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ECOLOGY AND REPRODUCTION OF FIVE SPECIES  
OF ANOSTRACA IN OKLAHOMA

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ECOLOGY AND REPRODUCTION OF FIVE SPECIES  
OF ANOSTRACA IN OKLAHOMA

APPROVED BY

*Howard P. Clemens*

*Carl D. Rogge*

*A. P. Brown*

*Wm T. Penford*

*George H. Woodman*

DISSERTATION COMMITTEE

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ECOLOGY AND REPRODUCTION OF FIVE SPECIES  
OF ANOSTRACA IN OKLAHOMA

INTRODUCTION

At least 28 species, representing six families, of Anostraca are known to occur in North America. Approximately one-third of these species are known to be present in Oklahoma and Kansas (Dexter 1959), but virtually nothing is known of their ecology. Thus, the relatively rich fairy shrimp fauna of this part of the United States offers a fertile area for ecological research.

My problem consisted of two major phases, the field phase and the laboratory phase. Major objectives of the former are listed below.

1. To locate habitats of different species in Oklahoma and to visit habitats throughout all seasons of the year to determine the seasonal occurrence of the respective species.
2. To measure certain physical-chemical conditions to reveal similarities and differences of the habitats of various species.

The major objectives of the laboratory phase were as follows.

1. To study the production of eggs by each species with respect to the frequency of clutch deposition, clutch size, and the duration of the productive period.
2. To observe the developmental history of each species and to determine the instar at which sexual maturity is attained.



3. To study the hatching of fairy shrimp eggs to determine how soon after deposition hatching would occur and to obtain preliminary information concerning factors influencing hatching.

## REVIEW OF LITERATURE

The history of the study of the Anostraca in North America is generally considered to have begun with the publication of A. S. Packard's monograph of the phyllopod of North America (Packard 1883). Although earlier works exist, Packard's monograph was the most extensive to appear up to that time. Most literature during the next fifty years was concerned with taxonomy or distribution of species. Shantz (1905), Johansen (1922), Van Cleave (1928), Creaser (1930a, 1930b), Ferguson (1935a, 1935b), and Mattox (1936) are representative of such works.

The ecology of all but a very few species is poorly known. Apparently water temperature is an important limiting factor to the occurrence of Eubranchipus vernalis (Verrill) and E. serratus Forbes. These species appear in Illinois and Ohio during the early spring but disappear soon after water temperatures increase to approximately 23° C (Ferguson 1939; Dexter and Ferguson 1943; Dexter and Sheary 1943; Dexter 1943, 1946; Dexter and Kuehnle 1948, 1951).

Streptocephalus seali Ryder has also been the subject of several studies. In Louisiana, Moore (1951, 1955) has collected this species from roadside ditches and pine forest pools throughout the year. Moore reported that the Louisiana habitats were characterized by a low alkalinity (4-15 ppm) and low pH (4.9-6.2). In Kansas (Leonard and Ponder 1949, Prophet 1959) and Oregon (Coopey 1946) the habitats of this species tend

to be more alkaline (pH usually 7.2 to 7.8) with total alkalinities of about 50 to 100 ppm. Streptocephalus similis Baird occurs in northeastern Mexico and southwest Texas. Moore (1958) reported that habitats of this species rarely exhibited a pH of less than 8.2 or a total alkalinity of less than 100 ppm.

Eubbranchipus holmani Ryder inhabits the heavily shaded brown-water cypress-sour gum pools of southwestern Louisiana (Moore 1959b). Known habitats of this species were also inhabited by S. seali but at different times of the year. Like other members of the genus Eubbranchipus, this species is a cold water form. Moore stated that when winter flooding of these pools resulted in the hatching of both species the streptocephalids were soon eliminated or greatly reduced in numbers by some unknown factor or factors. The more rapid growth rate of E. holmani at winter temperatures probably contributed to the disappearance of the streptocephalids.

Coopey (1950) found Eubbranchipus oregonus Creaser in mildly acid temporary pools (pH 5.8 to 6.2) in Washington during the winter and early spring. Lynch (1958, 1960) reported that several species of Branchinecta were found in small, alkaline pools in the northwestern United States.

The reproductive habits and complete developmental histories are known for only a few species. Parthenogenesis can occur in the brine shrimp, but internal fertilization is necessary in the fresh-water species (Dexter 1959, Pennak 1953). The sperm of E. vernalis are amoeboid (Baker and Rosof 1927).

Linder (1959, 1960) studied the structure and histochemistry of the female reproductive organs and egg membranes of Chirocephalus bundyi

(Forbes). He stated that the ability to withstand desiccation and the hatching behavior of eggs were dependent upon the amount of material secreted by the shell glands. In C. bundyi the shell glands are functional when the first eggs enter the ovisac and secrete a complete outer shell. The eggs are fertilized by stellate-shaped sperm in the egg sac. In Artemia, egg production may commence before the shell glands have matured, and the first eggs to be produced receive little, if any, shell material. Development of such eggs is rapid with the nauplii often emerging while still within the ovisac. Later clutches receive a copious supply of shell material and are capable of withstanding long periods of desiccation (Lochhead 1950).

The immature fairy shrimp escapes the egg as either a free-swimming nauplius or a more advanced metanauplius and passes through a series of immature stages. The number of instars and rate of development varies from one species to another. Heath (1924) reported that Artemia salina (L) was sexually mature by the 12th instar while Branchinecta (now Pristicephalis) occidentalis (Dodds) was capable of copulation during the 16th instar. The development of other species is poorly known.

Formerly, it was believed that anostracan eggs required long periods of desiccation and/or freezing before hatching would occur. Due to the temporary nature of most fairy shrimp habitats, eggs are usually subjected to such conditions, but drying or freezing are not indispensable to the hatching of the eggs of some species (Avery 1939, Lundblad 1921). Weaver (1943) reported that eggs of E. vernalis failed to hatch unless subjected to drying or freezing conditions. To the contrary, Hay and Hay (1889) reported that the eggs of this species would hatch without

freezing, and Castle (1938) observed hatching of E. vernalis eggs which had neither been dried nor frozen. Moore (1957) has observed the hatching of undried S. seali eggs in the laboratory on numerous occasions. In the case of Chirocephalus diaphanus Prévost, a European species, hatching is retarded by short periods of drying (Mathias 1926, Hall 1953).

In addition to temperature and desiccation, water depth, osmotic pressure of the external medium, and the accumulation of metabolites are thought to influence the hatching of anostracan eggs (Moore 1957; Hall 1959a, 1959b, 1959c, 1961).

Numerous organisms are known to prey upon fairy shrimps. Known predators include water birds, leeches, fishes, amphibians, crayfishes, dytiscids, and notostracans (Johansen 1922, Creaser 1929, Mozley 1932, Pacaud 1935, McCarraher 1959). Pacaud (1936) believed that amphibians and dytiscids became serious predators only when the milieu was modified to the extent that the fairy shrimps were weakened.

The food habits of most species are little known. In general, fairy shrimps are filter feeders and utilize bacteria, algae, protozoans, pollen, and bits of detritus (Despax 1924, Pennak 1953, Dexter 1959).

Virtually nothing is known of the parasites of anostracans. Creaser (1929) reported that structures thought to be cysts of trematode cercariae have been observed in some species such as E. vernalis. However, this has never been confirmed. Young (1952) reported that Artemia salina was an intermediate host of the cestode, Hymenolepis californicus. The infected Artemia were found in Mono Lake and salt ponds near Chula Vista, California. Mono Lake is inhabited by a colony of California gulls (Larus californicus) during the summer. Young demonstrated that it was

possible to infect laboratory-hatched gulls with this tapeworm by feeding the young gulls infected brine shrimp. The transfer of the parasite eggs from the gulls to the brine shrimp occurs in some unknown manner.

## METHODS AND MATERIALS

Field reconnaissance was initiated during September 1959 to establish the location of fairy shrimp habitats. Early trips were conducted to localities in Oklahoma from which other workers had collected anostracans (Mackin 1936 and personal communication from Dr. Arthur N. Bragg, University of Oklahoma). Exploratory work was conducted in all parts of the state with the exception of the panhandle and northeastern corner. In all, 46 of the 77 Oklahoma counties were visited. Field work was also conducted in Sumner County, Kansas, in the vicinity of my home, Caldwell.

Habitats located in Sumner County, Kansas, and Tillman, Comanche, McClain, and Cleveland counties in Oklahoma were selected for study. The above areas were selected on the basis of the number of species and abundance of habitats that were found. The study habitats were visited throughout all seasons of the year. The frequency of visits to any one area was determined primarily by the occurrence of precipitation and the duration of the period that the habitats were inundated.

A dip net was used to collect specimens. Samples for identification purposes were preserved in 70% alcohol. In the laboratory, individuals were examined with the aid of a binocular microscope to determine their sex and body length. Length was measured from the front of the head to the tip of the cercopods but exclusive of the terminal

setae. Nomenclature used is after Dexter (1959).

Live animals to initiate stock cultures or for other laboratory use were transported from the field in gallon jars containing habitat water. Usually 25 to 50 adults or up to approximately 100 immatures were placed in each jar. They were divided into groups of 25 or fewer individuals after returning to the laboratory. During the warm months an air conditioned vehicle facilitated the transportation of large numbers of individuals over relatively long distances.

#### Culture Techniques

Techniques used for the culturing of fairy shrimps were modified after Moore (1957). Gallon jars were used as culture vessels. Cultures usually consisted of 15 to 25 adults in each jar of about 1.5 liters of aerated water. A culture medium prepared from soil extract and distilled water was generally used since the natural habitat waters were often very turbid. The extract was prepared by mixing a handful of garden soil with one liter of distilled water. After several days the liquid was decanted, filtered, and sterilized. The extract was refrigerated until needed at which time about one part extract was added to each 100 parts of aerated distilled water. A few drops of thick yeast suspension was added daily to each culture. With the exception of the single cold water species which was cultured at 10-15° C, all stock cultures were kept in an air conditioned room at temperatures of 20-22° C.

#### Physical-Chemical Measurements

Surface water temperatures were measured with either a thermistor or a standard laboratory thermometer which was shaded during measurements.



Hydrogen-ion concentrations were measured with the aid of a Beckman Model N pH meter, and an Industrial Instruments Model RC-7 conductivity bridge was used to determine resistance. Resistances were converted to reciprocal megohms and expressed as micromhos per cm at 18° C.

Dissolved oxygen was determined electrometrically with a platinum-silver oxide electrode modified after Kanwisher (1959) and by the unmodified Winkler method. Chemical analyses of dissolved oxygen and alkalinity were according to Standard Methods (1955). The proportions of the total alkalinity attributed to hydroxides, normal carbonates and bicarbonates were determined and all alkalinities were reported as either carbonate or bicarbonate alkalinity in ppm of  $\text{CaCO}_3$ .

Relative concentrations of calcium, sodium, and potassium were measured with a Perkin-Elmer Model 1146 Flame Photometer. Water samples for the measurement of cations were kept refrigerated in both the field and the laboratory until analyses could be made.

## RESULTS

The physical-chemical characteristics of habitats and observations of the life histories of five species of fairy shrimps are presented. Field observations were started during September 1959 and were continued until January 1962. Analyses of alkalinity, calcium, sodium, and potassium were not made until the spring of 1961. The decision to include these measurements, especially the cation determinations, was made after preliminary attempts to culture specimens in the laboratory revealed that the relative concentrations of the cations might influence survival. For example, I found that S. seali would survive more than a week in a saline solution (4444 micromhos at 18° C) but they rarely lived more than two or three days in tap water. It was noted that there was relatively less potassium to sodium and calcium in the tap water than in the saline solution. When the potassium level of the tap water was adjusted to approximately that of the saline solution survival was prolonged. The relative ratios of these cations in the three media are shown below. Ten individuals were placed in approximately one liter of each medium. After three days all individuals in the tap water had died. Eight animals in the saline solution and three in the modified tap water were still alive after one week.

	Saline	Tap HOH	Modified Tap HOH
Ca:Na	1:24	1:32	1:32
K:Na	1:20	1:115	1:17
Ca:K	1:1	1:0.3	1:1.8

If species differ in their tolerance to the relative concentration of various ions such measurements may prove valuable in determining the distribution of species in certain regions.

Streptocephalus seali Ryder

Physical-Chemical Conditions

Streptocephalus seali was collected during all seasons of the year at temperatures which ranged from 1.0° to 34° C. Dissolved oxygen concentrations in small temporary pools inhabited by S. seali varied from about 5% to 130% saturation (Tables 1 and 12). Hydrogen-ion concentrations ranged from 5.8 to 8.7, but variations within the individual habitats were not so great. In general, habitat waters tended to be slightly alkaline. With few exceptions, alkalinity was due entirely to bicarbonates and ranged from 22 to 211 ppm. Specific conductance ranged from 43 to 471 micromhos.

Calcium was usually more concentrated than either sodium or potassium and ranged from three to 57 ppm. Sodium and potassium ranged from one to 20 ppm and two to 11 ppm respectively. Sodium was more concentrated than potassium with about the same frequency as the converse. With respect to the relative concentrations of these cations, S. seali habitats were characterized by the relationship  $Ca > Na + K$ .

General Observations

This species was most commonly found in roadside ditches and small shallow ponds virtually devoid of aquatic vegetation. Except for two ditch habitats in Kansas (Table 12), this species was not found associated with other anostracans. In the above two ditches S. seali was

Table 1.--Physical-chemical conditions in Streptocephalus seali habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk <sup>3</sup> ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
	10-31-59	12	82	6.7					149
	1-30-60	12	130	7.7					112
	4- 2-60	16	87	7.2					234
	4- 4-60	25	95	7.8					420
	6- 2-60	33	90	7.7					433
	2-11-61	8	98	8.2	118	20	5	2	118
Ditch	4- 7-61	15	87	7.8	114	20	8	3	192
Comanche Co.,	5-25-61	30	105	8.4	138	30	5	3	412
Okla. 0.2mi east	6- 2-61	29	55	8.2	120	12	2	2	337
of Jct 49-62	6-10-61	34	70	8.2	110	18	5	4	368
	6-15-61	24	74	7.5	80	6	1	2	172
	7-26-61	33	102	7.7	132	28	1	4	433
	9-14-61	20	69	8.3	114	22	5	6	200
	10-17-61	21	77	8.2	174	45	7	8	343
	10-24-61	18	85	8.1	175	45	7	7	322
	11- 7-61	10	63	7.2	100	30	4	9	200
	10-17-59	18	75	7.6					278
Ditch	9-14-61	17	63	8.0	45	25	13	7	220
Comanche Co.	10-17-61	17	51	8.1	164	47	19	8	377
Elgin, Okla.	10-24-61	16	44	8.6*	180	57	20	8	369
	11- 7-61	9	77	7.7	102	32	13	6	183

Table 1.--Continued. Physical-chemical conditions in Streptocephalus seali habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk <sup>3</sup> ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Pond	4-23-60	27	75	5.8					244
Comanche Co.	5-25-61	26	99	6.1					172
2.7mi west of	6-10-61	34	87	7.6	50	12	3	2	216
Jct 49-62, south	6-26-61	26	50	6.9	70	4	2	2	166
side of road	10-17-61	20	73	7.4	76	19	4	8	168
Pond									
Comanche Co.	6-10-61	32	15	7.6	42	6	2	5	150
2.7mi west of Jct	6-15-61	24	5	6.8	60	3	2	4	134
49-62, north side									
Pond 1mi north	10-29-60	19	95	6.9		3	5	5	51
3.5mi west	11-25-61	14	87	6.9	22	4	4	11	43
Caldwell, Kans.	12-24-61	5	84	6.7	29	3	2	10	101
Ditch	11-25-61	8	65	7.9	113	24	4	8	149
1.5mi south	12-24-61	3	86	8.0	95	28	15	8	303
South Haven, Kans.									
Pond	11-25-61	9	115	8.7**	211	40	7	7	367
1.6mi south	12-24-61	1	81	8.5***	155	36	16	6	471
South Haven, Kans.									

\* 20 ppm CO<sub>3</sub>

\*\* 56 ppm CO<sub>3</sub>

\*\*\* 48 ppm CO<sub>3</sub>

present only during the spring to early fall while Eubbranchipus serratus was present during the colder periods.

No intensive study of population dynamics was undertaken but samples from young broods usually consisted of nearly equal numbers of males and females. Females often outnumbered males in samples from old broods but the converse was occasionally true. Males tended to be slightly larger than females. Adults often reached lengths of 25 to 30 mm. The largest individuals collected measured 36 mm. Conditions being favorable, it was not uncommon for broods to survive three months or more.

#### Developmental History

This species escapes the egg as a nauplius which averages approximately 0.3 mm in length. At 20° C ecdysis occurred about once every 24 hours. Four days after hatching the length of 35 individuals averaged 2.4 mm (Figure 1). The compound eyes and all eleven pairs of trunk appendages were present. The first four to six pairs of legs had developed the structure characteristic of the adult. The remaining five to seven pairs of legs were in various stages of development. Length averaged 6.2 mm on the eighth day and the second antennae and genital appendages were sufficiently differentiated to determine sex. On the twelfth day length averaged 10.3 mm and the first evidence of initial egg production was observed. The paired oviducts of some females, 10.5 to 11 mm in length, contained a few ova. Although the reproductive organs of 11 to 12 mm males were probably functional, the appearance of their second antennae and cercopods was not yet adult-like.

Length increased from nearly 0.5 to 1.0 mm per day during the first 12 days of the life span. During the period of sexual maturation

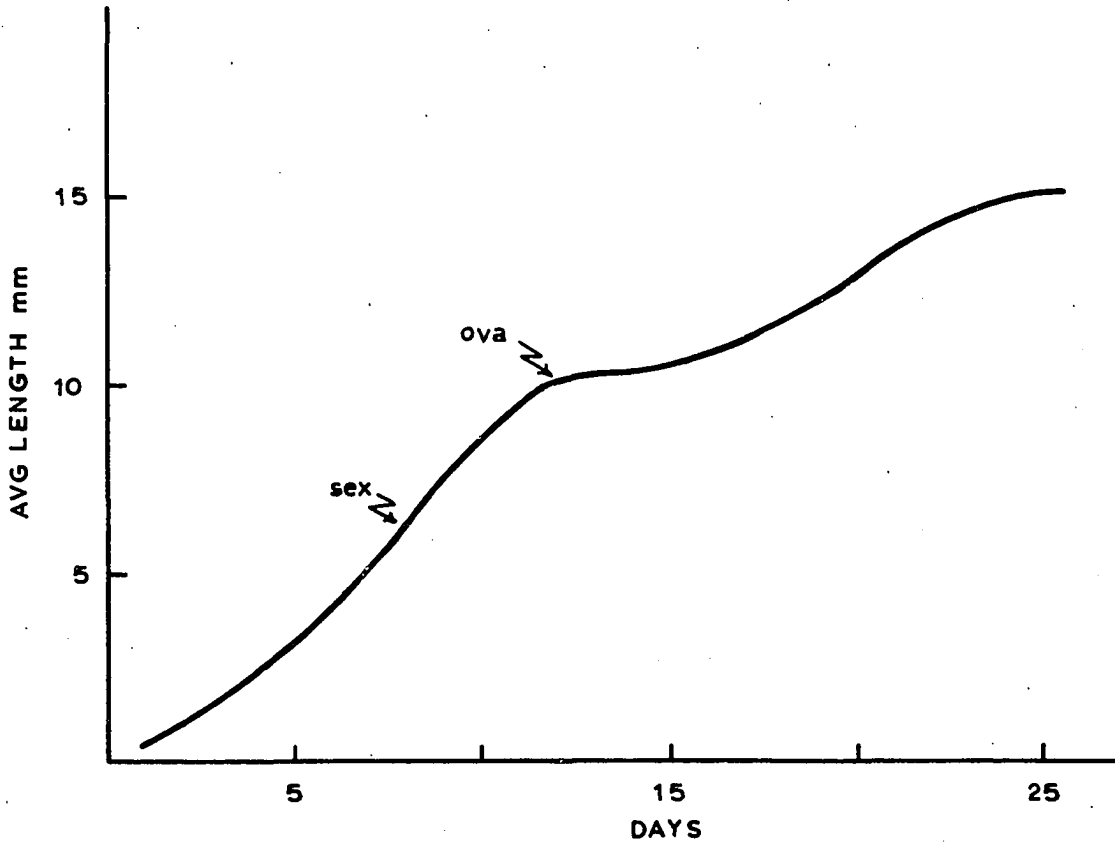


Figure 1. Growth of Streptocephalus seali cultured at 20° C.

growth was retarded but later increased to 0.5 to 1.0 mm per day for approximately one week and then began to level-off as an average length of about 15 mm was attained.

#### Egg Production

Egg production usually commenced after females reached a length of about 11 to 12 mm and continued throughout most of the adult life (Table 2). The duration of productive periods listed extended from the onset of egg production to the death of the female. Clutches were deposited at intervals of one to three days, and each clutch was fertilized during a separate copulation. Initial clutches usually consisted of fewer than 25 eggs. In several cases the first one or two clutches were reabsorbed after entering the egg sac. When this happened the first clutch to be deposited consisted of more than 50 eggs. Egg production continued in the absence of males but these unfertilized eggs retained a whitish color and smooth texture and were usually reabsorbed while in the egg sac. Apparently shell material was not secreted by the shell glands in these instances. The appearance of dark eggs in the egg sac and their eventual deposition were used as criteria to establish the occurrence of copulation and the subsequent fertilization of all or at least a part of the clutch.

Female SS-11 exhibited the shortest productive period and the lowest production, depositing 133 eggs in three clutches during 11 days. The most eggs deposited by a single female was 1791 by SS-2. This total was deposited in 13 clutches over 36 days. The maximum number of eggs deposited in a single clutch was 306. Field-collected females often deposited larger clutches. One female 32 mm in length deposited 718



Table 2.--Egg production by eleven mated Streptocephalus seali females cultured at 20° to 22° C.

Culture Number	Number Clutches Deposited	Number of Eggs/clutch Min.--Max.	Total Eggs Deposited	Duration Productive Period, Days
SS-1	4	6 147	247	21
SS-2	13	17 306	1791	36
SS-3	10	69 282	1330	41
SS-4	5	56 137	394	19
SS-5	14	75 185	1718	40
SS-6	7	1 83	266	17
SS-7	3	20 70	142	20
SS-8	6	10 74	233	14
SS-9	6	11 85	256	24
SS-10	5	59 114	416	29
SS-11	3	13 54	133	11

eggs in a single clutch. Other females, 25 to 27 mm in length, were observed to produce clutches containing 400 to 524 eggs.

#### Hatching of Eggs

Numerous egg cultures were prepared and observed in the laboratory to determine how soon after deposition hatching would occur and the effect of temperature, drying, and dilution of the external medium. Eggs were removed from the parent culture within one day of deposition and subjected to various treatments.

Numerous samples of eggs were placed in small vessels containing about 75 ml of water from the parent cultures and kept at 20° to 22° C. Later the eggs were dried at room temperature for three to 16 days and then immersed in aerated distilled water for one week. A total of 18% of the eggs hatched during the time these cultures were continued (Table 3). Total hatch in individual samples ranged from 2 to 60%. Hatching prior to drying was observed in 15 of the 25 samples and constituted approximately 64% of the total hatch. Hatching of these undried eggs occurred four to 11 days after deposition. In some cases, as few as three days drying at room temperature was sufficient to stimulate some eggs to hatch. Dried eggs consistently began hatching 12 to 18 hours after immersion in aerated distilled water.

Temperatures as low as 10° and as high as 32° C were found to be unfavorable for hatching. For example, one clutch was divided into three samples of 100 eggs each which were immersed in small vessels of water from the parent culture. One sample was kept at 20° as a control, and one sample was placed at 10° and the other at 32°. Additional culture medium

Table 3.--Hatching of Streptocephalus seali eggs at 20° to 22° C.

Sample Number	Number Eggs in Sample	Days Wet Before Drying	Per Cent Hatched Before Drying	Days Dried	Per Cent Hatched After Drying	Total Per Cent Sample Hatched
15	25	33	4	3	40	44
23	125	5	0	7	2	2
27	298	9	1	6	7	8
28	135	17	11	18	1	12
32	53	43	38	12	0	38
33	20	22	5	7	0	5
39	36	5	0	7	3	3
41	70	30	0	12	26	26
44	41	13	5	12	22	27
45	151	28	60	9	0	60
46	79	36	9	16	38	47
55	18	30	5	6	0	5
56	115	29	0	16	2	2
58	30	27	0	16	3	3
59	65	37	6	16	3	9
64	70	37	0	16	3	3
65	131	36	24	16	7	31
66	172	35	14	16	0	14
75	50	19	0	7	2	2
76	50	19	0	7	16	16
83	109	16	14	5	0	14
85	115	9	22	3	0	22
89	52	12	0	10	27	27
98	40	15	0	7	2	2
99	40	9	20	9	2	22

was added as needed to prevent drying of the eggs. After 40 days 31% of the control sample had hatched but there was no hatch in either test sample. The test samples were then moved to the control temperature and all samples were continued for another 24 days. During this time 24% of the 10° sample, 23% of the 32° sample, and another 20% of the control sample hatched.

Hatching was often induced by dilution of the external medium and by drying. However, hatching was inhibited if the eggs were subjected to such treatment within one day of deposition. For example, clutches from two females were combined and divided into five samples of 100 eggs. The control sample was immersed in 75 ml of water from the parent culture. When the water level was reduced to about half its original state aerated distilled water was added. The four test samples were placed in empty vessels and were dried for periods of either 4, 7, 14, or 28 days and then immersed in 75 ml of aerated distilled water. All samples were kept at room temperature. After 48 days 75% of the controls had hatched. None of the eggs dried four days hatched, 3% of the samples dried 7 and 14 days hatched, and 2% of the sample dried 28 days hatched.

### Streptocephalus texanus Packard

#### Physical-Chemical Conditions

This species was present at water temperatures of 13° to 37° C. Dissolved oxygen concentrations were usually near saturation levels at the time of measurements. Hydrogen-ion concentrations varied from 4.7 to 9.5. Habitats in which S. texanus was the only anostracan present or in which B. lindahli also occurred were typically alkaline (Tables 4 and

12), but pools inhabited by mixed populations of S. texanus and Thamnocephalus platyurus were usually acid (Table 7).

Total alkalinity was usually due to the presence of bicarbonates. Bicarbonate alkalinity in pools inhabited by S. texanus ranged from 10 to 270 ppm. Specific conductance ranged from 26 to 555 micromhos. Variation in the concentrations of calcium, sodium, and potassium from one habitat to another was also great. Overall, calcium ranged from two to 57 ppm and was usually more concentrated than either sodium or potassium. Sodium ranged from one to 33 ppm and potassium ranged from one to 16 ppm. The relationship  $Ca > Na + K$  appeared to be typical of most S. texanus habitats.

#### General Observations

This species was collected from roadside ditches, small shallow ponds, and flooded cultivated fields. It was present only from April to November. Thamnocephalus platyurus was associated with S. texanus in several roadside ditches in southwestern Oklahoma, and mixed populations of S. texanus and Branchinecta lindahli were observed in two Kansas ditch habitats. Although it was never observed concurrently with the latter two species, Eubranchipus serratus appeared in these same two ditches during the winter and early spring (Table 12).

More than one brood of S. texanus frequently appeared in a given habitat during the same season. Samples from young broods consisted of nearly equal numbers of males and females, but females appeared to outnumber males during the final stages of a brood's life span. Under favorable conditions broods of this species survived at least two months. Mature individuals frequently attained lengths of 15 to 18 mm with a maximum length of 25 mm being observed.

Table 4.--Physical-chemical conditions in Streptocephalus texanus habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk <sup>3</sup> ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
	6-10-61	34	80	8.4	95	18	3	3	300
	6-13-61	25	46	6.7	92	18	2	1	229
	6-14-61	21	44	7.3	90	12	2	1	188
	6-17-61	21	77	6.7	104	18	2	2	204
	6-23-61	23	50	7.2	132	18	2	2	306
Ditch	7-15-61	24	57	7.9	52	10	2	5	80
McClain Co.,	7-27-61	31	83	9.3*	87	25	2	5	256
Okla. 0.2mi east	8-14-61	30	16	6.8	32	12	1	9	153
Jct 9-74	8-16-61	27	34	8.0	71	20	1	9	203
	8-17-61	33	38	9.0***	69	22	2	8	262
	9- 6-61	37	130	9.5'	48	20	3	8	100
	9-13-61	22	54	7.8	64	12	1	5	111
	9-16-61	19	63	8.4	79	16	2	6	171
	9-19-61	36	128	7.3		22	1	6	
	9-21-61	22	65	8.1	87	20	1	7	200
	10-12-61	28	18	7.9	114	40	1	12	358
Ditch Cleveland Co.									
1.1mi west	6-14-61	29	57	7.7	110	30	8	4	517
Jct 74-Main St.	7-19-61	37	119	8.6''	158	20	2	8	461
Norman, Okla.									
* 16 ppm CO <sub>3</sub>	' 22 ppm CO <sub>3</sub>								
** 10 ppm CO <sub>3</sub>	'' 8 ppm CO <sub>3</sub>								

Table 4.--Continued. Physical-chemical conditions in Streptocephalus texanus habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Ditch									
Comanche Co., Okla.	10-17-59	18	90	7.5					
Jct 62-49	6-10-61	34	33	7.8	110	15	3	4	387
Pond	10-17-59	19	130	7.5					344
Comanche Co., Okla.	5-25-61	25	55	6.5	173	39	17	9	401
1mi south of	6-10-61	32	85	6.2	124	27	10	9	555
Jct 277-281	6-15-61	25	68	7.2	140	18	3	5	323
	10-17-61	17	57	7.8	270	57	33	12	408
Pond									
Comanche Co.	6-26-61	31	56	8.4	70	7	1	3	229
5.4mi west Cache, Okla.									
Marsh	4- 1-61	21	110	8.2	26	4	4	7	59
4.5mi north	5-13-61	23	90	5.6	18	6	4	10	233
Drury, Kans.	6-18-61	29	104	7.2	34	4	2	10	144
Ditch 1mi north									
Caldwell, Kans.	6-18-61	27	92	7.1	72	5	2	4	190

### Developmental History

Streptocephalus texanus hatched as a nauplius. During the early stages of development I was unable to distinguish between the young of S. texanus and those of S. seali.

Approximately 12 hours after hatching 30 nauplii averaged 0.39 mm in length (Figure 2); by the sixth day, individuals averaged 4.0 mm in length, and all trunk and abdominal segments had differentiated and the second antennae were starting to change. Length averaged 5.6 mm the next day and the appearance of the second antennae was sufficiently modified so that sex could be ascertained. Ten days after hatching the length averaged 9.4 mm and eggs were observed in a few females 9 to 10 mm in length. All females were gravid by the twelfth day.

Length increased about 0.5 to 1.0 mm per day during the first four days of the life span and up to 1.6 mm per day during the next week. During sexual maturation growth was slightly retarded for two days. It then increased to about 1.0 mm per day but gradually decreased as individuals reached lengths of 14 to 16 mm.

So far as could be ascertained, ecdysis occurred once each day. On this basis sexual dimorphism was apparent by the 7th instar and individuals were mature and copulated by the 11th instar.

### Egg Production

The production of eggs by mated S. texanus females was observed in the laboratory (Table 5). The first five couples listed were field-collected individuals and the last five were reared from eggs in the laboratory. The field-collected individuals were at least two weeks old



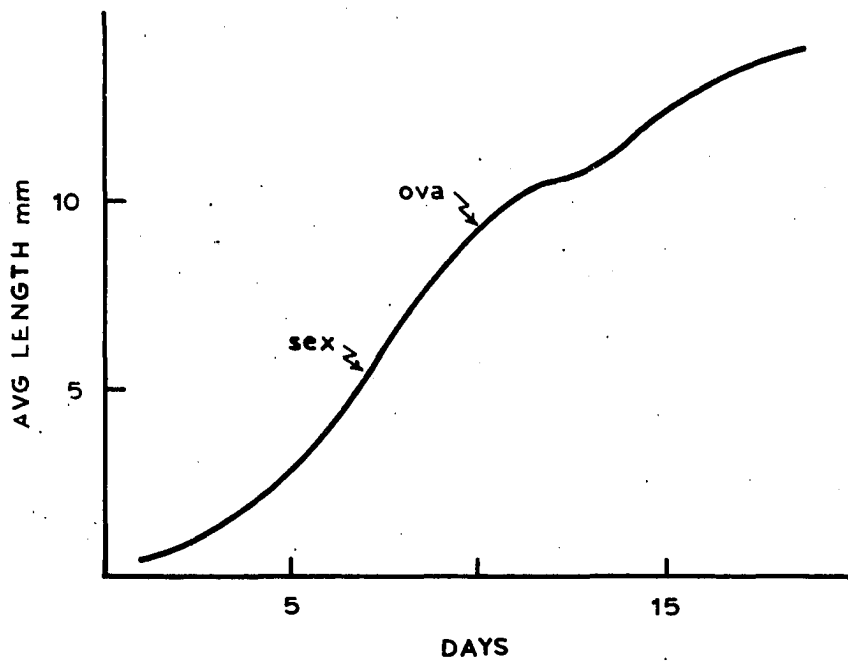


Figure 2. Growth of Streptocephalus texanus cultured at 20° C.

when collected. These females were gravid at the time they were isolated in the laboratory and it is possible that production of one or more clutches had occurred prior to isolation.

The maximum total production by a field-collected female was 1635 eggs. This total was deposited by ST-2 in seven clutches during 27 days. Maximum production by a laboratory-reared female (ST-9) was 867 eggs deposited in nine clutches during 15 days. The smallest total produced was 133 eggs by ST-10. These eggs were deposited in three clutches during 11 days.

In all cases clutches were deposited at intervals of one to two days. A separate copulation was required to fertilize each clutch. Overall, clutch size varied from 3 to 654 eggs per clutch. The largest clutches usually occurred towards the end of the productive period. The maximum clutch sizes deposited in the laboratory appeared to approximate clutch sizes of individuals in nature.

#### Hatching of Eggs

Hatching of eggs prior to drying was observed in 7 of 22 samples kept at 20° to 22° C, but it represented only 6% of the total hatch. In all cases hatching of these undried eggs occurred four to seven days after deposition. Drying for one day at room temperature was sufficient to stimulate the hatching of some eggs (Table 6). In all, 26% of the 3186 eggs shown hatched during the time the cultures were continued. The total hatch in individual samples varied from one to 91%.

Eggs dried or immersed in aerated distilled water immediately after deposition failed to hatch. Hatching was never observed at

Table 5.--Egg production by ten mated Streptocephalus texanus females cultured at 20° to 22° C.

Culture Number	Number Clutches Deposited	Number of Eggs/clutch Min.--Max.	Total Eggs Deposited	Duration Productive Period, Days
ST-1	8	58 183	887	22
ST-2	7	33 654	1635	26
ST-3	3	24 148	291	16
ST-4	3	44 288	496	17
ST-5	4	48 243	548	16
ST-6	5	3 226	448	17
ST-7	7	4 230	692	27
ST-8	5	67 205	504	16
ST-9	9	7 174	867	15
ST-10	3	13 66	133	11

temperatures below 15° nor as high as 32° C. Hatching could sometimes be induced by diluting the culture medium after the eggs had aged for at least one week.

Thamnocephalus platyurus Packard

Physical-Chemical Conditions

Physical-chemical data of habitats of this species are presented in Table 7. Water temperatures at the time of observations ranged from 17° to 36° C. Hydrogen-ion concentrations ranged from 4.7 to 8.2. Carbonates were never present and bicarbonate alkalinity tended to be low, ranging from 10 to 42 ppm. Dissolved oxygen concentrations ranged from 37 to 120% saturation. Specific conductance reflected a low dissolved salt content, ranging from 26 to 126 micromhos.

In addition to low alkalinities and low conductances these habitats were characterized by relatively high potassium and low calcium and sodium values. Potassium was the dominant cation and ranged from 4 to 16 ppm. Calcium and sodium never exceeded 4 and 2 ppm, respectively. With respect to the relative concentrations of these three cations, habitats of T. platyurus were characterized by the relationship  $K > Ca > Na$ .

General Observations

Thamnocephalus platyurus was collected during the summer and early fall from roadside ditches and flooded cotton fields in southwestern Oklahoma. All habitats were located in Tillman County near Manitou and Tipton. Streptocephalus texanus was present in all T. platyurus habitats although the converse was not true.

Sandy soil and high evaporation rates greatly limited the duration of the inundated period of these habitats. Although extensive

Table 6.--Hatching of Streptocephalus texanus eggs at 20° to 22° C.

Sample Number	Number Eggs in Sample	Days Wet Before Drying	Per Cent Hatched Before Drying	Days Dried	Per Cent Hatched After Drying	Total Per Cent Sample Hatched
1	97	15	0	7	12	12
2	116	13	0	1	22	22
6	64	13	0	1	19	19
7	119	20	32	2	18	50
8	162	15	2	15	6	8
9	158	15	2	7	21	23
10	151	15	0	7	91	91
11	170	9	0	12	66	66
12	108	14	1	7	3	4
15	87	18	1	13	46	47
16	106	18	0	6	1	1
17	180	17	0	6	33	33
21	243	17	1	6	2	3
35	55	10	0	13	2	2
37	572	22	0	1	11	11
46	26	11	0	6	19	19
51	100	9	0	4	63	63
55	100	4	0	2	9	9
57	272	11	0	3	21	21
58	100	8	4	2	25	29
64	100	14	0	9	18	18
66	100	14	0	7	56	56

dip netting of habitats was carried out during visits, samples contained too few individuals of this species to determine variation in sex ratios. This is probably the largest species of fairy shrimp in the central part of the United States. The largest male I collected measured 40 mm in length and the largest female 42 mm.

#### Developmental History

Individuals hatched in the laboratory rarely survived more than three days. Less than one day after hatching ten immatures averaged 0.8 mm in length and exhibited up to seven trunk segments and pigmented compound eyes. Three days later only three individuals averaging about 5.0 mm in length were still alive. The next day, length averaged 7.5 mm and sexual dimorphism was evident. On the seventh day, length averaged 12.2 mm and the egg sacs of the two surviving females each contained a few ova. One week later one male and one female were still alive and averaged approximately 20 mm.

#### Egg Production

Observations of egg production by this species in the laboratory were very limited. Only two females and one male were reared to adulthood in the laboratory. Although each of the two females produced several clutches the eggs were usually reabsorbed after entering the egg sac. Both females produced at least two clutches before one was fertilized. The first clutches to be deposited consisted of 96 and 106 eggs. One female died before depositing another clutch. The second clutch deposited by the remaining female contained 155 eggs. Field-collected females, 40 to 42 mm in length, were found to deposit from 611 to 875 eggs in a single clutch.

Table 7.--Physical-chemical conditions in Thamnocephalus platyurus habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk <sup>3</sup> ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
	10-17-59	17	113	6.8					26
	10- 8-60	29	120	4.7					67
Ditch 3.6mi west Manitou	6-26-61	31	65	6.7	16	2	1	8	42
	7-26-61	34	100	7.3	40	2	2	15	84
	9-14-61	22	105	8.2	18	4	1	6	27
	11- 7-61*	13	100	7.7	14	4	1	6	29
Ditch 4.5mi west Manitou	6-26-61	31	52	6.9	24	2	1	13	45
	7-26-61	36	91	7.5	40	2	1	16	115
	9-14-61	22	107	7.8	25	4	1	13	126
Ditch 8mi north 2mi east Tipton	6-26-61	32	76	8.2	40	3	2	15	107
Flooded field 4mi west Manitou	6-26-61	31	67	4.7	10	2	1	4	27
Ditch 3.5mi west Manitou	6-26-61	33	37	6.9	28	2	1	8	82
Flooded field 3.6mi west Manitou	6-26-61	31	67	6.8	26	3	1	14	42

\* Only S. texanus present

## Hatching of Eggs

Eggs from several field-collected females were divided into smaller samples and immersed in vessels of habitat water for two to 42 days and then dried at room temperatures for one to seven days. Aerated distilled water was then added to each sample.

Hatching most commonly occurred after samples were dried at least one day (Table 8), but eggs in four samples hatched five days after deposition. Only 7% of the 1379 eggs hatched prior to drying compared to 23% hatching after drying. The total hatch in individual samples varied from 3 to 75%. Eggs dried three to seven days began hatching six to nine hours after immersion in aerated distilled water. Samples tested at 10° and 32° C failed to hatch. Eggs dried or placed in distilled water immediately after deposition failed to hatch. Dilution of the hatching medium with distilled water often induced hatching provided that the eggs had been aged at least five days.

Branchinecta lindahli Packard

## Physical-Chemical Conditions

This species was collected at water temperatures which ranged from 13° to 36° C. When measured, dissolved oxygen ranged from 51 to approximately 150% saturation. Habitats were typically more alkaline than those of the other four species. The hydrogen-ion concentrations in B. lindahli habitats ranged from 6.0 to 9.8 (Tables 9 and 12) but were usually above 8.3. Because of the small volume of water present in most rock hole habitats, alkalinity determinations were often omitted. When measured, carbonate alkalinity ranged from zero to 40 ppm while bicarbonate alkalinity ranged from 10 to 178 ppm. Specific conductance



Table 8.--Hatching of Thamnocephalus platyurus eggs at 20° to 22° C.

Sample Number	Number Eggs in Sample	Days Wet Before Drying	Per Cent Hatched Before Drying	Days Dried	Per Cent Hatched After Drying	Total Per Cent Sample Hatched
1	150	2	0	7	34	34
2	150	12	0	3	22	22
3	150	12	14	7	0	14
4	150	30	0	7	16	16
5	150	14	0	3	40	40
6	150	41	9	6	0	9
7	60	11	3	4	0	3
8	60	42	0	1	75	75
9	100	22	0	1	67	67
10	104	30	0	2	27	27
11	155	28	41	3	2	43

varied much from one habitat to the next and from one visit to another. Overall, conductance ranged from 22 to 516 micromhos.

With respect to relative concentrations, calcium was the dominant cation and ranged from two to 60 ppm. Sodium and potassium were usually present in approximately equal quantities and each ranged from one to 16 ppm. In general, B. lindahli habitats were characterized by the relationship  $Ca > Na + K$ .

#### General Observations

Branchinecta lindahli was collected primarily from small granite rock holes in the Wichita Mountains Wildlife Refuge in southwestern Oklahoma. I also found it associated with S. texanus in two ditch habitats near Caldwell, Kansas (Table 12).

The rock holes were situated in Lugert granite of Proterozoic age near the summit of Mount Scott at an altitude of approximately 2464 feet. They were small and shallow, the largest being less than three feet in diameter and not more than six inches deep. Although fairy shrimps were frequently abundant the individuals were rather small, seldom exceeding 10 mm in length. Individuals cultured in the laboratory often reached lengths of 13 to 15 mm. Males and females were generally present in about equal numbers. Individuals usually congregated along the edges of the holes feeding on an algal growth and detritus. Copulation was often observed, with males attempting to copulate with any individual, male or female, which could be grasped. A life span of approximately one month seemed typical of this species.

Table 9.--Physical-chemical conditions in Branchinecta lindahli rock hole habitats.

Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk ppm	CO <sub>3</sub> Alk ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Habitat # 1									
5-25-61	30		8.4	86		27	6	7	306
6-10-61	35	75	9.3	30	10	6	1	1	126
6-26-61	28	64	9.2			2	1	1	105
7-26-61	32	90	9.1	52	10	20	4	8	286
9- 7-61	28	73	7.7	49		16	4	4	132
9-14-61	21	90	8.9	22	2	2	1	2	22
Habitat # 2									
4- 7-61	14		9.0						252
5-25-61	31		8.3	115		27	11	7	329
6-10-61	35	80	8.9			12	2	1	108
6-15-61	22	90	7.8	20		3	2	4	42
6-26-61	28	65	9.0			6	2	1	78
7-26-61	36	96	8.3	32		12	4	5	181
9- 7-61	30	75	8.6	44	2	12	2	3	101
9-14-61	22	101	9.0	18	10	4	1	2	83
10-17-61	22	105	7.6	62		20	4	5	155
Habitat # 3									
4- 7-61	14	150	9.4			37	16	10	262
5-25-61	30		8.4	75		23	8	9	306
6-10-61	35	84	9.3			6	4	1	151
6-15-61	22	90	8.7			12	2	1	109
7-26-61	34	90	9.5	10	40	22	4	5	283
9- 7-61	28	76	9.7	32	20	12	4	4	132
9-14-61	21	95	9.4	10	8	2	2	2	154

Table 9.--Continued. Physical-chemical conditions in Branchinecta lindahli rock hole habitats.

Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk ppm	CO <sub>3</sub> Alk ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Habitat # 4									
4-7-61	13					20	2	1	49
5-25-61	28		8.4	33		12	2	3	87
6-10-61	35	77	9.0			6	2	1	86
6-15-61	21	97	8.4	14		6	1	1	40
6-26-61	26	62	9.3			6	2	1	59
7-26-61	33	90				10	4	5	178
9-7-61	28	95	9.8	31	20	5	2	3	70
9-14-61	19	87	8.7	14	1	2	2	2	23
Habitat # 5									
4-7-61	15		8.9			50	9	4	317
5-25-61	30		7.7	88		30	13	8	411
6-10-61	35	80	8.9	40	16	15	5	2	200
6-15-61	21	90	8.3	64		9	3	1	154
6-26-61	30	65	8.7			15	4	5	329
7-26-61	33	117	9.2	26	30	25	10	10	324
9-7-61	29	97	9.4	48	34	22	10	10	151
9-14-61	20	86	9.3	32	16	16	3	3	85
Habitat # 6									
5-25-61	29		7.7	178		46	12	13	516
6-10-61	35	70	8.9			15	4	3	270
6-26-61	28	51	8.8			18	2	3	278
7-26-61	30	95	8.6	114	12	50	6	10	438
9-7-61	28	85	8.9	113	8	40	4	9	180
9-14-61	20	86	9.3	32	16	16	3	5	85
10-17-61	22		9.2	156	32	60	9	16	388

### Developmental History

Branchinecta lindahli escapes the egg as a nauplius which averages about 0.3 mm in length. At 20° C, development is rapid and reproduction commences seven to nine days after hatching (Figure 3). As far as could be ascertained, ecdysis occurred about once every 24 hours.

Thirty individuals averaged 0.8 mm in length during the second day. Four days after hatching the length averaged 3.1 mm and all trunk and abdominal segments had formed. Sexual dimorphism was apparent on the fifth day at which time length averaged 5.8 mm. All individuals were sexually mature one week after hatching and averaged 10.3 mm in length.

As can be seen, the growth curve of B. lindahli was sigmoid and did not exhibit a period of retarded growth during sexual maturation. During the first week length increased up to 3.0 mm per day and then decreased to about 1.0 mm per day during the following four to five days. Finally, growth decreased to 0.5 mm per day or less as individuals attained lengths of 14 to 16 mm.

### Egg Production

The relatively short productive periods exhibited by the B. lindahli females in Table 10 reflects a short life span which appeared to be typical of this species. Each female deposited from two to seven clutches, each being fertilized during a separate copulation. Initial clutches generally consisted of one to five eggs. Clutches were most commonly deposited at daily intervals. Overall, clutches consisted of from one to 87 eggs. The lowest total production observed was nine eggs

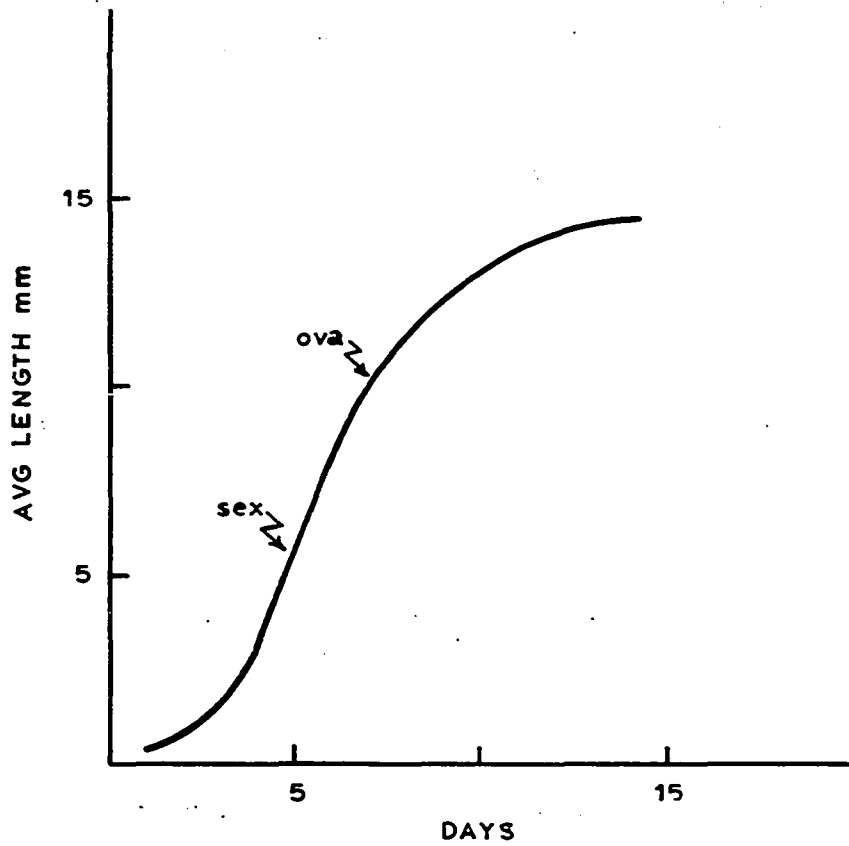


Figure 3. Growth of Branchinecta lindahli cultured at 20° C.

which was deposited by BL-8 in three clutches during a productive period of four days. The largest total was 258 eggs deposited in six clutches by BL-9 during eight days. Usually the maximum clutch produced by each female was deposited near the end of the productive period. The largest clutch observed was 120 eggs deposited by a 15 mm female.

#### Hatching of Eggs

Hatching before drying was observed in four of the twelve samples of B. lindahli eggs (Table 11). In these instances hatching occurred on the third day after deposition; other samples were dried at least four days before hatching commenced. Only 4% of the 410 eggs hatched prior to drying compared to a total hatch of 16%. Hatching in individual samples ranged from 4 to 54%. Eggs kept at temperatures below 15° and above 30° C failed to hatch. Samples dried at least four days at room temperatures often exhibited hatching within eight hours after immersion in aerated distilled water. Drying or dilution of the hatching medium immediately after deposition inhibited hatching.

#### Eubbranchipus serratus Forbes

##### Physical-Chemical Conditions

Eubbranchipus serratus was found at water temperatures ranging from near freezing to 24° C (Table 12). Dissolved oxygen was usually near saturation levels. Habitat waters tended to be slightly alkaline; observed hydrogen-ion concentrations ranged from 6.0 to 8.3. Bicarbonate alkalinity equaled total alkalinity and ranged from 18 to 122 ppm. In most cases, alkalinities were less than 70 ppm. Specific conductance ranged from 40 to 312 micromhos.

Table 10.--Egg production by ten mated Branchinecta linda females cultured at 20° to 22° C.

Culture Number	Number Clutches Deposited	Number of Eggs/clutch Min.--Max.		Total Eggs Deposited	Duration Productive Period, Days
BL-1	4	1	17	35	4
BL-2	5	1	24	66	9
BL-3	4	1	27	41	7
BL-4	3	5	46	56	7
BL-5	6	5	53	117	13
BL-6	7	1	87	128	9
BL-7	2	2	11	13	6
BL-8	3	2	4	9	4
BL-9	6	6	66	258	8
BL-10	6	7	60	216	10



Table 11.--Hatching of Branchinecta lindahli eggs at 20° to 22° C.

Sample Number	Number Eggs in Sample	Days Wet Before Drying	Per Cent Hatched Before Drying	Days Dried	Per Cent Hatched After Drying	Total Per Cent Sample Hatched
1	13	22	0	7	54	54
2	32	12	0	4	6	6
3	25	11	0	6	12	12
19	24	14	4	7	17	21
20	25	14	0	7	4	4
22	34	13	0	10	32	32
31	16	6	0	4	12	12
32	13	4	0	5	46	46
40	60	25	18	2	0	18
41	68	24	3	4	7	10
42	50	22	4	7	0	4
43	50	20	0	4	14	14

Calcium was usually more concentrated than either sodium or potassium, and potassium tended to be slightly more concentrated than sodium. However, the combined quantities of sodium and potassium usually exceeded that of calcium. The respective ranges for calcium, sodium, and potassium were two to 22 ppm, two to 23 ppm, and one to 13 ppm. With respect to the relative concentrations of these three cations, E. serratus habitats were characterized by the relationships  $Ca > K > Na$  and  $K + Na > Ca$ .

#### General Observations

Eubbranchipus serratus was a frequent inhabitant of roadside ditches and small marshy pools in Sumner County, Kansas, during the late fall to early spring. It was never observed concurrently with other species; but S. seali, S. texanus, and D. lindahli were known to occur in several E. serratus habitats during the warmer months. Samples from young broods of E. serratus consisted of approximately equal numbers of males and females, but samples from more mature broods usually contained a predominance of one sex. Individuals were commonly found dispersed among the sparse marginal vegetation or under leaves and other bits of detritus on the bottom of the pool.

#### Developmental History

Eubbranchipus serratus hatched as a metanauplius. A few hours after hatching 25 metanauplii cultured at 15° C averaged 0.49 mm in length and possessed up to four trunk segments as well as pigmented rudiments of the compound eyes. Length of the first antennae was about half that of the total body length. First antennae of the other four

Table 12.--Physical-chemical conditions in Eubranchipus serratus habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk <sup>3</sup> ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Ditch 4mi east Caldwell	3-19-60	4	88	6.7					60
	10-29-60*	19	51	6.9					85
	12-26-60	9	106	7.9					77
	4- 1-61	22	93	6.8		22	23	9	61
	6-18-61*	29	113	7.8	58	6	2	3	160
	9- 9-61*	32	122	7.8	65	10	12	3	159
	10-11-61*	16	60	7.9	61	10	12	4	120
	12-24-61	2	85	7.2	38	5	3	8	84
Ditch 1mi south 2mi east Corbin	6-18-61*	29	86	7.2	74	9	2	3	188
	10-14-61*	18	25	7.4	92	8	5	13	175
	11-24-61	11	108	7.2	41	4	4	9	72
	12-24-61	3	75	7.2	41	3	2	6	99
Ditch 4.5mi east Caldwell	3-19-60	4	83	6.7					
	10-29-60**	18	73	6.7					
	12-26-60	6	73	7.9					65
	5-13-61**	22	80	6.0		6	3	4	104
	6-18-61**	29	138	7.7	40	3	2	1	116
	11-24-61	13	53	7.2	18	3	4	4	42
	12-27-61	3	85	6.9	19	5	10	5	150
Ditch 0.2mi west Perth	4- 3-61	16	112	7.6		10	4	6	123
	11-25-61	14	98	7.9	87	18	4	6	128

Table 12.--Continued. Physical-chemical conditions in Eubbranchipus serratus habitats.

Habitat and Locality data	Date	HOH Temp °C	Per Cent Saturation Oxygen	pH	HCO <sub>3</sub> Alk ppm	Ca ppm	Na ppm	K ppm	Conductance Micromhos 18° C
Marsh 4.5mi east Caldwell	3-19-60	4	75	6.7					49
	12-26-60	6	67	7.8					40
	4- 1-61	22	88	6.5	35	10	6	3	64
	5-13-61**	22	72	6.0	27	6	4	3	104
	6-18-61**	28	114	7.5	50	3	2	4	139
	11-24-61	11	94	6.6	32	15	5	9	53
	12-24-61	1	80	7.2	27	4	5	7	131
	12-27-61	3	95	6.7	30	3	5	6	124
Ditch 5mi east Caldwell	3-19-60	6	83	6.8					
	12-26-60	4	60	7.7	82	22	2	9	72
	5-13-61	22		6.2	6	6	4	3	217
	6-18-61	28	107	7.4	122	6	2	5	312
	11-24-61	9	69	7.8	78	15	5	9	103
	12-27-61	2	79	6.9	36	8	8	6	159
Animal Wallow 3 north 2 east Caldwell	4- 1-61	24	116	6.9	26	2	4	5	52
Ditch 0.3mi east 1mi north Corbin	11-25-61	12	75	8.3	78	15	5	9	149

\* Only S. seali present

\*\* Only S. texanus and B. lindahli present

species during the first few days did not exceed one-third the total body length. Sexual dimorphism was evident by the ninth day at which time length averaged 5.5 mm. Incipient egg production was observed on the 13th day in females at least 10 mm in length. The length averaged 9.3 mm after 13 days (Figure 4).

Length increased about 0.5 mm per day during the first five days and up to 1.0 mm during the next 11 days. The rate of growth gradually dropped to less than 0.5 mm per day as individuals attained an average length of about 14 mm.

#### Egg Production

Egg production by ten mated E. serratus females was observed (Table 13) in the laboratory. The first five couples were mature when isolated so their totals must be considered as minimal. The other couples were immature when isolated. All cultures were kept at 10° C.

Multiple clutches were characteristic of this species. Clutches were deposited every two to four days and each was fertilized during a separate copulation. The first clutch to be produced usually consisted of less than 20 eggs. In some cases the first clutch was not fertilized but was reabsorbed in the egg sac. This accounted for the slightly larger initial clutches deposited by ES-6 and ES-8. The most eggs deposited in a single clutch was 290 eggs by ES-3. The largest clutch produced by a female which matured after isolation was 207 eggs by ES-9. Female ES-10 deposited the smallest total, 42 eggs in 3 clutches during 15 days, and ES-6 the largest total, 640 eggs in 8 clutches during 22 days.

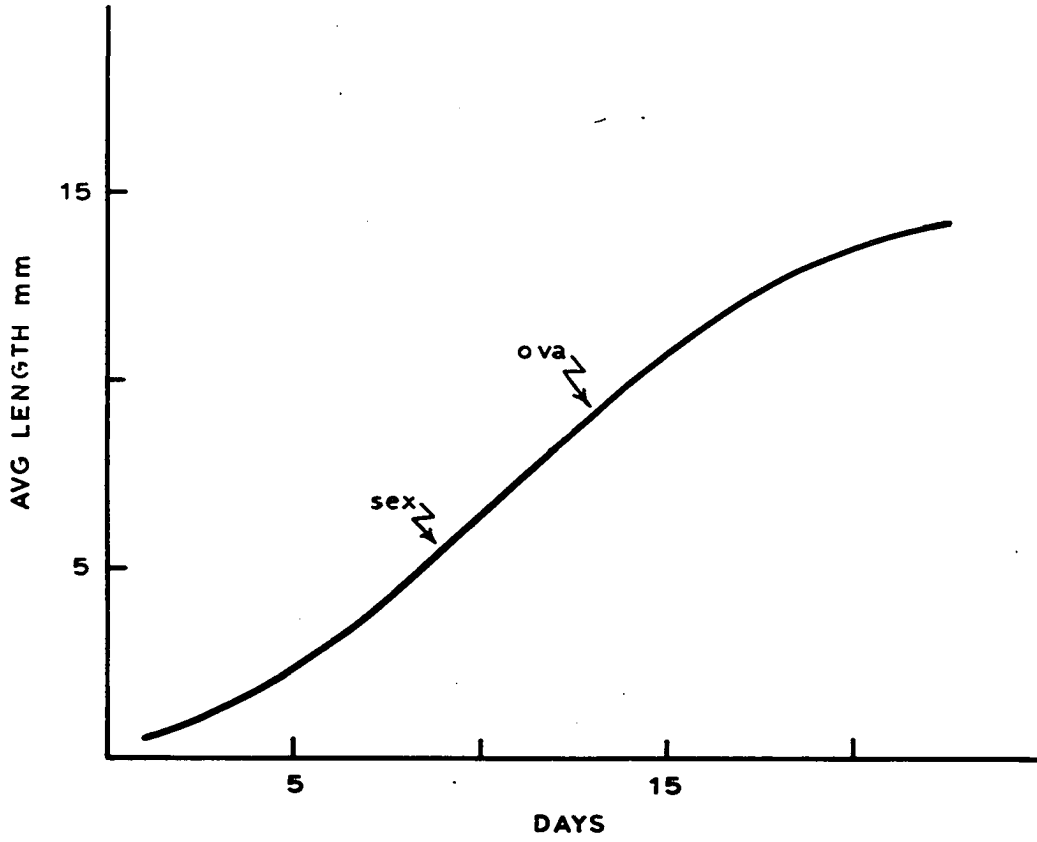


Figure 4. Growth of Eubranchipus serratus cultured at 15° C.

Table 13.--Egg production by ten mated Eubbranchipus serratus at 10° C.

Culture Number	Number Clutches Deposited	Number of Eggs/clutch Min.--Max.		Total Eggs Deposited	Duration Productive Period, Days
ES-1*	4	46	253	428	14
ES-2*	2	97	232	329	13
ES-3*	4	4	290	622	15
ES-4*	5	94	128	531	24
ES-5*	2	21	270	291	6
ES-6	8	33	114	640	22
ES-7	7	14	59	264	20
ES-8	3	42	66	161	12
ES-9	4	1	207	350	15
ES-10	3	1	39	42	15

\* Mature when isolated

## Hatching of Eggs

In general, Eubbranchipus serratus eggs required relatively long periods of drying and temperatures of about 10° to 15° C before hatching commenced. Hatching did not occur at temperatures of 20° C or higher. Even after drying, eggs at 15° C were immersed six days or more before hatching began. Hatching often continued over a period of three to five days. Several days usually elapsed between the time the egg shell cracked and the metanauplius emerged from the inner membranous sac.

Fourteen samples were subjected to initial wet periods at either 20°, 15°, or 10° C and then dried at 20° unless otherwise indicated (Table 14). After drying, samples were immersed in aerated distilled water at 15° C. The total hatch in individual samples ranged from 2 to 86%. It appeared that hatching required fewer days immersion if drying occurred at 20° and/or the eggs were previously aged at 15° or less. Eggs of E. serratus were observed to hatch at temperatures as low as 6° C.



Table 14.--Hatching of Eubbranchipus serratus eggs at 15° C.

Sample Number	Eggs Per Sample	Days Wet and Temp. Prior to Drying	Days Dried	Days Immersed at 15° Before Hatching Began	Per Cent Sample Hatched	Remarks
1	69	48 at 20°	2	16	55	
2	87	42 at 10	7	7	47	
3	29	32 at 20	16	11	86	dry at 10°
4	50	32 at 10	12	9	80	
5	60	37 at 15	19	6	18	
6	60	27 at 10	20	8	25	dry at 15
7	50	23 at 10	12	22	10	dry at 10
8	50	41 at 10	8	6	10	
9	50	17 at 20	19	14	36	dry at 15
10	50	17 at 20	19	15	36	dry at 10
11	50	33 at 20	20	10	30	
12	100	18 at 20	24	9	70	dry at 15
13	174	5 at 20	15	11	2	
14	170	12 at 20	11	20	6	dry at 10

## DISCUSSION

### Seasonal Occurrence

Although fairy shrimps are categorized repeatedly as vernal inhabitants of temporary pools such statements are misleading. Most species do occur during the vernal months but many are also present during other seasons.

Actually the annual precipitation and thermal patterns greatly influence the occurrence of the species in a given region. In Kansas and Oklahoma, precipitation is normally greatest during the spring and least during the winter. With respect to precipitation patterns, spring is the most favorable and winter the least favorable season for the occurrence of fairy shrimps. One might expect the same to be true for temperatures, but the effects of temperature on hatching, development, and survival vary from one species to the next. Thus temperatures conducive to hatching and survival of one species may be unfavorable for another species.

Based on the monthly occurrence of species in the field and the hatching and survival of species at different temperatures in the laboratory, I have designated species as either year-round, spring to early fall, or fall to early spring forms.

## Year-Round Species

I collected Streptocephalus seali during all seasons of the year. Moore (1955) reported that it was present throughout the year in Louisiana, and locality data published by Dexter (1953, 1956) indicate that it is probably a year-round form throughout most of its known range.

It is a eurythermic species. Adults have been observed at temperatures ranging from near freezing to 42° C (Moore op. cit., Prophet 1959). In the laboratory, I have hatched eggs at temperatures as low as 13° C, and the immatures survived exposure to 10° C for more than three weeks. Moore (op. cit.) found that development of S. seali occurred at a very slow rate at temperatures which varied between 10° and 15° C.

The ability of S. seali to tolerate a wide range of environmental conditions is reflected by its wide distribution. It is perhaps the most widely distributed species of fairy shrimp in North America. To date, S. seali has been reported from 23 states throughout all parts of the United States and from Canada and Mexico (Dexter 1959).

## Spring to Early Fall Species

Three species, S. texanus, T. platyurus and B. lindahli, were present only during the warmer months. All are common to the central and southwestern states (Dexter 1959), and the little that has been published concerning the occurrence of these species supports the above designation of spring to early fall species (Moore 1950, Leonard and Ponder 1949, Dexter 1953, 1956).

I consider these species to be warm stenothermic forms. In general, hatching of their eggs was inhibited by temperatures below 15° C. In the laboratory, adult B. lindahli survived a constant

temperature of 10° C for more than two weeks while T. platyurus and S. texanus usually died within three to five days.

#### Late Fall to Early Spring Species

Eubbranchipus serratus is a cold stenothermic species which occurs primarily in the northern half of the United States (Dexter 1959). In Illinois, Dexter and Ferguson (1943) found that E. serratus disappeared when water temperatures approached 23° C. Most published records for this species in other parts of its range indicate an early spring occurrence (Dexter 1953, 1956).

Temperatures below 20° C are apparently necessary for the hatching and prolonged survival of this species. I have observed hatching of E. serratus eggs at temperatures of 6° to 15° C but never at 20° C or higher. McGinnis (1911) reported that temperatures of about 14° to 17° C appeared to be optimal for adults of this species. Dexter and Ferguson (1943) reported that individuals kept at a constant temperature of 9° C lived more than 43 days compared to a survival of 14 days at a constant temperature of 16° C.

#### Physical-Chemical Conditions

The limnological features of small temporary pools have been studied very little. The chemical composition of temporary pools is, like that of permanent waters, influenced by the geochemistry of the surrounding terrain. However, physical-chemical conditions in the small temporary pools are subject to more rapid and extensive fluctuations. The fauna of such waters must tolerate a wide range of environmental conditions or else be restricted to a particular period when conditions are favorable.

Knowledge of the limnological features of the habitats of most species of fairy shrimps is very limited. Because of their shallow nature and large exposed surface area the temperatures within anostracan habitats tend to follow the air temperatures rather closely. It is probable that dissolved oxygen rarely becomes limiting. Even at maximum temperatures concentrations of dissolved oxygen in the study habitats were generally sufficient for survival of fairy shrimp. Approximately 4 ppm or more of dissolved oxygen were usually detected in the study habitats.

In one case I did suspect an oxygen deficiency as being the primary cause for the disappearance of a brood of immature S. seali. On June 10, 1961, a small recently flooded pond contained an abundance of S. seali. This observation was made at 11:00 AM on a clear day at which time only 1.1 ppm (15% saturation) of dissolved oxygen was present. The entire bottom of the pond was matted with decaying matter. Five days later, this pond was again visited at the same hour. Fairy shrimps were absent and only 0.5 ppm (5% saturation) of oxygen was detected. The water was darkly stained by the decomposition of the abundant plant material.

Temperature, pH, alkalinity, and dissolved oxygen fluctuate hourly as well as seasonally in large bodies of water. For example, the pH and dissolved oxygen in Buckeye Lake, Ohio, were found to be lowest during the early morning hours (Tressler, et al. 1940). There was a gradual increase until the maximum levels were reached during the late afternoon hours. On the other hand, free CO<sub>2</sub> concentrations were greatest during the early morning. The CO<sub>2</sub> level decreased during the

photosynthetic period and increased during the hours of darkness. Gasaway (1961) reported similar fluctuations in small fish-culture ponds in Oklahoma. Similar extensive studies of diurnal fluctuations of the physical-chemical conditions in temporary pools might be valuable in determining which environmental factors exert the most influence on the life span and occurrence of fairy shrimps.

Specific conductance measurements were excellent for demonstrating the variability of the habitat waters. Such measurements not only approximated the total dissolved salts in the water but indicated habitat age. A low conductance was typical of recently formed pools. Increased conductance between successive visits to habitats resulted from the concentration of salts by evaporation and leaching. Marked decreases in conductances were due to dilution resulting from additional rainfall.

When the evaporation rate is high, the conductance of small pools changes rapidly. Rzóska (1961) found that conductances changed rapidly in rainpools in Northern Sudan. For example, rain water pouring from a roof had a conductance of 35 micromhos; a few hours later the conductance of a newly formed rainpool was 140 micromhos. During the next 11 days the conductance of this pool increased to 1130 micromhos. Although such marked variations in conductance were not observed in the study habitats, it was evident that the conductance of the habitats varied with age. Gasaway (op. cit.) found that conductance closely followed the curve for photosynthetic activity in small fish-culture ponds.

Although the mater has been studied only slightly, there is evidence that the distribution of some aquatic organisms may be related

to the chemical nature of the habitat water. Hutchinson (1957, see p 571) stated that the ionic ratios of waters may have ecological value. Reynoldson (1956, 1958a, 1958b) reported a broad relationship between calcium and dissolved matter and the composition of triclad populations in certain European waters. Boone and Boas-Becking (1931) noted that Artemia nauplii failed to survive in KCl solutions more concentrated than 0.1M but that the toxic effect of the potassium could be antagonized by the addition of sodium and to a lesser extent by calcium and magnesium. Croghan (1958) found that Artemia would survive for several days in 0.5M NaCl solutions while death occurred within 24 hours in KCl solutions. However, survival in the potassium solution could be prolonged by the addition of sodium salts.

The tolerance to total dissolved solids as well as to relative concentrations of individual ions probably varies from species to species. The brine shrimp, Artemia, can tolerate extremely brackish conditions (Carpelan 1957). Branchinecta campestris has only been found in extremely alkaline waters (pH 9.5-10) or waters having such a high dissolved salt content that only Artemia can develop abundantly in the same habitat (Lynch 1960). Some species which have a wide distribution tolerate such a wide range of chemical conditions that it is nearly impossible to describe a single distinctive characteristic of their habitats.

On the basis of the physical-chemical conditions studied, the habitats of S. seali and S. texanus were similar in many ways. Both species have been collected within a wide range of pH and alkalinity. I found that calcium was usually more concentrated than either sodium

or potassium in their habitats, but S. texanus was also present in waters in which potassium was the dominant cation.

One of the most distinctive features of B. lindahli habitats was the tendency for the pH to be greater than 8.4. As a result, a large proportion of the total alkalinity was due to the presence of normal carbonates. Calcium was more concentrated than either sodium or potassium in these habitats.

Thamnocephalus platyurus habitats typically had a pH of less than 7.3 and a total alkalinity of less than 30 ppm. The most striking feature of these habitats was the comparatively high potassium and low calcium and sodium levels. Potassium was always more concentrated than either calcium or sodium. Whether other T. platyurus habitats would exhibit this same relationship is not known. Published locality data for T. platyurus in Oklahoma are all from the western third of the state (Mackin 1936). Soils in western Oklahoma contain more exchangeable potassium than soils in the central and eastern part of the state (Harper 1950). Thus, temporary pools in western Oklahoma should be relatively rich in potassium. The wide occurrence of S. texanus in the western half of Oklahoma may indicate that it can also tolerate a rather high level of potassium. On the other hand, S. seali is found mainly in central Oklahoma where there is less exchangeable potassium in the soil. Results are incomplete but experiments indicate that S. texanus can tolerate nearly three times the relative K: Ca+Na ratio tolerated by S. seali or B. lindahli.

Although levels were not as great as in the T. platyurus habitats, the waters inhabited by E. serratus were also characterized



by the relatively high potassium values. Calcium was more concentrated than potassium which in turn was more concentrated than sodium. But the combined quantities of the monovalent cations usually exceeded that of calcium so that the relationship  $K+Na > Ca$  was typical.

The chemical nature of habitats of these species in other parts of their ranges must be studied before it can be determined whether the differences mentioned above are real or apparent.

#### Developmental History

Although the number of instars and the rates of growth varied from species to species, the general pattern of development was similar. Fairy shrimps hatch as either a nauplius or a more advanced metanauplius. Both of these larval forms possess a median ocellus and paired first and second antennae and mandibles. The metanauplii also exhibit rudiments of the compound eyes and two or more trunk segments. My observations of recently hatched individuals indicated that E. serratus emerges as a metanauplius while S. seali, S. texanus and B. lindahli hatch as a nauplius. I did not observe the young of T. platyurus emerging from the egg, but I believe that this species hatches as a metanauplius since the young possessed rudiments of the compound eyes and at least three trunk segments in less than 24 hours after hatching.

The second antennae serve as the primary locomotor organs during the first few days of the life span. There is a progressive appearance of trunk segments in an anterior to posterior direction. Each of the first 11 trunk segments possesses a pair of swimming legs. The last nine segments constitute the abdomen. The terminal abdominal segment is modified to form a biramous telson. As the trunk appendages become

adult-like they take on the functions of locomotion, feeding, and respiration and the second antennae undergo a gradual transformation to the adult condition. Concurrently, a bulge appears on the first two abdominal segments and differentiates into the ovisac of the female or penes of the male.

Moore (1957) demonstrated that the developmental rate of S. seali was influenced by temperature and food. Such is undoubtedly true of other species. I found that under the culture conditions used each species molted approximately once each day. Streptocephalus seali exhibited sexual dimorphism by the 8th instar and eggs appeared by the 11th instar. Although the male second antennae were adult-like by this time, it was not until the 16th instar that the posterior part of the male cercopods developed the spiny appearance characteristic of the adult male. In S. texanus reproduction commenced as early as the 11th instar. The pre-adult stages of these two species are so similar that it is almost impossible to distinguish one species from the other. The diagnostic feature most commonly used to separate these species is the appearance of the male cercopods, which in S. texanus have setiferous margins but are spined distally in S. seali. However, cercopods of immature male S. seali have setiferous margins like those of S. texanus. Gaulin (1960) reported that juveniles could be distinguished on the basis of the appearance of the second antennae of males. He found that the front margin of the posterior spur on the outer branch of the "scissors" forms a sharp angle in S. texanus but is rounded in S. seali. I found that this feature was readily apparent in S. texanus by the 9th instar and as early as the 12th instar in S. seali.

Under the same culture conditions the development of E. lindahli was more rapid than that of either S. seali or S. texanus. Sexual dimorphism was evident by the 5th instar and copulation was first observed during the 7th instar. The low number of pre-adult instars observed for this species may indicate that a few stages were not detected. It is probable that a shorter developmental sequence would be beneficial in view of the type of habitat in which this species is frequently found. Small rock holes do not remain flooded for exceptionally long periods. Thus, only species which mature rapidly would have a good chance of invading such ephemeral pools.

Eubbranchipus serratus exhibited sexual dimorphism by the 9th instar and commenced egg production by the 13th instar. Metanauplii of this species could be distinguished from the early stages of S. seali, S. texanus and E. lindahli by the relative length of the first antennae which in E. serratus was equal to half the body length. In the other species the length of the first antennae was equal to about a third of the body length.

#### Egg Production

Moore (1955) postulated that S. seali produced numerous clutches of eggs during its adult life. This was later demonstrated by Gaulin (1960) and Moore (1959a). My results show that S. texanus, E. lindahli, T. platyurus, and E. serratus also produce numerous clutches during their adult lives.

Each clutch is fertilized during a separate copulation. Copulation must occur just before or soon after the eggs are moved into the egg sac. The factor or factors which attract the male to

the female, or vice versa, and which trigger the copulatory act are not known. I have rarely observed the copulatory act in S. seali, S. texanus, and E. serratus and never in T. playturus. If females of these species were isolated from males egg production continued, but shell material was not deposited around the eggs after they entered the ovisac. These unfertilized eggs remained whitish and were soon reabsorbed. If males were then introduced the succeeding clutches received a shell coat which soon became light tan to reddish brown. Such egg masses were deposited one to three days after they entered the egg sac.

#### Hatching of Eggs

The continued survival of fairy shrimps in ephemeral pools is possible as a result of their comparatively rapid maturation and the production of eggs which are capable of withstanding the intervening dry periods. Mathias (1937) believed that two types of eggs are produced, those that developed without drying and others which could withstand prolonged periods of desiccation. Hall (1959c) believed that Chirocephalus diaphanus produces only one type of egg, but that it is capable of delayed or continuous development, depending upon environmental conditions. Weaver (1943) stated that not all eggs of E. vernalis were equally sensitive to the "shock" of drying and/or freezing. All three of the above statements draw attention to the variability of the hatching of anostracan eggs. I failed to note any difference in the appearance of eggs which hatched without previous drying and those that hatched after drying. I feel that the explanation is more nearly a combination of the latter two statements. That is,

after fertilization development continues up to a point and then the embryo may become dormant. The eggs vary in their resistance to hatching. Some eggs undergo a continuous development and hatch relatively soon or require only a slight change in some environmental condition, such as temperature, to stimulate hatching. Others need a more severe "shock" to stimulate further development and hatching.

With the exception of E. serratus some eggs of all species considered in this study were observed to hatch within 3 to 11 days after deposition. In these instances the eggs apparently underwent continuous development and the time to hatching was rather constant for a given species. For example, hatching of some B. lindahli eggs would occur on the third day after deposition while some S. seali and S. texanus eggs would hatch 5 to 7 days after deposition. The eggs which did not hatch within one week or more after deposition could often be stimulated to hatch by either drying or diluting the culture medium. In either case, the hatching time was again similar. That is, if hatching occurred in either dried or diluted samples, it invariably occurred within 24 hours after the addition of the aerated distilled water to the sample vessels (E. serratus eggs thus treated did not begin hatching for at least six days). The hatching of these eggs so soon after treatment indicates that development was nearly completed before the addition of distilled water.

Although drying and dilution appeared to stimulate hatching, the age of the eggs at the time these conditions occurred was important; i. e., when eggs were either dried or placed in aerated distilled water immediately after deposition hatching rarely occurred. Hatching was

more likely to occur after the eggs were first allowed to "age" in culture medium for at least 3 to 7 days.

Moore (1959a) found that S. seali eggs stored in ampules of water for at least 30 days began hatching as soon as 15 hours after immersion in aerated demineralized water. He reported that hatching was greatest 15 to 24 hours after immersion and rarely continued more than two days. This closely agrees with the hatching of S. seali eggs in nature. My results indicated that this is also true of S. texanus, T. platyurus, and E. lindahli eggs. In fact, eggs of these species which were first aged in culture medium for at least one week and then dried three to seven days began hatching as soon as six hours after immersion in aerated distilled water. On the other hand, dried E. serratus eggs usually required 6 to 16 days immersion at 15° C before hatching commenced. Weaver (1943) reported that E. vernalis eggs dried and/or frozen up to 46 days required 12 to 13 days immersion before hatching would start (temperatures not reported).

The matter of temperature requirements for the development and hatching of anostracan eggs needs much additional research. My results indicate that E. serratus eggs require temperatures of about 6° to 15° C before development and hatching will occur. Temperatures below approximately 15° or as high as 32° C inhibit development and hatching of the eggs of S. seali, S. texanus, T. platyurus, and E. lindahli.

## SUMMARY

1. Temporary pools inhabited by one or more species of fairy shrimps were studied during the period from September 1959 to January 1962. Water temperature, dissolved oxygen, pH, alkalinity, specific conductance, and the relative amounts of calcium, sodium, and potassium were measured during all seasons. These data were used to determine similarities and differences in the general physical-chemical characteristics of the waters inhabited by each species. The developmental history and production of eggs of each species were observed in the laboratory, and the effects of certain environmental conditions on the hatching of eggs were studied.

2. Streptocephalus seali, Streptocephalus texanus, Thamnocephalus platyurus, Branchinecta lindahli, and Eubbranchipus serratus were found in the study habitats which were located in Sumner County, Kansas, and Cleveland, McClain, Comanche, and Tillman counties in Oklahoma.

3. Specific conductance was a good indicator of the age of habitats. Conductance was lowest in recently flooded habitats, and it gradually increased due to the concentration of solutes by leaching and evaporation.

4. The relative concentrations of calcium, sodium, and potassium were thought to be especially important in influencing the

distribution of species. Habitats of T. platyurus were located in soils high in exchangeable potassium; temporary pools in such soils were relatively high in potassium (4-16 ppm) and low in calcium (2-4 ppm) and sodium (1-2 ppm). Habitats of T. platyurus were characterized by the relationship  $K > Ca > Na$ , a pH less than 7.3 and a total alkalinity of less than 40 ppm.

5. Both S. seali and S. texanus were commonly found in waters having a pH of less than 8.4, a total alkalinity of at least 50 ppm, and in which calcium tended to be more concentrated than either sodium or potassium. With respect to cation relationships, E. lindahli habitats were similar to those of the above two species but this species was most commonly found in waters having a pH greater than 8.4. The pH and total alkalinity of E. serratus habitats were similar to those of S. seali and S. texanus, and the latter two species frequently inhabited E. serratus pools during the warmer months. Calcium was more concentrated than either sodium or potassium in E. serratus pools but the combined concentrations of the monovalent cations usually exceed that of the former so that the relationship  $K + Na > Ca$  was characteristic.

6. Ecdysis occurred approximately once each day in S. seali, S. texanus, and E. lindahli at 20° C and in E. serratus at 15° C. The development of E. lindahli was very rapid with copulation occurring as early as the 7th instar. Egg production by S. seali commonly began during the 14th instar and males exhibited all adult features by the 16th instar. Reproduction by S. texanus commenced as early as the 11th instar and by the 13th instar in E. serratus.

7. The length of the first antennae relative to total body length can be used to distinguish between the metanauplii of E. serratus



and those of S. seali, S. texanus, and B. lindahli. The first antennae of E. serratus metanauplii were equal to approximately half the total body length but tended to be less than a third of the total body length in the other three species.

8. Egg production continued throughout most of the female's adult life. In general, females of each species deposited one clutch every one to three days. Each clutch was fertilized during a separate copulation. Egg production continued in the absence of males but the unfertilized eggs were reabsorbed after entering the egg sac. In general, the number of eggs produced per clutch increased with parental age.

9. Total production per female was influenced by food, length of the productive period, and the frequency at which males failed to fertilize the clutches. The largest number of eggs deposited by a female of each species was 1791 by S. seali, 1635 by S. texanus, 640 by E. serratus, and 258 by B. lindahli.

10. Temperature greatly influenced the hatching of eggs. Eggs of E. serratus hatched at temperatures of 6° to 15° C but not at 20°. Eggs of the other four species most commonly hatched at 20° C or slightly higher, but temperatures as high as 32° C inhibited hatching. Drying for one to seven days would often stimulate hatching if the eggs were first allowed to "age" in small vessels of parent medium. Dilutions of the hatching medium would also stimulate the hatching of "aged" eggs. Drying or immersion of the eggs in distilled water immediately after deposition inhibited hatching.

11. Evidence indicated that development of the eggs may be either continuous or delayed. Some eggs hatched three to seven days

after deposition. Often development was delayed and hatching did not occur until the eggs were subjected to some environmental "shock".

12. Based on their natural monthly occurrence from September 1959 to January 1962 and the observed influence of temperature on the hatching of eggs in the laboratory, species were grouped according to seasonal occurrence as year-round, S. seali; spring to early fall, S. texanus, T. platyurus, and E. lindahli; and late fall to early spring, E. serratus.

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