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Photoperiodic Effects on Salinity Selection in the Gulf Killifish, *Fundulus grandis*

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tern. It thus appears that ictalurids are characterized by low bile K^+ relative to the plasma.

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PHOTOPERIODIC EFFECTS ON SALINITY SELECTION IN THE GULF KILLIFISH, *FUNDULUS GRANDIS*.—Many estuarine fishes make both seasonal and daily movements through waters of differing salinities (Gunter, 1967; Blancher, 1974). With the exception of spawning migrations, these movements generally have been considered to be responses to exogenous cues such as temperature (Gunter, 1967) or salinity changes (Perez, 1969), and tidal flow (Butner and Brattstrom, 1960). The influence of changes in the length of the daily photoperiod on estuarine movements apparently has received little attention. In contrast, spawning migrations into either more or less saline waters have been correlated with changes in the daily photoperiod (Baggerman, 1957). Spawning migrations also have been associated with changes in titers of several endogenous hormones including corticosteroids (Heyl and Carpenter, 1972) and prolactin (Lam, 1972; McKeown and Overbeeke, 1972). These two hormones have been implicated repeatedly in osmoregulation in a variety of fishes (Johnson, 1973).

In addition to seasonal variations, circulating levels of cortisol (Garcia and Meier, 1973) and prolactin (Leatherland and McKeown, 1973; Spieler, 1975) undergo circadian variations. Circadian changes in hormone concentrations are greater than mean seasonal changes in the mullet, *Mugil cephalus* (Spieler, 1975). Thus, if the blood concentrations of cortisol and/or prolactin directly modulate salinity preferences, it should be possible to demonstrate circadian variations in salinity preference. Furthermore, changes in the daily photoperiod that cause changes in the circadian rhythm of plasma prolactin in *Fundulus grandis* (Spieler, 1975) might also be expected to induce similar changes in the daily pattern of salinity preference.

The present study was carried out with a brackish water killifish, *Fundulus grandis*. Salinity preferences were ascertained throughout a day in order to determine whether there were circadian changes. Salinity preferences were also examined in fish maintained on daily photoperiods of differing lengths in order to explore a possible photoperiodic role in seasonal preferences.

Gulf killifish (*Fundulus grandis*) of mixed sexes with regressed gonads and averaging 6 cm in length were purchased in August from bait dealers who collected them from brackish water near Grand Isle, Louisiana. The fish

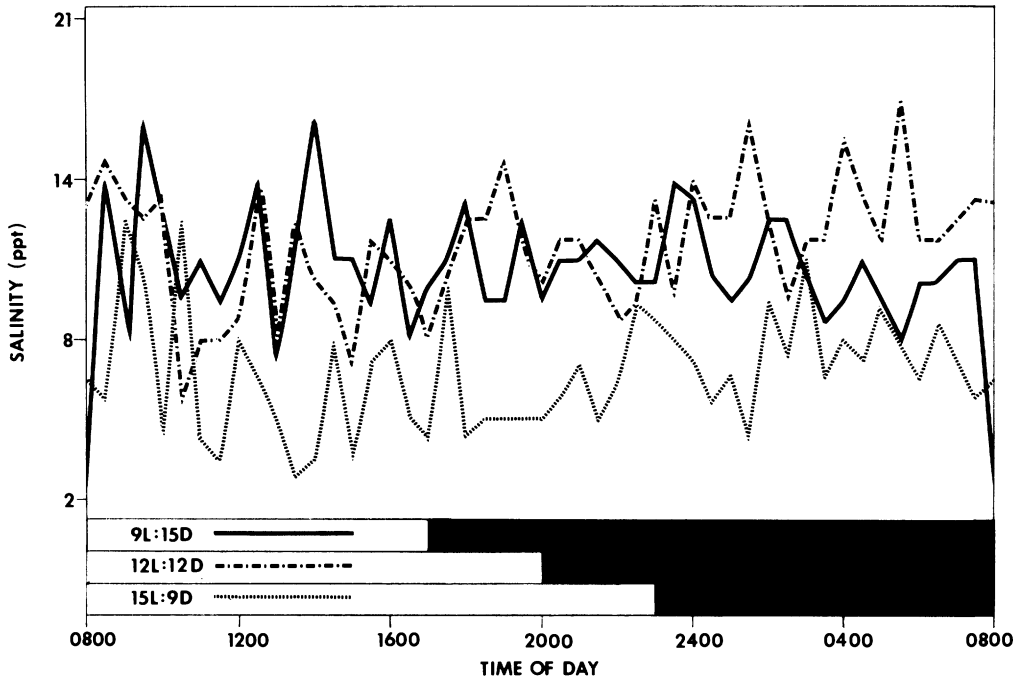


Fig. 1. Salinity selection of *Fundulus grandis* acclimated to one of the three different daily photoperiods. Eight fish per photoperiod were observed individually at half hour intervals throughout a day, beginning at the onset of light.

were acclimated in 80-liter aquaria for a minimum of three weeks to three different photoperiodic regimens (9L:15D, 12L:12D, 15L:9D). The photoperiods were selected to cover the maximum seasonal difference that Gulf killifish might experience, the 9L:15D photoperiod offering even less light than the normal minimum (10 hr) at this latitude (30° N). There were 8 fish in each group. To maintain consistent water quality, salinity (2‰) and temperature (21 ± 2 C) for the three groups, all aquaria were attached to a common biological filter. The fish were fed daily a commercial flake food (TetraMin) except during the day of testing. A single fish was placed, at the beginning of the light period, in a salinity gradient chamber (modified Staalnd device, Staalnd, 1969; Spieler, 1975). The fish were placed in the chamber at the salinity concentration to which they had been acclimated (2‰). The gradient contained six different salinities (2, 8, 14, 21, 28, 35‰) along a horizontal axis through which the fish were free to move. The position of the fish was monitored by photographs taken every 30 min throughout a day. The gradient was maintained at the same light-

dark cycle as that to which the fish were acclimated. Dim light (less than 2 lumens/cm²) was substituted for total darkness to permit photography. The readings of less than 2 lu/cm² were taken directly in front of the chamber, the actual reading in the chamber should have been less, probably about full moonlight (.5 lu/cm²). Fish from different photoperiod regimens were tested alternately so that every series of three tests had a fish from each of the three photoperiods. Statistical analyses of the data were made using an analysis of variance (ANOVA), Duncan's New Multiple Range, and the Student's *t* test.

No circadian rhythms of salinity preference were found in fish maintained on any one of the three different photoperiodic schedules (Fig. 1). Apparently circadian rhythms of hormones such as cortisol and prolactin do not exert a direct influence on salinity preference in *Fundulus grandis* under the conditions of this experiment.

Although circadian variations were not observed, there were distinct differences in salinity preference of fish acclimated to different photoperiodic schedules (Fig. 1). A significant in-

teraction ($P < .05$ ANOVA) existed among the salinity selections of the three groups (21 of 48 observations intervals had differences, $P < .05$: Duncan's New Multiple Range). The fish maintained on a 15-hr daily photoperiod selected less saline waters ($6.7 \pm 1.5\%$) than those kept on daily photoperiods of 9 or 12 hours ($11.3 \pm 1.3\%$) ($P < .05$: Student's t).

Although many estuarine fishes move into less saline waters during spring and summer when the daily photoperiod is relatively long, this movement generally has been attributed to increasing temperature (Gunter, 1967). In the Louisiana estuaries near Grand Isle where the *Fundulus grandis* were acquired, most fish have moved, or are moving, into fresher water in April when the water temperature approximates 21 C (Day et al., 1973), the holding temperature for our experimental fish. Our results suggest that temperature alone may not account fully for movements of fishes into water of lower salinities, and that an increasing daily photoperiod can have a significant role in determining changes in the salinity preference of some fishes. This conclusion is consistent with Baggerman's (1957), who found that higher temperatures changed salinity preference of three-spined sticklebacks, *Gasterosteus aculeatus*, only at specific times of year. Baggerman concluded that the physiological condition of the fish determined whether or not they responded to the rise of temperature. Presumably, changes in physiological conditions may be accounted for by seasonal changes in the photoperiod.

In conclusion, our results indicate that salinity preference is not a direct reflection of circulating levels of a specific hormone but is probably a more complex neural and/or endocrine phenomenon. This system is influenced by changes in the daily photoperiod, suggesting that some seasonal movements in water of differing salinities are attributable in part to changes in day-length. As McHugh (1967) cautioned previously, it is dangerous to draw causal relations from simultaneous natural events (i.e., fish migrations and temperature changes). Experimental evidence is an indispensable ingredient of scientific inquiry.

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DIURNAL MEASUREMENTS OF PLASMA CHLORIDE IN CATFISH.—Plasma chloride concentration is thought to be held relatively stable in vertebrates in spite of well documented diurnal changes in their excretion rates (Mills, 1951). A diurnal rhythm of plasma chloride, and a seasonal shift in the phase of this rhythm has been suggested for *Fundulus* (Meier et al., 1973). This paper presents diurnal plasma chloride concentrations from catfish under a variety of conditions and during several seasons to extend the sparse information on diurnal plasma chloride regulation in teleosts.

All fish used in these experiments were supplied by the Fish Farming Experimental Station, Stuttgart, Arkansas. Samples were taken from actively feeding fish, but food was withheld 24 hr prior to the beginning of each experiment. Blood was obtained from unanesthetized animals by puncture of the caudal vessel. Each fish was bled only one time and

lithium heparin was used as an anticoagulant. Plasma chloride levels were determined from a 50 μ l sample with a Bucheler-Cotlove automatic titrator.

Channel catfish (*Ictalurus punctatus*) or blue-channel catfish hybrids (*I. furcatus* \times *I. punctatus*) were held in flow-through raceways, recirculating systems or open ponds. Water was provided either from a well or from a large outdoor reservoir.

Blood was taken from at least six fish every six hr and continued for 24 hr. The initial sample time was repeated the following day and the sequence of bleeding each fish was recorded to detect any change due to disturbance of the fish during the experiment. Fish sampled at densities from 22 to 220 fish/M³ were compared to detect any effect due to crowding. Diurnal measurements were made in January, February, May, October and November. In one study (November) fish were removed from cages in a lake and transferred to a large indoor recirculating system. Diurnal sampling was begun 24 hr after transfer. Groups of fish were always held under conditions of natural day length.

Diurnal variation of plasma chloride was not found during any season nor under any of the environmental conditions examined (Table 1). The daily mean (mean of all daily values) in May was lower than that during any other season; however, due to the different environ-

TABLE 1. DIURNAL PLASMA CHLORIDE CONCENTRATIONS OF CHANNEL CATFISH AND BLUE \times CHANNEL CATFISH HYBRIDS DURING SEVERAL MONTHS AND UNDER VARIOUS ENVIRONMENTAL CONDITIONS. Values represent the mean of at least six fish in meq/l \pm SE.

	Sunrise	S + 6	S + 12	S + 18	Daily Mean
29 Jan	Hybrids in indoor raceways with heated recirculated water ($Cl^- = 0.42$ meq/l).				
146 fish/m ³	119 \pm 0.7	119 \pm 0.3	117 \pm 1.6	117 \pm 1.0	119 \pm 0.5
22 fish/m ³	123 \pm 1.3	120 \pm 1.0	122 \pm 1.3	123 \pm 0.7	122 \pm 0.6
26 Feb	Channel catfish in indoor raceways ($Cl^- = 0.42$ meq/l).				
220 fish/m ³	128 \pm 3.3	126 \pm 5.8	123 \pm 4.1	132 \pm 3.6	127 \pm 2.2
55 fish/m ³	133 \pm 5.6	130 \pm 1.5	132 \pm 2.4	130 \pm 2.3	131 \pm 1.6
18 May	Hybrids in outdoor tanks supplied with well water ($Cl^- = 0.42$ meq/l).				
	113 \pm 2.0	115 \pm 1.6	112 \pm 1.5	115 \pm 2.3	114 \pm 1.0
23 Oct	Channel catfish in cages in 0.1 hectare outdoor pond ($Cl^- = 0.3$ meq/l).				
	119 \pm 0.9	117 \pm 6.3	125 \pm 1.0	126 \pm 1.2	122 \pm 1.4
16 Nov	Channel catfish in indoor recirculating system ($Cl^- = 0.28$ meq/l). Sampling begun 24 hr after transfer from cages in a lake.				
	119 \pm 1.2	123 \pm 0.6	118 \pm 0.7	120 \pm 2.2	120 \pm 0.7