Hematocrit, osmolality, and ion concentration in fishes: consideration of circadian patterns in the experimental design

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Abstract: Significant circadian patterns in plasma osmolality and chloride ion concentration were documented in the sailfin molly *Poecilia latipinna* (Baird & Girard) and the common snook *Centropomus undecimalis* Bloch; however, significant patterns were not found in the sheepshead minnow *Cyprinodon variegatus* Lacepede. There were, however, no distinct patterns in hematocrit for any of the three species. Circadian patterns, although important in ion and osmoregulation studies are usually not considered. Furthermore, the time of day the fishes are tested are typically not mentioned. Questionable results and difficulty in making comparisons with previously published data can occur if the patterns are present and not considered in the experimental design.

Key words: Chloride; Circadian; Euryhaline; Hematocrit; Osmolality

INTRODUCTION

Ion and osmoregulation studies of fishes have typically been designed to search for patterns which aid in understanding habitat use or compare responses in different environmental situations. These data, in conjunction with oxygen consumption data, can lead to an understanding of the energetics of habitat use patterns.

The majority of these studies, however, usually do not state the time of day their determinations were made and thus overlook the importance of circadian cycles in their experimental design (e.g., Valentine & Miller, 1969; Barton, 1979; Nordlie, 1985, 1987). Furthermore, comparison of their data with other published data from confamilial species may yield distinct differences or similarities based solely on the time of day the samples were taken.

Studies on diurnal and circadian cycles or rhythms in osmolality, ion or hematocrit concentrations are few. To date, studies document significantly elevated values in sodium, hematocrit (Hannah & Pickford, 1981) and osmolality (Bulger, 1986) between 1400–1800 in *Fundulus heteroclitus*. Moreover, daily chloride cycles for *F. grandis* and

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F. chrysotus have also been documented (Meier *et al.*, 1973; Spence *et al.*, 1977) but these cycles do not occur for the freshwater channel catfish *Ictalurus punctatus* and *I. punctatus* × *I. furcatus* hybrids (Davis & Simco, 1976).

The purpose of this paper is two-fold: (1) to determine if there are circadian patterns in hematocrit, osmolality and chloride concentrations in three euryhaline fishes: the sheepshead minnow *Cyprinodon variegatus* Lacepede; the sailfin molly *Poecilia latipinna* (Baird *et* Girard); and the common snook *Centropomus undecimalis* (Bloch); and (2) to discuss the importance of these patterns in documenting secondary stress responses to experimental manipulation. We do not purport to determine the functional importance of these patterns (see Bulger, 1986) but wish to establish their importance when designing experiments to examine these parameters in different treatments or when comparing the values with other published data.

Materials and Methods

We collected fishes from impounded mangrove marshes in the Indian River Lagoon, Florida, U.S.A., and tested them between 7 December 1987 and 21 January 1988. Fishes were transported to the laboratory in styrofoam coolers containing impoundment water where they were held in their ambient environmental temperatures (19–24 °C) overnight under aeration. The laboratory temperature was 25 ± 2 °C and the holding water always increased to that temperature. The fish were then transferred to 76-l aquaria (at 25 ± 2 °C) equipped with individual filters, aerators and heaters. The fish were held in 30 ± 1‰, 30 ± 2 °C (increased from 25 °C over a 24-h period) and under a 12-L : 12-D photoperiod centered at 1230, for 7 to 14 days prior to experimentation (normal day length during the testing periods ranged from 1029 to 1037). Light intensity at the waters surface was 0.65×10^{16} quanta $\cdot s^{-1} \cdot cm^{-2}$. *Cyprinodon varigetus* and *P. latipinna* were fed flaked food twice daily, whereas *C. undecimalis* were fed live fish once daily. The sex of the fishes was not considered. Experimental salinities were produced using filtered (5 μm) Atlantic Ocean seawater diluted with aged reverse osmosis (RO) water. Salinities were checked daily using an AO refractometer.

All fish were fasted for 24 h prior to testing. Ten individuals were tested at 4-h intervals from 0400 through 2400. Fish tested at night (2000, 2400, 0400) remained in darkness until they were netted and tested under dim light (intensity at water surface $= 0.01 \times 10^{16}$ quanta $\cdot s^{-1} \cdot cm^{-2}$). Blood of all individuals was collected and centrifuged within a 30-min period, centered at one of the previously mentioned test times. Fish were netted (see results concerning potential effects of netting) and immediately measured to the nearest mm standard length (SL). Blood samples were obtained by first blotting each individual dry and severing its caudal fin with a razor (Barton, 1979). The incision was immediately blotted and blood from the caudal artery was drawn into a heparinized micro-capillary tube and centrifuged for 4 min at 11500 rpm in an International micro-capillary centrifuge (Model MB) for hematocrit (%) determination. Individual blood collections were completed within one minute to reduce handling

effects on blood constituents (Chauvin & Young, 1970; Robertson *et al.*, 1987). Plasma osmolality (mmol · kg^{-1}) was then determined on a 10-μl sample with a Wescor vapor pressure osmometer (Model 5500). Chloride ion concentration (meq · l^{-1}) was determined from a 10-μl sample on a Buchler digital chloridometer (Model 4–2500). A total of 60 individual determinations per blood constituent and species (10 per time period) were made. All blood constituents were determined for each individual.

Fish size, hematocrit, osmolality and chloride ion concentration were analyzed by ANOVA ($\alpha = 0.05$) and a Student–Newman–Keuls multiple comparison test (SNK) (Klockars & Sax, 1986). These analyses were performed using the SPSSX 2.1 program package (SPSSX, 1985).

Results

Individuals ranged between 24–41 mm SL for *C. variegatus*; between 32–56 mm SL for *P. latipinna*; and 54–120 mm SL for *C. undecimalis*. There were no significant differences in size for either *P. latipinna* or *C. undecimalis* (ANOVA; $P > 0.05$; Table I); however, the 2000 mean ($\bar{x}$ = 37.0 mm SL) was marginally larger (ANOVA; $P = 0.052$) than the 0800 mean ($\bar{x}$ = 33.2 mm SL) for *C. variegatus*. There were no significant differences (ANOVA; $P > 0.05$) in hematocrits across all time periods for all three species (Table I).

Significant circadian patterns in osmolality and chloride ion were found for *P. latipinna* and *C. undecimalis* (Table I), whereas only Cl^- showed a significant elevation for *C. variegatus* (Table I). Osmolality during the 1200 and 1600 time period was significantly elevated for *C. undecimalis* (Table I; SNK, $P < 0.05$). Values ranged from 327–407 mmol · kg^{-1}. Chloride, however, was significantly elevated only during the 1200 period and ranged from 112–191 meq · l^{-1} (Table I; SNK, $P < 0.05$). For *P. latipinna*, the 1200 osmolality value was significantly higher than the 0400 and 0800 time periods whereas the 1600 value was higher than the 0400 value (SNK; $P < 0.05$) and ranged from 301–401 mmol · kg^{-1}. The 0400 chloride value, however, was significantly lower than all other time periods except 1600 (SNK; $P < 0.05$). The chloride values ranged from 106–134 meq · l^{-1}. There were no significant differences (ANOVA; $P > 0.05$) in osmolality across all time periods for *C. variegatus*; however, the 0800 and 2000 chloride values were significantly lower than the 2400 value (Table I). Osmolality values ranged from 312 to 368 mmol · kg^{-1} whereas Cl^- values ranged from 109 to 145 meq · l^{-1}. At all time periods for all species, hematocrit, osmolality, and chloride values among the 10 individuals sampled showed no effect of sampling order and, therefore, no indication of capture stress effects.

Discussion

We documented distinct circadian patterns in osmolality in *P. latipinna* and *C. undecimalis* but not in *C. variegatus*. Significant chloride concentrations were docu-

TABLE I

Statistics for parameters examined at one of six times of day ($\bar{x} \pm$ SD). Each mean value is based upon 10 individuals. Entries within a parameter having a common superscript or no superscript are not different ($P > 0.05$).

Time of day (h)	Size (mm SL)	Hematocrit (%)	Osmolality ($mmol \cdot kg^{-1}$)	Chloride ($meq \cdot l^{-1}$)
Cyprinodon variegatus				
0400	$35.0 \pm 1.6^{1,2}$	24.9 ± 7.3	333.7 ± 12.1	$125.6 \pm 4.6^{1,2}$
0800	33.2 ± 4.5^{1}	28.7 ± 3.4	340.4 ± 9.5	119.3 ± 4.7^{1}
1200	$36.0 \pm 2.0^{1,2}$	23.3 ± 4.2	337.5 ± 8.8	$125.6 \pm 3.9^{1,2}$
1600	$35.3 \pm 2.3^{1,2}$	24.3 ± 5.2	345.6 ± 16.3	$122.6 \pm 3.9^{1,2}$
2000	37.0 ± 1.5^{2}	27.2 ± 3.5	344.3 ± 5.5	118.0 ± 7.9^{1}
2400	$35.5 \pm 2.3^{1,2}$	28.3 ± 4.0	340.5 ± 13.1	127.6 ± 8.7^{2}
Poecilia latipinna				
0400	41.8 ± 7.5	26.9 ± 3.8	316.8 ± 4.1^{1}	116.1 ± 5.9^{1}
0800	43.8 ± 5.2	24.7 ± 4.4	$329.0 \pm 4.1^{1,2}$	123.1 ± 2.3^{2}
1200	42.1 ± 7.1	30.2 ± 5.0	350.9 ± 18.8^{3}	124.0 ± 3.2^{2}
1600	43.4 ± 6.5	28.5 ± 9.6	$341.6 \pm 30.4^{2,3}$	$120.5 \pm 6.1^{1,2}$
2000	44.2 ± 6.9	27.4 ± 4.6	$335.3 \pm 5.8^{1,2,3}$	124.0 ± 6.5^{2}
2400	43.9 ± 5.8	23.6 ± 3.8	$332.8 \pm 11.3^{1,2,3}$	123.8 ± 5.7^{2}
Centropomus undecimalis				
0400	75.3 ± 8.3	32.1 ± 3.5	342.2 ± 6.9^{1}	121.6 ± 5.1^{1}
0800	83.1 ± 15.9	28.6 ± 3.0	346.5 ± 9.6^{1}	135.5 ± 7.1^{1}
1200	81.1 ± 15.9	32.9 ± 2.9	382.1 ± 15.9^{3}	154.7 ± 22.7^{2}
1600	81.7 ± 24.2	30.0 ± 3.8	366.9 ± 6.5^{2}	130.7 ± 8.8^{1}
2000	74.4 ± 3.1	32.5 ± 5.8	337.8 ± 8.9^{1}	126.0 ± 5.4^{1}
2400	72.2 ± 5.4	33.1 ± 6.4	342.2 ± 8.6^{1}	124.3 ± 7.1^{1}

mented for all three species. Hematocrit, however, was not significantly different for any species. Although there were variations within the 10 individuals sampled for each time period, there was never a trend indicating secondary stress responses resulting from the 30-min period of sampling in which aquarium captures were made. The range in chloride and osmolality observed in each of these species comprised a major portion of the values reported for other euryhaline fishes (Holmes & Donaldson, 1969). To date, similar chloride and osmolality diel patterns have been documented in some euryaline cyprinodontids (Meier *et al.*, 1973; Spence *et al.*, 1977; Bulger, 1986). Furthermore, Hannah & Pickford (1981) also documented significantly elevated hematocrits and sodium at 1600 in *F. heteroclitus* but no patterns were documented for chloride or potassium. The sodium cycle in *F. heteroclitus* seems to be in phase with the elevated values of osmolality documented by Bulger (1986) for the same species. Additionally, Ikeda *et al.* (1976) detected diurnal peaks in chloride in the yellow-tail *Seriola quinqueradiata* which varied depending upon the type of food fed to the fish. Leatherland *et al.* (1974), however, failed to detect a significant pattern in hematocrit for juvenile kokanee salmon *Oncorhynchus nerka*. All of these studies suggest that there are species specific differences in diel patterns in osmolality, ion concentration and hematocrit. However, the apparent wide variation in diel or diurnal study responses within congeneric species (see Meier *et al.*, 1973, Figs. 1, 2) simply may be due to juxtoposition of the time axis.

It is interesting to note that all of the species previously studied are euryhaline and inhabit estuarine habitats which present complex ambient physio-environmental conditions to the fish. Freshwater channel catfish *Ictalurus punctatus* and the hybrid *I. punctatus* × *I. furcatus* that occur in less fluctuating habitats apparently do not have chloride cycles (Davis & Simco, 1976). This suggests that the phenomena of circadian patterns in osmolality and at least chloride ion concentration may be most distinct in euryhaline fishes that inhabit biotopes that fluctuate in temperature (see Bulger, 1986, for lengthy discussion).

Of major importance in ion and osmoregulation studies is the time of day the blood samples were obtained, yet few studies indicate this important point. Comparison of treatment data within a study may show a significant effect simply due to differences in the time of day samples were collected. Additionally, comparisons of data among studies almost never consider differences in time of collection, making such comparisons suspect. Future studies of this nature should take into account the time of day samples will be collected of if patterns have already been established, collections should be made in light of this published data. Finally, we feel that future studies should state the time of day their animals were tested so as to facilitate easy comparison of data sets.

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