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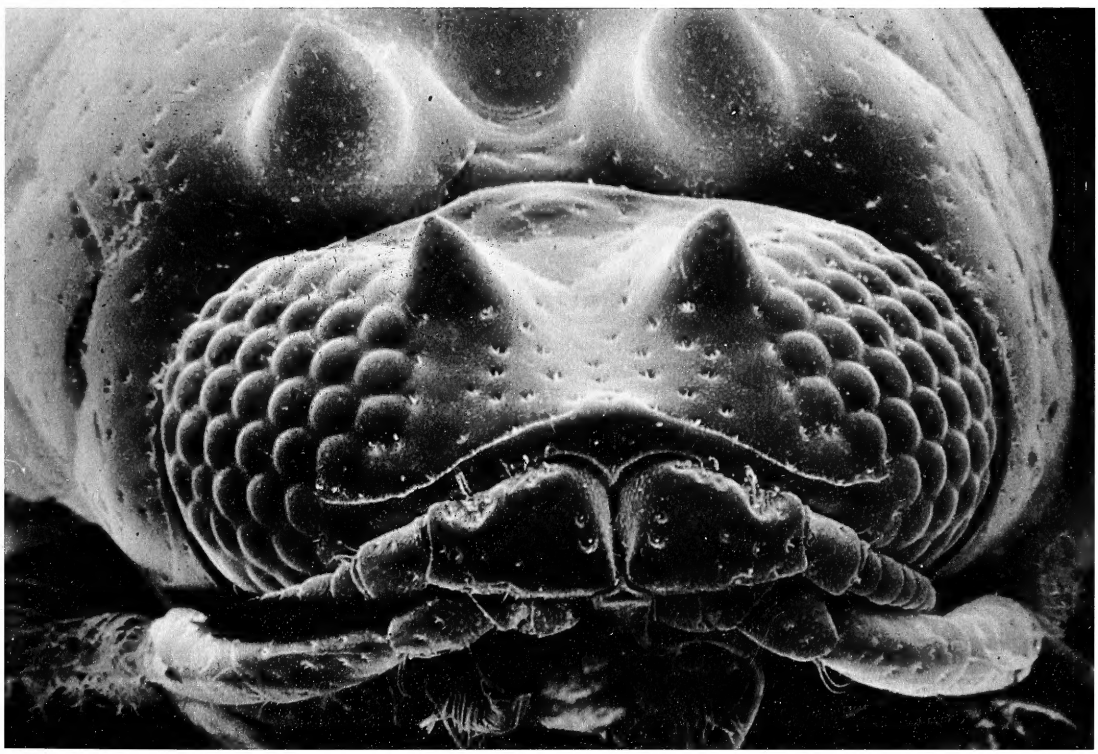

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Water Relations of an Annual Grass, *Bromus diandrus*, in the Central Valley of California

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Abstract.—Annual grasses dominate uncultivated land in the Central Valley of California. The Mediterranean climate constrains growth of grasses to winter and early spring when most precipitation occurs and temperatures are moderate. Water is the key limiting resource, yet its dynamics in the annual grasses is not well understood. We determined diurnal and seasonal patterns in water potential and relative water content (RWC) in *Bromus diandrus*, evaluated the degree of coupling between water potential and RWC and also tracked growth of the plants. The work was conducted in a 8.0 ha exclosure which has had minimal disturbance since its establishment in 1974. Predawn water potential remained mostly unchanged and high through about 80% of the growing season. Midday water potential was consistently lower than at predawn and showed greater fluctuation. Relative water content declined to low values of 73% and 56% at predawn and midday, respectively. This condition was reversible since plants were able to recover to higher RWC levels following rainfall. Predawn coupling of water potential and RWC was variable. The two were tightly coupled early and late in the growing season but diverged during the middle of the season. Alternatively, water potential and RWC were tightly coupled throughout the growing season at midday. Growth followed a sigmoid function $Y = 0.00923 + 0.00232x - 0.0000985x^2 + 0.00000193x^3$ ($r^2 = 0.95$). We conclude that the growth of *Bromus diandrus* is linked to water availability but that there is a predetermined time span for the life cycle. The end of the growing season then, is likely more a function of the onset of germination in the fall/winter than a consequence of the water environment in the spring.

Primary productivity and species composition vary dramatically from year to year in the annual grassland of the Central Valley of California. Establishment and growth of introduced annual grasses, the dominant vegetation (Heady 1977) is largely dictated by water availability (Pitt and Heady 1978). The Mediterranean climate of the region constrains annual grass growth to winter and early spring when most precipitation occurs and temperatures are moderate. Despite the apparent governing role of water (Ewing and Menke 1983), there remain questions about inconsistencies in productivity forecasting models (Duncan and Woodmansee 1975; Murphy 1970).

Accurate productivity forecasts are, in part, dependent on measurement of plant water status as it regulates growth. Kramer (1988) has long perceived measurement

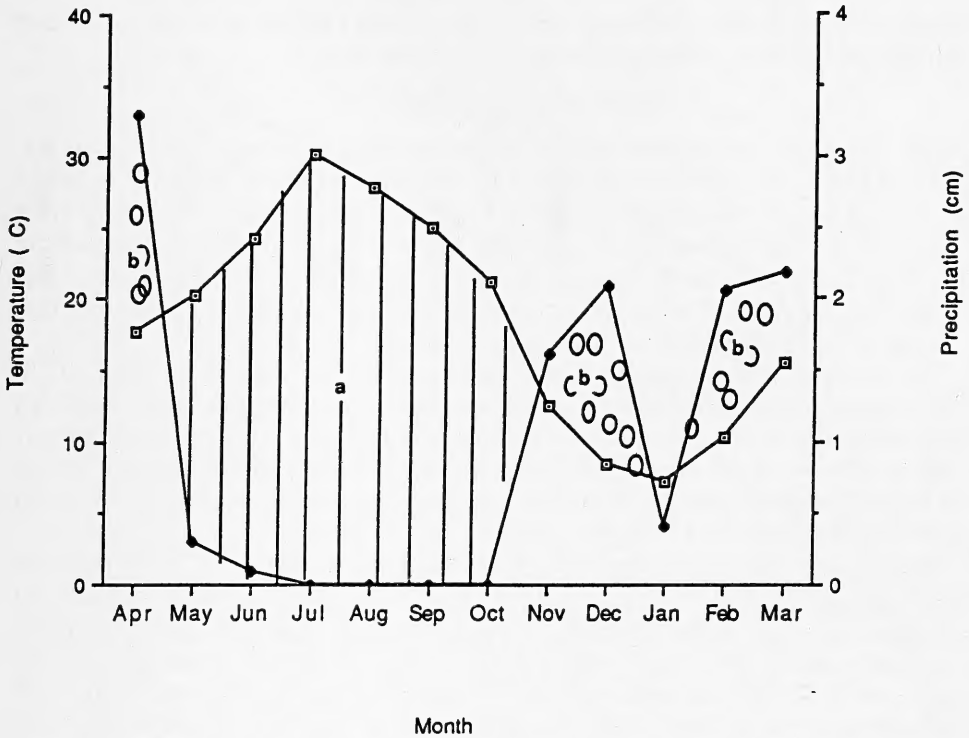

Fig. 1. Climate diagram with precipitation (◆) and temperature (⊡) patterns during 1988–1989. Vertical shaded areas (a) represent a drought period when temperature > precipitation at approximately the 2:1 scale, and circular shaded areas (b) represent wet periods when precipitation > temperature at approximately the 2:1 scale.

of plant water potential as the reliable basis for inferring physiological status of plants. Shulze et al. (1988) caution against reliance on a "most important factor" and advise an integrative approach that considers additional variables, including relative water content, in working out mechanisms of plant response to the external water environment. Water potential and relative water content may be tightly or loosely coupled depending upon time of day and stage of plant development. Analysis of the degree of coupling between these two variables affords an opportunity to more accurately predict primary productivity and its relation to variations in precipitation.

Timing of precipitation, for example, apparently is at least as important as amount in determining plant productivity. Understanding growth of annual grasses has important theoretical implications in predicting patterns in annual grassland ecosystems. There is practical significance since many ranchers rely on annual plants for grazing during winter months (Barbour and Major 1977). Also, the end of the growing season signals the start of the fire season; a problem of considerable concern since the Valley is characterized by intense multiple human use.

This work was initiated to better understand the patterns in water relations and growth of a dominant annual grass in the southern Central Valley of California. Objectives were to: 1) determine the diurnal and seasonal patterns of water potential and relative water content in *Bromus diandrus*; 2) evaluate the degree of

coupling between water potential and relative water content of *B. diandrus*; and 3) track growth of *B. diandrus* during the growing season.

The Study Site

The study site was located on the California State University campus in Bakersfield (35°20′N, 119°05′W) and had an elevation of about 150 meters above sea level. The soil was predominantly a Cajon fine sandy loam, the surface layer ranging from about 18 to 60 cm in depth. The soil was deposited as outwash on the Kern River fan, rarely formed clods and permitted water percolation and permeation by roots. The study site exclosure encompassed 8.0 ha and has had minimal disturbance since its establishment in 1974.

The local climate is Mediterranean, arid and highly variable (Major 1977). Precipitation varies both spatially and temporally with the typical pattern of high temperatures and low precipitation through the summer months. Cool temperatures and most of the year's precipitation prevail in the winter and early spring, the experimental period during 1989 (Fig. 1). This climatic diagram is modeled after that described by Lauenroth (1979).

The pristine California steppe (Kuchler 1964) has, in large part, been replaced by annual species and cultivation (Heady 1977). The composition of introduced annual grasses varies dramatically in the region (McNaughton 1968). Our site was dominated by *Bromus diandrus, B. tectorum,* and *Hordeum jubatum.* A very small portion of the foliage cover was *Erodium cicutarium* and other forbs. The grass canopy was uniform except in areas used by the Beechey ground squirrel (*Spermophilus beecheyi*) and the San Joaquin kit fox (*Vulpes macrotis*). The landscape in these areas was a matrix of patches or grassy areas used heavily, intermittently, or not at all by the squirrels and foxes. The patches were intercepted by trails or corridors which connected burrows. This heterogeneity in the landscape established distinct microhabitats for *B. diandrus* which were absent in the uniform areas not used by animals.

Methods

Water potential of *B. diandrus* was measured weekly during the growing season from 16 January to 4 April 1989. Ten replicate plants were randomly selected for measurement at predawn (0400 to 0600 hours) and also at midday (1200 to 1400 hours). The pressure bomb technique (Scholander et al. 1965) was used (Soilmoisture Equipment, Inc., model 3005, Santa Barbara, CA) and whole plants were cut just above the crown and immediately inserted in the chamber. Predawn and midday were selected to correspond with periods of maximal and minimal water potential respectively in the grass (Sala et al. 1981). A dissecting scope (magnification = ×20) was mounted over the chamber to confirm the bubbling endpoint. Total water potential of the plant was assumed equal to the pressure required to induce bubbling at the cut tip of the plant since most of the water moved through xylem tissue. The xylem water has very few solutes and osmotic potential can routinely be assumed to be negligible (Salisbury and Ross 1985).

Relative water content (RWC) of *B. diandrus* was measured weekly during the growing season from 2 February to 12 April 1989. Ten replicate plants were randomly selected on most dates for measurement at predawn and also at midday. Whole plants, excluding the root system, were weighed to the nearest 0.1 mg on

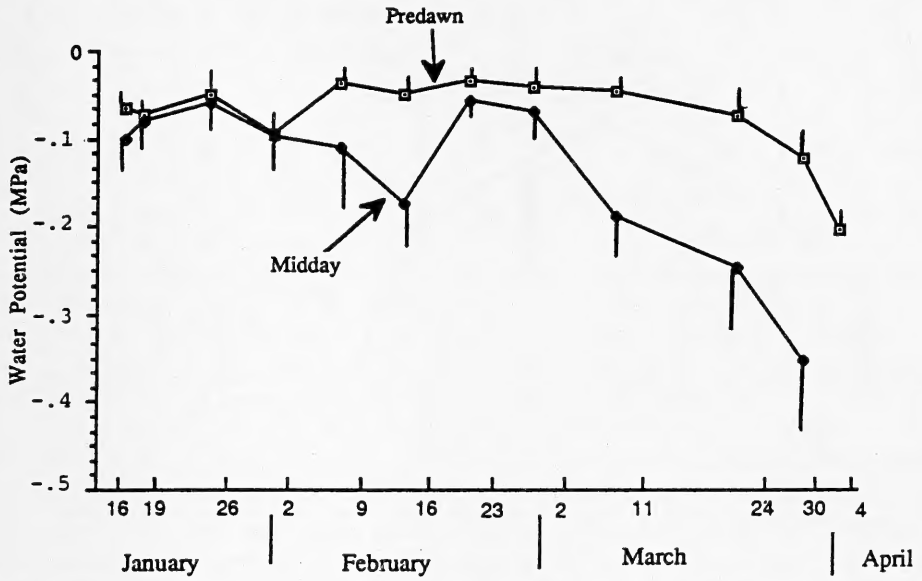

Fig. 2. Water potential (MPa) of *Bromus diandrus* through the course of the growing season. The upper and lower lines represent values at predawn and midday, respectively. Vertical bars are 95% confidence intervals.

an analytical balance. Mean fresh weights were determined immediately after plant sample collection. Plants were then placed in distilled water for a minimum of 3 h and saturated weights were determined. Barrs and Weatherley (1962) reported nearly all the water uptake by excised plant parts occurs in the first three hours of submersion. Finally, the same plants were placed in an oven at 60°C and dried for 24 hours to a constant weight. RWC in percent was calculated as:

$$\text{RWC} = \frac{\text{Mean fresh wt.} - \text{Dry wt.}}{\text{Saturated wt.} - \text{Dry wt.}} \times 100$$

The calculated RWC was theoretically independent of dry weight (Moore and Chapman 1986).

Soil moisture measurements were made at 5 cm and 20 cm throughout the experimental period. A "Quickdraw" Soilmoisture Probe was used (Soilmoisture Equipment Corp., model 2900F, Santa Barbara, CA). The instrument acted as a tensiometer so that water was extracted from the porous sensor by the soil. When the vacuum created in the tensiometer was just sufficient to overcome extraction of water by the soil, an equilibrium and an estimate of "soil suction" were achieved.

Growth was estimated from dry weight data collected throughout the experimental period and reported per plant for the aboveground whole plant component.

Significant differences in water potential and relative water content were determined by assessing separation in 95% confidence intervals. Growth of *B. diandrus* was tracked by using best-fit polynomial regression models.

Results

No rainfall event larger than 2.0 mm occurred in the 21 days prior to the start of the experiment on 16 January 1989. Water potential decreased slightly in both

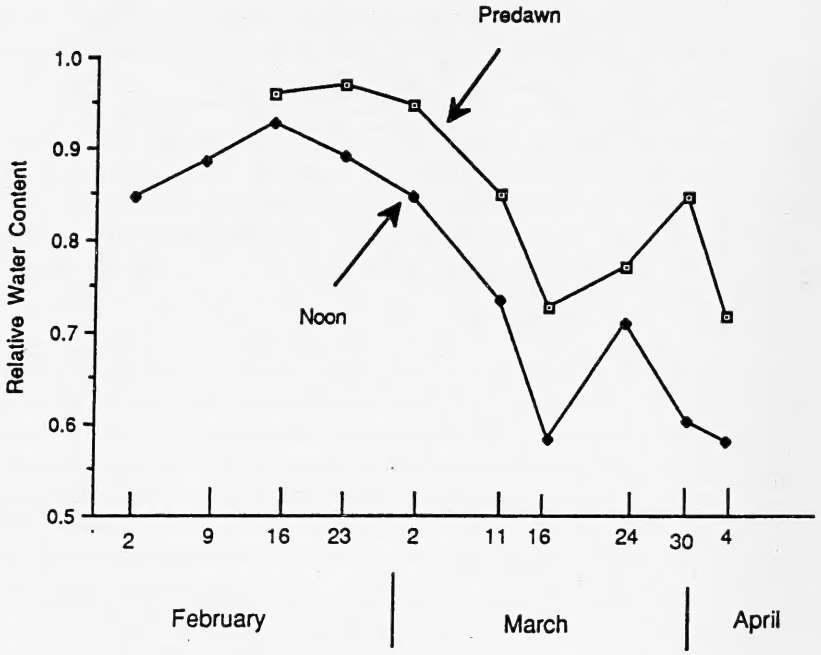

Fig. 3. Relative water content of *Bromus diandrus* through the course of the growing season. The upper and lower lines represent values at predawn and midday, respectively.

predawn and midday measurement conditions through the next 21 days (Fig. 2). Following 19.5 mm of rain on 5 February, predawn water potential recovered to a level near −0.05 MPa and remained unchanged for the next 38 days. In contrast, midday water potential continued to decline for an additional 10 days and then recovered to around −0.07 MPa before declining for the next 23 days. By 16 March, both predawn and midday water potentials began to decline and did not recover by the end of the experiment. Fluctuation in midday water potential is likely attributable to factors which influence atmospheric demand such as rainfall (5 February), wind (7–9 February), and cloud cover (16 March). Despite 14.2 mm of rain on 24–25 March, there was no recovery in plant water potential. This appeared to signal the end of the growing season and/or the overriding of plant response to the water environment by a genetic trigger for senescence. Both predawn and midday water potential declined to lowest levels at the end of the experiment; −0.27 MPa on 4 April and −0.36 MPa on 30 March, respectively. Midday measurements were unreliable after 30 March since water columns were broken and cells were plasmolyzed. Estimates of several transects revealed that living plants were less than 10 percent of the cover at that time in the year. Individual plant variability was most pronounced during midday and near the end of drying intervals.

Relative water content was highest (0.97) in plants at predawn on 23 February and decreased steadily to 0.73 on 16 March (Fig. 3). Midday relative water content peaked at 0.93 on 16 February and also decreased steadily to 0.56 on 16 March. Following 14.2 mm of precipitation on 24–25 March, predawn and midday relative water content rose to 0.85 and 0.73, respectively. Thereafter, relative water content decreased dramatically to 0.73 at predawn and 0.58 at midday by 4 April.

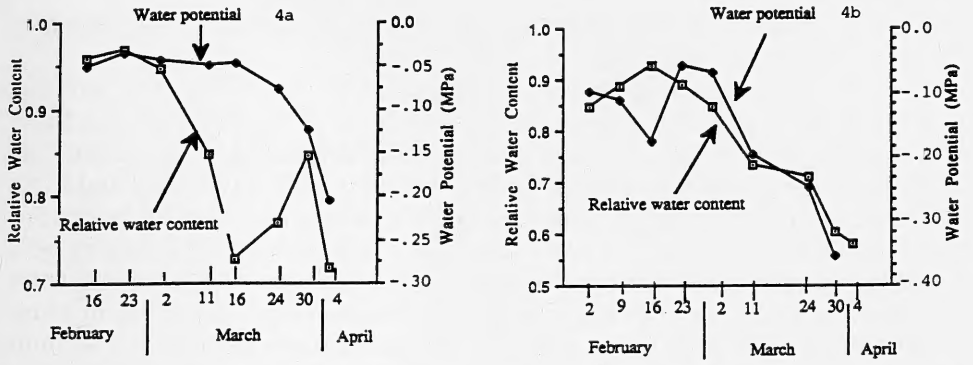

Fig. 4. Water potential (MPa) and relative water content through the growing season. Fig. 4a represents predawn measurements, and Fig. 4b represents midday measurements.

By 16 March, most plants were in the inflorescence stage. Comparison of flowering and nonflowering plants revealed a significant difference ($t = 2.306$, $P \leq 0.05$) in relative water content with mean relative water content higher (0.66) in flowering plants than in nonflowering (0.58). This may be attributable to reduced functioning, including lower transpiration, from leaves of plants in which the inflorescence is mature (Gepstein 1988).

Available soil water ranged from −0.01 to −0.04 MPa at a depth of 5 cm and −0.02 to −0.04 MPa at 15 cm throughout the experiment. Soil water was not likely limiting during the experiment since these values were routinely higher than plant water potential values.

Water potential and relative water content were tightly coupled early in the growing season at predawn (Fig. 4a). During the middle of the growing season however, through March, relative water content declined dramatically and increased only following the 24–25 March rain. During the late portion of the growing season, water potential and relative water content were again tightly coupled. Alternatively, water potential and relative water content were tightly coupled through most of the growing season at midday (Fig. 4b). The correlation coefficient between water potential and relative water content was $r = 0.58$ at predawn and $r = 0.85$ at midday.

Discussion

Diurnal and Seasonal Patterns

Water potential of *Bromus diandrus* was always higher at predawn than at midday. This reflected the dark period recovery in plant water status and was consistent with the results of Redman (1976) who worked with the perennial grass, *Agropyron dasystachyum*. Like Redman's measurements, variability increased in our data as water potential declined and was most apparent near the end of the growing season. Unlike Redman, however, we found RWC to be a more sensitive indicator of plant water status than water potential. Large diurnal changes in RWC, 0.97 to 0.73 at predawn and 0.93 to 0.56 at midday, were contrasted with relatively low variation in water potential, −0.036 MPa to −0.275 MPa at predawn and −0.048 MPa to −0.350 MPa at midday. Furthermore, RWC rebounded following 14.2 mm of precipitation on 24–25 March while water potential con-

tinued to decline. Differences in annual and perennial grasses may be related to variable morphology in the two.

The variation in coupling between water potential and relative water content at predawn may be attributable to shifts in plant growth and development. Early in the growing season, the growth of grass plants is dominated by increase in cell volume. Turgor pressure and cell volume are linearly related (Gardner and Ehlig 1965). Consequently, relative water content is closely allied with the water potential which is the capacity of water to do work or essentially fill enlarging cells to maintain turgor. Later in the growing season, however, growth is predominantly by differentiation and specialization of tissues. The measurement of xylem water potential may reflect high pressure in this conducting tissue but may not account for proportionally lower relative water content with increased dry weight. Dry weight accumulation is a consequence of cell wall thickening, uptake of osmotic substances and synthesis of secondary compounds (Salisbury and Ross 1985). Finally, water potential and relative water content are tightly coupled later in the growing season as growth decreases and cells plasmolyze. Xylem water potential then closely parallels relative water content of the grass plant. Relative water content and water potential both decline with maturation of the flower. This may be a consequence of decreased elasticity and increased osmotic concentration. Millar et al. (1968) reported two relationships between RWC and water potential during annual plant development: 1) tight coupling during vegetative growth or tillering of barley and 2) loose coupling with higher RWC at lower water potential during flowering. We also observed a loose coupling with inflorescence but with lower RWC and higher water potential. Growth phases may have been tied to RWC and water potential at midday but were less distinguishable than at predawn.

Seasonal osmotic adjustment likely influences degree of coupling between RWC and water potential. Kramer (1988) maintains that while RWC is a useful parameter, water potential has wider application in understanding plant water stress. Yet, Sinclair and Ludlow (1985) report there is increasing evidence cell volume or RWC is possibly a key regulator of metabolic activity. Osmoregulation by plants ensures maintenance of constant turgor and/or volume (Schulze et al. 1988), a prerequisite for growth and development. High concentrations of osmotic agents like sugars and ions may compensate for reduced RWC in plant cells in maintenance of turgor. This can occur without substantially altering the soil-plant-atmosphere water continuum and hence, water potential. This may explain the discrepancy between RWC and water potential in our study, notably following the 5 February and 24–25 March rains.

Sala et al. (1981) reported an equilibrium between leaf water potential of blue grama (*Bouteloua gracilis*) and the wettest soil layer at predawn which was independent of atmospheric demand. They suggest that root surface limited water uptake at midday when soil water potential exceeded −2.0 MPa. Our study also suggests that plant and soil water potential approach an equilibrium at predawn. Alternatively, there was no evidence for a threshold response at midday. That coupling is consistent and tight between water potential and relative water content at midday likely reflects an overriding influence of environmental factors, notably water and temperature. Variation in plant water potential and relative water content values are lower at midday.

Soil moisture never dropped below −0.048 MPa and water potentials remained

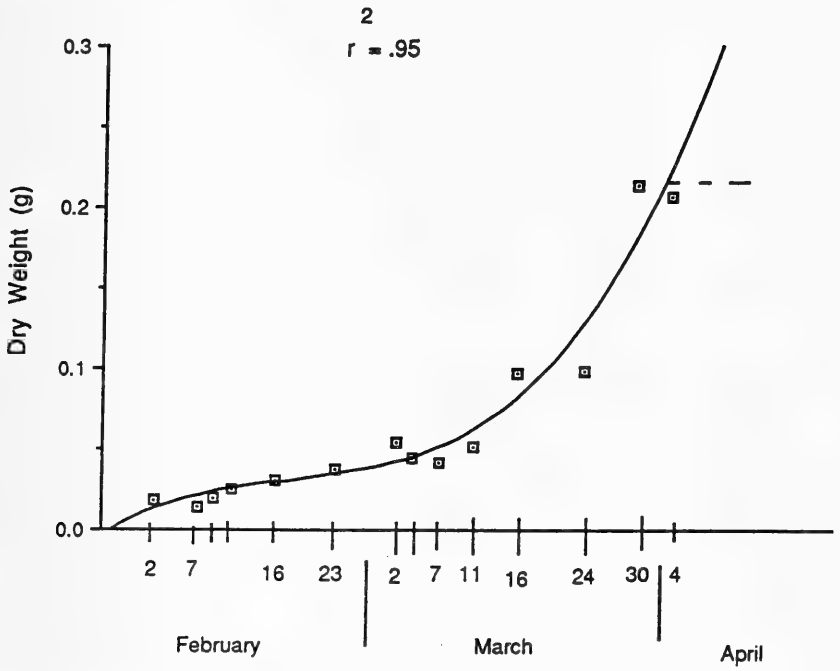

Fig. 5. Growth of *Bromus diandrus* through the course of the growing season. The function is $Y = 0.00923 + 0.00232x - 0.0000985x^2 + 0.00000193x^3$ where Y is weight (grams) and x is time during the growing season (days) ($r^2 = 0.95$).

uniformly high through the course of the growing season. Nevertheless, RWC declined and by the end of the experiment, less than 10% of the plants in the canopy cover were green. Water, including subsurface soil moisture, and moderate winter temperatures regulate growth through the season as earlier modeled (Pendleton et al. 1983), but the life cycle ends shortly after inflorescence maturation. This may be independent of water status of the soil and merely natural senescence of the plant.

Growth

Growth of *Bromus diandrus* is clearly weather sensitive. Pitt and Heady (1978) reported a range in cover of the grass from a mean of 4.5% from 1955–1975 to a high of 18.5% in 1957 at the Hopland Field Station in the central portion of the coastal mountains, Mendocino County, California. In general, growth followed a sigmoid function (Fig. 5). The polynomial which best fits our dry weight data is:

$$Y = 0.00923 + 0.00232x - 0.0000985x^2 + 0.00000193x^3 \qquad (r^2 = 0.95)$$

where Y is weight (grams) and x is time during the growing season (days). While the model predicts near exponential increase in growth, in fact, dry weight is expected to plateau (dashed line) since leaves are dry, flowering is complete, and there is little evidence of growth in the few remaining live plants. This classic growth curve (Leopold and Kriedemann 1975) corroborates the tiller growth of *Bromus mollis* and *Avena barbata* reported by Ewing and Menke (1983). They also reported an extended early period of slow growth followed by rapid growth.

Ecological Implications

Growth of *Bromus diandrus* is tightly coupled with the water environment. Unlike certain perennial grasses which efficiently exploit available water throughout a soil profile, however (Sala et al. 1982), this annual grass only takes up water and therefore grows under relatively high soil moisture conditions. It functions as a drought evader with likely little negative feedback to the plant from drying conditions. Monson and Smith (1982) reported a general lack of adaptation of the annual forbs *Amsinckia* and *Erodium* species to arid conditions in the Mojave Desert. Water potential remained high throughout the life cycles of these plants, as it did in ours; yet water stress, also as in our experiment, was apparent during the reproductive phase.

Levitt (1972) classified true drought avoiders as (1) the water savers, and (2) the water spenders. *Bromus diandrus* clearly functions as a water spender relying on regular and substantial extraction of water from the soil to maintain relatively high plant water potential. Since soil moisture remained uniformly high throughout the experiment, length of life cycle likely signals the drying of the grass. Water potential was not measured below −0.04 MPa. Plants began to dry, however, and relative water content declined to 0.56 at midday near the end of the experiment. This implicates a genetic trigger (Murphy and Thompson 1988) in completion of the life cycle. Water to initiate seed germination in the fall/winter therefore may not necessarily determine timing of inflorescence and senescence in the spring when soil water is available.

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X-ray Microanalysis of the Cuticle Surface of Two Southern California Marine Isopod Species

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Abstract.—Energy dispersive (EDS) x-ray microanalysis (XRMA) was used to determine the elemental composition of thoracic (pereonal) epicuticle in two isopod crustacean species, *Colidotea rostrata* and *Excorallana tricornis occidentalis*. This represents the first study analyzing the cuticle of marine isopods using EDS techniques. The most common elements (with an atomic number greater than sodium) in both species were calcium, magnesium, potassium, silicon, phosphorus, and aluminum, with calcium making up 86–95% of total elemental composition in all analyses. There were significant interspecific differences in elemental composition. Other elements identified in these species include the heavy metals strontium, neobium, molybdenum, dyprosium, lanthanum, and platinum. These heavy metals have not been reported as normal constituents of isopod cuticle, and may be indicative of marine pollution in the coastal waters of southern California and northern Baja California.

This study used energy dispersive x-ray microanalysis (EDS XRMA) to identify and compare the elemental composition of thoracic epicuticle from two common southern California shallow water marine isopods, *Colidotea rostrata* (Valvifera, Idoteidae) and *Excorallana tricornis occidentalis* (Flabellifera, Corallanidae). *C. rostrata* occurs only as a commensal of two eastern Pacific sea urchins, *Strongylocentrotus purpuratus* and *S. franciscanus* (Stebbins 1989), while *E. tricornis occidentalis* occurs as both free-living and symbiotic individuals in a number of marine habitats (Delaney 1984; Guzman et al. 1988).

Use of x-ray microanalysis with marine invertebrates has been limited mainly to bioassays of crustaceans and molluscs such as oysters (Pirie et al. 1984; Thomson 1985), mussels (George and Pirie 1980; Marshall and Talbot 1979; Chassard-Bouchard et al. 1985), and gastropods (Mason and Nott 1980). Previous x-ray microanalyses of crustaceans include: wavelength dispersive analysis (WDS) of elemental composition in the internal organs of the marine shrimp *Crangon crangon* (Elkaim and Chassard-Bouchaud 1978); electron probe analysis of calcium concentrations in various cuticle layers during molting in *C. crangon* (Hubert and Chassard-Bouchaud 1978); electron energy loss spectroscopy (EELS) and EDS analysis of calcium transport mechanisms in molting crayfish (Mizuhira and Ueno 1983); the detection of heavy metals in the digestive systems of terrestrial isopods via EDS analysis of thin sections (Hopkin and Martin 1984); and EDS analysis of the surface cuticle of the terrestrial isopod *Porcellionides pruinosus* (Hadley and Hendricks 1987).

Materials and Methods

Specimens of the marine isopod *Colidotea rostrata* (Valvifera, Idoteidae), a symbiont of the common Pacific sea urchin *Strongylocentrotus purpuratus,* were collected at Punta Salsipuedes, Baja California Norte, Mexico in February 1984. Specimens of *Excorallana tricornis occidentalis* (Flabellifera, Corallanidae) from waters near Santa Catalina Island, California were given to the author by the National Marine Fisheries Service (NOAA) as part of a program documenting the biological effects of "El Nino" in California. All specimens were initially preserved in a 70% ethanol-seawater solution, then later transferred to 70% ethanol. The cuticle of the second thoracic segments (first pereonites) of male specimens were dissected and prepared for electron microscopy using standard ethanol dehydration series and critical point drying in liquid carbon dioxide (Felgenhauer 1987). These "bulk" specimens (as distinguished from ultrathin sections of specimens) were carbon-coated in an Edwards vacuum-evaporator, mounted on a beryllium wafer using colloidal graphite, and the wafer in turn mounted on a Cambridge S4-10 specimen stub. To identify all elements with an atomic number greater than sodium, EDS XRMA was then performed on the specimens using a Tracor Northern EDS system.

Analyses of both isopod species were done at constant operating parameters of 20 KeV (accelerating voltage of the primary electron beam) for 200 seconds (sec). On three specimens of each species three replicate analyses of the same tissue area were performed sequentially to verify accuracy of element identification. A separate study of *Colidotea rostrata* was performed with parameters of 20 KeV for 100 sec, using one analysis of each of five separate specimens.

Magnification setting was $\times 5000$ in all analyses. Based on raster size, accelerating voltage, and isopod cuticle thickness, it is estimated that the microvolumes sampled in this study would include all the epicuticle and a portion of the upper exocuticle.

Results of the two-species study were analyzed statistically for mean elemental composition (%), 95% confidence limits of the means, and variances. In addition, elements present in more than one specimen of both species were compared for significant differences using the *t*-test (see Cox 1972).

Voucher specimens of both species are deposited in the collections of the Natural History Museum of Los Angeles County.

Results

Table 1 summarizes the range of element percents in the epicuticle from both *Excorallana tricornis occidentalis* and *Colidotea rostrata,* using operating parameters of 20 KeV for 200 sec. Element percentages were calculated as percents of total k-alpha emissions only. The epicuticle examined for both species contained predominantly calcium (Ca), with much smaller amounts of magnesium (Mg), potassium (K), silicon (Si), aluminum (Al), and phosphorus (P). *E. tricornis occidentalis* also contained relatively large amounts of strontium (Sr) (4.1–5.5%). *C. rostrata* also contained molybdenum (Mo) and neobium (Nb) (Table 1). Elemental composition in *E. tricornis occidentalis* differed significantly from *C. rostrata* ($P < 0.05$, *t*-test), for Ca, Mg, K and P, but not for Si, at the operating parameters of 20 KeV for 200 sec (Table 2).

A separate analysis of *Colidotea rostrata* using parameters of 20 KeV for 100

Table 1. Range of element percents in analyzed areas of thoracic epicuticle for *Colidotea rostrata* (N = 9, 3 isopods) and *Excorallana tricornis occidentalis* (N = 9, 3 isopods), using x-ray microanalysis parameters of 20 KeV accelerating voltage for 200 seconds.

	Species			
	Excorallana tricornis occidentalis		*Colidotea rostrata*	
Range of percent element composition	Ca	86.4–87.2	Ca	92.5–94.8
	Mg	4.5–6.1	Mg	1.4–1.5
	Si	1.8–2.4	Si	2.3–3.0
	K	0.2–0.4	K	0.6–0.7
	P	0.1–0.2	P	0.7–1.3
	Al	0.3*	Al	1.0*
	Sr	4.1–5.5	Mo	0.1*
			Nb	0.1*

* No range indicated for single-element identifications.

sec identified the following elements (from most to least common): Ca, Mg, K, Si, Sr, zirconium (Zr), argon (Ar), dyprosium (Dy), lanthanum (La), and platinum (Pt). The latter five elements were each identified only once in the analysis. In addition, La and Pt were identified by less than 100 x-ray counts; the identifications of these two elements may be erroneous since the low count rate is at the approximate limit of accuracy of the x-ray analyzer.

Discussion

The main components of the isopod exoskeleton are chitin, protein and calcium carbonate (Sutton 1972). Various aspects of the exoskeleton have been studied in terrestrial isopods of the suborder Oniscidea, such as hindgut cuticle (Holdich

Table 2. Comparison of element composition in pereonal epicuticle of *Colidotea rostrata* (N = 9, 3 isopods) and *Excorallana tricornis occidentalis* (N = 9, 3 isopods).

	Element percent in analyzed samples					
Species	Ca	Mg	K	Si	P	Sr
C. rostrata						
Mean	93.65	1.45	0.65	2.65	1.0	—
95% confidence limit (±)	10.33	0.45	0.45	3.15	2.69	—
Variance	1.32	0.01	0.01	0.12	0.09	—
E. t. occidentalis						
Mean	86.8	5.3	0.30	2.1	0.15	4.8
95% confidence limit	0.81	1.62	0.20	0.61	0.10	1.42
Variance	0.11	0.43	0.01	0.06	0.01	0.33

Species were compared for significant differences in element percents ($P < 0.05$) using the t-test (+ indicates a significant difference). Only elements detected more than once were analyzed for significant differences.

	Elements				
	Ca	Mg	K	Si	P
t-value:	10.49	7.89	5.19	2.12	3.78
S.D. at 95% confidence level:	+	+	+	−	+

and Mayes 1975), epicuticle surface (Holdich and Lincoln 1974; Schmalfuss 1978), integument (Price and Holdich 1980a, b); cuticle surface (Hadley and Hendricks 1987); and exoskeleton chemical composition (Wood and Russell 1987).

The cuticle of three oniscid species, *Porcellio laevis, P. lamellatus,* and *Armadillidium vulgare,* contains 80–85% by weight of calcium carbonate (Lagarrigue 1968). Wood and Russell (1987) determined the chemical composition of exoskeleton in *Oniscus asellus* to be primarily amorphous calcium carbonate, which is prevented from crystallizing to calcite by association with skeletal proteins. Amorphous calcium carbonate is also of widespread occurrence in the majority of decapod Crustacea studied by Pobeguin (1954).

Energy dispersive X-ray spectroscopy (EDS) has been used in relatively few studies of chemical composition in arthropods, and even more rarely in studies of terrestrial isopod crustaceans. EDS techniques have been used to measure heavy metals in the hepatopancreas of the isopod *Oniscus asellus* (Hopkin and Martin 1982, 1984), and the element composition of the cuticle surface and associated microstructures of *Porcellionides pruinosus* (Hadley and Hendricks 1987). This latter study was the first application of EDS to analyze the element composition of surface cuticle in an adult arthropod species. The present study is believed to be the first application of EDS to study the element composition of surface cuticle in adult marine crustaceans.

The inorganic constituents in arthropod cuticle have been determined primarily by studies of marine decapod crabs. Typically large to trace amounts of Ca, P, Mg, Al, iron (Fe), sulfur (S), and zinc (Zn) have been reported, with Ca by far the most abundant element. Calcium and Mg are usually present as both carbonates and phosphates (Richards 1951; Hackman 1984). Elements detected in the cuticle of a terrestrial isopod by Hadley and Hendricks (1987) included Ca, S, K, P, chloride (Cl), Si, Al, Mg, and sodium (Na). In the present study the most common elements (with an atomic number greater than Na) present in the epicuticle of *Colidotea rostrata* and *Excorallana tricornis occidentalis* were Ca, Mg, K, Si, P, Al, and Sr, with Ca making up 86–95% of total elemental composition in all analyses (Table 1). Statistical analyses showed significant interspecific differences in the percentage of Ca, Mg, K, and P in the epicuticle of these two marine isopod species (Table 2).

Certain heavy metals (Sr, Nb, Mo, Zr, Pt, Dy, and La) identified in these two species of marine isopods have not been previously reported as normal constituents of crustacean cuticle. It is not known whether these are indicative of marine pollution off the coasts of northern Baja California and southern California. However, the Southern California Bight is known to contain a high content of heavy metals relative to other coastal regions of the United States (Bascom et al. 1979).

EDS techniques can detect less than 10^{-16} g of an element (Barbi 1978). As noted by Hadley and Hendricks (1987), EDS analysis of bulk specimens (as in the present study) is considered a semiquantitative technique, due to the lack of control of specimen geometry. Specimen geometry is critical for fully quantitative analysis, which is performed using flat, ultrathin biological specimens (Hall 1979). EDS analysis of bulk specimens, such as pieces of thoracic epicuticle, involves simpler specimen preparation procedures, and thus allows rapid analysis of replicate samples, though at a comparatively gross morphological level. EDS techniques should be useful for environmental applications such as monitoring relative

heavy metal pollution between different sites, as well as the study of relative differences in element composition between species and between higher taxa.

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Does Honey Bee Nasanov Pheromone Attract Foragers?

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Abstract.—Conventional wisdom accords to the honey bee Nasanov gland pheromone a forager attractant function. Our experiments failed to support three predictions of that hypothesis. 1) Fragrant components of Nasanov gland secretion were not more effective than other scents as recruitment incentives for new (naive) foragers to a food source. 2) Honey bees harvesting scented rewards did not choose "Nasanov mixture" scent in preference to other odors. 3) After training to "mixed scent," containing Nasanov and nonNasanov components, bees did not prefer Nasanov pheromonal components. Our experiments did not support the conclusion that Nasanov gland secretions function as a "forager attractant" pheromone.

The Nasanov (scent) gland of the worker honey bee, *Apis mellifera,* consists of several hundred cells located just beneath the sixth intertergal membrane, near the dorsal surface of the abdomen (Snodgrass 1956). When a bee raises its abdomen and flexes the terminal segment, that membrane is exposed. Volatile secretions of the Nasanov gland are released.

Nasanov secretion includes the fragrant alcohols, geraniol and nerol (trans-3,7-dimethyl-2,6-octadien-1-ol and its cis-isomer), citral (their mixed aldehyde isomers), and other oxidation products (Boch and Shearer 1962; Shearer and Boch 1966; Pickett et al. 1980, 1981). These terpene derivatives also contribute to the characteristic odors of several plant species.

The Nasanov scent may function as a pheromone during swarm settling (Sladen 1901; Morse and Boch 1971; Witherell 1985); trap boxes baited with a blended "Nasanov mixture" of fragrant compounds effectively capture swarms (Schmidt and Thoenes 1987; Schmidt et al. 1989). Nasanov scent is also released at the colony entrance when lost or dislocated worker bees are attempting to orient (Sladen 1901; Ribbands and Speirs 1953; Renner 1960).

A possible role for Nasanov scent as a "forager attractant" pheromone has a more checkered history. Von Frisch (1923) advanced that hypothesis to explain the distribution of newly recruited foragers in one of his early experiments. He found that a food source visited by "sealed-gland" bees received fewer recruits than one visited by normal control bees. When later experiments gave a quite opposite result (von Frisch 1947), he dismissed that considerable body of negative

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Table 1. Recruitment of naive bees by foragers harvesting 1.25 M sucrose rewards that were unscented, or perfumed with 100 μl/l cinnamon oil, citral, cajeput oil, geraniol or nerol.

Sucrose reward	Expt. days	Forager visits	Naive recruits	Recruits/ 100 visits
Unscented*	2	258	3	1.2
Cinnamon	3	822	110	13.4
Citral**	3	888	99	11.1
Cajeput	2***	611	70	11.5
Geraniol**	3	648	64	9.9
Nerol**	3	914	100	10.9

* Presented on the first and last days of the experiment.
** Nasanov scents.
*** One day rained out.

evidence (Wenner and Wells 1990); von Frisch continued to regard forager attraction as a well-established function of Nasanov gland scent (1967).

Several attempts to verify the "forager attractant" hypothesis have used dish preference tests. For example, more bees "hovered or landed" at geraniol-scented dishes than at control scents or unscented control dishes; the sum of activity at control dishes, however, exceeded that at the experimentals (Free 1962). A very similar result was obtained when excised Nasanov glands, rather than geraniol, provided the experimental scent (Free 1968).

In other experimental designs, more honey bees were captured in insect traps baited with a mixture of Nasanov compounds, a "Nasanov lure," than in unbaited (odorless) control traps (Free et al. 1984). Similarly, Mayer et al. (1989) reported that a commercially available pheromone mixture, "Bee-Scent" (Scentry Corp.), when sprayed on blooming fruit trees, increased numbers of foraging bees over those observed in untreated orchard plots. Neither of these studies included alternative (nonbee) scents as additional control treatments.

Despite von Frisch's ambiguous results, alternative interpretations of dish preference study results and a paucity of controls in trap and field tests, the body of evidence cited above has been viewed as generally supportive of the Nasanov "forager attractant" hypothesis. Many contemporary accounts of honey been natural history accept that function for Nasanov pheromone (e.g., Seeley 1985; Free 1987; Winston 1987; Gould and Gould 1988).

On the other hand, citral and geraniol failed to attract bees in an olfactometer (Woodrow et al. 1965). Neither did these fragrances regularly increase bee populations when sprayed on test plots of alfalfa, unless coupled with sucrose rewards (Waller 1970). Indeed, in Waller's (1973, Table 1C) "dish preference" experiments, results seem to refute the "forager attractant" hypothesis.

Also, foragers seldom expose their Nasanov glands when visiting natural flowers (Free 1968), but they often do so at experimental feeders containing unscented sucrose solutions (Wenner et al. 1969; Wells and Wenner 1971). Paradoxically, when scent levels in food rewards were increased in those experiments, Nasanov exposure decreased and recruitment increased.

Thus, some published reports support the Nasanov "forager attractant" pheromone hypothesis, while others do not. Additional experimental studies may help to resolve the issue. If Nasanov secretion were in fact a forager attractant pher-

omone one might expect that: 1) one or more of its fragrant constituents would be more effective than other scents in recruitment of foragers to a food source; 2) honey bees harvesting scented rewards should choose a "Nasanov mixture" scent in preference to alternative odors provided after removal of the training scent; 3) honey bees harvesting a "mixed scent" reward, containing both Nasanov and control components, should choose Nasanov compounds when the training mixture is replaced by a set of "single scent" rewards that each contain only one of the training mixture components.

We have experimentally examined these three predicted properties of a presumptive Nasanov gland "forager attractant" pheromone by testing the null hypotheses of equality among scents under the challenge of these predictions.

Materials and Methods

All experiments were done on the Occidental College Campus, Los Angeles, California. Individually-marked foragers from a colony of approximately sixty thousand honey bees were fed 1.25 M sucrose solution from syracuse watch glasses at a distance of 50 m from the hive. The sucrose rewards were unscented or were scented with Eastman Organic Chemicals No. T 378 geraniol, Sigma Chemical Company No. N-7761 nerol, No. C-1645 citral, No. A-6769 anise oil, No. B-4258 bay oil, No. C-7517 cajeput oil, No. C-7267 cinnamon oil, No. C-8392 clove oil, or mixtures of the above at rates specified below. In control experiments excised Nasanov glands or Scentry Inc. "Bee-Scent" commercial pheromonal mixture was used.

Experiment 1.—Eleven individually paint-marked foragers, initially trained on clove-scented sucrose solution, were given two-hour feedings of unscented 1.25 M sucrose for three days prior to the experiment. Then, and throughout the experiment, all unmarked bees (newly recruited "naive" bees) that landed on the feeding dish were captured and killed. Hence, only the marked foragers made repeated trips from hive to feeder.

Food was provided only from 10:00 AM–12:00 M during each day of the experiment. All scented solutions were at the rate of 100 μl fragrant oil/l. Unscented sucrose reward was provided on control days 1 and 22 of a 22-day experiment. On each of days 2–21, a sucrose solution scented with cinnamon, citral, cajeput, geraniol, or nerol was provided, sequentially in that order, one scent per day, with four repetitions of the sequence. Days on which a given scent was provided were separated by four feedings of other odors. During one sequence (days 14–19), however, recruitment could not be monitored because of intermittent rain. Thus, we recorded recruitment on two days each for unscented and cajeput-scented rewards and on three days each for rewards containing cinnamon, citral, geraniol, or nerol scent.

Experiment 2.—Ten individually-marked foragers were initially trained to 1.25 M clove-scented sucrose and were fed daily from 10:00 AM to 12:00 M during each day of the experiment. On day 1 of the experiment, instead of the training scent, separate dishes of cajeput-, anise-, bay- and "Nasanov mixture"-scented sucrose were provided. The specific nonNasanov scents were at the rate of 100 μl/l; the "Nasanov mixture" included 100 μl geraniol, 100 μl citral, and 50 μl nerol/l.

The scent at which each marked forager first landed and drank, and its choices

at subsequent visits were recorded. On day two the most popular scent was omitted; only the three less favored scents were provided. On day three the new most popular scent was omitted, leaving two choices. On day four, the remaining (least popular) scent was pitted against clove, the original training scent. On each day, first and subsequent visits of the marked foragers were recorded. This experiment was repeated thrice with new sets of marked bees each time, and with minor variations (once without "day three," once without "day four" and once with "day one" only).

Experiment 3.—Ten individually-marked foragers were trained to a mixture of scents that included 100 μl/l each of anise oil, bay oil, citral, geraniol and nerol.

On day one of the experiment, instead of the mixture, individual scents (100 μl/l, as in the mixture) were provided in separate dishes. The scents at which the experimental bees first landed and drank, and their choices on subsequent visits, were recorded. These bees were again offered the array of scented-sucrose rewards in separate dishes on the second day of the experiment, and their choices were recorded.

Control experiments.—Three additional experiments were run as controls. In the first, ten bees were trained on 100 μl/l clove-scented sucrose. Then, on the day of the experiment, they were offered separate dishes containing either unscented, 100 μl/l anise-scented, 100 μl/l cajeput-scented or, as a fourth choice, unscented 1.25 M sucrose to which eight fresh surgically excised Nasanov glands had been added. Data were recorded as in experiment 2.

In a second control, bees were trained on 100 μl/l anise-scented sucrose and then tested on separate dishes of 100 μl/l bay-scented, 100 μl/l cinnamon-scented rewards or, as a third option, 1.25 M sucrose solution to which 100 μl/l of Scentry "Bee-Scent" had been added. The scent(s) chosen by each bee were recorded.

As a variation of this experiment, these same bees were then fed bay-scented sucrose for two days. Then, on the third (test) day, they were offered a choice of 100 μl/l clove-, 100 μl/l cinnamon-, or 200 μl/l Scentry "Bee-Scent"-perfumed 1.25 M sucrose. Foraging visits by each bee to the test scents were recorded.

A third control experiment measured Nasanov gland exposures by foragers harvesting 1.25 M sucrose scented with 100 μl/l of "Bee-Scent," 100 μl/l of bay oil, or the unscented sucrose reward. Twelve bees were allowed to harvest one of the above solutions for an hour, then, during the next thirty minutes, numbers of visits and observable Nasanov gland exposures at the feeder were tallied. Two trials were run for each reward type; the same set of marked foragers was used throughout the experiment.

Results

Experiment 1.—Results recorded on separate days when the reward had a given scent did not differ systematically. Therefore, cumulative visits to the feeder by marked foragers, total numbers of naive recruits, and a ratio of recruits to visits are reported in Table 1. Marked foragers readily harvested unscented sucrose and each scented reward when it was offered. On days when unscented reward was provided, the first visits of some foragers were delayed, resulting in fewer cumulative visits. Once started, however, those foragers made repeated trips to the feeder.

Each of the scents we used was effective in the recruitment of naive foragers.

Table 2. Reward choices by honey bee foragers. After training to clove-scented sucrose, bees were allowed to choose bay-, anise-, cajeput- or "Nasanov mixture"-scented rewards. On day 2 only the latter three scents, and on day 3 the latter two scents were provided. On day 4, "Nasanov mixture" was pitted against clove-scented reward. N = number of trained bees.

Day	N	Bay	Anise	Cajeput	Clove	"Nasanov mixture"	Did not visit
1	40	29	4	0	—	0	7
2	30	—	20	1	—	0	9
3	20	—	—	13	—	0	7
4	20	—	—	—	11	0	9

Recruitment rates among citral-, geraniol-, nerol-, cajeput- and cinnamon-scented rewards did not differ significantly ($\chi^2 = 3.894$, df = 4). Neither did recruitment to the three Nasanov scents, as a group, differ significantly from recruitment to the grouped control scents ($\chi^2 = 2.363$, df = 1). Recruitment did differ significantly between scented sucrose rewards and the unscented control ($\chi^2 = 23.176$, df = 1, $P < .01$).

Experiment 2.—Data from four stepwise repetitions of this experiment are summarized in Table 2. Prior to the first test, the individually-marked foragers harvested clove-scented sucrose from a feeder dish. On the first experimental day, when they were offered an array of separate dishes containing bay-scented, anise-scented, cajeput-scented and "Nasanov mixture"-scented sucrose rewards, respectively (but none with the clove-scented training solution), forager choices among the test solutions were not equal ($\chi^2 = 70.879$, df = 3, $P < .01$). Most of the foragers harvested the bay-scented rewards.

When the experiment was repeated on day 2, without bay, significant inequality of visitation again occurred ($\chi^2 = 36.286$, df = 2, $P < .01$). Most of the foragers harvested anise-scented sucrose. Similarly, on day three, numbers of foragers visiting the two remaining test dishes were not equal ($\chi^2 = 13.000$, df = 1, $P < .01$). With neither bay nor anise present, foragers chose cajeput. On day 4, clove, the control scent, was preferred to "Nasanov mixture" ($\chi^2 = 11.00$, df = 1, $P < .01$).

The null hypothesis of equal visitation to all dishes was not supported in any of the experiment 2 tests, and "Nasanov mixture" was never the scent of choice. Under these experimental conditions, bees were constant foragers on their chosen scents, with a cumulative error rate (imbibe from any other dish) of only one percent.

Experiment 3.—During the training period, marked foragers readily harvested sucrose reward perfumed with a blend of anise, bay, citral, geraniol and nerol scents. On test days 1 and 2 when sets of individual dishes, each containing only one of those scents, were provided, they drank from the dishes on which they landed.

Visitation patterns of seven experimental bees are summarized in Table 3, with an asterisk indicating the dish first visited by each bee. Bay and geraniol were favored scents, both for first visits and largest numbers of harvesting trips. Bees also drank from anise and nerol, but never from citral. Neither the "forager attractant" nor the null hypothesis predicted this result ($\chi^2 = 19.891$, df = 4, $P < .01$ of equal visitation, bees 1–6).

Table 3. Individual scents visited by bees first trained to a mixture of all scents. A = anise, B = bay, C = Citral, G = geraniol, N = nerol. * = first visit.

Bee	Day 1					Day 2					Total				
No.	A	B	C	G	N	A	B	C	G	N	A	B	C	G	N
1	—	—	—	—	—	0	0	0	19*	3	0	0	0	19	3
2	0	15*	0	0	0	0	12	0	16	0	0	27	0	16	0
3	0	13*	0	0	0	0	23	0	0	0	0	36	0	0	0
4	0	6*	0	6	1	0	0	0	8	1	0	6	0	14	2
5	0	4	0	6*	1	0	3	0	17	2	0	7	0	23	3
6	0	11*	0	2	2	4	4	0	10	4	4	15	0	12	6
7	—	—	—	—	—	0	11	0	1*	0	0	11	0	1	0

Only bee No. 3 was completely constant to one scent. The others drank from two or more differently scented dishes during the two-day experiment. The cumulative error rate (imbibe from a dish other than that most frequently visited) in experiment 3 was 29 percent, but it varied considerably among bees.

Control experiments.—When ten marked foragers that had been harvesting clove-scented sucrose were offered a choice among three test dishes, one containing freshly excised Nasanov glands and two with control scents, six bees chose the anise control and two chose cajeput. No bees landed at the dish of fresh Nasanov glands. Two of the marked foragers did not visit any dish on the test day.

When ten marked foragers, trained on anise-scented rewards, were offered a choice of Scentry "Bee-Scent"-, bay- or cinnamon-scented sucrose, six bees chose bay, two landed on cinnamon, and two did not visit. Eight of these same bees, after two days of harvesting bay-scented sucrose, chose clove, while the other two visited cinnamon. None of the bees regularly visited the dish perfumed with "Bee-Scent." Individual forager constancy to chosen scent was high, as in experiment 2, but when bees landed on the "Bee-Scent" dish or the "excised Nasanov gland" dish, they drank from it.

In the third control experiment (sums of two trials for each reward type), 106 visits to Scentry "Bee-Scent"-scented reward yielded ten Nasanov gland exposures (9%); 87 visits to bay-scented sucrose yielded nine gland exposures (10%); and 104 visits to unscented sucrose yielded 34 gland exposures (33%). When added to a sucrose reward, "Bee-Scent" suppressed Nasanov gland exposure as effectively as did bay oil.

Discussion

Naive bees, seeking a food source for the first time, rely heavily on odors to which they have been introduced in the hive by the experienced foragers that recruit them (Wells and Wenner 1971, 1973; Wenner 1974; Wenner and Wells 1990). Recruitment of new foragers to an unscented source is negligible (Wenner et al. 1969; Wells and Wenner 1971; Friesen 1973; our experiment 1), and foragers may be individually constant to specific scents in an array of food sources (plastic flowers) polymorphic for color and odor (Wells and Wells 1985).

Nasanov scent experiments which used unscented controls have confirmed the importance of odors to field bees (e.g., Free et al. 1984; Mayer et al. 1989; experiment 1). However, these experiments do not establish that Nasanov components are unique "forager attractant" pheromones. In our experiment 1, for

instance, Nasanov scents did not differ significantly from control fragrances in attractant properties. Our results, and those of others (e.g., Mamood et al. 1992), do confirm conditioned responses by bees to odors associated with food rewards; Nasanov scents are effective conditional stimuli.

In our experiment 2, on redistribution of experienced foragers to an array of unfamiliar scents at a feeder location, "Nasanov mixture" was never the option of choice. Even in experiment 3, when foragers were allowed to choose among the individual components of an odor mixture to which they had been trained, all bees did not gravitate to Nasanov compounds. Some did prefer geraniol, but others chose bay, and forager constancy to individual scents was low.

The results of experiment 2 argue against the notion that components of the Nasanov secretion only acquire effective forager attractant properties when mixed together. Furthermore, neither excised Nasanov glands nor Scentry "Bee-Scent" gave results appreciably different from those we obtained with specific Nasanov components; suppression of Nasanov gland exposure shows that bees perceived Scentry "Bee-Scent" at our experimental concentrations.

Neither the existing body of evidence nor the results reported here justify the conclusion that Nasanov gland secretions function as a "forager attractant" pheromone.

Acknowledgments

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The Spinicaudatan Clam Shrimp Genus *Leptestheria* Sars, 1898 (Crustacea, Branchiopoda) in California

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Abstract.—The spinicaudatan clam shrimp genus *Leptestheria,* the only genus of the family Leptestheriidae known from North America, is reported for the first time from California. *Leptestheria compleximanus* (Packard, 1877), which earlier had been erroneously reported from California (based on specimens collected in Baja California, Mexico), was found in two locations in the western Mojave Desert of California, where it occurs sympatrically with notostracans and anostracans. Some brief notes on natural history of the species, and a taxonomic synonymy, are provided.

The branchiopod crustacean orders Spinicaudata and Laevicaudata (formerly united as the order Conchostraca; see Fryer 1987; Martin and Belk 1988; Martin 1992) currently include five extant families commonly called clam shrimp. The Laevicaudata contains only the family Lynceidae, with three known genera, two of which are known from North America (see Martin and Belk 1988). The Spinicaudata contains four families (Cyclestheriidae, Cyzicidae, Leptestheriidae, and Limnadiidae), all of which contain at least some species known from North America.

In California, only two spinicaudatan families have previously been reported. The Limnadiidae is represented by several species in the diverse genus *Eulimnadia* (Belk 1989; Sassaman 1989). The taxonomically confusing family Cyzicidae is also relatively common (Mattox 1957a; Wootton and Mattox 1958), although species and genera in this family are badly in need of systematic reevaluation (e.g., see Straškraba 1965).

The family Leptestheriidae, of which the only species known from North America is *Leptestheria compleximanus* (Packard, 1877), has not been previously reported from ephemeral ponds in California. However, because of the following set of circumstances, the species has often been listed as occurring in this state. In two similar publications that appeared in 1895, Jules Richard (1895a, b) listed "Basse-Californie" (Lower California), Mexico, for one collection of *L. compleximanus.* Richard (1895a) stated that his material came from "l'arroyo de la Purissima (Basse-Californie)." Although there is a similarly spelled locality of Purisima Creek, California, on the San Francisco peninsula, it is not in an optimal habitat for ephemeral pond species. In contrast, Richard's Arroyo Purissima [sic] in Baja California ("La Purisima creek" of Maeda-Martinez 1991), site of a former mission, is located in an area of abundant ephemeral ponds (Clay Sassaman, pers. comm.). Furthermore, Richard (1895a: 107) stated that the species was collected along with *Eocyzicus digueti,* a spinicaudatan species known at that time only

from Baja California,[1] strongly suggesting that Richard's specimens of *Leptestheria compleximanus* were from Baja California, and not California. (*Eocyzicus digueti* has since been reported from Kansas and Nevada (Wootton and Mattox 1958), the Sonoran and Chihuahuan deserts of northern Mexico (Maeda-Martinez 1991), Arizona (Belk 1992), and San Diego County, California (Simovitch and Fugate, in press)).

Richard's (1895a, b) Baja California record of *Leptestheria compleximanus* was based on collections made by "M. Diguet." Part of this collection found its way to the Paris Museum, where it was later studied by the great Hungarian limnologist Eugene Daday (Daday 1923), as evidenced by his mentioning specimens collected by Diguet from the "Mares de l'Arroyo de la Puressima" [sic] in "California" in his monograph on the family Leptestheriidae (Daday 1923: 319). These are undoubtedly the specimens listed by Forró and Brtek (1984: 99), in their account of the Hungarian Natural History Museum's collection of Anostraca and Conchostraca taxa described by Daday, as *Leptestheria compleximana* [sic] (the same incorrect spelling used by Daday) from "California." Prior to Daday's (1923) monograph, Dodds (1915) and Pearse (1918: 674) (in the first edition of Ward and Whipple's "Fresh-Water Biology") had correctly listed Richard's record of the species from "Lower" California. However, probably based on Daday's monograph, both Creaser (1935) and Mattox (1959), in two popular texts on freshwater biology, omitted the word "Baja," thereby mistakenly crediting the collecting site to California, and several subsequent compilations (e.g., Pennak 1978, 1989; Moore 1965; Fitzpatrick 1983; Saunders and Wu 1984) have perpetuated this error.

Creaser (1935) and Mattox (1959), it turns out, were correct in reporting the species from California, albeit for the wrong reason. *Leptestheria compleximanus* (Packard, 1877) does indeed occur in California, and in this paper we report two geographically close areas in the western Mojave Desert of California where the species is found in relatively large numbers.

Materials and Methods

The species first came to our attention in the form of dried mud samples collected on 13 October 1990 from Amboy Crater, Mojave Desert, California (located just southeast of Amboy, California, and approximately 500 m south of Route 66 in San Bernardino County) (Fig. 1A, B) by Dr. Edward Wilson of the Earth Sciences Division of the Natural History Museum of Los Angeles County. Dr. Wilson also reported having seen live conchostracans and notostracans at times when the crater contained water. These dry mud samples were packed with shells of a spinicaudatan clam shrimp, and obviously were collected not long after the population's demise, as the valves were in reasonably good condition (Fig. 1D). In the laboratory, we added dechlorinated water to the samples on 8 November 1990, and soon obtained a series of small conchostracans easily identifiable as *Leptestheria compleximanus* (Packard, 1877). Specimens of these labo-

[1] Although Richard (1895a) suggested that *E. digueti* might be the same as Baird's (1866) *Estheria newcombi,* described from California (and of historical interest as being the first report of any species of conchostracan from California), *E. newcombi* was incompletely described, is possibly synonymous with *Cyzicus californicus* (see Wootton amd Mattox 1958), and is no longer considered a valid species.

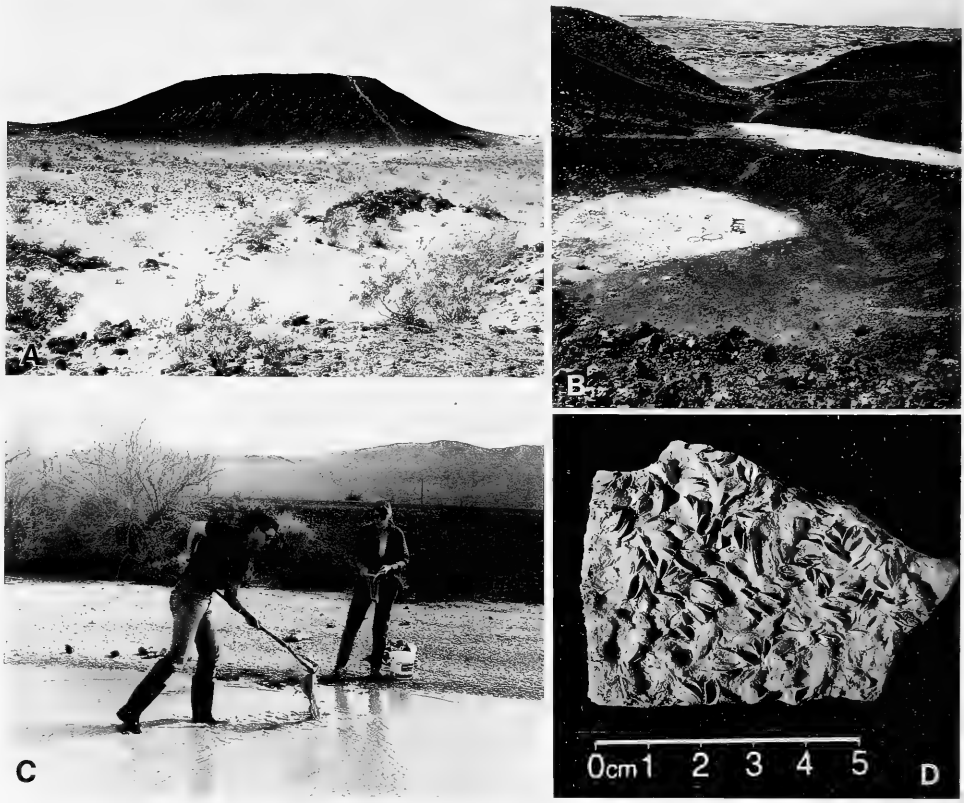

Fig. 1. Collecting localities (Amboy Crater and Troy Dry Lake, western Mojave Desert) for *Leptestheria compleximanus* in California. A, Amboy Crater as seen from state road 66. B, view of crater floor from northeastern rim of crater, showing two small areas (light colored) where temporary pools form. Depression in far crater wall is area of lava outflow to the southwest. C, collecting area at Troy Dry Lake. Road in background is Interstate 40. D, dried mud sample collected from Amboy Crater in 1990, containing numerous valves of *Leptestheria compleximanus.*

ratory-reared samples were preserved on 29 November 1990, when the animals were 21 days old and measured up to 4.70 mm in valve length in both males and females. The animals were not yet mature, as no females were observed with an egg mass visible beneath the shell. In the laboratory this species can reach maturity in only 9 or 10 days (Clay Sassaman, pers. comm.), so it is possible that our rearing conditions were suboptimal.

Live individuals of *Leptestheria compleximanus,* as well as live notostracans (*Triops longicaudatus*) and anostracans (*Branchinecta mackini* and *B. lindahli*), were observed in the field on 18 March 1992 in shallow, very muddy pools (Fig. 1C) on either side of west Interstate 40 at Troy Dry Lake, Mojave Desert, just east of mile marker 26, 1.9 miles east of the Ft. Cady exit to Newberry Spring (which is at mile marker 23.5). These individuals were slightly larger than the laboratory reared specimens from Amboy Crater. Additionally, dry mud from around these pools, and from the easternmost of two small craters within Amboy Crater (Fig. 1B), was returned to the laboratory.

Natural History Observations

The roadside site (Troy Dry Lake) (Fig. 1C) was filled with water so muddy that visibility was minimal; clam shrimp could be seen only as they neared the water surface, which they did often, sometimes to the point that the highest part of the carapace valves extended slightly above the surface. Other branchiopods seen and collected included the anostracans *Branchinecta mackini* and *B. lindahli* and the notostracan *Triops longicaudatus.*

Leptestheriids were active and were observed mating in the field and later in the laboratory, where they lived only about 24 hours. Mating involves clasping of the female's shell by the male's first two pairs of thoracopods so that she is held in front of him in a horizontal plane (with her carapace hinge directed forward while his is directed upward) and is propelled by his motion, adding no appreciable component to movement herself. The female is positioned far toward the front of the male, so that from above the couple resembles a swimming letter T, the horizontally positioned female being the cross of the T (similar to mating in *Cyzicus* as illustrated by Mathias 1937: 44, fig. 3). A photograph of a mating pair of *L. compleximanus* (although referred to only as "clam shrimp" the species is recognizable) appeared in the October 1975 issue of National Geographic magazine (Findley and Sisson 1975: 578–579). Males at Troy Dry Lake were very active in their pursuit of, and attempts to clasp, females. Clasping occurred with egg-less females as well as with females already carrying an extruded egg mass visible beneath the thin shell.

Burrowing is common. This activity was at first thought to be an artifact of the shallow habitat, but even in deeper waters, in the field and in the laboratory, clam shrimp were seen to burrow into the underlying mud, and then to emerge at a point near where they entered. It is possible that they actively ingest mud and derive some nutrition from organic matter contained therein, as it is difficult to envision effective filtering in water so thick with suspended mud and clay. Tasch (1964) did not mention burrowing in a culture of *L. compleximanus* from Mexico, which he maintained in the laboratory for over three and one half months, but commented on their swimming "with ventral valves upward and agape" just below the surface of the very turbid water. We did not see any similar activity in our population.

Females are slightly smaller than males. Size of the Troy Lake specimens, measured from the maximum length and height of the valves, ranged from 6.10 mm long and 3.18 mm high in females (N = 15) to 6.48 mm long and 3.68 mm high in males (N = 15). This is larger than specimens reared from dried mud in 1990 from Amboy Crater, but is smaller than reported lengths of the species (e.g., up to 9.3 mm long in males from Colorado (Saunders and Wu 1984) and up to 11 mm long in Kansas specimens (Packard 1883)). Richard's (1895a) specimens from Baja California are significantly smaller; he lists a carapace length of only 6.5 mm, yet all 36 females in that collection were ovigerous. The Troy Dry Lake specimens are also smaller than those in an unlabeled lot housed at the Natural History Museum of Los Angeles County. That lot, the collecting locality of which is unknown, contains males up to 12.93 mm long and 7.41 mm high. Of the 92 animals we collected at Troy Dry Lake, males (N = 77) outnumbered females (N = 15) in a ratio of approximately 5:1. This is opposite to what Richard (1895a) reported from Baja California (37 females and 6 males). The skewed ratio in both

Richard's and our collections may indicate differential survival or differential susceptibility to capture, because in laboratory rearings the sex ratio in this species invariably approaches 1:1 (Clay Sassaman, pers. comm.) as is the case with many (but not all) other spinicaudatans (e.g., see Sassaman 1989; Sassaman and Weeks, in press) including members of the Leptestheriidae (Scanabissi Sabelli and Tommasini 1992). No females were seen to be carrying eggs within the thoracopodal epipods as has been described for another species in this genus (*Leptestheria dahalacensis*; Tommasini and Scanabissi Sabelli 1989, 1992).

Belk (1992) noted that in Arizona, where this species is the most common conchostracan, the species can be found from mid-June through October at pool temperatures of 19.5 to 27°C. The species is also common in Mexico, where it has the widest distribution of any known conchostracan (Maeda-Martinez 1991). Horne (1967) considered *L. compleximanus* a eurythermal species, as he found it in Wyoming in pools ranging from 1 to 32°C. Additional habitat notes are given by Sublette and Sublette (1967) for populations in playa lakes in Texas and New Mexico. Co-occurrence with other large branchiopod species is common. As one example, in a temporary pond just north of Jimenez, Chihuahua, Mexico, *Leptestheria compleximanus* was found to co-occur with the anostracan species *Streptocephalus moorei, S. mackini,* and *Thamnocephalus platyurus,* the notostracan *Triops longicaudatus,* and the spinicaudatan *Eocyzicus digueti* (Belk 1973).

Descriptive Notes

Specimens from the Troy Dry Lake site (Fig. 2) and those reared from mud from Amboy Crater agreed in almost all morphological respects with each other and with previous descriptions of *L. compleximanus* (e.g., figures in Packard 1883, figs. 8, 9 and plate 5 (Kansas); Daday 1923, fig. 94 (Baja California); Mattox 1957a, figs. 5, 14 (Texas); Saunders and Wu 1984, figs. 10–14 (Colorado); Martin 1989, fig. 1C (Arizona); Dodson and Frey 1991, fig. 20.74B, Mexico (?)). The paired rows of spines along the posterior margins of the telson (Fig. 2C, D) are smaller than those illustrated by Saunders and Wu (1984) for Colorado specimens, but this may be a factor of size, as their specimens were larger than ours.

Daday's illustrations indicate minute spinules along the length of the furcal rami, whereas these are absent in the figures of Packard (1883) and Dodson and Frey (1991). The discrepancy is probably accounted for by the fact that the furcal rami are slightly rotated inward, with the result that the row of minute spinules often seen along the dorsal border in other species is, in this species, nearly hidden from lateral view and more visible only in dorsal view (Fig. 2C, D). Packard's (1883) illustration (his plate 5, fig. 1) of the entire animal is slightly misleading, in that the male claspers are obviously very stylized in that drawing. The basal protrusion of the clasper "hand" and the spine-covered pad that opposes the movable finger (Fig. 2E) are not as stalked or as distant from the clasper as depicted by Packard's illustrator. Other minor discrepancies were noted between our Troy Dry Lake specimens and previous descriptions of this species, but they seem to fall within the range of acceptable variation in this morphologically rather plastic family (Straškraba 1966).

The species is easy to identify and distinguish from all other clam shrimp species (orders Laevicaudata and Spinicaudata) in North America by its possession of an acute angle on the extremity of the "head" region just cephalad to the occipital

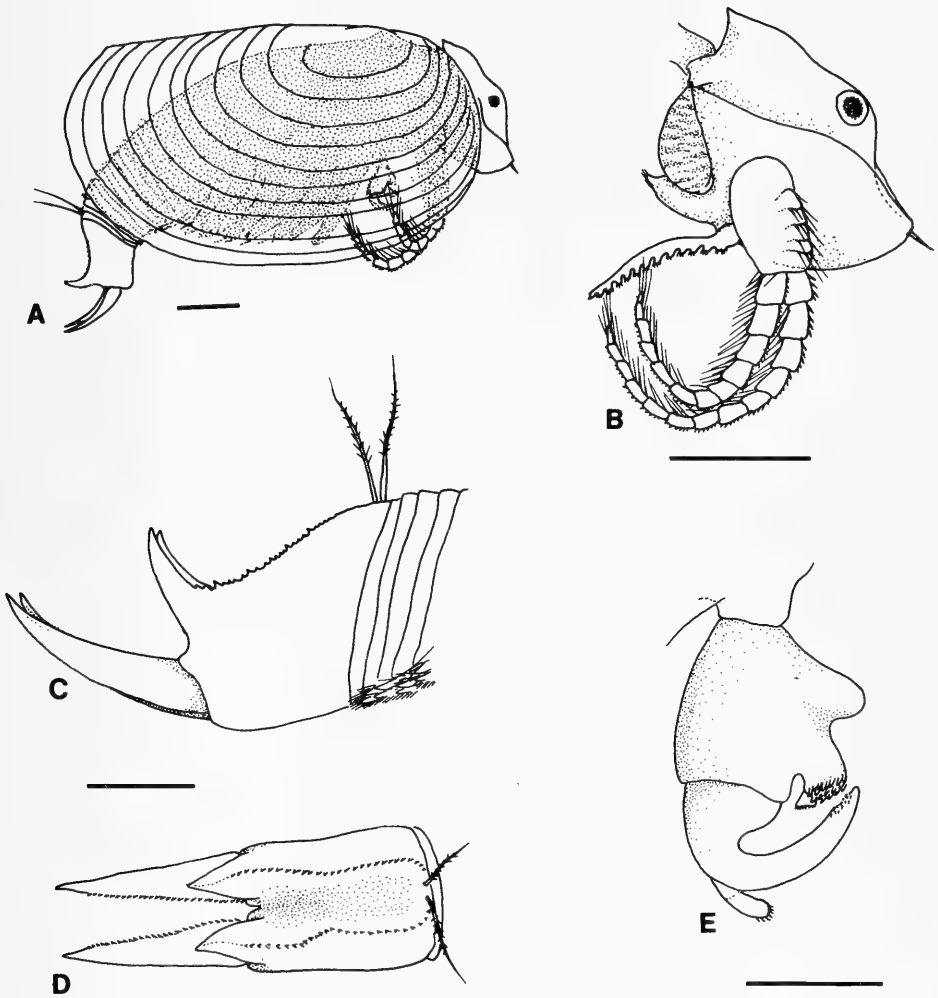

Fig. 2. Selected diagnostic features of a 6.1 mm long male *L. compleximanus* (Packard) from the Troy Dry Lake site. A, entire animal, lateral view. B, higher magnification of head region; note frontal seta projecting from apex of rostrum. C and D, lateral (C) and dorsal (D) views of the telson and furcal rami. E, lateral view of right first thoracopod (first clasper). Scale bars = 1.0 mm for A, B; 0.5 mm for C–E.

notch, a long and somewhat quadrate shell with a more or less flattened dorsal line (Fig. 2A), possession of a well developed fornix (which leptestheriids share with the cyzicids), distinctive male first claspers with proximal protuberance (Fig. 2E), and a diagnostic frontal seta on both males and females (Fig. 2B). The frontal seta, often considered an apomorphy of the family Leptestheriidae, also has been reported in developing juveniles of the family Cyzicidae and in a reduced state in adult female cyzicids (e.g., Barnard 1929), again underlining the close phylogenetic relationship of these two families.

When first proposing the new genus *Maghrebestheria,* Thiery (1987) mentioned the similarity between the only known species of that genus (*M. maroccana*) and *L. compleximanus,* but no mention was made of this in his formal erection of the genus (Thiery 1988).

Taxonomic History

The taxonomic history of this species, like that of so many other species of the Branchiopoda, is convoluted and confusing. The species was first described as a member of the genus *Eulimnadia* (which today is in the family Limnadiidae), and later assigned to *Estheria,* a name that had to be dropped as it had been previously employed for a group of parasitic dipterans. The history of the genus was discussed by Mattox (1957a, b). In the synonymy that follows, we list the major publications that treat this species, while acknowledging that this probably is not an exhaustive list.

Leptestheria compleximanus (Packard, 1877)

Eulimnadia compleximanus Packard, 1877, p. 174, fig. 13a, b (Kansas).—Packard 1879, 1880, Zoology for Colleges and High Schools, 1st and 2nd editions (no description), fig. on p. 302 (not seen)[2].

Estheria compleximanus Packard, 1883, p. 305, figs. 8a, 8b, 9, plate V figs. 1–7, plate XXIV figs. 8, 10, plate XXV fig. 6 (Kansas) (described as a "new species" in this work, although citing his previous mention of it above).—Richard 1895a, p. 104; 1895b, p. 107 (Baja California, Mexico).—Cockerell 1912, p. 43 (Colorado).—Pearse 1918, p. 674, fig. 1046.—Dodds 1915, p. 275 (key), fig. 16 (Colorado).—Dodds 1917, p. 73 (Colorado).—Dodds 1920, p. 96 (Colorado).

Estheria compleximana.—Simon 1886, p. 453 (list).

Leptestheria compleximana.—Sars 1898, p. 10.—Daday 1923, p. 391, fig. 94a–s.—Wootton and Mattox 1958, p. 122 (Mexico).—Forró and Brtek 1984, p. 99 (in reference to Daday's collections).—Thiery 1987, p. 192.

Leptestheria compleximanus.—Creaser 1930, p. 7 (Utah).—Creaser 1931, p. 267 (Mexico).—Creaser 1935, p. 380, fig. 512.—Moore 1950, p. 655 (Texas).—Moore 1965, p. 41 (Oklahoma).—Mattox 1957a, p. 367, figs. 5, 14A, B (Texas).—Mattox 1959, p. 583, fig. 26.8.—Tasch 1964, p. 128 (Mexico).—Tasch and Shaffer 1964, p. 806 (Mexico).—Horne 1967 p. 474 (Wyoming).—Horne 1974, p. 476 (Texas).—Sublette and Sublette 1967, p. 383 (New Mexico, Texas).—Belk 1973, p. 509 (Mexico).—Belk and Cole 1975, p. 211 (Arizona).—Oldham 1978, p. 50 (Kansas).—Pennak 1978, p. 344 (key), fig. 243C (after Packard).—Pennak 1989, p. 362 (key), fig. 17C (after Packard).—Hartland-Rowe 1982, p. 175.—Fitzpatrick 1983, p. 49.—Saunders and Wu 1984, p. 11, figs. 10–14, 23 (map) (Colorado).—Chengaleth 1987, p. 15 (Manitoba, Canada).—Martin 1989, figs. 1, 2D, 3F, 4A, B, 5C, 6A (Arizona).—Debrey et al. 1991, p. 399 (Wyoming).—Maeda-Martinez 1991, p. 215, fig. 7 (map) (Mexico).—Belk 1992, p. 123 (Arizona).

Leptestheria compleximannus.—Slack 1967, p. 1021 (Lake Winnipeg, Canada) (misspelling).

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[2] Packard (1883) mentioned two other appearances of the name *Eulimnadia compleximanus,* in the first and second editions of "Zoology for Colleges and High Schools" (1879, 1880), subsequent to his original 1877 description of the species. According to Packard (1883), no description was included, although a figure on p. 302 is mentioned. We have not been able to locate these text books and cite them here following Packard (1883: 305).

Unnamed "clam shrimp" in photograph. Findley and Sisson 1975, p. 578–579 (Utah).

Leptisthera compleximanus.—Dodson and Frey 1991, p. 774, fig. 20.78 (misspelling) (Mexico [?]).

Leptistheria.—Dodson and Frey 1991, p. 772 (key) (misspelling).

Discussion

As currently recognized (Thiery 1988) the family Leptestheriidae consists of five genera: *Eoleptestheria* Daday, 1914, *Leptestheria* Sars, 1898, *Leptestheriella* Daday, 1923, *Maghrebestheria* Thiery, 1988, and *Sewellestheria* Tiwari, 1966. The last two genera are monotypic, but the first three contain a large number of species, over 30 worldwide (unpublished data).

Although the genus *Leptestheria* Sars, 1898, is widely distributed on all continents except Antarctica (Marincek and Petrov 1985), *Leptestheria compleximanus* (Packard, 1877) is the only species known from North America (i.e., the United States, Canada, and Mexico). The occurrence of this species in southern California is not surprising in light of its known presence in Arizona and in Baja California. Debrey et al. (1991), in a brief and error-filled account of branchiopods in southeastern Wyoming, believed their record of *L. compleximanus* from Wyoming to be the first; it had previously been reported from that state by Horne (1967). The record of the species in Lake Winnipeg, Canada (Slack 1967) should be verified, as large permanent lakes are not usually the habitat of this species or of many other spinicaudatans. To date, the species has been reported from Canada (above record), from 9 states in Mexico (Baja California Sur, Chihuahua, Coahuila, Distrito Federal, Durango, Estado de Mexico, San Luis Potosi, Sonora, and Zacatecas), and from the following states in the United States: Arizona, California (this study), Colorado, Kansas, New Mexico, Oklahoma, Texas, Utah, and Wyoming. References for the above records are listed in the synonymy.

Intensive sampling of many Mojave Desert ephemeral ponds, and the subsequent laboratory rearing of eggs from dried mud taken from many Mojave sites, has not previously revealed the presence of leptestheriids (Clay Sassaman, pers. comm.). We are unsure as to whether *L. compleximanus* is confined to a restricted area in California or is abundant only for a short time, or both. A brief population duration might be indicated by its potential for rather rapid development (9 to 10 days from hatching to adulthood) in the laboratory (C. Sassaman, pers. comm.). More sampling to determine the exact extent of the range of the species, and its habitat and physiological requirements, is needed, as is also the case with many other clam shrimp and indeed other branchiopods in North America (e.g., see Martin et al. 1986).

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Laboratory Tests of Species Discrimination in Three Species of *Peromyscus*

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Abstract.—Species discrimination by *Peromyscus boylii* (brush mouse), *P. maniculatus* (deer mouse), and *P. californicus* (california mouse) was investigated in the laboratory using a computerized data collection system that recorded time spent adjacent to each of two stimulus mice. In tests between *P. maniculatus* and *P. boylii,* male and female *P. boylii* chose significantly to spend more time adjacent to the conspecific stimulus mice. Other groups of test mice did not spend significantly more time next to either stimulus mouse. Behavioral differences in relation to captivity may be correlated with differences in tendency to associate with conspecifics in laboratory experiments.

The process by which animals recognize individuals of their own species is of interest in studies of reproductive isolation and its role in speciation. Behavioral isolation may be important in *Peromyscus* since some species can hybridize under certain conditions (Dice 1933, 1937; McCarley 1954), but do not seem to do so in nature. Species discrimination studies with *Peromyscus* have attempted to study in the laboratory the process by which these mice recognize and choose to associate with their own species. These experiments have allowed the mice to interact freely in multi-chambered cages (Blair 1953; Smith 1965; Tamsitt 1961), or have presented the mice with a choice between odors only (Doty 1972; McCarty and Southwick 1977; Moore 1965), or between mice separated from the experimental subject by wire mesh (Carter and Brand 1986; McCarley 1964).

Cross-fostering studies, in which infants are reared by parents of another species, have been done to analyze the extent to which learning influences species or subspecies discrimination in *Peromyscus* (Carter and Brand 1986; McCarty and Southwick 1977) or other rodents (McGuire and Novak 1987), and in birds (Clayton 1990). Cross-fostering has also been used in the study of other aspects of reproduction in rodents (Hawkins and Cranford 1992; McGuire 1988).

The purpose of the research reported here was to test the hypothesis that sympatric populations of *P. boylii* (brush mouse), *P. maniculatus* (deer mouse), and *P. californicus* (california mouse) will recognize and choose to associate with conspecific mice in the laboratory. This research will allow comparisons of the behavior of these species with species discrimination behavior of other *Peromyscus* species that have been studied in the laboratory, and will provide the background for further study of the behavioral mechanisms involved in species recognition, using cross-fostering.

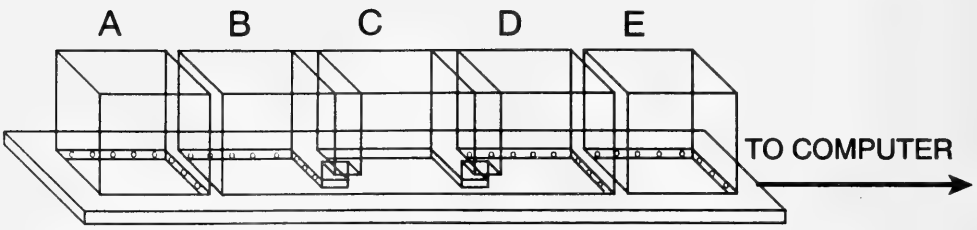

Fig. 1. Diagram of a species recognition experimental unit, with chambers A–E. Chambers B–D are connected by tunnels, and chambers A and E are separated from the others by wire mesh.

Materials and Methods

Peromyscus were live-trapped in western Riverside County and southwestern San Bernardino County, California and transferred to the animal care facility. *P. boylii* and *P. maniculatus* were trapped in areas where they are sympatric near mountain streams. *P. californicus* and some *P. boylii* were trapped in sympatric areas along the border between Yellow Pine forest and chaparral. Each mouse was kept in a separate cage. If females were pregnant when captured, their offspring were also placed in separate cages after weaning. Most experimental subjects were field caught.

Species discrimination experiments were performed in three experimental units, each consisting of five linearly arranged plexiglass chambers with wire mesh tops. The two outer chambers each housed one stimulus mouse (heterospecific and conspecific mice of the opposite sex from the test mouse) and were separated from the center three chambers by 0.64 cm wire mesh. The test mouse was able to move freely through the three central chambers (Fig. 1, B–D), and thus it could spend time in the chamber (Fig. 1, B or D) next to the heterospecific or conspecific stimulus mouse (Fig. 1, A or E), or to avoid them both by staying in the center chamber. Food and water were available only in the central chamber (Fig. 1, C).

Table 1. Results of species recognition experiments, in number of minutes spent next to each stimulus mouse.

	Stimulus mouse						
	P. maniculatus		*P. boylii*				
Test mouse	$\bar{x}$	S.E.	$\bar{x}$	S.E.	N	t	*P*
Male *P. maniculatus*	20.28	12.72	6.84	2.62	8	1.12	.299
Female *P. maniculatus*	19.90	10.52	27.15	10.82	9	0.42	.684
Male *P. boylii*	18.47	6.60	53.73	9.10	15	2.71	.017*
Female *P. boylii*	6.81	1.78	62.24	14.99	9	3.74	.006*
	P. californicus		*P. boylii*				
	$\bar{x}$	S.E.	$\bar{x}$	S.E.	N	t	*P*
Male *P. californicus*	8.44	1.49	39.03	13.98	9	2.21	.058
Female *P. californicus*	22.84	10.35	25.93	10.77	10	0.18	.858
Male *P. boylii*	16.01	6.89	6.61	2.12	12	1.68	.121
Female *P. boylii*	15.97	6.57	34.87	8.97	11	1.60	.141

* Significant at $P < .05$.

Amount of time spent in the chamber next to each stimulus mouse was used as an indication of degree of preference to associate with that species. Eight experimental conditions were used, defined by the identity of the test mouse and stimulus mice (Table 1). After a mouse was used as the test mouse in an experiment it was used as one of the stimulus mice in one or more other experiments.

The three units were housed in two 1.82 × 2.74 m rooms. Two units in one room were on plywood shelves which did not allow the experimental animals to see from one unit to another. The third experimental unit was in an adjacent room, monitored by a video camera. The animal care facility and the experimental rooms were at room temperature with continuous ventilation, and the lights were controlled by a time clock. The light cycle was 12 hours of light and 12 hours of darkness.

A computerized infrared photodetector system monitored mouse positions in chambers A, B, D, and E, 10 times each second. If the test mouse was not in B or D, it was assumed to be in chamber C. A Commodore 64 computer monitored each experimental unit and recorded data on disc. This system was described in more detail by Rouse et al. (1988). An infrared sensitive video camera was positioned above one experimental unit, with a wide angle lens that allowed the entire unit to be viewed simultaneously. Light was provided by an infrared light source, which is invisible to the mice and is not expected to affect their behavior.

Preparation for an experiment began in the evening, when one previously untested mouse was placed in chamber C of each of the three units, for a 24 hour acclimation period. During this time removable barriers prevented the test mouse from leaving chamber C. At the end of the 24 hours, the barriers were removed and chambers A and E were put into position, each containing a stimulus mouse of the opposite sex from the test mouse. Test mouse activity was recorded for two hours, which Carter and Brand (1986) found to be the most effective experiment duration. After the experiments the mice were replaced in the animal rooms and all chambers were washed with water and detergent in preparation for the next experiment.

Data analysis included a calculation of time spent by the test mouse in the chamber next to each stimulus mouse. Each two hour trial was divided into 30 minute time blocks and the results computed for each time block and for the entire trial. The data for the entire trial were analyzed statistically with paired-t comparisons for time in the conspecific chamber versus time in the heterospecific chamber. The video record available for one of three experimental units was examined for confirmation of computer records.

Species Discrimination Tests Between P. maniculatus and P. boylii

Male *P. maniculatus* spent little time in the end chambers next to the stimulus mice and showed no preference to associate with either conspecific or heterospecific mice (Table 1). Female *P. maniculatus* spent more time next to the stimulus mice than was true for the males, but also exhibited no preference for either of the stimulus mice (Table 1). In contrast, male and female *P. boylii* both spent much more time next to stimulus mice than did either sex of *P. maniculatus* and exhibited a strong and statistically significant pattern of association with conspecific stimulus mice (Table 1).

Species Discrimination Tests Between P. boylii and P. californicus

In these tests female *P. boylii* spent the greatest percentage of time next to the conspecific mouse (68.6%), and the male *P. californicus* spent the most time next to the heterospecific mouse (82.2%), but none of the four test groups demonstrated a significant preference to associate with either the conspecific or heterospecific stimulus mouse (Table 1).

Discussion

Both male and female *P. boylii,* when tested with *P. maniculatus,* made a significant choice to associate with the conspecific stimulus mouse. In all of the other combinations the test mice did not choose to spend a significantly greater amount of time adjacent to either the conspecific or heterospecific mouse. The failure of *P. maniculatus* to demonstrate significant preference to associate with conspecific mice in this study contrasts to previous studies of this species when tested against *P. polionotus* (Moore 1965) or *P. leucopus* (Doty 1972). Other species which have not demonstrated significant preference for association with conspecifics in laboratory studies include *P. polionotus* when tested with *P. maniculatus* (Moore 1965), *P. leucopus* males when tested with *P. gossypinus* (McCarley 1964), and *P. comanche, P. nasutus* and *P. truei* when tested together (Tamsitt 1961). However, in experiments by Blair (1953) *P. nasutus* and *P. truei* did prefer to associate with their own species. Other species which have exhibited significant preference to associate with conspecifics include *P. truei* and *P. nasutus* when tested together (Blair 1953) and *P. californicus* tested against *P. eremicus* (Carter and Brand 1986; Smith 1965), *P. leucopus* when tested with *Onychomys* (McCarty and Southwick 1977), and *P. gossypinus* and *P. leucopus* males when tested together (McCarley 1964). In studies of species recognition and behavioral reproductive isolating mechanisms it is important to know whether these species differences in degree of preference for associating with conspecifics in the laboratory are a reflection of an animal's ability to recognize conspecifics in nature, or a result of species differences in behavioral response to the laboratory environment.

Our general observations of our experiments indicated that *P. boylii* actively investigated the test cage environment during the experiments while *P. maniculatus* and *P. californicus* were much less active. *P. maniculatus* were the least active, and often stayed in one part of a test chamber throughout the experiment. These differences in activity may have influenced the results. In further research with these mice it would be beneficial to attempt to determine whether the amount of activity by the stimulus mice, irrespective of the species of the stimulus mice, influences the choice shown by *P. boylii* and/or *P. maniculatus.* It appears that the behavior of *P. boylii* is not affected as much by the laboratory conditions as the behavior of *P. maniculatus,* and perhaps as a result the *P. maniculatus* were not as active in seeking out conspecifics.

When *P. boylii* was tested with *P. californicus,* neither species demonstrated a clear preference to associate with their own species. This lack of discrimination is apparently not the result of the type of experimental apparatus or procedure, since the tests with *P. boylii* and *P. maniculatus* used the same apparatus and procedure, and did indicate a consistent significant conspecific choice by *P. boylii.* Also, in previous experiments with *P. californicus* and *P. eremicus* (Carter and

Brand 1986) using the same apparatus (but not computerized) both species showed significant preference for conspecifics.

In our experiments the estrous state of the females was not monitored, since previous experiments by Carter and Brand using the same experimental approach (1986) indicated no significant difference between choice behavior associated with estrous and non-estrous females of *P. eremicus* and *P. californicus.*

In nature, the species that we studied apparently do not interbreed. The absence of any documented discrimination in favor of conspecifics by some species in these experiments does not necessarily mean that they have no behavioral reproductive barriers or that they cannot recognize conspecifics. The experiments only show that any behavioral preference that they may normally have for associating with conspecific mice does not show up in the laboratory situation, as it does in some other species.

Observation of our laboratory colonies revealed species differences in behavior. When a cage was opened, the *P. maniculatus* and *P. californicus* almost always crouched in a corner or crawled under the litter, while *P. boylii* were very active and often jumped for the opening. It is likely that there are other species differences in their response to captivity, which affect their behavior during species discrimination experiments. Of the species studied in these experiments, *P. boylii* seems to adjust better to the laboratory than the other species. Experiments of the general type reported here are a necessary first step in any laboratory study of interspecific behavioral interactions. Species that do not adjust well to laboratory conditions will not be suitable subjects for further study of these behavioral interactions. A challenge for future experiments of this type will be to attempt to differentiate between the effects of experimental condition and the effects of captivity on the animal's behavior.

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Research Note

Lead in the Feathers of the California Least Tern (*Sterna antillarum browni*)

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As noted by Pattee et al. (1990) "recovery of endangered species populations requires that the variables responsible for their decline be identified and controlled." This view is particularly applicable for the California Least Tern (*Sterna antillarum browni*), an endangered species of bird that nests primarily in the Southern California area (Massey 1989). With the steady increase in human population in this area over the past several decades, there has been a substantial decline in the population of Least Terns. It has been presumed that one of the major reasons for this decline has been the destruction of nesting habitat; traditionally Least Terns nest on coastal sandy beaches adjacent to a river or channel mouth (Massey 1974, 1981). However, in the highly urbanized Southern California area these terns are also being exposed to a number of environmental contaminants, including lead and other toxic metals. The presence of these contaminants in Least Terns has not been extensively surveyed and thus have a yet undetermined impact on the reproductive success of this species.

Lead poisoning can have serious effects on an individual and a population (Eisler 1988). Symptoms of lead poisoning include: emaciation, weakness, paralysis, and death (Anderson 1975; Coburn et al. 1951; Cook and Trainer 1966). Sub-lethal exposure to lead can cause abnormal behavior in birds (Burger and Gochfeld 1986). In this study we wanted to determine: (1) if California Least Terns are accumulating a lead burden, (2) if terns from metropolitan areas have a higher burden than terns from non-metropolitan areas, and (3) if adults have a higher burden than locally raised chicks.

For this analysis, we obtained feathers from six adult California Least Terns and 10 chicks or fledglings (Table 1). All were from individuals which had been found dead either in a breeding colony or at a post-breeding foraging site. The breeding colonies were: Venice Beach, Los Angeles County; Bolsa Chica Ecological Reserve and Huntington Beach State Park, Orange County; Camp Pendleton and Chula Vista, San Diego County. The post-breeding foraging site was Harbor Lake Park in Wilmington, Los Angeles County. Detailed accounts of these sites can be found in the California Least Tern Recovery Plan (California Least Tern Recovery Team 1980). For purposes of analysis, only Venice Beach, Bolsa Chica, Huntington Beach and Harbor Lake were considered to be in metropolitan areas. All carcasses were frozen in plastic bags until feathers were removed for analysis.

Lead residues were determined following the procedures outlined by Jan and Hershelman (1980) in which about 1 gram of feathers was cut with a carbon steel

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Table 1. Lead in California Least Tern feathers.

Sample no.	Lead conc. (ppm)	Source	Location[1]
1.	0.31	adult	Bolsa Chica
2.	4.77	adult	Camp Pendleton
3.	2.91	adult	Camp Pendleton
4.	2.76	chick	Camp Pendleton
5.	0.43	chick	Camp Pendleton
6.	1.56	chick	Camp Pendleton
7.	0.05	chick	Camp Pendleton
8.	0.05	adult	Chula Vista
9.	1.71	fledgling	Harbor Lake
10.	3.13	fledgling	Harbor Lake
11.	1.18	adult	Harbor Lake
12.	2.99	adult	Harbor Lake
13.	8.75	chick	Venice Beach
14.	1.04	chick	Venice Beach
15.	2.11	chick	Venice Beach
16.	0.80	chick	Venice Beach

[1] Localities mentioned in text.

blade, washed in methyl alcohol, digested in nitric acid, and then evaporated to near dryness. The nitric acid digestion was repeated, hydrochloric acid added and the digested feather residue decanted into polyethylene vials prior to analysis with an atomic absorption spectrometer with graphite furnace. The values presented are the mean $\pm$ 1 standard deviation. Significant differences were determined using Student's t test with a rejection level of $P < 0.05$.

The mean lead level for all 16 samples was 2.14 ppm $\pm$ 2.20 (range 0.05–8.75). There was no significant difference in the lead levels in feathers of chicks and fledglings (2.23 ppm $\pm$ 2.49) and those of adults (2.04 ppm $\pm$ 1.83). Similarly, there was no significant difference between the lead level in feathers from birds in the metropolitan areas of Los Angeles and Orange County (2.01 ppm $\pm$ 2.48) and other areas in San Diego County (1.74 ppm $\pm$ 1.16).

The overall mean lead level found in all sixteen California Least Terns is substantially higher than levels found in two other terns. Stoneburner and Harrison (1981) found a mean of 0.03 ppm lead in feathers of Sooty Terns (*Sterna fuscata*) from the Gulf of Mexico and north central Pacific Ocean. Howarth et al. (1981) found a mean value of 0.66 ppm lead in feathers of Crested Terns (*Sterna bergii*) from an industrialized area of Australia and a mean of 0.78 ppm in feathers from a non-industrialized area; this difference was not significant. More recently, comparable lead levels in feathers have been reported for similarly coastal nesting Common Terns (*Sterna hirundo*; 1.49–1.79 ppm) and Black Skimmers (*Rynchops niger*; 2.68 ppm) in New York and New Jersey (Burger and Gochfeld 1991; Gochfeld et al. 1991).

Higher lead levels have been found in other birds especially those that are hunted, live in urban areas or forage in areas contaminated with lead shot. Feathers of an upland game bird, the Ruffed Grouse (*Bonassa umbellus*), contained a mean of 6.4 ppm lead (Scanlon et al. 1980). Two ducks, Northern Pintail (*Anas acuta*) and Scaup (*Aythya* sp.) had mean lead levels of 2.9 ppm and 29.1 ppm respectively (Hall and Fisher 1985). House Sparrows (*Passer domesticus*) from rural areas had

a mean of 27.0 ppm of lead in their feathers while those from an urban area had 158.3 ppm (Getz et al. 1979). Even values as high as those found in the urban sparrows in these studies did not seem to hinder the reproductive ability of these birds. On the other hand three of four California Condors (*Gymnogyps californianus*) found dead in 1981–1986 died from lead poisoning (5.7–35.0 ppm wet weight) (Janssen et al. 1986; Pattee et al. 1990). All avian species appear to be vulnerable to effects of lead but with wide differences among individuals and species (Pattee et al. 1981; Beyer et al. 1988).

Upland game birds and waterfowl can be exposed to lead through hunting (Eisler 1988). Waterfowl and scavenging species can be contaminated by the direct ingestion of metallic lead shot (Bellrose 1964; Pattee et al. 1990). The California Least Tern would not be exposed to lead by either of these routes as their diet consists entirely of small fish such as Northern Anchovies (*Engraulis mordax*) and Killifish (*Fundulus* sp.) captured alive in coastal marine and estuarine waters (Atwood and Kelly 1984). Presumably the lead residues in the terns were derived from lead in the tissues of the fish in their diets. This in turn is related to uptake from polluted coastal waters which receive runoff from sewer outfalls and storm drains (Harrison and Laxen 1981). Automobile exhaust gas appears to be the major source of lead in the urban environment (Grue et al. 1986) and could easily contribute to the contaminant levels in runoff waters (Waldron and Stofen 1974). At the Bolsa Chica Ecological Reserve the outer bay receives direct runoff from the Wintersburg Flood Control Channel while the inner bay has only a muted tidal flow due to tidegates between the two areas. Riznyk and Mason (1979) determined that the level of lead in the sediments of the outer bay (64 ppm) were significantly higher than the sediment levels from the inner bay (9 ppm). This suggests that the surface runoff from adjacent urban areas and freeways was responsible for the higher sediment lead levels in the outer bay. Least Terns nesting at Bolsa Chica forage for fish in both of these areas. The lack of any significant difference between lead levels from terns from the more metropolitan colonies may mean that what we categorized as non-metropolitan areas in fact are still influenced by surface runoffs containing lead despite at least Camp Pendleton being in an obviously more rural area.

The lead detected in the tern chick feathers is derived from contaminants ingested at the breeding sites during the first three to four weeks of life when chicks are growing their first coat of feathers, the juvenal plumage. The similarity of adult and chick lead levels suggests that the source of lead contamination in the adults is the food supply obtained in the local Southern California coastal waters and not in some more distant migration or wintering area.

A wide array of environmental contaminants has been implicated in the decline of various avian populations. Certainly the best known are DDT and other chlorinated hydrocarbons, although lead has also become increasingly recognized as being important. Eisler (1988) provides a synoptic review of the impact of lead on wildlife systems. California Least Tern eggs contain 11.79 ± 6.67 ppm wet weight DDT, including all metabolites (Boardman 1988), and in some cases appreciable levels of several metals including lead (Collins 1992). These levels are high enough to cause concern in other species and we view them with some alarm. Although the level of lead in California Least Tern feathers is substantially higher than in other tern species there do not seem to be overt signs of significant

reproductive impairment that can be related directly to these contaminant levels. However, systematic analysis of contaminants of all sorts in this endangered species and their possible influence on its recovery has until recently been distinctly lacking. The quantification of all factors, especially environmental contaminants, potentially limiting populations of endangered species is essential if those responsible for their recovery are to make appropriate allocation of limited funding and undertake proper management actions in the coming years.

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Research Note

COVER: Scanning electron micrograph of the marine isopod *Excorallana bruscai,* 120×, p. 64.