RESISTANCE OF THE PLAINS KILLIFISH *FUNDULUS KANSAE* (CYPRINODONTIDAE) TO COMBINED STRESSES OF TEMPERATURE AND SALINITY

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Transference experiments were used to evaluate the resistance of *Fundulus kansae* to the combined stresses of temperature and salinity. Salinities ranging from 0.5% to 1.5% provided maximum protection against death from heat shock. Higher salinities presumably minimize the survival of the killifish when exposed to heat shock. As temperatures decreased optimum salinities for survival decreased. Young-of-year fish manifested greater resistance to test factors than did adults. The habitat preference of a particular life history-form appeared to correlate with its relative resistance to stresses of temperature and salinity.

The plains killifish, *Fundulus kansae* Garman, occurs from South Dakota and Wyoming south to Texas (1). It is frequently found in large numbers in small rivers and creeks throughout the plains region. During periods of drought and submodal stream discharge, these rivers and creeks become quite shallow, dissolved salt concentration increases and the water becomes quite warm. Water temperatures of 39 C are not uncommon.

The objective of this study was to determine if the plains killfish, which lives within this severe habitat, has evolved high resistance to heat shock, and to evaluate the effects of various salinities upon its survival.

METHODS

Collection of specimens

Specimens of *F. kansae* were collected from three Oklahoma sources: Sand Creek, Marshall County, Elm Fork of the Red River, Greer County, and North Fork of the Red River, Jackson County. Collections were made the latter half of June and the first half of July, 1967. Water depths ranged from 4 in to 3 ft, and water temperatures ranged from 30-39 C. It was observed that greater numbers of young-of-year *F. kansae* were collected in areas of shallow water and high temperatures. No adults were collected in July. The highest salinities were noted in the Elm Fork and North Fork of the Red River.

Laboratory experiments

The fish were transported to the laboratory and were held in 50 gal aquaria. Specimens used in experiments were exposed to 30 C for 3 to 7 days prior to experimentation. No attempt was made to treat fish from the three sources separately. Aquaria were continuously aerated, and a diet of commercially prepared food was fed daily. No attempt was made to control the photoperiod. No deaths were recorded in the acclimation aquaria.

Water from Lake Texoma, an impoundment formed by Denison Dam on the Red River was used. To obtain desired salinities, NaCl was added to this water. Natural salinity present within the lake water was not taken into account.

Fish were transferred directly from acclimation to test temperatures. Shock experiments were performed in 2 gal jars in which water temperatures varied less than ± 0.5 C. Introduced air was used to agitate the water to prevent thermal stratification. A constant watch was maintained on the fish during the first 12 hr of each experiment; subsequently, periodic checks were made. Fish which survived 3 days (4,320 min) were considered to survive indefinitely, in reference to the test-factors. Fish were considered dead when all movement ceased. Time of death, weight, and, when possible, sex were recorded for each fish.

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RESULTS

Reactions of the plains killifish to combined stresses of temperature and salinity are presented in Tables 1, 2, and 3. The average survival time, in minutes, of *F. kansae* at various temperatures and salinities is depicted in Table 1. These data indicate that the pattern of average death time is not consistent, yet a trend is obvious. Resistance to death from heat shock is influenced by the presence of salt in solution. The degree of influence, however, varies with the temperature. Salinity optimum for survival decreased with a decrease in temperature.

The average survival time of young-of-year and adult *F. kansae* exposed to the combined stresses of temperature and salinity was compared (Table 2). Results indicated that the average survival time of young-of-year *F. kansae* was considerably greater than the average lifetime for adults, under similar conditions. As the temperature decreased, the salinity concentration optimum for survival also decreased.

TABLE 1. *Average survival time in minutes for* F. kansae *exposed to various temperatures and salinities. Sample sizes are indicated in parenthesis.*

Temp. (C)	Lake Water	Salinity 0.5%	1.0%	1.5%	2.0%
37	3,578	3,711	1,779	3,459	405
	(15)	(10)	(19)	(10)	(7)
38	1,589	1,629	1,852	1,540	232
	(10)	(10)	(10)	(9)	(9)
39	680	764	952	468	136
	(15)	(10)	(10)	(10)	(9)
40	58	58	113	103	34
	(11)	(10)	(9)	(9)	(9)
41	20	20	17	35	22
	(10)	(10)	(10)	(10)	(10)

TABLE 2. *Average survival time, in minutes, of adult and young-of-year* F. kansae *exposed to various temperatures and salinities. Sample sizes are indicated in parenthesis.*

	Salinity									
	Lake Water		0.5%		1.0%		1.5%		2.0%	
Temp. (C)	Adults	Young-of-year	Adults	Young-of-year	Adults	Young-of-year	Adults	Young-of-year	Adults	Young-of-year
37	2,531	4,320	3,396	3,790	2,445	1,462	2,730	3,641	468	357
	(6)	(9)	(2)	(8)	(6)	(13)	(2)	(8)	(3)	(4)
38	1,338	1,757	540	2,086	1,172	2,306	1,076	1,911	139	244
	(4)	(6)	(3)	(7)	(4)	(6)	(4)	(5)	(1)	(8)
39	199	856	283	969	885	981	633	427	109	145
	(4)	(11)	(3)	(7)	(3)	(7)	(2)	(8)	(2)	(7)
40	47	67	38	71	59	140	—	103	29	37
	(5)	(6)	(4)	(6)	(3)	(6)		(9)	(3)	(6)
41	14	25	5	27	14	18	—	35	28	21
	(4)	(6)	(3)	(7)	(2)	(8)		(10)	(1)	(9)

TABLE 3. *Range of survival times, in minutes, of* F. kansae *exposed to combined stresses of salinity and temperature.*

Temp. (C)	Lake Water Range	Salinity 0.5% Range	1.0% Range	1.5% Range	2.0% Range
37	1,345 - 4,320	1,931 - 4,320	1,205 - 4,320	1,140 - 4,320	221 - 750*
38	128 - 4,320	440 - 4,320	954 - 4,320	1,026 - 4,320	96 - 391*
39	102 - 1,108*	239 - 4,320*	640 - 4,320*	448 - 4,320	93 - 195*
40	38 - 122*	36 - 154*	44 - 241*	57 - 144*	14 - 50*
41	13 - 45*	15 - 62*	11 - 53*	23 - 80*	12 - 32*

* Young-of-year, only

The ranges of survival times, in minutes, of *F. kansae* exposed to combined stresses of temperature and salinity are presented in Table 3. The maximum survival time for killifish exposed to 37 C in combination with lake water was 4,320 min, which, for purposes of this study, was considered to be indefinite. This resistance, also, was observed at 37 C with combinations of 0.5, 1.0, and 1.5% salinities. The maximum survival time of specimens exposed to 37 C and 2% salinity was 750 min. The presence of high salinity was presumably a casual factor in the death of these specimens. Data obtained at 38 C, and various salinity combinations, closely correlated with the pattern observed at 37 C. Resistance was least at 2% salinity. The pattern obtained when killifish were exposed to 39 C and various salinities was considerably different from the previous patterns. The maximum survival time recorded at 39 C in lake water was 1,108 min; however, with combinations of 0.5, 1.0, and 1.5% salinities, survival for some was considered to be indefinite (4,320 min). Resistance at 39 C in 2% salinity dropped precipitously, to 195 min. A temperature shock resulting from direct transfer of fish from a temperature of 30 C to 39 C, in the absence of dissolved salt, was apparently detrimental to the survival of this species. Resistance increased as the salinity concentration increased. However, salinity concentrations of 2% and above when treated as a shock factor, were detrimental to the plains killifish. The ranges of survival time determined for *F. kansae* subjected to 40 and 41 C, in combination with various salinities, were quite comparable. Increased resistance to the stress factors was evident with combinations involving 0.5, 1.0, and 1.5% salinities. As in previous experiments, least resistance was obtained at 2% salinity. Temperatures of 40 - 41 C, when treated as thermal shock apparently exceeded the resistance for this species. Under such conditions, survival time of the plains killifish was only slightly extended by the presence of dissolved salt.

DISCUSSION

These data indicate that salinities ranging from 0.5 - 1.5% provided maximum protection against death from heat shock. Higher salinities presumably minimize the survival of the plains killifish when exposed to heat shock. As the temperature of the water decreases, the optimum level of salinity for survival also decreases. This type of an environment is the assumed habitat of freshwater fish. Investigations by Strawn and Dunn (2) indicated that the presence of some dissolved solids (salt) in the water benefited both freshwater and salt-marsh fishes during exposure to high temperatures. It was also demonstrated that as temperatures decreased optimum salinities for survival decreased for freshwater fishes and increased for salt-march fishes.

Undoubtedly, for any particular acclimation temperature, every species of fish has a temperature range within which resistance for an indefinite period of time is possible. This range has an upper lethal temperature, above which the animal cannot survive indefinitely but may live for some limited time. This time is dependent upon many factors including the physical state and condition of the fish. Loeb and Wasteneys (3), experimenting with cyprinodontiform fishes, found that fish not previously acclimated to the test factors died within 4 min when exposed to distilled water at 35 C. Fish maintained for 30 hr or more at 27 C were, however, more or less tolerant to 35 C. Hathaway (4) found that within several species of centrachids, different age groups appeared to have approximately the same temperature limits, and that a change in the temperature survived could be manifested by experimental acclimation of each species. Other investigators (5-7) express the importance of acclimation to thermal resistance. Specimens used in this study were not acclimated to various test temperatures or salinities prior to the transference experiments. By experimentally acclimating killifish to various salinities and temperatures, the optimum salinity or temperature required to maximize expected survival time at any given salinity or temperature could be determined.

Fishes undoubtedly acclimate to temperatures under natural conditions. Brett (8) showed that seasonal variations in the thermal tolerance of the brown bullhead, *Ictalurus nebulosus*, corresponded to concomittant variations in the average temperature of Lake Apeongo, Ontario. It seems reasonable to assume that *F. kansae* may also express variable thermal resistance with seasonal variations of water temperatures through natural acclimatization. The killifish may, however, avoid heat shock in other ways. Avoidance by the killifish of shallow waters, where temperatures are higher, seems reasonable. In nature, it was observed that young-of-year *F. kansae* were present in shallow waters in greater numbers than were adult killifish. Experimentally, young-of-year specimens manifested greater resistance to the combined stresses of temperature and salinity than did adults. Undoubtedly, the absence of adult killifish from shallow waters involves several intrinsic and extreme factors; however, decreased resistance of adults to high temperatures may also be involved. Moreover, this phenomenon may be related to the thermal history of the two life history-forms. Spawning and subsequent appearance of young-of-year killifish within streams occurs during late spring and early summer conditions. Young-of-year killifish would have been exposed, primarily and, hence, preadapted, through natural acclimatization to warm water temperatures. Conversely, sub-adults and adults are exposed, during winter conditions, to extremely low temperatures and, presumably, acclimatize to such conditions. These forms, once acclimatized to exceedingly low temperatures, may require more time to acclimatize sufficiently to high temperatures. Brett (8) found that the fathead minnow, *Pimephales promelas*, required over 20 days to become completely acclimated to 16 C after previously living at 24 C, but became acclimated to 28 C from 20 C within 24 hr. However, this process may proceed at different rates in different species. Also, the experimental results were not obtained with temperatures corresponding to the extremes found in nature. A second way to survive high temperatures is to remain in deeper water where thermal stratification offers protection from lethal temperatures (2). Where both young and adult forms of *F. kansae* are present, the adults are invariably found in the deeper pools. A behavior of *F. kansae* is almost complete submersion under the sand, debris or vegetation. During summer conditions, this behavior is most common to adults. It is conceivable that by this behavior the plains killifish receive protection from exposure to high temperatures. In these areas, circulation of the water would be limited and thermal stratification undoubtedly occurs, even in relatively shallow water.

It seems reasonable to assume that the resistance of *F. kansae*, determined experimentally, to the combined stresses of temperature and salinity may differ considerably from the temperature and salinity zones optimum for survival, growth and reproduction of the species in nature. There is evidence to indicate that temperature not sufficiently high to cause death may, over an extended period of time, have adverse effects upon a fish. Investigation by Brett (9) indicate that sockeye salmon may survive in temperatures well above the zones for adequate growth and reproduction. Thus, temperatures and salinities which effect the life of the plains killifish may well be considerably lower than the lethal conditions determined experimentally in this study.

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