DIURNAL PERIODICITY OF ACTIVITIES IN THE PLAINS KILLIFISH, *FUNDULUS ZEBRINUS KANSAE*

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The activities of *Fundulus zebrinus kansae* were monitored in natural conditions. Daily differences in absolute levels of activity and in times of peak activities were related to meteorological conditions. High positive correlation was observed between the levels of reproductive and agonistic activities. Feeding increased gradually from a minimum in early morning to a maximum in mid-afternoon. A shift from primarily afternoon spawning in the winter to primarily morning spawning in the summer was postulated. The observed patterns of activity were examined for possible causative factors.

Most studies of diurnal periodicity of behavior in fishes have related primarily to laboratory observations or have dealt specifically with feeding activity. Our primary purpose is to present an analysis of hourly changes in the behavior of the plains killifish, *Fundulus zebrinus kansae* (Family: Cyprinodontidae) in natural conditions. (We follow the inference of Miller (1) and the conclusions of Drewry (2) in regarding *kansae* a conspecific of *zebrinus*.) Koster (3) described spawning and other activities for *kansae* in a pool of a small tributary of the Cimarron River in New Mexico on two days in August. To our knowledge there are no other published accounts of behavior in this species except a note by Minckley and Klaassen (4) on burying behavior.

METHODS

Observations were conducted as a project of the fish ethology class (summer, 1970) of the University of Oklahoma Biological Station on July 17 and 21, 1970 at Coffeepot Creek, a small freshwater stream one mile east of Rubottom, Love Co., Oklahoma. The water was less than 10 cm deep at the observation sites and current ranged from moderate in small raceways to none in small backwater areas. The bottom was sandy with little rooted vegetation. The killifish and *Notropis lutrensis* were the only fishes observed in the area, with the former being the most abundant. July 17 was warm and cloudless; July 21 was heavily overcast and considerably cooler.

Observations were made along a 40-m length of stream at four stations on July 17 and three stations on July 21. The stream bank was sharply incised, and enabled observation from almost directly above the water at heights of 2 to 3 m. Behavior was quantified by a team of two workers (observer and recorder) at each station. The activities of males which could be observed easily at each station were recorded for a 30 min period each hour from 0830 to 1430. At each station the data for each 30 min period were corrected to represent the activity level of five males. At hourly intervals, the frequency of feeding acts in one minute periods was recorded individually for five females at each station. Measurements of O_2 concentration, light intensity, and temperature were made hourly.

Feeding consisted primarily of "nipping" and "digging" on the bottom. In nipping the mouth is opened, pressed against the bottom substrate or other surface, and quickly closed. In digging the fish tilts head downward as if to nip the bottom and then forcibly plunges its head, often past the eyes, into the substrate. These acts were easily discerned with the aid of binoculars. Feeding was scored for each nipping or digging movement. Male behavior was enumerated according to the frequencies of agonistic, courtship, and spawning interactions. These behaviors are described in detail by Koster (3) and Foster (5). Agonistic activity was scored once for each agonistic bout between a male and another fish. A courtship was recorded for each interaction, excluding schooling behavior, in which it appeared that a male attempted to maintain close association with a female. A spawning was recorded for each interaction in which a male and female clasped and vibrated on the bottom in the spawning attitude described by Koster (3). When clasping and vibrating occurred sev-

eral times in quick succession in a given pair the bout was scored as a single spawning. Similarly, prolonged interaction between male-female pairs was scored as a single courtship, including those in which courtship activities were interspersed with several spawning acts.

RESULTS AND DISCUSSION

There was close agreement between observers regarding the relative frequencies of agonistic and reproductive activities at the various stations (Tables 1 and 2). The greatest difference was in the agonistic levels recorded at stations I and III as compared with station II from 1000 to 1230 on July 21. Characteristics of feeding activity were also found to be similar between stations. The data from all stations were lumped in calculating relative frequencies (see Fig. 1) for each behavioral category.

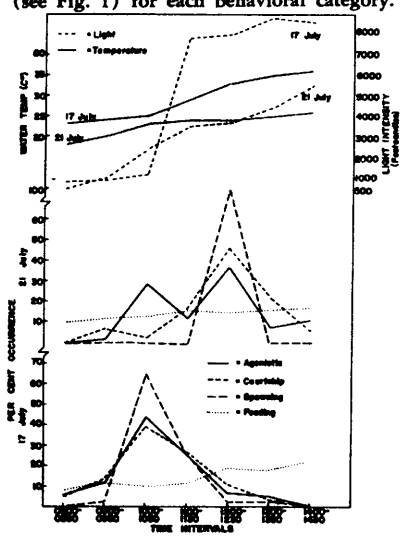

FIGURE 1. Hourly changes in activities of *Fundulus zebrinus* in Coffeepot Creek, July 17 and 21, 1970. On July 17 the light meter was shaded in the morning and in direct sunlight in the afternoon, accounting for the abrupt increase in light intensity measurements.

In early morning, killifish were widely dispersed in the stream and feeding was the predominant activity. Aggression and courtship were absent or at minimal levels. By midmorning the males tended to restrict their activities to particular areas and began defending territories; aggression was directed toward *N. lutrensis* as well as conspecifics. This transition occurred with the initiation of courtship behavior. Certain males showed little or no aggressive tendency; these were feeding and moving freely about, occasionally with females and other males.

Territorial males were dark in general coloration with bright red pectorals and pelvics, reddish-black anal fin, and dark vertical bars on the body; the iris of the eye was darkened and the caudal fin was chalky white. Nonaggressive males were drab with silvery irises and dark gray caudal fin. The digging act often appeared exaggerated in territorial males. The male would tilt downward and, with tail held high, plunge the head into the substrate, hold the vertical position briefly, and then return to the horizontal. Their digging was more forceful than that in females and nonterritorial males, and it created a "flagging" effect of the whitened caudal fin. The high levels of aggressive and reproductive activity combined with bright body colors and exaggerated feeding movements made territorial males conspicuous to the observer and may have served to ward off intruders and/or attract sexually motivated females.

Territorial males were located in backwater areas and in raceways primarily at the stream edge or near beds of algae and emergent vegetation. The territories were in open situations and were centered about various salient features of the environment, such as alongside a dead tree limb and around bottom irregularities. This observation contrasts with Koster's (3) statement that "no specific territory seemed to be guarded for any great length of time but rather a certain amount of 'elbow room' was maintained." The territorial organization at Coffeepot Creek was less rigid than that described for cyprinodontids of the genus *Cyprinodon, e.g.*, by Barlow (6) and by Echelle (7). In Coffeepot Creek, there was considerable overlap between territories, males were more tolerant of intruding fish, and resident males often left the territory to move several meters up- or downstream, usually in pursuit of females.

The daily increase and subsequent decline in reproductive activity (Fig. 1) may have

reflected a motivational flux that occurred as environmental factors (*e.g.*, temperature and light) approached and then passed optimum conditions for reproduction. This could explain the pattern of sexual activity on July 17, when temperature and light intensity both reached high levels, but seems inadequate for July 21, when neither factor attained the level at which sexual activity began to decline on the former day. Oxygen concentration seems negligible as an influencing factor since it remained at a constant level each day, *i.e.*, 8 ppm on 17 July and 7 ppm on 21 July.

Certain observations indicated that males had lower thresholds and wider ranges of permissive environmental factors for reproductive activity than females. First, at all times males were more persistent in courtship and more active in initiating courtship interactions than females. Second, males began courting well before females showed notable sexual tendencies and continued after females had stopped exhibiting sexual activity. These considerations imply that female receptivity was a major factor governing daily patterns of reproductive activity. Hypothetically, reproductive motivation reached a maximum in both sexes (although more slowly in females) perhaps as a result of increasingly favorable environmental factors as the morning progressed (Fig. 1) and heightened sexual stimulation derived from increased levels of social interaction. It declined, conceivably, as a consequence of motivational effects of the performance of sexual activities. An example of the latter is the possibility that sexual motivation in females wanes with depletion of the number of large, mature eggs in the ovaries. The direct relationship between spawning (a measure of female receptivity?) and courtship (a measure of male sexual arousal?) may be obtained from feedback relationships based on the level of female receptivity. With increasing sexual arousal in females, the males may receive greater positive feedback *via* reciprocated sexual behavior, which, in turn, might increase the frequency of male-initiated courtships. Conversely, in periods of low female receptivity the balance may shift toward negative feedback causing a decline in the frequency of courtship.

On July 17, the killifish spawned from approximately 0900 until 1330 at water temperatures ranging from 24 to 34 C, with peak activity from 1000 to 1030 at temperatures near 25 C. On July 21 spawning occurred only from 1200 to 1230 at temperatures near 25 C. The peak of reproductive activity occurred later in the day on July 21 than on July 17, probably due to cooler temperatures and lower light intensities on the former day (Fig. 1). Koster (3) noted that reproduction occurred primarily between 1105 and 1310 on August 18 at water temperatures above 82 F (27.8 C), and he observed neither spawning nor agonistic bouts on August 19 when observations began at noon and the water temperature was 88 F (31.1 C). Besides July 17 and 21, 1970, we have recorded spawning (clasping and vibrating) on the following dates in southwestern Oklahoma (time and temperature in parentheses): 25 March 1968 (1545, 25 C); 8 June 1969 (1450, 23 C); 12 June 1969 (1400, 30 C); 14 July 1970 (0930-0955, near 25 C); October 1969 (1120, 24 C); 27 October 1969 (1239, 23 C). Koster (8) reported summer as the spawning season for *kansae* in New Mexico; Minckley and Klaassen (9) estimated that in Kansas spawning commenced in April and all females were spent by the end of August. Hubbs and Ortenburger (10) found that young-of-year occurred in Oklahoma collections by June 1 and that most adults had spawned by the end of July. Our data suggest a somewhat more prolonged spawning season than is indicated by the literature, but summer seems to be the period of peak spawning activity. We propose that, as a result of seasonal change in thermal regime, there is a shift from primarily afternoon spawning at the start of the spawning season to primarily morning spawning in the summer. Theoretically, there would be days of moderate temperatures, particularly in the spring and fall, when permissive temperatures for spawning would exist all day. Day-long spawning was observed in the spring by Echelle (7) in the Red River pupfish, *Cyprinodon rubrofluviatilis*.

Tables 1 and 2 demonstrate that absolute levels of reproductive and agonistic activities were notably higher on 17 July than on 21 July. This probably derived largely from the lower temperatures and light intensities on the latter date, but the possibility exists that spawning had passed its peak by then. No spawnings were observed at Coffeepot Creek from 0730 to 1200 on July 31,

1970, though water temperatures (24-28 C) were within the permissive range, and there was little or no courtship or aggression. Locally, day to day spawning intensities may vary considerably, with times of profuse spawning followed by periods of lesser activity during which egg complements are reconstituted.

TABLE 1. *Percentage occurrence of agonistic bouts recorded in 30 min periods for* Fundulus zebrinus *males at various Coffeepot Creek stations on two days in July, 1970.*[a]

Time	17 July				21 July		
	Station						
	I	II	III	IV	I	II	III
0800-0830	2	2	6	11	0	0	0
0900-0930	10	22	19	13	0	5	0
1000-1030	44	28	25	50	21	47	14
1100-1130	28	30	35	18	24	7	13
1200-1230	12	13	10	7	45	22	50
1300-1330	5	3	6	2	6	4	14
1400-1430	0	2	0	0	3	16	9
Total bouts observed	217	60	132	121	33	77	90

[a] Data for each observation period adjusted to represent five males at each station.

TABLE 2. *Percentage occurrence of courtship and spawning sequences recorded in 30 min periods for* Fundulus zebrinus *males at various Coffeepot Creek stations on two days in July, 1970.*[a]

Time	17 July				21 July		
	Station						
	I	II	III	IV	I	II	III
0800-0830	4(0)	0(0)	7(0)	10(0)	0(—)	0(—)	0(0)
0900-0930	16(8)	0(0)	4(0)	15(0)	9(—)	8(—)	0(0)
1000-1030	46(77)	35(50)	53(40)	33(100)	6(—)	0(—)	0(0)
1100-1130	23(15)	67(50)	18(40)	28(0)	33(—)	4(—)	0(0)
1200-1230	10(0)	0(0)	11(10)	0(0)	45(—)	38(—)	69(100)
1300-1330	2(0)	0(0)	4(10)	14(0)	6(—)	47(—)	19(0)
1400-1430	0(0)	0(0)	0(0)	0(0)	0(—)	8(—)	16(0)
Total sequences observed	113(13)	21(4)	68(10)	75(4)	33(0)	23(0)	16(6)

[a] Data for each observation period adjusted to represent five males at each station. Numbers in parentheses, spawning sequences; numbers not in parentheses, courtship sequences.

There was high positive correlation between the levels of courtship and agonistic activity on both days of observation (Fig. 1), with the only exception occurring from 1000 to 1030 on July 21. The males at station II were the source of this discrepancy, for those at stations I and III showed low agonistic activity and correspondingly low courtship activity during the 1000-1030 period (Tables 1 and 2). The afternoon decline in agonistic interactions and courtship activity was correlated with gradual breakdown of territoriality; the males lost much of their bright coloration and began to mingle with the general population. The high correlation between courtship and agonistic activity is attributable to competition for females between courting males. Males were relatively tolerant of each other when no courtship was occurring, but aggression intensified when one began courting a female. Courting males frequently entered neighboring territories and this usually led to agonistic confrontations. Interference between males frequently led to aborted courtships.

A major function of aggression and territoriality in reproductive males may be the provision of space, from which other males are excluded, for the male to court females with relative freedom from intruders. Echelle (7) proposed this and protection of fertilized eggs as the major functions of territoriality in the Red River pupfish. Male pupfish tend to defend territories against all intruders from daylight until dark whether reproduction is occurring or not, and, therefore, afford considerable protection for eggs fertilized in the territory. Male killifish defend areas primarily against other males and then only during the reproductive period of the day; thus egg protection may be of minor importance as a function of territoriality. Compared to the pupfish, the vibrating movements of spawning killifish tend to bury the female's ventral surface deeper during oviposition; perhaps this makes the eggs less accessible to the feeding population.

Feeding activity in females increased gradually from early morning into mid-afternoon (Fig. 1), which, perhaps, reflected the increasing temperatures and correspondingly increasing metabolic demand. Though not quantified, a similar pattern was observed in breeding and non-breeding males. Echelle (7) noted similar behavior in the Red River pupfish; feeding increased gradually until mid-afternoon, reached a plateau of maximum activity, and remained high until just before dark when it showed a decided decline. Like the pupfish, the killifish does not seem to feed after nightfall. The average total number of feeding movements recorded during the day at various stations was 1,030 on July 17 and 920 on July 21. Feeding activity was more intense on the warmer day.

By 1230 on July 17, when the water temperature was 33 C, the killifish, particularly the adults, began to move into areas that afforded protection from increasingly higher temperatures. By 1400 adults were virtually absent from areas in the mainstream where they had been abundant earlier in the day. At 1440 the temperature was 38 C or above in sunlit areas of flowing water; such waters supported no adults and only small numbers of juveniles. Numerous killifish of all sizes were in two small coves, one shaded (water temperature, 37 C) and the other (water temperature, 34.6 C) sunlit but receiving seepage from an adjacent embankment. On July 21, when afternoon temperatures were considerably lower, there was no notable tendency for the population to congregate in these areas.

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REFERENCES

1. R. R. Miller, Occ. Pap. Mus. Zool. Univ. Mich. 568: 1-25 (1955).
2. G. E. Drewry, Ph.D. Dissertation, Univ. Texas, Austin, 1962.
3. W. J. Koster, Copeia 1948: 25-33 (1948).
4. C. O. Minckley and H. E. Klaassen, Copeia 1969: 200-201 (1969).
5. N. R. Foster, Ph.D. Dissertation, Cornell Univ., Ithica, N. Y., 1967.
6. G. W. Barlow, Am. Midl. Nat. 65: 339-359 (1961).
7. A. A. Echelle, Ph.D. Dissertation, Univ. Oklahoma, Norman, 1970.
8. W. J. Koster, *Guide to the Fishes of New Mexico*, Univ. New Mexico Press, Albuquerque, 1957.
9. C. O. Minckley and H. E. Klaassen, Trans. Am. Fish. Soc. 98: 460-465 (1969).
10. C. L. Hubbs and A. I. Ortenburger, Publ. Univ. Okla. Biol. Surv. 1: 15-43 (1929).