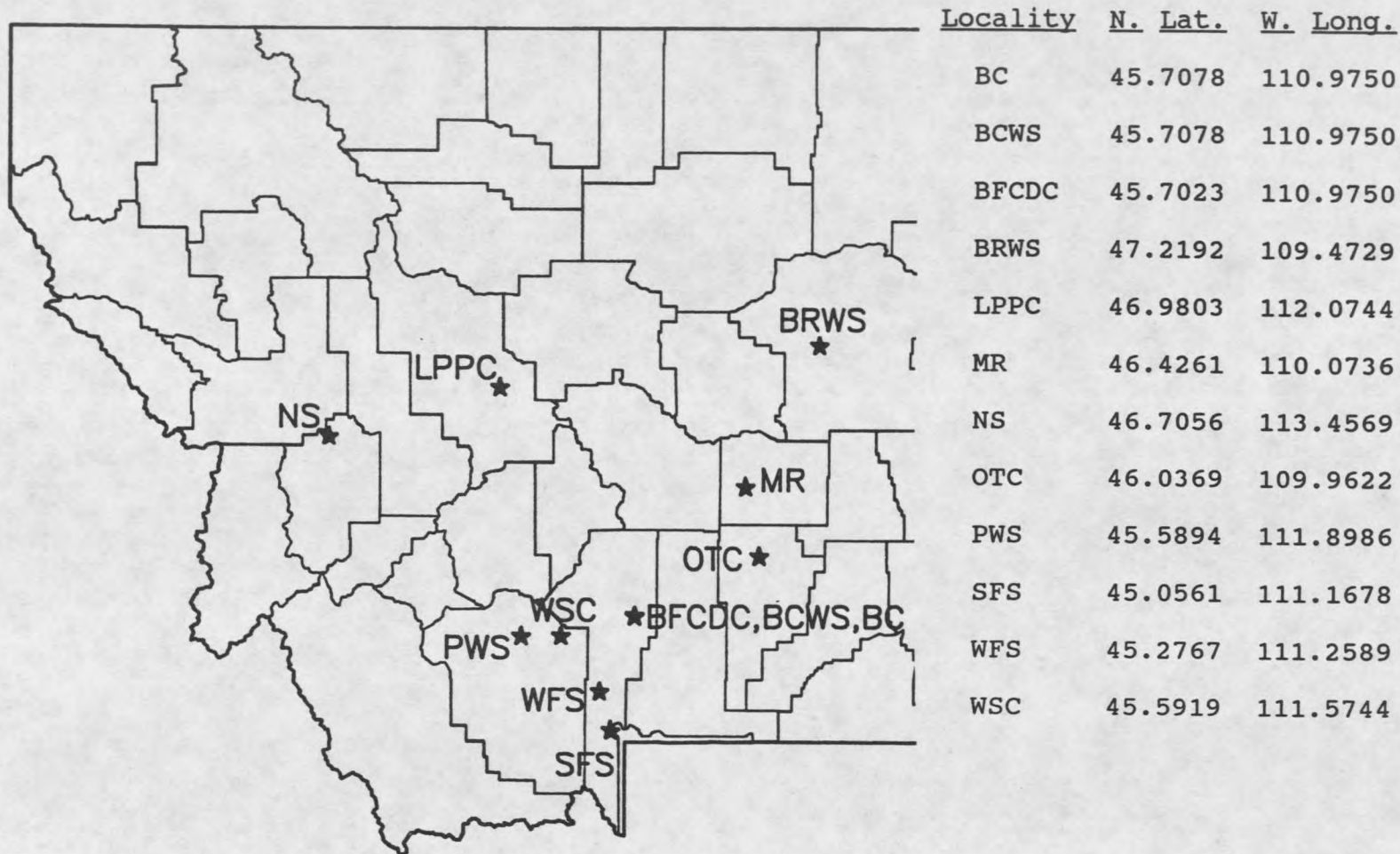


Figure 1. Map of Western Montana showing localities of collection of *Zaitzevia* spp. See list of textual acronyms (page ix) and text for further explanation.

limited to divergent morphological characters, with additional mention of genitalia.

Morphometric Analysis

In addition to standard morphological techniques, morphometric analyses were used to measure potential differences in overall size and proportion between populations. Six mixed-sex populations of Zaitzevia spp. were used for these analyses, in order to cover the breadth of temperature regimes that Zaitzevia occupies. Two cold stenothermic populations (WFS and SFS), two cold eurythermic populations (BC and Musselshell River (MR)), and two warm stenothermic populations (BCWS and BRWS) were represented. Twenty-five specimens were measured from WFS, SFS, BC, and BCWS, eleven from MR, and seventeen from BRWS. All specimens represent point-specific localities, yet most are from variable dates of collection (Appendix B). This was done purposefully to avoid bias of potential seasonal effects.

Measurements included pronotal length and width, as well as elytral length. Pronotal length was measured from the posterior edge of the pronotum at the anterior-most point adjacent to the scutellum, to the apex of the anterior pronotal curve. Pronotal width was measured at the widest point. Elytral length was measured from the median pronotal juncture to the posterior tip of the elytra. Specimens were measured with an ocular micrometer under 40X magnification

with 10X oculars on a Nikon dissecting microscope and calibrated with a Minitool device. Measurements were accurate to within $\pm 1/2$ of an empirical unit (1 unit=0.0194 mm). All specimens examined were dry when measured.

All measurements were added to achieve a gross measure of individual size (referred to herein as total size), and pronotal length was divided by pronotal width to determine relative proportional size. ANOVA models were employed to analyze the data on MSUSTAT (Lund 1988). Mean population total size measurements were analyzed using a fixed model one-way ANOVA. Multiple comparisons of means were analyzed utilizing LSD procedures following the ANOVA. Proportional values were transformed by $2\arcsin(\text{square root} [\text{proportion}/2])$ and analyzed under the same assumptions as above. Values under this transformation are positively correlated with increasing proportion. Null hypotheses of no significant total size and proportion differences between populations were tested.

Larval Instar Analysis and Morphology

Morphological examination of larvae was necessarily preceded by an analysis of larval instars so that larvae of the same developmental stage could be examined. One hundred sixty-two and four hundred fifty-two larvae of Z. thermae collected by D.L. Gustafson on June 20 and November 1, 1987, respectively, were examined for head capsule diameter. Head

capsule measurement techniques for the determination of instars in Coleoptera larvae were reviewed by Bartell and Roberts (1974) and Crowson (1981). A multimodal distribution of head width measurements is hypothetically indicative of distinct developmental stages (Crowson 1981).

Larvae were removed from alcohol and blotted on Kimex paper to remove excess liquid. Head capsules were removed under a Wild dissecting scope and placed in rows in a thin, uncovered, glycerine slide mount. An ocular micrometer was placed in the Wild dissecting scope with 20X oculars and calibrated using a Minitool device. Head capsules were measured under 50X across the occipital bulge and widths were recorded on an empirical scale from 7 to 22. Translation of empirical widths is from 0.128 to 0.403 mm (0.0183 mm per empirical unit). Histograms of measurement data were constructed to indicate larval instar classes of Z. thermae.

Results

Standard Analysis

Analyses of the skeletal features revealed three anatomical markers distinguishing Z. thermae and Z. parvula. These include differential curvature of the pronota, and variations in the form of the wings and metendosternites. Individual features are discussed below.

Pronotum. The pronotum of Z. thermae is strongly

downcurved at the lateral margins. When viewed from above, the tooth of the anterior angles appears acutely produced from the anterior pronotal margin (Figure 2). Lateral rotation of the pronotum reveals this illusion, showing the tooth to be, in truth, sinuously produced.

The pronotum of Z. parvula is less strongly downcurved at the lateral margins. When viewed from above, the tooth of the anterior angles appears smoothly produced from the anterior pronotal margin (Figure 2).

These differences appear to be consistent, even in relatively large Z. thermae and small Z. parvula, respectively.

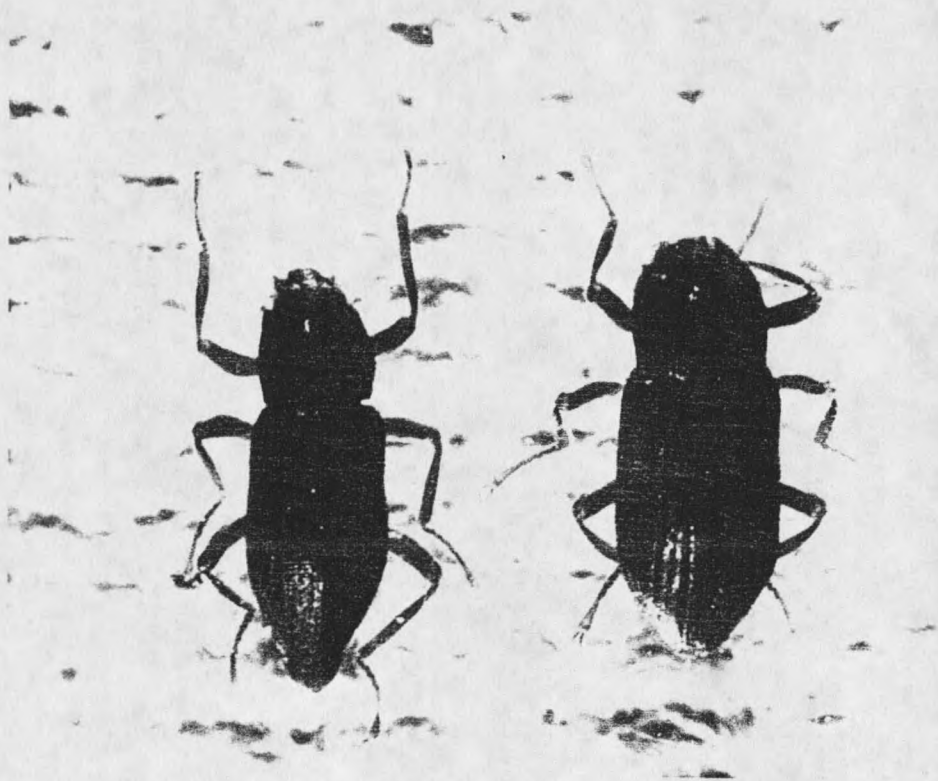
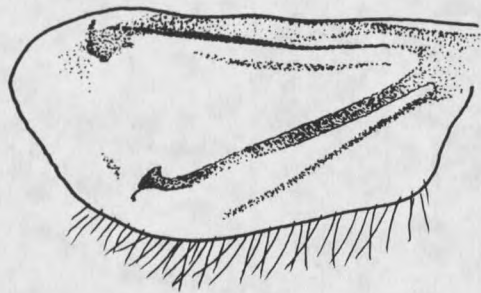


Figure 2. Habitus of Zaitzevia thermae and Z. parvula, NS. Note the slender form of Z. thermae. See text for further explanation.

Wings. Hind wings in Z. thermae are vestigial (Segal 1933) showing reductions in relative length, venation, and setation (Figure 3).



1 mm.

Figure 3. Wing of Z. thermae . See text for further explanation.

The distal membranous region is greatly shortened, with the loss of some thickened areas (flecks) found in the region in Z. parvula (Figures 4 through 7). Wings in Z. thermae are $\leq 2/3$ the length of the elytra. Those in Z. parvula are consistently $> 2/3$ the length of the elytra, with NS and SFS populations exhibiting the relative extremes of development (Figures 5 and 6, respectively). Zaitzevia parvula, PWS, BC, WFS, and BRWS exhibited intermediate wing development.

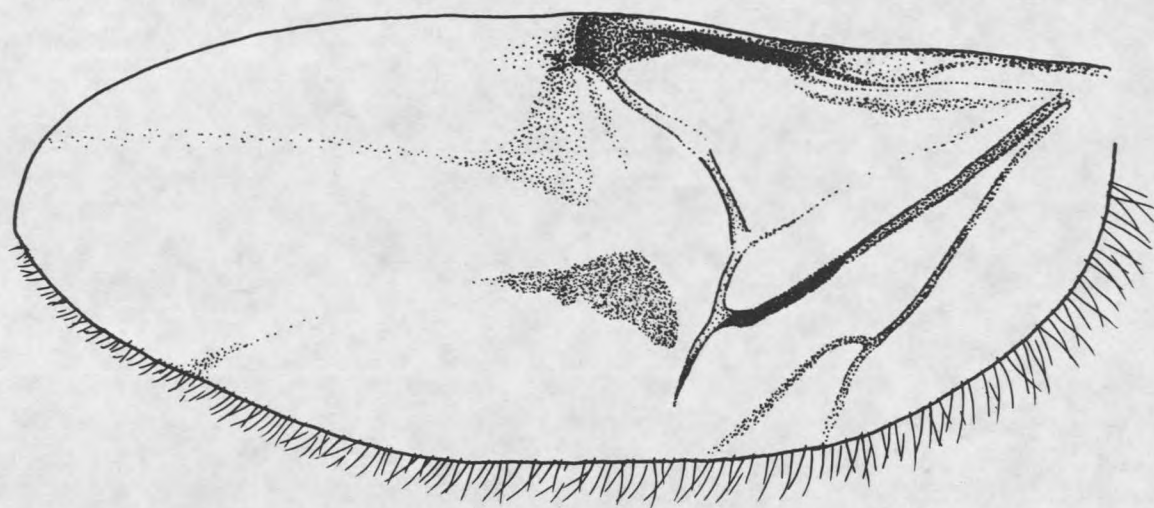
Venation in the wing of Z. thermae is also reduced, particularly in the radial region (sensu Crowson 1955). In all Z. parvula populations examined, complete wing venation appeared to be conserved in all size classes (Figures 4

through 7).

Wing surfaces of all Zaitzevia are microtrichiate, a feature omitted from illustration for the sake of clarity. Marginal setation follows the posterior edge of the wings from the proximal side of the anal angle proceeding distally. In Z. thermae, setation extends to the cubital region, thus not extending to the most distal tip of the wing (Figure 3) as it does in Z. parvula (Figures 4 through 7).

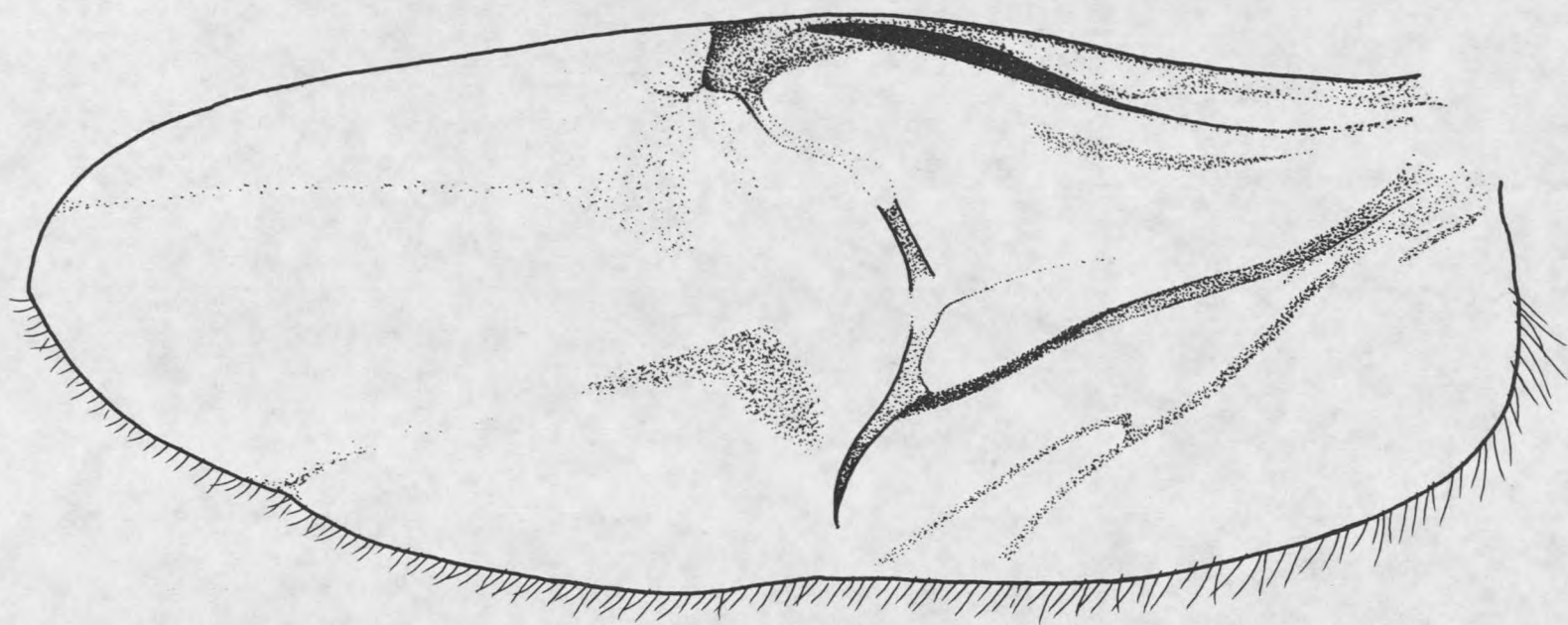
Metendosternite. Although typically used as a higher taxonomic character (Crowson 1938, 1955), the metendosternite proved to be a key character differentiating the sister taxa. The metendosternite in Z. thermae is reduced to a simple T-shaped structure, completely lacking lobes on the apophyseal arms (Figure 8). All Z. parvula examined showed marked lobation on the anterioventral side of the apophyseal arms (Figure 8). The size of the lobes appears to vary somewhat with wing size, as one may expect (Doyen 1966), yet Z. parvula exhibits apophyseal lobes across the range of variation.

Genitalia. Examination of the genitalia of both sexes revealed no significant differences between the taxa. In fact, it would appear that genitalia are mechanically compatible, possessing no "lock and key" mechanism (Shapiro and Porter 1989). Undoubtedly, this finding leaves open the



1 mm.

Figure 4. Wing of Z. parvula, BC population. See text for further explanation.



18

1 mm.

Figure 5. Wing of Z. parvula, NS population. See text for further explanation.

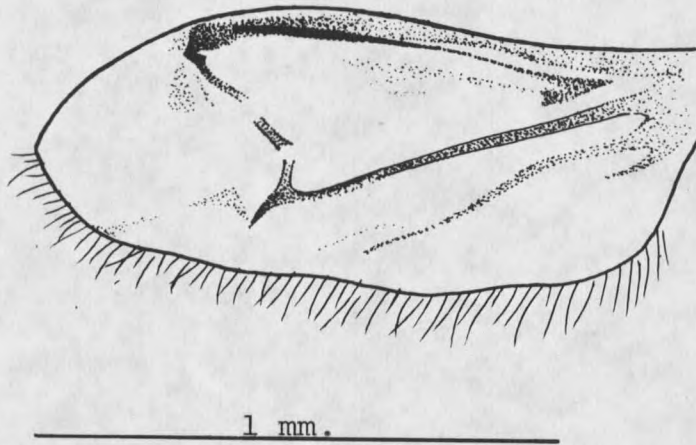


Figure 6. Wing of *Z. parvula*, SFS population. See text for further explanation.

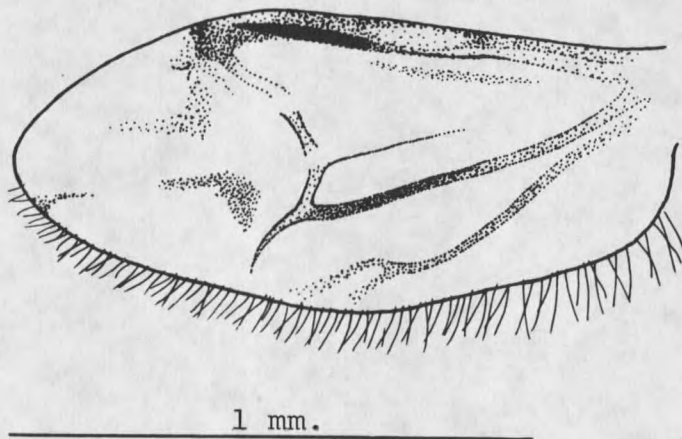


Figure 7. Wing of *Z. parvula*, BRWS population. See text for further explanation.

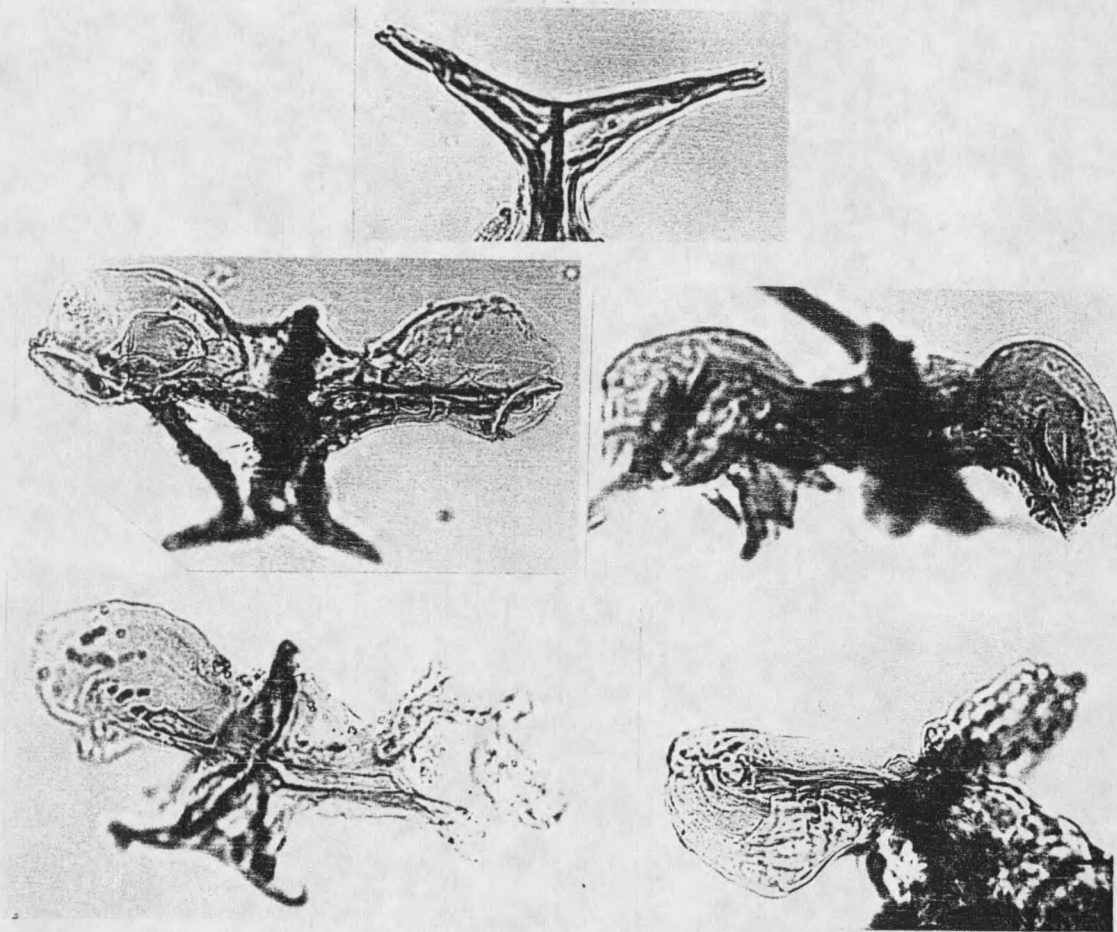


Figure 8. Oblique views of metendosternites of *Zaitzevia* spp. Top row: BCWS. Middle row: BC (left), NS (right). Bottom: SFS (left), BRWS (right). Apophyseal arms are simple in *Z. thermae*, BCWS (top), and lobed in all *Z. parvula*, (middle and bottom). See text for further explanation. Photographs taken at 400X magnification; enlargement = 4X.

question of simple developmental variability between ecotypes as Brown (1972) has remarked. This question is the topic of further investigation in the following two chapters.

It is noteworthy that in both *Z. thermae* and *Z. parvula*, sexes can be externally determined by the

presence of an emarginate pygidium in males and a complete pygidium in females.

Morphometric Analysis

ANOVA models revealed distinct differences in total size and proportion between populations, and with respect to the thermal environment of origin (i.e. warm or cold stenothermic, or cold eurythermic (Tables 1 and 3, respectively). LSD procedures were used to make pair-wise

Table 1. ANOVA on between-population variation in total size. Values are on an empirical scale. See text for further explanation.

Source	DF	SS	MS	F-value	P-value
Btw/populations	5	13109.0	2621.8	74.27	0.0000
Within pops.	121	4271.1	35.299		
Total	126	17380.0			

comparisons between populations. Results of pair-wise comparisons are presented in Tables 2 and 4 for volume and proportion, respectively. It is apparent that Z. thermae stands alone in total size and is functionally classified with warm water types, proportionally. Both measures, therefore, may be useful in its identification. It is noteworthy that Z. thermae is proportionally longer than any of the cold water Z. parvula, with the marginal exception of WFS beetles. This substantiates the observation of a relatively more strongly downcurved pronotum in Z. thermae for those Zaitzevia examined above.

Table 2. Summary of LSD comparisons of total size means of populations. Measurements are left on an empirical scale for comparison with values in ANOVA Table 1. Equal letter codes in column 4 indicate no significant difference between respective means. (Standard errors are shown in parentheses). See text for further explanation.

Population	N	Mean size: 1 unit = 0.0194 millimeters	Non-significant differences between populations
BC	25	152.1 (4.1)	C
MR	11	154.5 (6.7)	CD
SFS	25	164.8 (4.6)	E
WFS	25	156.5 (7.5)	D
BRWS	17	133.0 (5.6)	A
BCWS	25	141.6 (6.4)	B

Table 3. ANOVA on between population variation in proportional size (length/width) of pronota. Proportional values were transformed by $2\arcsin(\text{sq. root} [\text{prop.}/2])$. See text for further explanation.

Source	DF	SS	MS	F- value	P-value
Btw/populations	5	0.08845	0.01769	12.24	0.0000
Within pops.	122	0.17629	0.001445		
Total	127	0.26474			

Larval Instar Analysis

Figure 9 summarizes data collected from head capsule width measurements. The distribution is not clearly multimodal and is therefore subject to slightly different interpretations. In keeping with other studies on elmid larval development (White 1978a, Brown 1987), a five instar larval ontogenesis would appear to be a conservative hypothesis. Instar head widths correspond roughly to the

Table 4. Summary of LSD comparisons of population proportional means. Proportions are transformed by $2\arcsin(\text{sq. root} [\text{prop.}/2])$. Equal letter codes in column 4 indicate no significant difference between respective means. (Standard errors are shown in parentheses). See text for further explanation.

Population	N	Mean proportion (transformed).	Non-significant differences between populations
BC	25	1.461 (7.8E-3)	A
MR	11	1.489 (1.24E-2)	BC
SFS	25	1.487 (7.1E-3)	B
WFS	25	1.514 (5.4E-3)	CD
BRWS	17	1.534 (8.5E-3)	D
BCWS	25	1.528 (9.7E-3)	D

dimensions shown in Figure 9.

The lack of a distinctly multimodal distribution, especially with early instars, is, in all likelihood, an artifact of sampling techniques (net mesh diameter) and error in retrieving larvae from a bulk aquatic sample. In light of the potential effects of bulk sampling on the population of Z. thermae, no further samples were taken for this analysis.

Larval Morphology

Side-by-side morphological comparisons of approximately twenty larvae of Z. thermae and Z. parvula, WFS population, revealed no character differences between the species. This is similar to findings of earlier workers on the Elmidae (Brown 1972, Brown and White 1978, White 1978b).

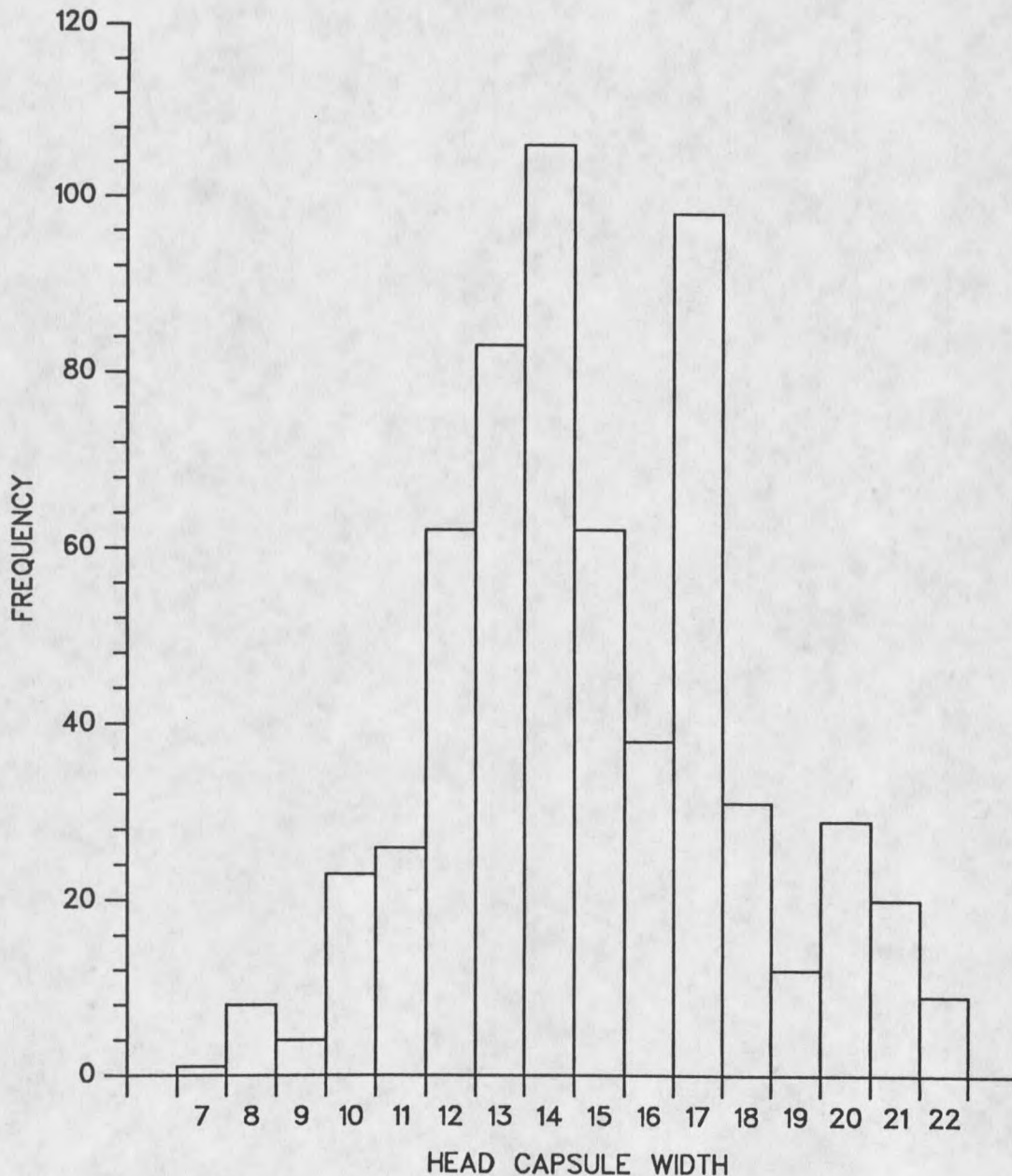


Figure 9. Distribution of head capsule measurements of *Z. thermae* for N=614 measurements. Frequency (Y axis) is the number observed in respective width classes (X axis). Widths are on an empirical scale of 1 unit=0.0183mm. Each class is accurate to within 0.00541mm. Hypothesized distribution of instars are: 1) 7-9; 2) 10-12; 3) 13-15; 4) 16-18; 5) 19-22. See text for further explanation.

ECOLOGY

Introduction

As with most elmids beetles, Z. thermae is a slow moving microphytophage on the gravelly substrate of its aquatic environment. Larvae and adults can be found virtually year-round in BCWS (see INTRODUCTION). Adults are probably long-lived (>1 year), as indicated by the presence of calcarious deposits on some individuals in their moderately calcium-rich environment (MBMG 1981, Brown 1987). Adults have been kept alive in an aquarium with little maintenance for six months (personal observation), not an exceptionally long time for elmids in captivity (White and Jennings 1973, Brown 1973).

I will consider five aspects of elmids beetle ecology and behavior. These include feeding, reproduction, respiration, avoidance, and dispersal. Mandibles of Zaitzevia spp. are typical of those of adult elmids, adapted for scraping microalgal deposits and diatoms from rock surfaces (Merritt and Cummins 1984). Feeding is consequently slow, and beetles have been observed grazing at a single pebble for hours (personal observation). Reproductive biology is, no doubt, complex, yet little or no work has addressed behavioral issues on this subject. It must suffice to say that Zaitzevia adults may be found in copula most of the year, and the males cling tenaciously to

their mates (Brown 1987, personal observation).

Respiration, avoidance, and dispersal are complex issues, yet some background is pertinent to this study of Zaitzevia.

Respiration

Elmid beetles (with the exception of the Larini) remain submerged all of their adult life except during a brief dispersal flight following eclosion (in flighted species). They respire via a plastron, a mechanical "gill" created by a dense layer of hydrofuge setae (10^5 to 10^6 setae/cm² (Thorpe and Crisp 1949)) on their abdomen, thorax, and femora. A number of behaviors are associated with the maintenance of, and oxygen exchange within, the plastron. Combing the plastron with femoral and tibial combs is common in maintaining setal associations and to aid in replenishing the plastron with new air (Thorpe and Crisp 1949). Additionally, elmids will often associate themselves with aquatic vegetation taking advantage of gas bubbles produced by the plants. By first seizing the bubble with their mouth, then utilizing their combs, they move the bubble along their ventral surface until the oxygen-rich gases can be worked mechanically into the space between their elytra and the dorsal surface of their abdomen. The plastron is thickest (and best protected) and is most highly active in gas exchange on the dorsum of the abdomen (Thorpe and Crisp 1949).

Elmids in poorly oxygenated waters may actively force

water around their body. By adopting an erect posture they may be able to increase water circulation around their body (Thorp and Crisp 1949). This behavior was commonly observed in the afore-mentioned aquarium cultures of Z. thermae, as was "push-up" behavior. This may well have been an additional way of forcing water past the plastron just as stoneflies force water past their gills in poorly oxygenated environments (Merritt and Cummins 1984). Push-ups were observed in solitary animals as well as those in relative proximity to others, thus it would not appear to be a social or reproductive behavior. The above behaviors were subsequently observed in situ, but warrant further investigation.

Zaitzevia thermae is commonly associated with mosses (Bryophyta) and watercress (Nasturtium officinale), a nearly ubiquitous inhabitant of springbrooks (Pennak 1990). Vegetational "mats" are a seasonal phenomenon in BCWS, restricted to summer, autumn, and winter. Zaitzevia thermae is commonly found amongst the vegetation in BCWS and is by no means restricted to the lithic substrate. Thermal spring waters are naturally low in dissolved oxygen, thus the beetles' associations with aquatic plants is not surprising.

Avoidance

The water temperature of Z. thermae's environment is, perhaps, the most influential factor in the species' biology (Vannote and Sweeney 1980, Ward and Sanford 1982). An

investigation into how temperature may influence behavior and development seems paramount to an investigation of a thermophilic species. First, however, a more detailed background on BCWS and natural events affecting it is important.

Flow rates and temperature of BCWS are affected seasonally by ground water infiltration rates from surface sources (spring melt, summer rains). Flow and temperature appear to be inversely related. Flow is lowest in winter and highest in early summer; the reverse is true for temperature (see Results, Observational).

Seasonal variation in creek level and temperature dramatically affect surface temperatures in the warm spring. Thermal outlets below creek level (low rises) are affected on an ongoing basis, while those above mean summer creek level (high rises) are affected most dramatically by annual floods. Flooding of high rises can occur year-round but primarily from late March through June. Floods reach their maximum in mid-to-late spring and have been known to inundate the spring box (USFWS records). Flood water is cold, near 0°C, and has enormous scouring effects on BCWS.

The topographic character of BCWS changes annually due to the scouring process as well as mass wasting from the adjacent cliff-side. Mass wasting tends to affect the lithic texture of the spring by adding stones of 10 cm diameter and less, in large quantities. Large boulders

occasionally tumble into BC which adds barriers to flow through the low rise areas (personal observation).

Large boulders disrupt creek flow thus creating thermal "reservoirs" midstream. These reservoirs may be beneficial to Z. thermae since they are occupied by the beetles during, at least, the months of summer and autumn. Low rises that are relatively unprotected do not display the frequency of use by Z. thermae. Floods reduce or eliminate thermal reservoirs at both low and high rises, and force Z. thermae to seek refuge (personal observation).

Beetles avoid flood waters by retreating into the hyporheic zone, where thermal gradients are, no doubt, retained and more constant (Ward and Stanford 1982). Survival for long periods in the hyporheic zone is apparently possible for Z. thermae, since relatively severe flooding can last for three to four months. In California, adults of Z. parvula have been observed alive at 40 cm depth in the hyporheos where they maintained contact with water of a drying stream bed. (Bell 1972).

Aquatic insects commonly retreat into the hyporheic zone in order to avoid extreme conditions of temperature, flow, shifting substrates, and heavy sediment lode (Hynes 1970, McClelland and Brusven 1980, Ward and Stanford 1982, Fisher et. al. 1982).

The habit of cold water avoidance is, in all likelihood, strongly developed in Z. thermae (see Results,

Observational). Additionally, drift, a behavior common to elmids (Brusven 1970, Newman and Funk 1984, Brown 1987), would, hypothetically, be counterproductive for this species, given its apparent restriction to thermal water. However, cold water can be tolerated by Z. thermae for at least brief periods with no mortality (24 hr. refrigeration, personal observation).

Dispersal

In addition to its probable lack of drift behavior, Z. thermae is further restricted in its dispersal capabilities by flightlessness. Vestigial and brachypterous wing conditions are common for insects occupying predictable habitats, such as head waters (Greenwood 1988, Dingle 1989, Gatehouse 1989), and thermal environments (LaRivers 1950, Brown 1987, Dingle 1989). Flight in most elmids (and dryopoids) generally follows eclosion (Murvosh 1971, White 1978, Brown 1987). Those elmids that are flightless, however, would simply have to crawl back into the water whence they came in order to increase the probability of finding suitable habitat.

For Z. thermae, it is likely that mature larvae crawl out of the warm spring and pupate near its edge (Brown 1972, Brown, personal communication, White 1978a). Given the unpredictability of creek levels in spring and early summer, it is likely that pupation occurs in late summer and early fall. Some fresh and teneral adult specimens of Z. parvula

have been collected in pitfall traps near BC by D.L. Gustafson during the month of September (materials studied). Although pitfall traps were set at BCWS during this period, no migrations of mature larvae were observed outside of the spring. It is possible that pupation occurs within a few centimeters of the spring where there are near constant water levels. Thus pitfall trapping, which is constrained by mechanics for how close it may be done to the spring, may simply be ineffective for sampling larval movement. It is notable that no elmid pupae have ever been observed alive below water level (Holland 1972, Brown 1987).

Development and Thermal Ecology

Spring temperature has a profound effect on the development of Z. thermae. Species of insects inhabiting warm water tend to be smaller than related cold water inhabitants (Sweeney and Vannote 1978, Vannote and Sweeney 1980, Ward and Stanford 1982, McCafferty and Pereira 1984, Hyashi 1988).

This trend appears to hold true among the six populations of Zaitzevia examined (see Tables 2 and 4, MORPHOLOGICAL ANALYSIS, Results, Morphometric Analysis). Zaitzevia thermae generally exhibits the type of reduction in morphological features attributable to development in warm water. These include reduced overall size with a narrowing of body form, as well as the afore-mentioned reduction in wing size (Darlington 1928, LaRivers 1950,

Dingle 1989). Thus, one is left with the question, once again, of ecotypical variation of Z. parvula.

If Z. thermae is an ecotypical variant of Z. parvula, then one may expect that Z. thermae would select lower temperatures for development, if given the opportunity, and if a corresponding increase in individual body size and fecundity would result (Vannote and Sweeney 1980, Ward and Stanford 1982, McCafferty and Pereira 1984, Hayashi 1988). Because insects do not generally acclimate (Vannote and Sweeney 1980), microhabitat selection leading to an increase in body size (and vagility) may also lead to an increase in fecundity in females. In the same vein, ecotypical designation would suggest that Z. thermae originated from the same gene pool as that of local Z. parvula (a subject treated in the next chapter). However, this also implies that Z. parvula from cold water habitats could repeatedly reinvade warm springs. On this basis, Z. parvula would, hypothetically, be as likely to choose warm water as cold, if given the opportunity.

Thus, it is the basic hypothesis of this section that Z. thermae selectively avoids cold water and maintains occupancy of BCWS due to a shift in its behavior by natural selection. The null hypothesis is that, given a variable thermal environment, no difference will exist between Z. thermae and Z. parvula in their final thermal preference.

Methods and Materials

Observational

Temperatures were recorded over the course of one year with an iron-constantan thermocouple thermometer at the substrate/water interface in areas occupied by Z. thermae. Dissolved oxygen (DO) was measured at the same time in the warm spring pools with a submersible DO meter.

Experimental

Preferred temperature experiments for Z. thermae, BCWS, and Z. parvula, WFS, were conducted in the laboratory utilizing techniques similar to Crawshaw (1974) and Kavaliers (1980). A horizontal thermal gradient bar (Figure 10) was employed to cover the breadth of temperatures experienced in wild populations of Zaitzevia spp. The gradient bar measured 1061 cm. by 150 cm. and was cooled at one end by refrigerated isopropyl alcohol, and warmed at the other by heated water. Four gradient bars operated in series. As a consequence of this arrangement, temperatures at the extremes of the bars varied slightly, by no more than 2.2°C on the cold end and 0.9°C on the warm end for all trials.

A silicon bead, approximately 1 cm. in height, was laid around the edges of each gradient bar, creating a trough for water. Silicone was allowed to cure for 48 hours before water was placed in the trough and the gradient bar was

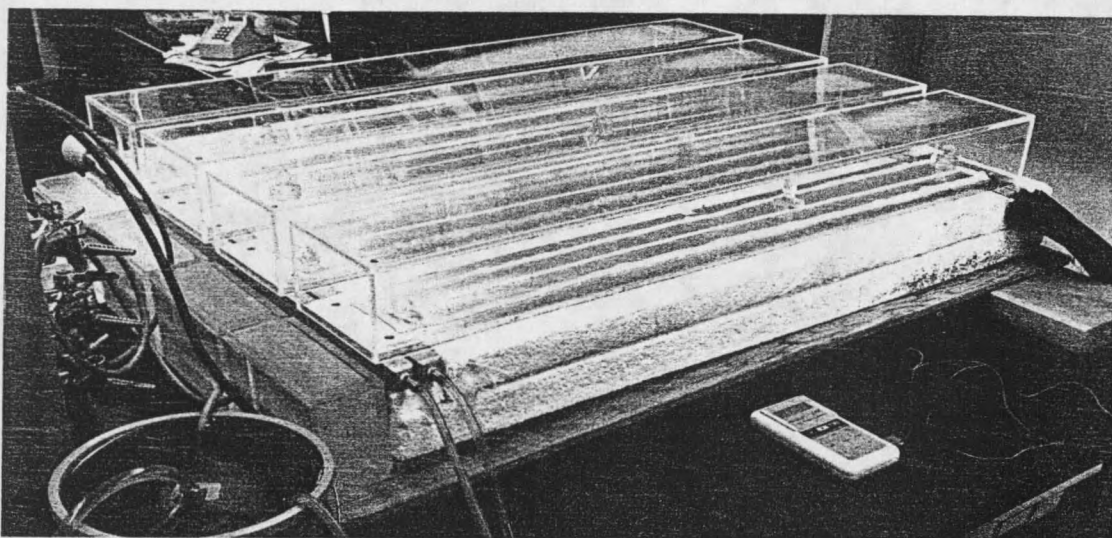


Figure 10. Gradient bar employed for preferred temperature experiments. Plexiglass covers are in place on all bars. Silicone bead for water trough and median paper strip are visible on bar 4. See text for further explanation.

turned on. Water was collected from the respective spring sites at the time of beetle collection, placed in the trough, and covered with a plexiglass cover (box) until a stable thermal gradient developed in the water (approximately one hour). A strip of acid-free wicking paper, 2 cm. wide was placed in the middle of the trough as a walking substrate for the beetles. These strips were held down by glass stoppers (Figure 10).

Beetles were collected from BCWS and WFS on the date of their experimental trials. Zaitzevia thermae were tested October 28 through October 29, 1990. Zaitzevia parvula, WFS, were tested November 27 through November 28, 1990 and November 28 through November 29, 1990. The WFS beetles were

subjected to more protracted experimentation than the BCWS beetles, as described below.

One hundred and one Z. thermae, BCWS, were collected from water registering 23°C and placed in each of four troughs at the point where the temperature was 23°C. Each trough received twenty-five beetles with the exception of one which received twenty-six. Plexiglass covers were placed over the individual troughs to minimize heat loss or gain, and evaporation. Beetles were placed in the trough at approximately 1700 hours. Measurements of the temperature at the location of individual beetles were made between 1100 h. and 1200 h. the following day. Individual trough covers were removed for location temperature measurements of beetles distributed in the trough. With an iron-constantan thermocouple thermometer, all measurements could be made in less than 10 minutes per trough. Water temperatures within uncovered troughs varied by less than one degree centigrade when measured at the extreme ends.

These same overall methods and materials were used for WFS Z. parvula, although they were collected at 12°C, and accordingly introduced on to the gradient bar at that temperature. The experiment was repeated, however, by reversing the gradient the next day (i.e. the day of measurement). After the first temperature measurements were made, the gradient bars were shut off from approximately 1200 h. to 1700 h. on November 28. The water in the troughs

equilibrated to nearly air temperature (23°C). The gradient bar was then reversed and allowed to operate over a second 18 hour period. Temperatures for the position of individual beetles were then recorded for this run.

Completely randomized block design and ANOVA procedures were employed for analyses of mean temperature selection between populations. Individual gradient bars were viewed as blocks. Multiple comparisons of means between runs were made utilizing LSD procedures on MSUSTAT. Null hypotheses of no significant differences between runs and blocks per run were employed for mean temperatures selected by beetles.

Results

Observational

Temperatures ranged from 16°C to 28°C on a seasonal basis, as described above, and varied from 3.4 mg/L to 6.0 mg/L, varying approximately with temperature (corr. = -0.89, temperature vs. DO, 23 paired observations). These dissolved oxygen values may be higher than actual values in the boundary layer occupied by Z. thermae, although diffusion would likely make the difference slight. Bridger Creek temperatures and dissolved oxygen were also measured in a similar fashion and were found to have the opposite seasonal variation pattern to BCWS, as one would expect. Creek temperatures varied from 16.6°C to 0.8°C, with dissolved oxygen being roughly in accordance from 6.4 mg/l

to 12.6 mg/l (corr. = -0.99, temperature vs. DO, 8 paired observations).

No Z. thermae were ever observed in water of less than 16°C throughout the course of this study and collection records (Appendix B) indicate no dispersal of individuals into BC despite the temperatures of BC approaching those of BCWS for brief periods in summer.

Experimental

Gradient bar number 1 for the BCWS beetles had an exceedingly high mortality (18/25 beetles) due to a leak in the trough and subsequent patching with fresh silicone. Results from this bar were omitted. Low temperature extremes on the thermal gradient differed between the BCWS and WFS runs. This was due to a deficiency in the cooling apparatus. Gradient temperatures varied between $0.8^{\circ}\text{C} \pm 0.9^{\circ}\text{C}$ at the low ends and $30.8^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$ at the high ends for the BCWS run. For both WFS runs, gradient temperatures varied between $9.4^{\circ}\text{C} \pm 0.6^{\circ}\text{C}$ at the low ends and $32.35^{\circ}\text{C} \pm 0.05^{\circ}\text{C}$ at the high ends. The discrepancy between low end temperatures for the BCWS versus WFS runs could have potentially created major difficulties in analysis. However, as will be seen below, no temperatures below 11.9°C were selected by any of the beetles from either population.

Histograms were constructed, lumping data from 5°C temperature classes beginning with 2.5°C on either side of the mean for each run (Figure 11). These figures make clear

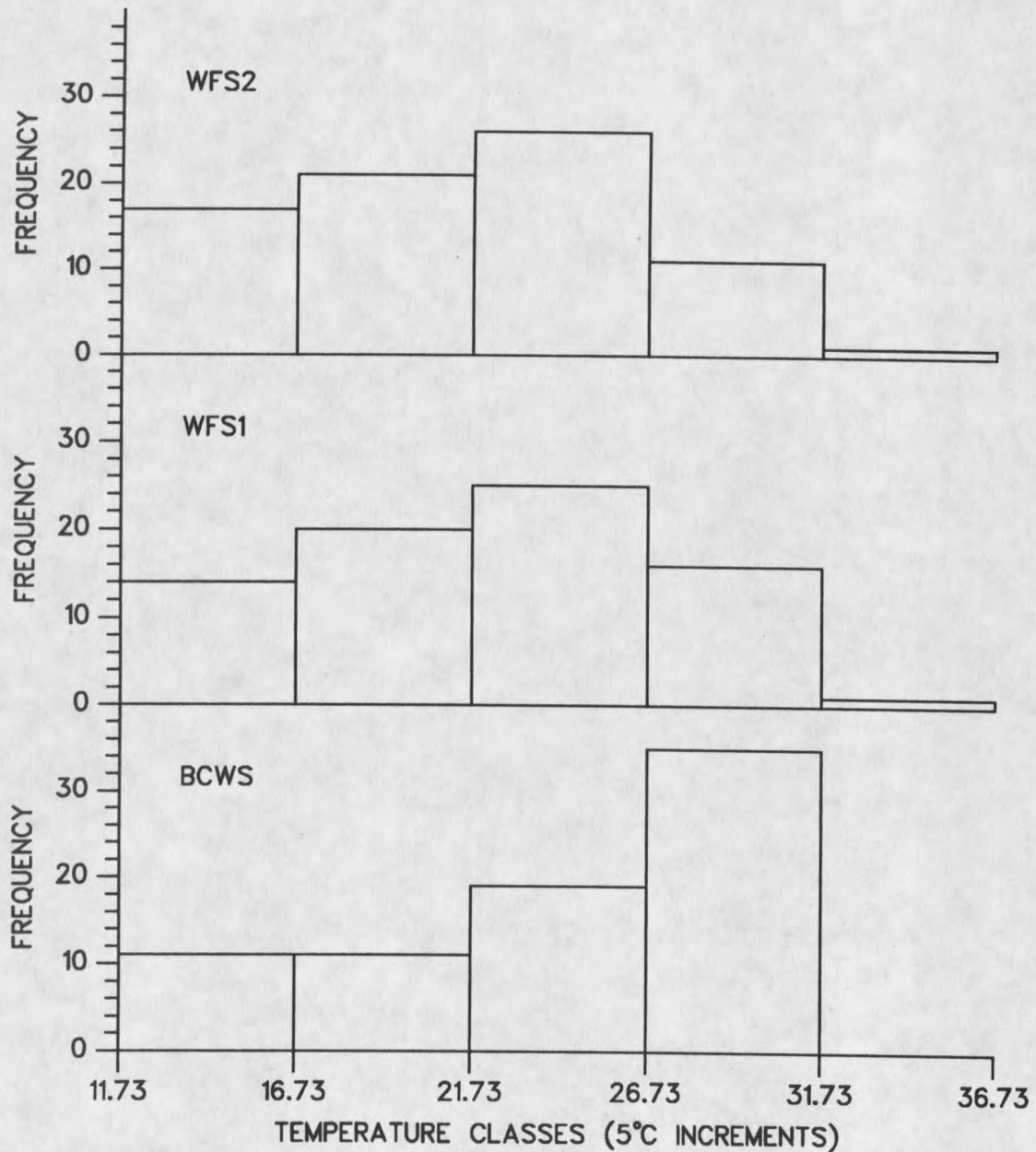


Figure 11. Histograms for thermal gradient trials. All histograms were constructed on the basis of the highest numerical mean per trial (BCWS run; $\bar{X}=24.23$). All histograms contain equal numbers of observations ($N=76$). WFS1=WFS population run number 1; similarly for WFS2.

the fact that beetles assorted to temperature classes in a non-random fashion.

Results from the ANOVA procedures are presented in Tables 5 and 6. Gradient bar number 1 was omitted from the results of all runs in order to conduct the analysis. Thus, all runs contained three blocks. It is clear from the analysis that no significant differences existed between blocks on a per-run basis. Since no Zaitzevia were observed below the highest low-end gradient temperature for any of the blocks or runs, no data were considered outliers. Thus, dependent variable affects were based on equal numbers of data points per trial and were significant at the $P=0.0009$ level. Table 6 shows that no significant difference between runs for the WFS Zaitzevia, while significant differences in mean temperature selected were observed between the BCWS run versus both WFS runs.

Table 5. ANOVA, completely randomized block design, on thermal gradient experiment runs. Runs include BCWS, WFS1, and WFS2. Significant P-value = *. See text for further explanation.

Source	DF	SS	MS	F-value	P-value
Between runs	2	394.04	197.02	7.19	0.0009*
Block (bar)	2	122.19	61.094	2.23	0.1100
Interaction	4	168.87	42.217	1.54	0.1915
Error	219	6001.6			

These results indicate that WFS Zaitzevia selected water temperature independently of the direction of the

gradient in a laboratory situation influenced by differential lighting and, potentially, electromagnetic forces. A slight shift to a lower mean temperature selected

Table 6. Summary of LSD multiple comparison of mean temperature selected per population per run. Equal letter codes in column 4 indicate no significant difference between respective means. (Standard errors are in parentheses). See text for further explanation.

Population/ run	N	Mean tempera- ture selected	Non-significant differences between populations (runs)
BCWS	76	24.23 (5.3)	A
WFS1	76	22.36 (5.0)	B
WFS2	76	21.03 (5.5)	B

by WFS beetles in run two may be a result of initial "overshoot" of thermal optimum in run one (Reynolds and Casterlin 1979). However, significant differences existed in thermal preference in all cases between BCWS and WFS Zaitzevia. This is highly suggestive that BCWS beetles preferred warmer water to that of WFS beetles.

Considering the above results, the null hypothesis of "no differential selectivity of thermal environments between Z. thermae and Z. parvula", may be tentatively rejected. The contingency of having only tested two populations must be weighed in the final analysis. It may be noted, however, that both populations tested were from stenothermic waters, more closely related in terms of their thermal regime than BCWS Zaitzevia and cold eurythermic Zaitzevia populations.

ELECTROPHORETIC ANALYSIS

Introduction

The subject of cryptic species amid insect populations was, until relatively recently, difficult to address due to the component of uncertainty with regard to reproductive isolation of sympatric sister-species. With the development of electrophoretic techniques and their application to population biology (Lewontin and Hubby 1966), reproductive isolation became more easily examined. Starch gel electrophoretic techniques have been employed for well over two decades in population studies of closely related organisms and the body of literature has become vast (Vawter and Brussard 1983, Nevo et.al. 1984, Brussard et.al. 1985, Foster and Knowels 1990, Howard and Shields 1990).

In a narrow sense, electrophoretic studies have examined phenotypic differences between taxa at the molecular level. Phenotypic expressions of enzymes and proteins can be directly interpreted in terms of the genotype of the organism (Munstermann 1980, Howard 1983, Pasteur et.al. 1988). Thus, the application of electrophoretic techniques to studies of cryptic species have sought, primarily, to demonstrate fixed or extreme allelic frequency differences at one or more loci between

populations or species (Nei 1972, Munstermann 1980, Thorpe 1982, Howard 1983, Snyder and Linton 1984, Brussard 1985, Crego et. al. 1990, Foster and Knowels 1990).

For taxonomic purposes, electrophoretic studies are best applied in the context of reproductive isolation and genetic distance and identity between populations and/or proposed taxa (Vawter and Brussard 1983, Anderson et. al. 1983, Brussard et. al. 1985). In particular, studies involving cryptic species should be born in context of morphological and ecological information (Nevo 1978, Thorpe 1982, Howard 1983, Crego et. al. 1990). In the case of Z. thermae, morphological and ecological evidence indicate the uniqueness of the Bridger Canyon Warm Spring (BCWS) Zaitzevia population (see MORPHOLOGICAL ANALYSIS, ECOLOGY). The most compelling questions, however, have been those addressing reproductive isolation of the BCWS population. In particular, has there been relatively recent reproductive overlap between the BCWS and Bridger Creek (BC) populations and does gene flow exist? In addition, how do these populations compare with other, more distant populations of Zaitzevia?

In this study, starch gel techniques were employed to test the null hypothesis that no reproductive overlap occurs between the BCWS and other Zaitzevia populations. In particular, one would expect to find no fixed allelic differences between the BCWS and BC populations if

reproductive exchange occurred between them. The likelihood of genetic exchange between these populations (vs. BCWS and other regional populations) is greatest since BC and BCWS are confluent, and one may, on a macrogeographic scale, consider these two Zaitzevia populations sympatric.

Materials and Methods

Specimens of Zaitzevia included in this study were taken from the following locations on the date(s) indicated: Bridger Canyon Warm Spring (BCWS), February 15 and October 28, 1990; Bridger Creek (BC), March 2 and October 7, 1990; West Fork Spring (WFS), February 15 and June 3, 1990; Snowflake Spring (SFS), March 12 and June 3, 1990, Little Prickly Pear Creek (LPPC), April 13, 1990; Nimrod Spring (NS), May 5, 1990; Warm Springs Creek (WSC), March 22, 1990; Brooks Warm Spring (BRWS), October 25, 1990; South branch of Otter Creek (OTC), March 2, 1990. (See Figure 1). Specimens were kept alive until returning to the laboratory, where they were stored at -80°C until use.

Horizontal starch gel electrophoretic techniques similar to Selander (1972), Vawter and Brussard (1975), and Pasteur et. al. (1988) were employed with some modification. Gel and tray buffer systems are listed in Table 7, along with corresponding loci examined for each system. A 12% starch mixture was used for each gel. Initial investigation revealed eleven loci, two of which were

Table 7. Tray and gel buffers utilized in electrophoretic analysis, with corresponding isozyme loci examined.

System		Buffer Solutions	Loci examined
R gel	1	0.030 M trizma base; 0.0046 M citric acid.	PGI, SOD, ME1, ME2, AAT
R tray		0.060 M LiOH; 0.300 M boric acid.	
C+ gel	2	0.009 M citric acid; 0.25% N-(3-aminopropyl)-morpholine.	LAP, IDH1, IDH2
C+ tray		0.037 M citric acid; 1.0% N-(3-aminopropyl)-morpholine.	
B gel	3	0.023 M trizma base; 0.013 M boric acid; 0.0005 M EDTA. *	AKP, GP, MDH
B tray		0.225 M trizma base; 0.125 M boric acid; 0.005 M EDTA. * *pH 8.6 adjusted.	

- 1) From Vawter and Brussard 1975.
- 2) Modified from Vawter and Brussard 1975.
- 3) From Pasteur et. al. 1988.

polymorphic. Polymorphic loci were SOD (superoxide dismutase) and PGI (phosphoglucose isomerase). Six of the nine populations (BCWS, WFS, SFS, LPPC, NS, and WSC) were examined at all loci. After it was determined that nine of the eleven loci were monomorphic, the other three populations (BC, BRWS, and OTC) were examined only for SOD, PGI, ME (malic enzyme) and AAT (aspartate aminotransferase), all of which stained on the same buffer system. For these populations, data were "scored" for the conservative (homozygous) condition at the nine monomorphic alleles. Interpretation of gels following staining followed that of Pasteur et.al. (1988).

Data were analyzed utilizing BIOSYS I (Swofford and

Selander 1981). Nei's (1978) unbiased genetic distance and identity as well as mean heterozygosity (unbiased estimate) were calculated for comparative purposes with other insect taxa. Roger's (1972) genetic distance was used in cluster analysis utilizing WPGMA, and distance Wagner procedures.

Results

Perhaps the most striking result of this study is the fixed allelic difference at PGI between the BCWS and BC populations (Table 8). It is also apparent that this condition is not simply one of a warm water versus cold water allelic shift. This is demonstrated by the heterozygous condition at PGI in the WSC population and the presence of both alleles in the OTC population, a cold eurythermic population (Table 8). If, indeed, the BCWS (warm stenothermic) and BC (cold eurythermic) populations were not reproductive isolates, one would expect in the BC population the heterozygous condition for PGI at nearly the same frequencies as those found in the WSC and OTC populations. However, this was clearly not the case, as outlined below.

At SOD, the presence of a rare heterozygote in the BCWS population is inconsequential and probably represents a vestige of an ancestral condition. The fact that BC Zaitzevia are fixed for the same allele as BCWS at SOD probably represents the relative isolation of these

Table 8. Allelic frequencies at all observed loci for nine populations of *Zaitzevia* spp. Allelic codes are as follows: b = fast anodal; c = slow anodal; f = cathodal. See text for further explanation.

Locus	Population								
	BCWS	BC	WFS	SFS	WSC	LPPC	NS	BRWS	OTC
PGI N=	52	61	57	48	20	20	11	5	5
b	-	-	-	-	-	.025	-	.100	-
c	1.00	-	1.00	1.00	.275	.975	1.00	.900	.200
f	-	1.00	-	-	.725	-	-	-	.800
SOD N=	52	62	37	46	18	20	11	5	5
b	.990	1.00	.311	.630	.833	.325	.727	.100	.900
c	.010	-	.689	.370	.167	.675	.273	.900	.100
AAT N=	20	41	33	29	20	20	11	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ME1 N=	51	62	63	49	20	20	11	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
ME2 N=	20	40	14	29	20	20	11	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IDH1 N=	20	20	20	20	20	20	2	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
IDH2 N=	20	20	20	20	20	20	2	5	5
f	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LAP N=	20	20	20	20	20	20	2	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
AKP N=	20	20	21	20	20	20	2	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GP N=	20	20	1	20	20	20	2	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
MDH N=	20	20	21	20	20	20	2	5	5
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

populations from other *Zaitzevia* in the region (Table 8).

Other populations examined are clearly differentiated at SOD owing to their substantially higher frequency of alleles at this locus.

Thus, when PGI and SOD are considered in concert, BCWS shows apparent reproductive isolation from other (regional) populations. Nevertheless, the genetic distances between the BCWS and other Zaitzevia populations examined are rather small (0.005 to 0.180) and similarities are conversely high (0.835 to 0.995) (Table 9; Nei 1978). One might expect such values, however, on the basis of sample sizes (both individuals and loci, Nei 1978) and/or relatively recent divergence of populations (Thorpe 1982). Heterozygosities for all populations are relatively low (0.000 to 0.063, Table 10) and two populations (WSC and OTC) deviate significantly from Hardy-Weinberg equilibrium (WSC: $\chi^2 = 5.50$, 1 d.f., $P < 0.025$; OTC: $\chi^2 = 1.23$, 1 d.f., $P < 0.35$) (see Table 10). These observations, however, are likely attributable to sample size (Nei 1978, King 1988).

A phylogenetic analysis was beyond the scope of this study, and with only two Zaitzevia species, inappropriate. Therefore, a cluster analysis was employed only to examine patterns amid populations that may share some ecological or evolutionary relationship. A number of clustering procedures were employed including UPGMA, WPGMA, and distance Wagner's analysis (Swofford and Selander 1981). Roger's (1972) genetic distance was used since it fits the metric criterion and is appropriate for use with the distance Wagner procedure (Swofford and Selander 1981, Howard 1983). UPGMA phenetic model assumes constant rates

Table 9. Genetic similarities and distances for all populations of Zaitzevia spp. utilized in electrophoretic analysis. Below diagonal: Nei (1978) unbiased genetic distance. Above diagonal: Nei (1978) unbiased genetic identity.

Population	1	2	3	4	5	6	7	8	9
1) BCWS	****	.909	.958	.988	.950	.960	.995	.835	.849
2) BC	.095	****	.864	.895	.992	.868	.901	.750	.905
3) WFS	.043	.147	****	.991	.925	1.000	.985	.903	.814
4) SFS	.012	.111	.009	****	.947	.992	1.000	.880	.840
5) WSC	.052	.008	.078	.055	****	.928	.950	.811	.906
6) LPPC	.041	.142	.000	.008	.075	****	.986	.902	.817
7) NS	.005	.104	.015	.000	.051	.014	****	.870	.845
8) BRWS	.180	.288	.103	.128	.209	.103	.139	****	.890
9) OTC	.164	.100	.206	.175	.098	.202	.169	.117	****

Table 10. Mean heterozygosities for all populations of *Zaitzevia* spp. utilized in electrophoretic analysis. Hardy-Weinberg expected values were calculated on the basis of observed values. Note that standard errors are large for most populations and that heterozygosities are generally quite low. See text for further explanation. (Standard errors are in parentheses).

Population	Mean sample size per Locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Direct count	Hdy-Wbg expected**
1) BCWS	28.6 (4.5)	1.1 (.1)	9.1	.002 (.002)	.002 (.002)
2) BC	35.1 (5.7)	1.0 (.0)	.0	.000 (.000)	.000 (.000)
3) WFS	27.9 (5.5)	1.1 (.1)	9.1	.037 (.037)	.039 (.039)
4) SFS	29.2 (3.7)	1.1 (.1)	9.1	.032 (.032)	.043 (.043)
5) WSC	19.8 (.2)	1.2 (.1)	18.2	.024 (.016)	.063 (.043)
6) LPPC	20.0 (.0)	1.2 (.1)	18.2	.055 (.050)	.045 (.041)
7) NS	6.1 (1.4)	1.1 (.1)	9.1	.033 (.033)	.038 (.038)
8) BRWS	5.0 (.0)	1.2 (.1)	18.2	.036 (.024)	.036 (.024)
9) OTC	5.0 (.0)	1.2 (.1)	18.2	.018 (.018)	.051 (.035)

* A locus is considered polymorphic if more than one allele was detected.

** Unbiased estimate (see Nei, 1978).

of divergence of isolated populations where the WPGMA and distance Wagner's models, though fundamentally different, do not. Additionally, the distance Wagner model groups on the basis of synapomorphic characters while arbitrating on the basis of parsimony (Wiley 1981, Howard 1983). The WPGMA model closely agreed with Wagner's distance model and was more easily interpretable in light of genotypic frequency data than a UPGMA model. Dendograms for the WPGMA and distance Wagner's models are presented for comparative purposes in Figures 12 and 13.

For the distance Wagner procedure, the WSC population was chosen as an outgroup based on the hypothesis that the heterozygous condition at PGI and SOD is the plesiomorphic state. Overall homoplasy was low for this procedure, 0.000 to 0.195 with all but one value below 0.029. The cophenetic correlation (the correspondence of the Wagner tree to the original data matrix (Wiley 1981)) was high, 0.910, a measure greater than those found for the Roger's (1972) distance metric for phenetic models (UPGMA=0.876, WPGMA = 0.795). In these trees, stenothermic groups and eurythermic groups tended to cluster. A more in-depth approach to ecological correlates with genetic distance/similarity measures is, therefore, suggested (see Nevo 1984).

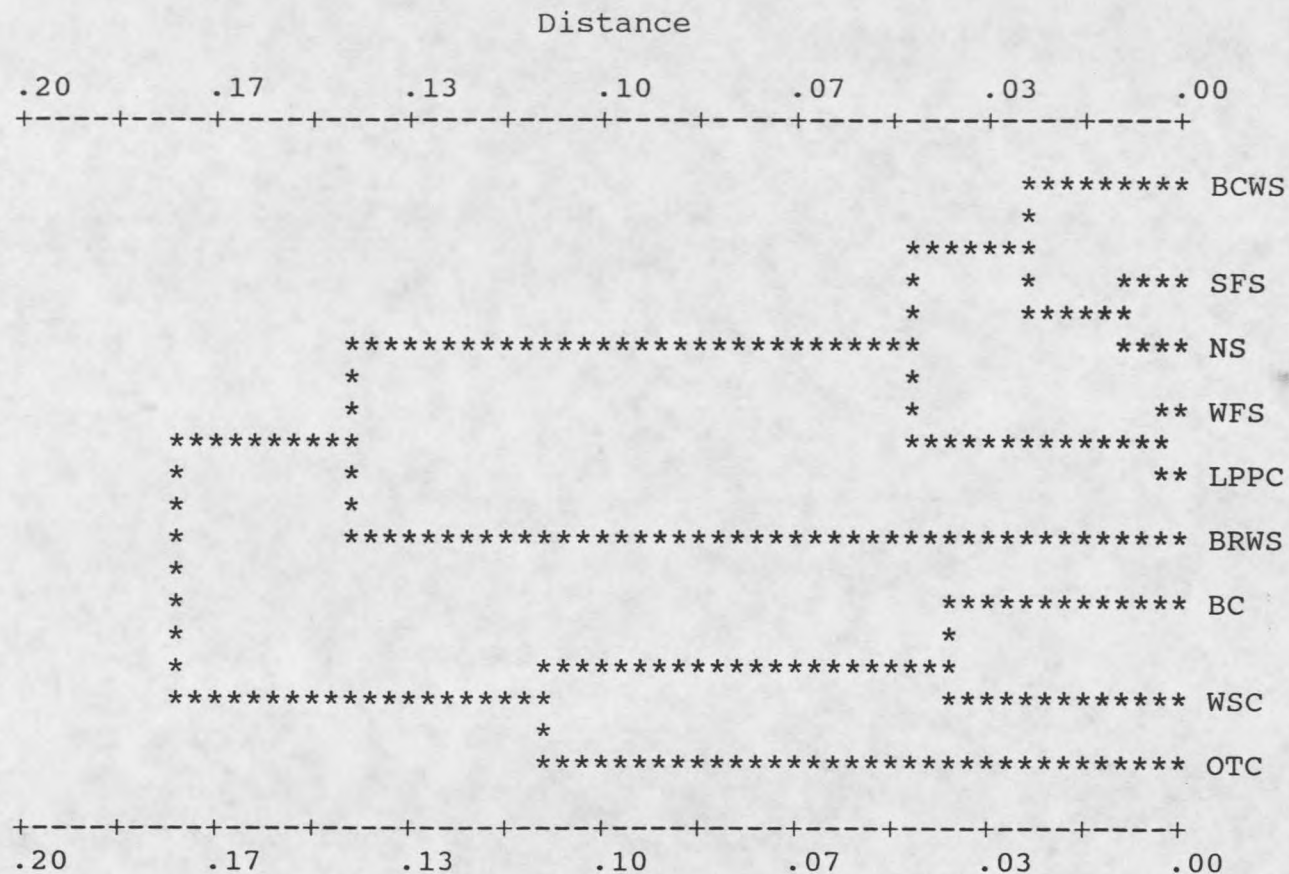


Figure 12. WPGMA tree for *Zaitzevia* populations examined, using Roger's (1972) genetic distance. Note the relationship between stenothermic ("spring") types and eurythermic types. NS and LPPC beetles are, however, of eurythermic origin and therefore confuse the relationship. Cophenetic correlation = 0.795. See text for further explanation.

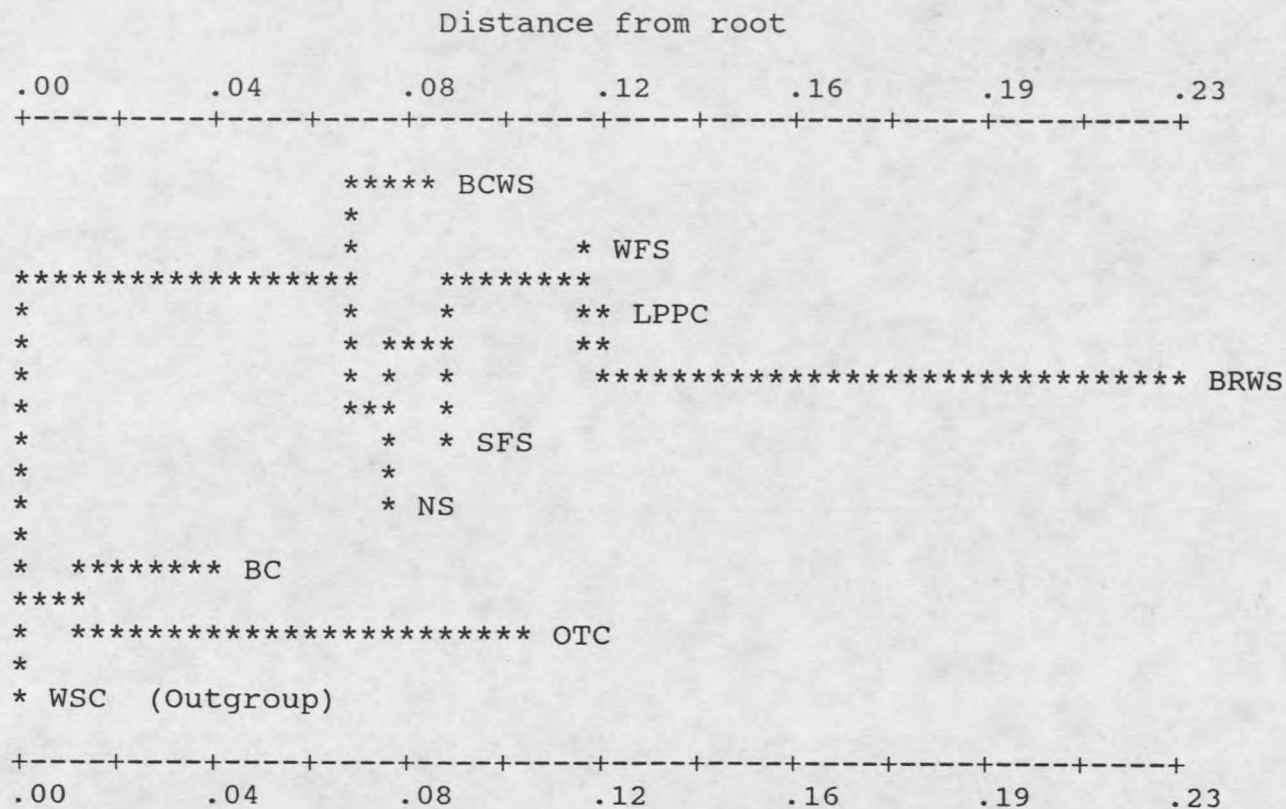


Figure 13. Distance Wagner tree for *Zaitzevia* populations examined, using Roger's (1972) genetic distance. WSC population is the chosen outgroup. Note the relationship between stenothermic ("spring") types and eurythermic types. NS and LPPC beetles are, however, of eurythermic origin and therefore confuse the relationship. Cophenetic correlation = 0.910. See text for further explanation.

CONCLUSION

Zaitzevia species are morphologically, ecologically and genetically variable in Western Montana. Total size and pronotal proportion vary widely between cold and warm water inhabitants. Additionally, specific morphological features appear to differ widely with habitat. On a metapopulation level, these differences may be exactly what one would expect given a relatively broadly adapted species. Upon closer inspection, however, populations show distinct morphological and ecological discontinuities. Lack of clinal variation in populations raises the basic question of the degree of divergence among populations. Identification of populations at the basic-taxon level (i.e. species), becomes the key step towards understanding metapopulation structure.

With Z. thermae, the difficulty lay in determining its identity as ecotype (ecological type), subspecies, or species. In this light, operational definitions, particularly of "ecotype" and "subspecies", will be prudent.

McCafferty and Pereira (1984) follow Ross (1974) in their definition of ecotype:

"Populations of a species that differ phenotypically as a response to different environmental conditions [that] they are subjected to during embryological or postembryological development...."

Furthermore, McCafferty and Pereira (1984) emphasize that variation is not based on genetic divergence of populations. Rather, the ecotype (ecomorph) arises from developmental factors influenced by the concerted action of environmental variables on within-population genetic variation. Consequently, organisms developmentally sensitive to a suite of environmental conditions will display a broad spectrum (cline) of morphologically correlated features.

Going one step further, McCafferty and Pereira (1984) follow Mayr (1965) in defining a subspecies as:

"...an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of the species."

Variation between subspecies is, by implication, genetically based. Reproductive isolation is not, however, a necessary condition of subspecies identity and the degree to which subspecies must be genetically divergent is subjective. In general, subspecies designations are geographically and/or morphotypically based, and the degree of genetic identity is slight ($I=0.775$ to 0.977 , Brussard et. al. 1985) with few major allelic frequency differences between conspecific morphs.

Defining a species becomes a far more difficult task since the affirmation of species status lies in demonstrating reproductive isolation between the populations. For animals that are not reproductively (mechanically or behaviorally), or ecologically distinct,

the difficulty of the task is amplified, especially with allopatric populations. Morphological novelties may be correlated entirely with environmental variables (Stock 1980, McCafferty and Pereira 1984, Greenwood 1988, Gatehouse 1989, etc.), thus forming clines. Alternatively, clines may be absent and populations may be regarded as cryptic species (Howard 1983, Crego et. al. 1990).

Behavioral adaptations that promote reproductive isolation, such as singing in crickets (Howard 1983) or temperature selection (May 1985), may be some of our best indicators of cryptic species. The timing of events such as oviposition, ontological events and eclosion, may strongly influence the success of a given population. On a regional scale, these events may vary widely for a given species (Whittaker 1975, Ward & Stanford 1982). However, on a local scale, these events may be strong indicators of the existence cryptic species. Thus, it would appear that the identification of cryptic species under localized conditions, is a simpler task than identification of those that are widely separated.

In the case of Z. thermae one might consider its population a "peripheral isolate" of the more wide-spread Z. parvula. This is hypothetically a special case of allopatric speciation (sensu Eldredge and Gould 1972) as discussed by Wiley (1981). A number of findings in this study tend to support this assumption.

Morphological dissimilarities of Z. thermae may be greatly attributable to ecological factors, particularly life in a relatively warm stenothermic environment. Certainly the BRWS and BCWS populations show similarities in size and proportion (see MORPHOLOGICAL ANALYSIS, Morphometric Analysis), yet BRWS Zaitzevia retain all other characters typical of those of Z. parvula. Reductions in total size of plastron-respiring organisms would appear to be related to relatively poorly oxygenated water (Thorpe and Crisp 1949), although accelerated development is likely to play a significant role (Sweeney and Vannote 1978, Vannote and Sweeney 1980, Ward and Stanford 1982, McCafferty and Pereira 1984, Hyashi 1988). Thus, conspecific size variation can be great among aquatic insects that occupy thermally variable habitats (Ward and Stanford 1982, McCafferty and Pereira 1984, Hayashi 1988).

If, however, development in thermal waters was a simple option available to Z. parvula, one would expect clinal size and proportional variation between warm stenothermic and cold eurythermic (and cold stenothermic) inhabitants. May (1985) points out that thermal gradients at warm/cold water interfaces or along springbrooks (Nielsen 1949) provide aquatic insects the relatively rare opportunity to thermoregulate with some effectiveness. In the case of BCWS, thermal gradients are ample due to its close association with BC. Thus, the question arises to why there

are no intermediate morphotypes present among either BC or BCWS Zaitzevia populations. The initial observation of no morphotypic overlap suggests that the two populations differ developmentally.

Correlated with the small size of Z. thermae is wing microptery. This condition is common with insects whose development has been accelerated by temperature (Stock 1980, Dingle 1989, Gatehouse 1989). Wing size reduction is also common in headwater inhabitants and, generally, in ecologically stable environments (Hynes 1970, Ward and Stanford 1982, Roff 1986, Gatehouse 1989). This reduction in wing size has two components, one behavioral and one physiological: 1) predictability of reproductive space; and 2) a corresponding increase in fecundity due to the decreased metabolic costs of wing development and maintenance (Vannote and Sweeney 1980, Ward and Stafford 1982, May 1985, Roff 1986).

Wing reduction may also be observed in cold stenothermic headwater populations of Z. parvula (i.e. WFS and SFS). However, venation is generally conserved in these populations (see Figure 6), where it is dramatically reduced in Z. thermae (Figure 2). Additionally, venation and relatively macropterous size appear to have been conserved in the warm water population of small Z. parvula from BRWS. This population merits further investigation.

In addition to reduction of wing size in Z. thermae,

there is a concomitant reduction in size and structure of the metendosternite. Although wing size reductions appear to be common among Z. parvula populations, and total body size reduction possible, it is noteworthy that all forms share similar metendosternite structure (see Figure 8). No doubt this structural reduction in Z. thermae further limits the dispersal capabilities of the beetles by negatively affecting their ability to fly. However, the ability for Z. thermae to disperse by means of flight may be maladaptive since the probability of finding a similar warm spring are extremely low (the nearest being some 30 km. distant). Therefore, a reduction in structure of the metendosternite, along with probable reductions of flight muscles, may be beneficial and selected for in Z. thermae by increasing the energy available for other anabolic processes (Vannote and Sweeney 1980, Ward and Stafford 1982, May 1985, Roff 1986). In light of the relatively well developed structures associated with flight in the, otherwise small, Z. parvula, BRWS population, it would appear that those reductions found in Z. thermae are not environmentally plastic, and therefore, not easily reversed (Mayr 1969). This argument is central to the hypothesis of monophyletic origin of Z. thermae.

Metabolically, Z. thermae must contend not only with critically low dissolved oxygen levels in warm water, but with the potentially high costs to fecundity due to rapid

development and subsequently reduced size (Vannote and Sweeney 1979). One of the compensatory factors might be reduction of structures associated with flight, already discussed, in order to increase energy available for gamete production. This is speculative, however, since this correlation was not measured in this study, but would appear to be reasonable in light of the literature (May 1985, Roff 1986, Gatehouse 1989).

Limited fecundity may be a critical factor for the successful occupation of thermal springs by Zaitzevia spp. This would not appear to be a relatively plastic characteristic of Zaitzevia spp. since they have been found to be reproducing in only two of sixteen warm springs sampled (BCWS and BRWS). It is noteworthy that thirteen of these springs are within the known geographic range and habitat type of Z. parvula. Two of these warm springs which have produced relatively large, long winged Z. parvula in bulk samples (NS and PWS), have no associated larvae. These adults would appear to have flown from nearby streams into the warm springs following eclosion. Undoubtedly, this kind of event is common, but colonizers must fail to recruit or larvae would be present.

Thus, the colonization, and subsequent reproduction in warm spring environments by Zaitzevia would appear to be a rare ecological event. This alone nullifies the concept of "ecotype" for Z. thermae.

Temperature gradient results indicate that Zaitzevia adults display a marked thermal preference (Reynolds and Casterlin 1979). Few studies have dealt with invertebrate thermophily or thermal preference. However, those that have tend to demonstrate distinct temperature preferences within the species tested (Crawshaw 1974, Kavaliers 1980). Similar tests with fish have shown that fish often choose temperatures optimum for feeding, resting or mating success (Badenhuizen 1967, Reynolds and Casterlin 1979, Magnuson et. al. 1979). The mobility of fish, however, enables them to thermoregulate more carefully than most aquatic insects (May 1985). For the most part, stream insects are limited to vertical movements in the hyporheic zone and water column to avoid upper or lower incipient lethal temperatures. Rarely do opportunities in nature allow stream invertebrates to choose a thermally optimal location.

Under the thermal equilibrium hypothesis proposed by Vannote and Sweeney (1979), a thermal optimum is where an individual's body size and fecundity are maximized. The geographic center of a species' range may be an equilibrium location (Vannote and Sweeney 1979). Suboptimal habitats are characterized by low density and depressed fecundity. Increased costs of maintenance metabolism at suboptimal temperatures appear to be responsible for decreased overall fitness. Consequently, when given the opportunity, it is reasonable to assume that evolution via natural selection

will lead to an ability to choose a thermal optimum. The thermal gradient analyses demonstrated that Z. thermae and Z. parvula show temperature preferences, but did not fully address the hypothesis of Vannote and Sweeney (1979) since reproductive success was not measured.

In light of the above, it is noteworthy that Z. parvula, WFS, is a spring inhabitant, albeit a cold stenothermic spring of approximately 12°C average temperature. This temperature is similar to SFS, yet SFS Zaitzevia display relatively greater size consistency. Conditions other than temperature may be at work during development.

Since Z. parvula, WFS, "chose" a temperature on a gradient bar higher than that which it resides at in situ, it may be a reflection of the species' final thermal preferendum. However, there are a number of thermal springs in Montana with springbrooks that offer a range of thermal conditions similar to that of the gradient bar analysis. As mentioned above, many are not inhabited by reproducing individuals of Z. parvula, thus temperature may or may not be the only limiting factor to Z. parvula's distribution. One may also observe that there was a slight shift on the reversed gradient for Z. parvula, WFS, to a slightly lower average temperature than during the first run. Although not statistically significant, this may indicate somewhat of an initial "overshoot" of preferred temperature as observed for

many fishes (Reynolds and Casterlin 1979).

Zaitzevia thermae displayed a significantly higher temperature preference than Z. parvula, WFS, even when comparing initial trials. The reversal of the gradient bars was not done with Z. thermae, yet it is unlikely that this would have resulted in significantly different final preferenda in light of the results of the Z. parvula, WFS, tests. It would appear, therefore, that Z. thermae is behaviorally adapted to at least cold water aversion (if not warm water attraction) and has a different average thermal niche than Z. parvula.

It is worth mentioning that cold eurythermic Z. parvula were not tested on a gradient bar. Under these conditions it may be speculated that these populations may have displayed a wider temperature tolerance and less tendency to clump into temperature classes (Figure 11) since they experience a greater breadth of water temperature conditions. The availability of sufficient numbers of Zaitzevia from cold eurythermic waters was a limiting factor in conducting these experiments.

Morphological and behavioral analyses of Z. thermae are complimented by genetic analyses to determine reproductive isolation. One would naturally predict that reproductive isolation would not occur in ecotypical populations or with narrowly adjacent "subspecies". (One may argue that geographic isolation, the basic premise of subspecies

designation, has already been violated by definition).

Differential enzyme activity has been linked with temperature and development in various insect taxa. Shifts in enzyme production may also be linked with shift in temperature in the insect's environment (Schott and Brusven 1979). Quantitative shifts in G-6-PDH (glucose-6-phosphate dehydrogenase) and qualitative shifts, i.e. phenotypic shifts in LDH (lactose dehydrogenase) and LAP (leucine aminopeptidase) were observed in Argia vivida (Hagen) (Zygoptera) in response to temperatures between 15°C and 40°C (Schott and Brusven 1979). These researchers claim that isozyme anabolism undergoes "acclimation" shifts to accommodate fluctuating temperatures. Their study, however, does not indicate shifts to fixation for alternate alleles in any of the above enzyme systems. Thus, neither quantitative nor qualitative shifts in enzyme activity would appear to produce the allelic frequency differences that one would expect to find between reproductively isolated populations or species.

For Z. thermae, functionally complete homozygosity at all alleles studied suggest a highly inbred population strongly influenced by conditions of genetic drift and selection, compounded by initial founder effects and/or bottlenecks (Menken 1987). In light of the relatively low levels of heterozygosity in other Zaitzevia populations, however, a more in-depth analysis may be more conclusive.

However, it is clear that nearly normal levels of heterozygosity at SOD (superoxide dismutase) and PGI (phosphoglucose isomerase) are conserved in most Zaitzevia populations. The presence of heterozygotes at PGI in the WSC population is a good indication that this is an ancestral state in cold eurythermic populations. However, the BC Zaitzevia population is fixed for the slow allele at PGI. Since the juxtaposed BCWS population is fixed for the fast allele, this indicates no, or exceedingly novel, genetic exchange between these two populations. Therefore, it appears that Z. thermae is reproductively isolated from Z. parvula. Furthermore, genetic independence of Z. thermae nullifies the hypothesis of ecotypy and makes the potential conclusion of subspecies designation fallacious. Additionally, the barrier between the BC and BCWS populations is, by all indication, ecological and not geographic. Thus, Eldredge and Gould's (1972) peripheral isolate model of novel species, as described in Wiley 1981, is supported.

Evidence gathered suggest that Z. thermae is of monophyletic origin and represents a distinct lineage. Therefore, it is my conclusion to reject the null hypothesis that Z. thermae is not a biological species.

Summary

Findings indicate that Z. thermae possesses novelties

that distinguish it morphologically, ecologically, and reproductively from Z. parvula. Thus Z. thermae may be regarded as a biological species, albeit cryptic in nature.

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APPENDICES

APPENDIX A

CONSERVATION MANAGEMENT OF
Zaitzevia thermae

Due to the restricted habitat of Z. thermae and the anthropogenic activities that have limited the availability of that habitat, one must consider strategies for the long-term management of the species. In light of the species' current Federal status (INTRODUCTION), it would appear imprudent to forego management considerations.

It is remarkable that Z. thermae has been able to survive dramatic alterations to its naturally restricted habitat. Nearly 90% of its original habitat has been lost to human-induced alterations. No doubt, these changes were unintentional with respect to the species' welfare. However, one must consider, too, at least one derived benefit to the species' welfare when considering the construction of the springbox. The simple fact that Bridger Canyon Warm Spring was made relatively inaccessible for recreational use (one of its historical uses (Ina Denton and Melvin Osborn, personal communication)) by construction of the springbox probably rescued BCWS from continuous human impact.

Anthropogenic uses aside, the springbox provides a physical barrier to swift currents during spring floods. This feature alone may be beneficial to Z. thermae, primarily in maintaining downstream warm water refugia similar to those created by boulders in the mass wasting process (ECOLOGY, Introduction).

Nevertheless, the springbox represents a large loss of

space that could still be viable habitat for Z. thermae. Comments from the daily logs at the BFCDC in the year 1938 (06/08/38) are indicative of the fact that the spring box could be managed as an open structure: "Ross ... cleaned moss from warm spring ...". As mentioned in the INTRODUCTION, collections from the springbox indicate the species' ability to persist under these conditions. Therefore, complete surrender of the spring to the beetle would appear unreasonable, and managerially unnecessary.

A natural first consideration for management would be to remove the roof from the springbox and reintroduce the beetle. Some ingenuity may benefit both current users of the impounded warm water and Z. thermae. Partial removal of the solid roof, approximately 2/3, replaced with a grate would increase available habitat for Z. thermae by 400%. A barrier to moss, eg. a heavy gauge grate, could be installed vertically in the springbox, at the limit of the open box, to keep moss from accumulating in the draft outlet. Periodically, moss could be removed manually to prevent overly heavy accumulation which might disrupt springbox function. Some moss is beneficial to Z. thermae (ECOLOGY, Introduction), thus complete moss removal would not be recommended, nor probably possible.

Naturally, some beetles would be lost during moss removal, as suggested above. However, in light of the history of the spring, it is apparent that Z. thermae can

maintain such mortality if its populations are allowed occupation of the springbox. Additionally, if moss were removed from the springbox during mid-to-late winter, mortality of beetles might be minimized. This is a reasonable postulate since beetles stay more closely associated with the lithic substrate during these months (personal observation), perhaps due to cold and/or a reduced productivity of moss during these months. Under Federal protection, provisions for this impact would be necessary.

Three additional recommendations appear necessary. First, foot traffic into the area of the spring needs to be restricted to minimize accidental or deliberate human alteration of the (currently) primary refugia of Z. thermae. Few solutions appear immediately, however, due to Montana stream access laws, and alternatives should be discussed with the USFWS. Perhaps an interpretive boardwalk could be constructed that allows stream access but averts trampling of the spring. This would be both educational and conservation-minded in addition to improving USFWS personnel access to the area.

Secondly, alterations to the upstream and adjacent channel of Bridger Creek need to be regarded with great caution. Although the warm spring is somewhat self-cleaning, heavy influxes of silt may have long-term effects on habitable space (McClelland and Brusven 1980), let alone the effects it may have on a plastron-respiring beetle

(Thorpe and Crisp 1950). Any alteration that affected the intensity of flooding in BC should be regarded with great caution since benthic organism populations may be severely affected by intense flooding (Fisher et. al. 1982).

Lastly, any activities potentially affecting the spring flow should be closely scrutinized by managers. Well drilling and aquifer modifications may be the most obvious activities in this category. However, road alteration or construction may also enter this category, in addition to affecting the stream channel of BC.

In summary, four basic conservation management recommendations have been proposed. They are: 1) replace 2/3 or greater of the solid portion of the springbox cover with a grate and reintroduce Z. thermae into this portion of its original habitat; 2) minimize foot traffic in the area of the spring, possibly by current interpretive methods; 3) scrutinize any activity affecting any of the adjacent and upstream environment of Bridger Creek to Bridger Canyon Warm Spring; 4) scrutinize any activity that could potentially affect the BCWS aquifer.

APPENDIX B

MATERIALS STUDIED OF
Zaitzevia Champion

Materials for electrophoretic analyses are listed in ELECTROPHORETIC ANALYSIS, Materials and Methods, and Table 8. Materials for temperature gradient analyses are listed in ECOLOGY, Materials and Methods. Unless otherwise stated, all materials listed below are from the MTEC or collections of D.L. Gustafson or M.M. Hooten.

Zaitzevia thermae (Hatch), Bridger
Canyon Warm Spring

Dissected Material. (Approx.) 25 males, 25 females; 20 June 1987 and 1 Nov. 1987; D.L. Gustafson. (Approx.) 25 larvae, mixed instars; 20 June 1987 and 1 Nov. 1987; D.L. Gustafson.

Morphometric Analysis. 1 male, 1 female; 22 Jan. 1987; D.L. Gustafson. 7 males, 9 females; 1 Nov. 1987; D.L. Gustafson. 2 males, 1 female; 15 Sept. 1988; M.M. Hooten.

Larval Instar Analysis. 162 mixed instars; 20 June 1987; D.L. Gustafson. 452 mixed instars; 1 Nov. 1987; D.L. Gustafson.

Collections Reviewed. (Sexes were, for the most part, not determined). 22 adults; 6 Mar 1938; D.J. Plétsch; Montana State University. 64 adults; 22 Jan. 1987; D.L. Gustafson. 6 adults; 12 Sept. 1987; D.L. Gustafson. 15 adults; 13 June 1987; D.L. Gustafson. 5 adults; 28 July 1987; D.L. Gustafson. 4 adults; 30 Mar. 1987; D.L. Gustafson. 25 adults; 22 Jan. 1987; D.L. Gustafson. 6

adults; 28 Feb. 1988; D.L. Gustafson. 7 adults; 30 Mar. 1987; D.L. Gustafson. 13 adults; 13 June 1987; D.L. Gustafson. 199 adults; 20 June 1987; D.L. Gustafson. 11 adults; 28 July 1987; D.L. Gustafson. 8 adults; 12 Sept. 1987; D.L. Gustafson. 15 adults; 15 Nov. 1987; D.L. Gustafson. 7 adults; 15 Sept. 1988; M.M. Hooten. 1 male, 1 female; 21 Sept. 1988; M.M. Hooten. 26 adults; 28 Oct. 1987; M.M. Hooten. Larvae: 12 Sept. 1987; 28 July 1987; 1 Nov. 1987; 30 Mar. 1987; 5 Feb. 1987; D.L. Gustafson.

Other Known Dates of Collection. Holotype; 6 Dec. 1936; C.D.J. Brown; United States Museum of Natural History collection.

Zaitzevia parvula (Horn), Montana

Dissected Material. (Approx.) 25, mixed sexes; Gallatin Co.; West Fork Spring; 26 Dec. 1987; D.L. Gustafson. (Approx.) 25, mixed sexes; Gallatin Co.; Snowflake Spring; 26 Dec. 1987; D.L. Gustafson. (Approx.) 10, mixed sexes; Gallatin Co.; Bridger Creek, 4800' elev.; 8 Nov. 1987; D.L. Gustafson. 2 males, 1 female; Gallatin Co.; Bridger Creek, 4800' elev.; 2 Mar. 1990; M.M. Hooten. 1 male, 2 females; Gallatin Co.; Bridger Creek, 4880' elev.; 2 Dec. 1989; M.M. Hooten. 1 males; Gallatin Co.; Bridger Creek, 4880' elev.; 21 Sept. 1989; M.M. Hooten. 11, mixed sexes; Granite Co; Nimrod Spring, 3800' elev.; 5 May 1990; D.L. Gustafson/M.M. Hooten. 3 males, 6 females; Fergus Co.;

Brook's Warm Spring, 3760' elev.; 25 Oct. 1990; D.L. Gustafson/M.M. Hooten. 1 female; Madison Co.; Potosi Warm Spring, 6100' elev.; D.L. Gustafson. (Approx.) 25 larvae, mixed instars; Gallatin Co.; West Fork Spring; 26 Dec. 1987; D.L. Gustafson.

Morphometric Analysis. 4 males, 7 females; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 8 Nov. 1987; D.L. Gustafson. 1 male; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 11 Sept. 1986; D.L. Gustafson. 1 male, 2 females; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 28 July 1987; D.L. Gustafson. 1 female; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 8 July 1987; D.L. Gustafson. 1 male; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; Sept. 1987; D.L. Gustafson. 1 male, 1 female; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 31 Mar. 1987; D.L. Gustafson. 1 male, 1 female; Gallatin Co.; Bridger Creek, 4800'-4900' elev.; 9 July 1986; D.L. Gustafson. 1 male, 1 female; Gallatin Co.; Bridger Creek, 4880' elev.; 2 Sept. 1989; M.M. Hooten. 1 male, 1 female; Gallatin Co.; Bridger Creek, 4880' elev.; 19 Oct. 1989; M.M. Hooten. 5 males, 5 females; Wheatland Co. at Two Dot; Musselshell R.; 19 Mar. 1988; D.L. Gustafson. 1 male; Golden Valley Co. at Ryegate; Musselshell R.; 1 Sept. 1989; D.L. Gustafson. 3 males, 2 females; Gallatin Co.; Snowflake Spring; 13 Nov. 1971; G. Roemhild. 3 males, 4 females; Gallatin Co.; Snowflake Spring; 11 Sept. 1987; D.L. Gustafson. 7 males, 4 females;

Gallatin Co.; Snowflake Spring; 19 Feb. 1987; D.L. Gustafson. 1 male, 1 female; Gallatin Co.; Snowflake Spring; 29 Aug. 1989; M.M. Hooten. 3 males, 4 females; Gallatin Co.; West Fork Spring; 11 Sept. 1987; D.L. Gustafson. 1 male; Gallatin Co.; West Fork Spring; 5 April 1987; D.L. Gustafson. 4 males, 7 females; Gallatin Co.; West Fork Spring; 19 Feb. 1987 D.L. Gustafson. 1 male, 5 females; Gallatin Co.; West Fork Spring; 26 Dec. 1987; D.L. Gustafson. 3 males, 6 females, 8 sex undet.; Fergus Co.; Brook's Warm Spring, 3760' elev.; 25 Oct. 1990; D.L. Gustafson/M.M. Hooten.

Collections Reviewed. (sexes not determined. Montana material, unless otherwise specified). 1 adult; Madison Co.; N. Fork Ruby River; 14 July 1958; coll. unk.; Montana State University (MSU) collection. 3 adults; Gallatin Co.; Snowflake Spring; 11 Sept. 1987; D.L. Gustafson. 8 adults; Gallatin Co.; Snowflake Spring; 19 Feb. 1987; D.L. Gustafson. 2 adults; Gallatin Co.; West Fork Spring; 19 Feb. 1987; D.L. Gustafson. 2 adults; Gallatin Co.; West Fork Spring; 11 Sept. 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bridger Creek, 4800' elev.; 8 July 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bridger Creek, 4800' elev.; 31 Mar. 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman Spring Creek; 11 July 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman, light trap; 16 Aug. 1937; D. Scharff. 1 adult; Broadwater Co.; 10 mi. E. Townsend, N.

Fork Deep Creek; 25-30 June 1958; coll. unk. 29 adults;
Gallatin Co.; Gallatin Canyon, 0.25 mi. N. Yellowstone N.P.
[Snowflake Spring]; 11 Sept. 1987; D.L. Gustafson, M.A.
Ivie. 2 adults; Wyoming; Yellowstone N.P.; Gibbon R., upper
sta.; E.R. Vincent. 67 adults; Gallatin Co.; Snowflake
Spring; 26 Dec. 1987; D.L. Gustafson. 4 adults; Gallatin
Co.; Snowflake Spring; 19 Feb. 1987; D.L. Gustafson. 2
adults; Gallatin Co.; Snowflake Spring; 13 Nov. 1971; G.
Roemhild. 29 adults; Gallatin Co.; West Fork Spring; 26
Dec. 1987; D.L. Gustafson. 1 adult; Gallatin Co.; West Fork
Spring; 19 Feb. 1987. 1987; D.L. Gustafson. 24 adults;
Gallatin Co.; Gallatin R., W. of Four Corners; 1 Sept. 1988;
D.L. Gustafson. 1 adult; Gallatin Co.; Gallatin R., 8 mi.
W. of Bozeman; 21 Aug. 1984; D.L. Gustafson. 1 adult;
Madison Co.; Warm Spring Creek, mouth; 17 July 1988; D.L.
Gustafson. 2 adults; Madison Co.; Warm Spring Creek, mouth;
22 Mar. 1988; D.L. Gustafson. 8 adults; Madison Co.; Warm
Spring Creek; 25 Apr. 1988; D.L. Gustafson. 7 adults; Teton
Co.; Muddy Creek at Hwy. 220; 21 Apr. 1988; C.E. Seibert. 7
adults; Madison Co.; Potosi Warm Spring, 6100' elev.; 9 Apr.
1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bridger Creek,
4800' elev.; 14 Mar. 1987; D.L. Gustafson. 1 adult;
Gallatin Co.; Bridger Creek, 4800' elev.; 31 Mar. 1987; D.L.
Gustafson. 1 adult; Gallatin Co.; Bridger Creek, 4800'
elev.; 26 Mar. 1987; D.L. Gustafson. 1 adult; Gallatin Co.;
Bridger Creek, 4800' elev.; 7 June 1987; D.L. Gustafson. 2

adults; Gallatin Co.; Bridger Creek, 4800' elev.; 8 July 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bridger Creek, 4800' elev.; 11 July 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bridger Creek, 4800' elev.; Sept. 1987; D.L. Gustafson. 7 adults; Gallatin Co.; Bridger Creek, 4800' elev.; 8 Nov. 1987; D.L. Gustafson. 11 adults; Madison Co.; Beartrap Canyon; 29 Dec. 1987; D.L. Gustafson. 3 adults; Jefferson Co.; Boulder R. at Basin; 30 Sept. 1988; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman, UV light; 25 July 1987; D.L. Gustafson. 4 adults; Gallatin Co.; Bozeman, UV light; 25 July-10 Aug. 1987; D.L. Gustafson. 1 adult; Sweetgrass Co.; Sweetgrass Cr. on U.S. Hwy. 191; 19 Mar. 1988; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman Creek, 5700' elev.; 4 July 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman Creek, 5700' elev.; 2 Sept. 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman Spring Creek; 12 Feb. 1987; D.L. Gustafson. 1 adult; Gallatin Co.; Bozeman Spring Creek; 6 Dec. 1987; D.L. Gustafson. 7 adults; Wheatland Co.; Fish Creek on U.S. Hwy 191; 19 Mar. 1988; D.L. Gustafson. 7 adults; Park Co.; Shields R. at Clyde Park; 19 Mar. 1988; D.L. Gustafson. 4 adults; Louis and Clark Co.; Dearborn R.; 23 Apr. 1988; C.E. Seibert. 2 adults; Missoula Co.; Clearwater R. above Salmon L.; 26 June 1989; D.L. Gustafson. 6 adults; Wyoming; Yellowstone N.P.; Little Firehole R.; 24 Aug. 1988; D.L. Gustafson. 3 adults; Wyoming; Yellowstone N.P.; Gibbon R., upper sta.; E.R.

Vincent. 2 adults; Gallatin Co.; Bridger Creek, 4800' elev.; 2 Mar 1990; M.M. Hooten. 1 adult; Lewis and Clark Co.; 2 mi. N. Wolf Creek, spring creek; 8 Apr. 1990; M.M. Hooten. 1 adult; Lewis and Clark Co.; 1 mi. N. Wolf Creek, Little Prickly Pear Creek; 13 Apr. 1990; M.M. Hooten. 2 adults; Madison Co.; O'Dell Creek, 0.5 mi. W. Ennis; 22 Mar. 1990; M.M. Hooten. 2 adults; Madison Co.; N. Meadow Creek, 10 mi. S. Ennis; 22 Mar. 1990; M.M. Hooten. 7 adults; Wheatland Co.; Fish Creek on U.S. Hwy 191; 15 Apr. 1988; C.E. Seibert. 4 adults; Meager Co.; S. Fork Musselshell R., 1 mi. SW. Lennep; 28 Apr. 1988; C.E. Seibert. 1 adult; Teton Co.; Muddy Creek at Hwy. 220; 26 Aug. 1988; C.E. Seibert. 1 adult; Lewis and Clark Co.; Sun River Game Range, NW. of Augusta, 68°F; 17 June 1988; C.E. Seibert. 4 adults; Gallatin Co.; Madison R. overflow E. of Three Forks; 27 Apr. 1988; C.E. Seibert. 2 adults; Granite Co.; Flint Creek, 3 mi. N. Philipsburg; 6 Apr. 1988; C.E. Seibert. 2 adults; Gallatin Co.; Brackett Creek, Bridger Canyon; 14 Apr. 1988; C.E. Seibert. 4 adults; Gallatin Co.; Sixteen Mile Creek at Maudlow; 25 May 1988; C.E. Seibert. Larvae; Gallatin Co.; Snowflake Spring; 19 Feb. 1987, 11 Sept. 1987, and 26 Dec. 1987; D.L. Gustafson. Larvae; Gallatin Co.; West Fork Spring; 19 Feb. 1987, 11 Sept. 1987, and 26 Dec. 1987; D.L. Gustafson. Larvae; Gallatin Co.; Bridger Creek, 4800' elev.; 5 Feb. 1987 and 14 Mar. 1987; D.L. Gustafson.

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