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Base and stressed ventilation rates for *Leiostomus xanthurus* Lacépède and *Morone americana* (Gmelin) exposed to strobe lights

By D. R. Sager[1], C. H. Hocutt[2] and J. R. Stauffer Jr[3]

[1] *Texas Parks and Wildlife Department, Austin, TX, USA,* [2] *Henson School of Science and Technology, Salisbury State University, Salisbury, MD, USA,* [3] *School of Forestry Resources, Pennsylvania State University, University Park, PA, USA*

Summary

A biomonitoring system interfaced with a microcomputer was used to monitor ventilation rates for white perch (*Morone americana*) and spot (*Leiostomus xanthurus*) under baseline and stressed conditions caused by strobe lights. Tests were conducted on light- and dark-acclimated specimens. These two estuarine species have been found to exhibit avoidance behavior to strobe lights. Potential accommodation to the strobe light stimulus was explored over a 24 h period. The biomonitoring system successfully recorded ventilation rates under baseline and stressed conditions. Baseline mean ventilation rates for 0.5 h intervals ranged from 1 count per minute (cpm) to 97 cpm for light-acclimated white perch with an overall mean for 24 h ($\bar{x}$) of 41 cpm. Mean stressed rates ranged from 1 to 100 cpm with an overall mean of 44 cpm. Baseline rates for dark-acclimated white perch ranged from 1 to 79 cpm ($\bar{x} = 35$ cpm), with stressed rates from 2 to 83 cpm ($\bar{x} = 30$ cpm). Light-acclimated spot had baseline ventilation rates ranging from 3 to 146 cpm ($\bar{x} = 42$ cpm), while stressed rates ranged from 2 to 134 cpm ($\bar{x} = 36$ cpm). Mean baseline rates for dark-acclimated spot ranged from 1 to 94 cpm ($\bar{x} = 40$ cpm), and stressed rates ranged from 1 to 72 cpm ($\bar{x} = 25$ cpm). The difference in ventilation rates between base and stressed conditions (as absolute values) for light-acclimated white perch over the 24 h experiments ranged from 0 to 43 cpm ($\bar{x} = 11.01$ cpm). Dark-acclimated white perch had differences ranging from 0 to 78 cpm ($\bar{x} = 11.13$ cpm). Light-acclimated spot had differences ranging from 0 to 101 cpm ($\bar{x} = 14.68$ cpm). Dark-acclimated spot had differences ranging from 0 to 70 cpm ($\bar{x} = 20.56$ cpm). Ventilation rates varied between species and among individuals within a species. Ventilation rates were generally lower for dark-acclimated specimens. For both species under all conditions, the base and stressed rates were significantly ($P < 0.05$) different during the 24 h period. However, dark-acclimated specimens exhibited a more distinct difference than light-acclimated specimens. The lack of accommodation to strobe light and a stronger reaction under dark conditions indicate that strobe lights continue to offer potential as behavioral guidance systems for these species.

Introduction

Gill ventilation rates have long been used to measure the stress fish experience or a reaction to a stimulus (Cairns et al. 1970, 1982; Spoor et al. 1971; Heath 1972; Szyper and Lutensky 1991; Wingard and Swanson 1992; Knoph 1996). Ventilation rates have also been used in studying physiological reactions to stimuli concerning such issues as oxygen consumption (Steffensen et al. 1984; Vanrooij and Videler 1996), temperature and salinity variations (Szyper and Lutensky 1991) and natural poisons (Zimmerman and Heatwole 1992).

Methods for this practice have progressed from direct observation of fish to the use of sophisticated electronic equipment to monitor electromyograms (EMG) generated from the electrical activity of opercular and buccal muscles during ventilation (Spoor et al. 1971; Heath 1972; Cairns et al. 1980; Capute 1980). With advances in electronics, the use of submerged electrodes, instead of electrodes attached to the fish to monitor EMG, electronic systems became accurate and automated through the use of better amplifiers (Gruber et al. 1977) and on-line computer systems (Cairns et al. 1980; Cairns and Gruber 1980) that do not need constant observation or supervision. Using an electrode chamber, Gruber et al. (1979) found that a bimodal signal was generated by the ventilation activity of freshwater fish (bluegill, *Lepomis macrochirus* Rafinesque; fathead minnow, *Pimephales promelas* Rafinesque), but the signals varied in waveform when the fish were under stress.

Such biomonitoring units offer advantages for experiments over long time periods. The use of a biomonitoring system is more cost-effective than constructing a large experimental system in the laboratory or environment, for conducting long-term experiments. A biomonitoring system can isolate the stimulus to be studied more easily than in a larger experimental apparatus or with an observer present. Using individual specimens in a biomonitoring unit also allows direct comparison of individual reactions to a stimulus. Biomonitoring systems have been used to document changes in gill ventilation rates by fish subjected to sublethal contaminant concentrations in water (Cairns et al. 1974, 1982; Capute 1980; Gruber et al. 1980; Wingard and Swanson 1992; Knoph 1996). Biomonitoring systems can be used for investigating alterations in fish ventilation rates from many chemical and other stimuli (Szyper and Lutensky 1991; Zimmer and Heatwole 1992). At present, few estuarine fish species have been used in such experimental systems (Cairns et al. 1980) and little information exists on their ventilation rates or reactions in such systems.

The use of behavioral guidance systems in fish management programs has received increased attention. Investigators have used behavioral systems to increase fish catches or to guide fish away from areas of concern. Behavioral guidance systems have been used to reduce fish impingement at water intake structures (Hocutt 1980; Hocutt and Edinger 1980; Sullivan 1997). Light is one of the most common behavioral altering stimuli used. Light intensities may alter behavioral actions by fish (Whitney 1969; Pavlov et al. 1972; Kwain and McCauley 1978; Rooney and Laming 1986). Lights have been used to increase commercial fish catches (Solov'ev 1971; Zilnov 1971; Yami 1976)

or to direct fish movements in open waters (Wickham 1973). Recent research on the use of strobe lights in behavioral guidance systems appears promising for freshwater and estuarine fishes (Patrick et al. 1985; Sager et al. 1987; Sullivan 1997). Strobe light systems elicit avoidance reactions by fish. Spot, *Leiostomus xanthurus* Lacépède, and white perch, *Morone americana* (Gmelin), have been found to react to strobe light guidance systems in hour long experiments (Patrick et al. 1985; Sager et al. 1987). For a guidance system based on avoidance reactions to be successful however, the reactions must be sustained over longer periods of time. If fish to be guided accommodate to the stimulus prior to reaching the desired location, the system is ineffective. Biomonitoring systems, as described above, may allow sensitive, cost-effective experimental systems on fish's long-term accommodation to behavioral stimuli.

This study examined the ventilation rates of two estuarine fish species at rest and subjected to stress by a strobe light stimulus. The two species used, *L. xanthurus* and *M. americana*, have been found to avoid strobe light stimuli (Patrick et al. 1985; Sager et al. 1987). This study determined the suitability of the biomonitoring system for estuarine fish species, the ventilation rates of the two species tested at rest and under stress, and whether the two species accommodated to the strobe lights over time.

Materials and methods

White perch and spot were collected from the Choptank River on the eastern shore of the Chesapeake Bay, USA. Specimens ranged in standard length from 85 to 180 mm for white perch and 73 to 163 mm for spot.

Experiments were conducted by monitoring changes in gill ventilation rates (breathing rates) of fish subjected to xenon strobe lights (300 flashes/min) or unexposed fish. Electrical impulses generated by gill ventilation were received via top and bottom submerged stainless steel electrodes in test aquaria in which a fish was held (Fig. 1). Signals (EMG) were amplified using an amplifier modified from Gruber et al. (1977), recorded on a Honeywell chart recorder, and interfaced (via a Starbuck analog–digital converter) with an IBM-compatible microcomputer. Ventilation rates were recorded every other minute. These data were stored on microcomputer disks for later analysis and a hard copy of the data was printed for QA/QC protocols. The experimental system was modeled after systems in use as biomonitoring stations (Cairns and Gruber 1980; Cairns et al. 1980), but was modified to use top- and bottom-mounted electrodes instead of electrodes mounted at aquaria ends. The new electrode arrangement was to eliminate the problem of signal polarity changing due to the orientation of the fish, thus making the system more accurate (Capute 1980). The amplifier was also modified to allow altering of the sensitivity of the system so that the best signal strength to count the bimodal EMG signal for each ventilation sequence could be obtained. This allowed adjustments for specimen and/or species differences in EMG strength (amplitude). The test aquaria were sized to allow specimens to turn around thus restricting swimming but allowing enough room to maintain the specimen for extended experiments with limited stress. As a result, the base ventilation rates reflect at rest conditions but not basal metabolic conditions.

The biomonitoring system allows accurate monitoring of ventilation rates without the presence of an observer or devices directly attached to the fish. Using direct observers relies on the various abilities of the observer (and fatigue) and introduces a factor that may alter the ventilation patterns by the presence of the observer. Electrodes attached to the fish alter the fish's behavior and ventilation rates due to stress and physical differences because of the attached instrument. The biomonitoring system may not capture all minor ventilation events if a specimen becomes more quiescent and the EMG signal amplitude decreases below that captured by the initial settings of the system. While the method may not identify all minor ventilation events when the fish are at rest and barely move their opercular and bucchal muscles, it is more accurate than most methods. This limitation may result in breathing rate estimates slightly lower than actual for some individuals as low rates may be slight underestimates. However, this methodology is the most accurate and cost-effective for long-term experiments. Statistical methods have been adopted to minimize the possibility of this limitation affecting data analyses (Gruber and Cairns 1981).

Specimens were tested under constant light conditions to

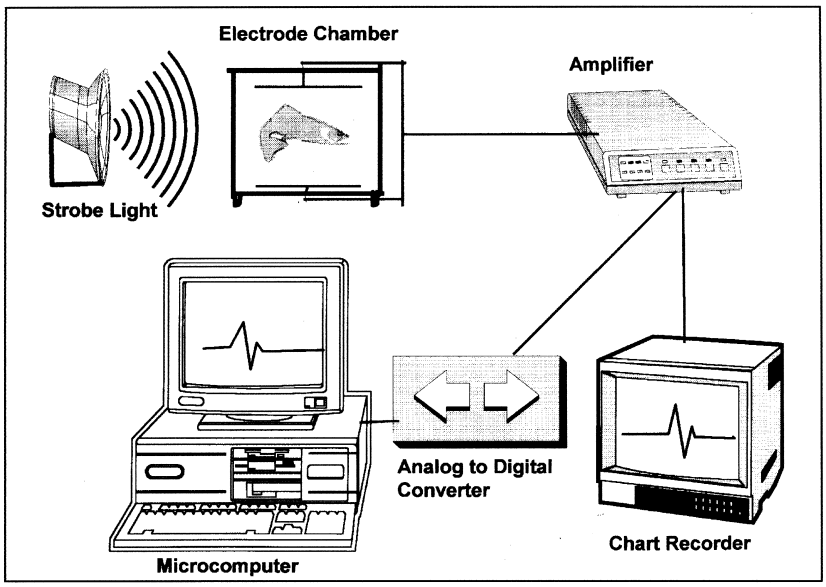

Fig. 1. Schematic of the biomonitoring system components

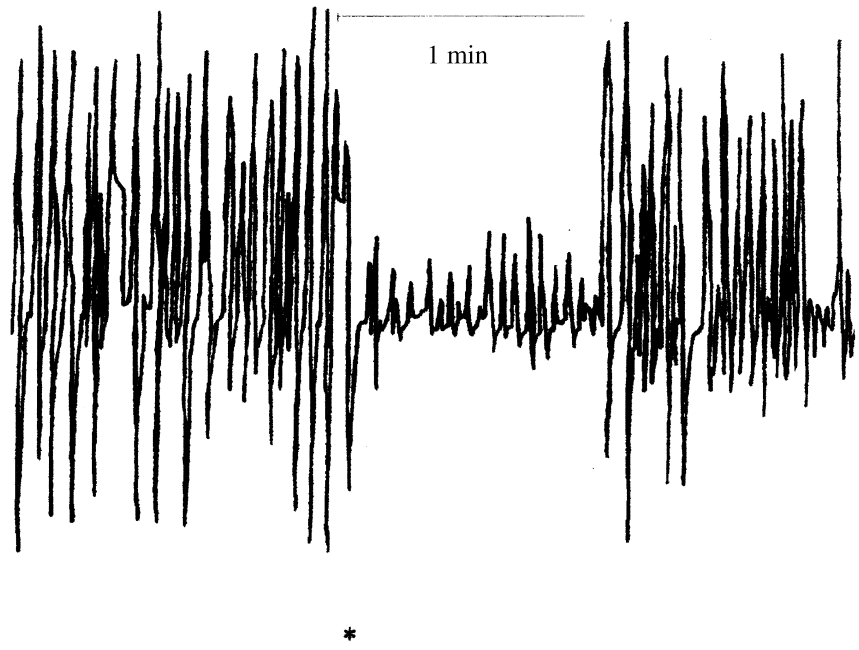

Fig. 2. Ventilation electromyogram (EMG) showing a bimodal signal that changes when stress is induced. The asterisk (*) indicates when the strobe light stress was initiated causing a change in ventilation rate and the amplitude of the EMG signal

eliminate changes in ventilation rates resulting from changes in light levels (photoperiod). Ten specimens of each species were tested under continuous light or dark conditions. Specimens were held for at least 3 days in freshwater conditions under the light conditions for the test to allow the specimens to acclimate. One specimen was introduced into the test aquarium and allowed to acclimate for 48 h, as recommended in Cairns and Gruber (1980). Thereafter, the amplifier, chart recorder and microcomputer were activated; the data handling program initiated; and the experiment started. The ventilation rate of the specimen was recorded for 24 h with the strobe light off to determine the base ventilation rate for that specimen. The strobe was then initiated and the ventilation rate recorded for 24 h to indicate the 'stressed' ventilation rate exhibited by the specimen. Experiments were conducted at a strobe flash frequency of 300 flashes/min because this frequency was found to elicit a consistent avoidance response by spot and white perch in other studies (Patrick et al. 1985; Sager et al. 1987).

A chi-square analysis was conducted on the change in mean ventilation rates for each specimen. Data were also analyzed by comparing the paired observation of the base ventilation rate with the 'stressed' rate for the same 30 min interval over the 24 h test periods. Differences between paired consecutive 30 min periods for each 24 h baseline (expected distribution) and 'stressed' (observed distribution) ventilation rate were analyzed by a chi-square analysis. Thirty minute intervals were used to reduce variation in the data set, as noted by Gruber and Cairns (1981). All analyses used a significance level of $P < 0.05$. Chi-square analyses have been used by other investigators for changes in ventilation rates (Cairns and Gruber 1980). Significant changes in the ventilation rate would indicate that the fish were stressed by the strobe light. If the 'stressed' ventilation rate returned to the base rate over time, accommodation to the strobe light would be indicated.

Results

Both white perch and spot ventilation rates changed when subjected to strobe lights, indicating that the fish did experience stress. The normal bimodal EMG signal changed in both amplitude and ventilation rate. The rates either decreased, increased, or both over time (Fig. 2).

M. americana

Light-acclimated white perch had mean baseline ventilation rates ranging from 1 to 97 counts per min (cpm) with an overall mean ($\bar{x}$) of 41 cpm (Figs 3a, 5a; Table 1). Mean stressed rates for light-acclimated specimens ranged from 1 to 100 cpm ($\bar{x} = 44$ cpm; Figs 3b, 5a, Table 1). Mean baseline rates for dark-acclimated white perch ranged from 1 to 79 cpm ($\bar{x} = 35$ cpm; Figs 3c, 5b, Table 2). Mean stressed rates for dark-acclimated white perch ranged from 2 to 83 cpm ($\bar{x} = 30$ cpm; Figs 3d, 5b, Table 2).

Presenting the change in ventilation rates from baseline to stressed conditions as an absolute value for the difference, aids in removing some difficulties in interpreting the data mathematically. Because individual specimens varied in how their ventilation rates were altered, using the absolute values eliminated negative and positive changes from canceling each other out in developing data means. Light-acclimated white perch had differences ranging from 0 to 43 cpm ($\bar{x} = 11.01$ cpm; Fig. 6a, Table 1). Differences for dark-acclimated ranged from 0 to 78 cpm ($\bar{x} = 11.13$ cpm; Fig. 6a, Table 2).

Chi-square analysis of the experiments indicated significantly different results ($P < 0.05$) for all white perch specimens tested, except for one light-acclimated fish. Chi-square analysis results were significantly different ($P < 0.05$) for all 0.5 h intervals of the light- and dark-acclimated experiments.

L. xanthurus

Spot had mean baseline ventilation rates ranging from 3 to 146 cpm for light-acclimated specimens with an overall mean of 42 cpm (Figs 4a, 5c, Table 3). Mean stressed rates for light-acclimated specimens ranged from 2 to 134 cpm ($\bar{x} = 36$ cpm; Figs 4b, 5c, Table 3). Dark-acclimated specimens had mean baseline rates ranging from 1 to 94 cpm ($\bar{x} = 40$ cpm; Figs 4c, 5d, Table 4). Mean stressed rates for dark-acclimated specimens ranged from 1 to 72 cpm ($\bar{x} = 25$ cpm; Figs 4d, 5d, Table 4).

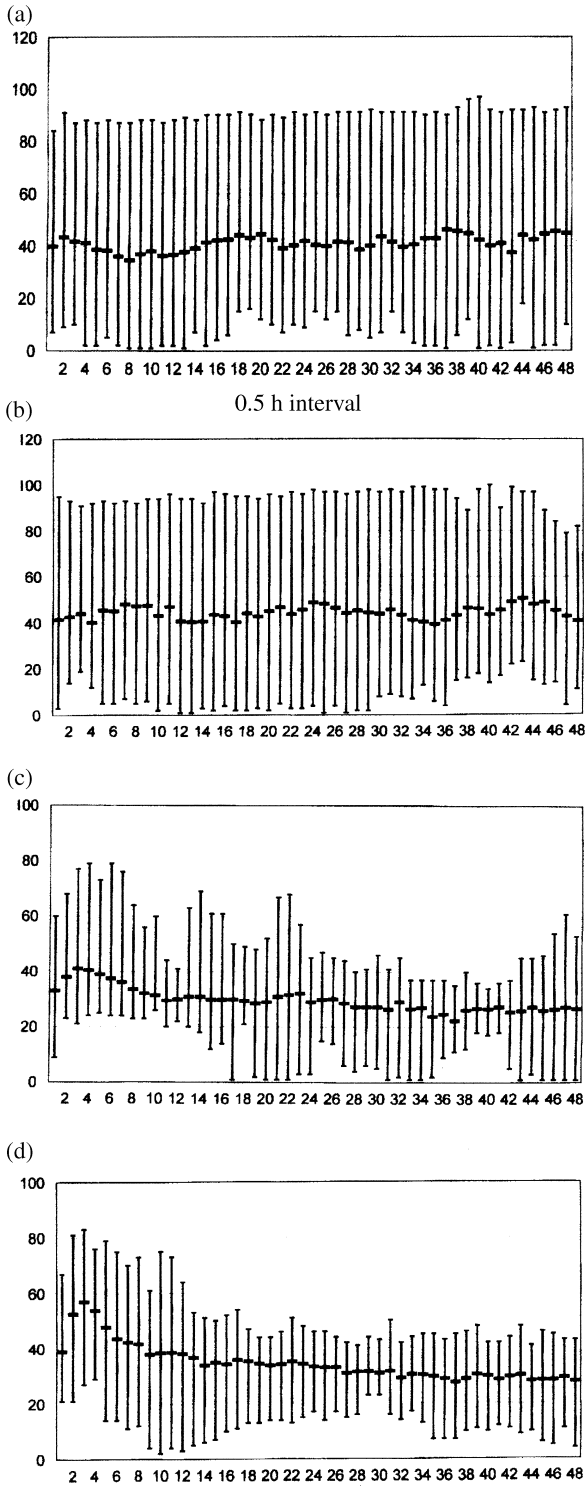

Fig. 3. Overall means and ranges for ventilation rates (cpm) during the 48 0.5 h intervals over the 24 h experiments for 10 white perch, *Morone Americana*, specimens under: (a) baseline light-acclimated conditions; (b) stressed light-acclimated conditions; (c) baseline light-acclimated conditions; (d) stressed dark-acclimated conditions

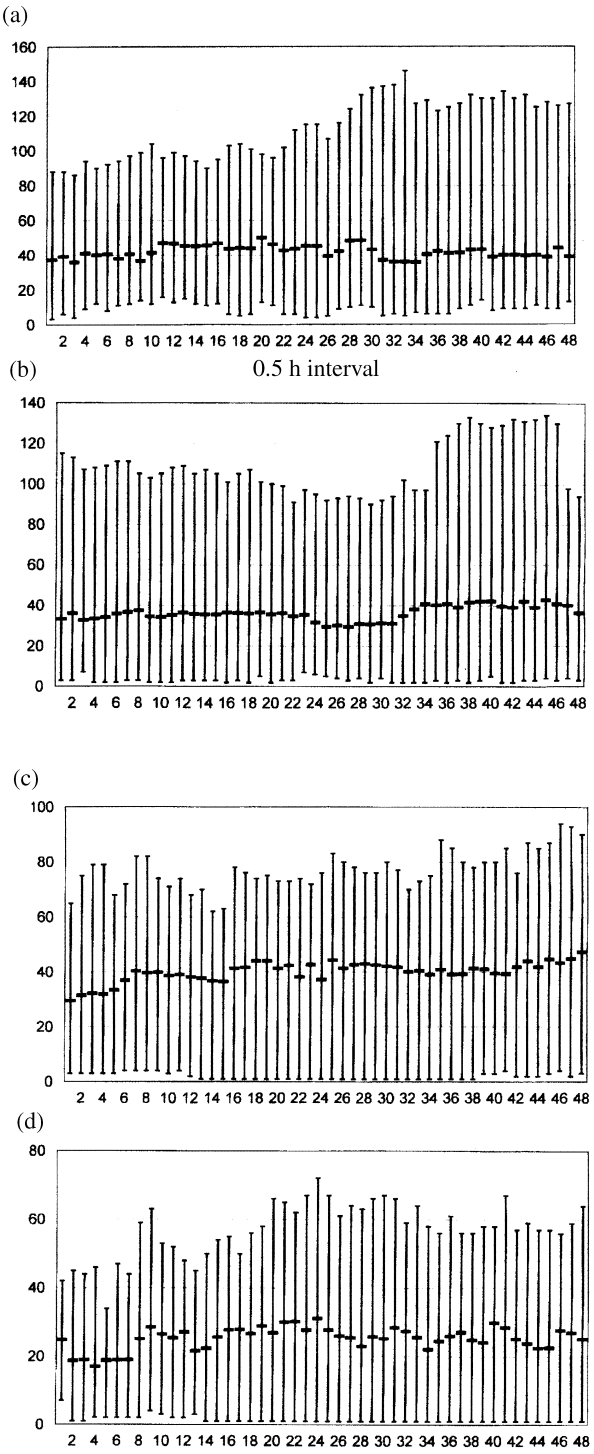

Fig. 4. Overall means and ranges for ventilation rates (cpm) during the 48 0.5 h intervals over the 24 h experiments for 10 spot, *Leiostomus xanthurus*, specimens under: (a) baseline light-acclimated conditions; (b) stressed light-acclimated conditions; (c) baseline dark-acclimated conditions; (d) stressed dark-acclimated conditions

Absolute values for the differences between baseline and stressed conditions for light-acclimated spot ranged from 0 to 101 cpm ($\bar{x} = 14.68$ cpm; Fig. 6b, Table 3). Differences for dark-acclimated spot ranged from 0 to 70 cpm ($\bar{x} = 20.56$ cpm; Fig. 6b, Table 4).

Chi-square analyses had significantly different results ($P < 0.05$) for all dark-acclimated specimens and all but one light-acclimated specimen. Chi-square analyses for the time intervals of the experiments had significantly different results ($P < 0.05$) for all intervals.

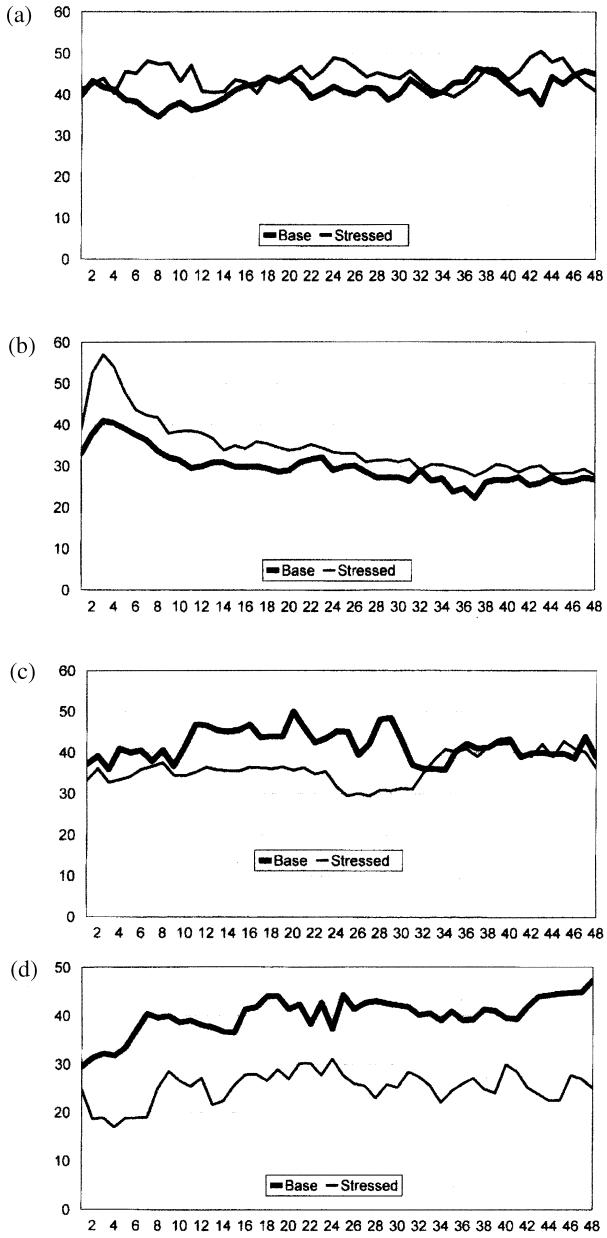

Fig. 5. Overall mean ventilation rates (cpm) for the 48 0.5 h intervals over the 24 h experiments for 10 specimens under baseline (thick line) and stressed (thin line) conditions for: (a) light-acclimated white perch; (b) dark-acclimated white perch; (c) light-acclimated spot; (d) dark-acclimated spot

Discussion

Biomonitoring system

Similar to findings by Spoor et al. (1971), Gruber et al. (1979), and Cairns et al. (1980), a bimodal signal was noted for the estuarine fish used in this study and the signal varied between baseline and stressed conditions (Fig. 2). The alterations to the biomonitoring system used in this study allowed the sensitivity of the amplifier to be varied for best ventilation determination. Also, the top and bottom electrode configuration recommended by Capute (1980) eliminated the loss of signal as the fish changed orientation in the chamber. These improvements may have yielded a system more accurate and versatile than the systems reviewed by Heath (1972) or Cairns and Gruber (1980). The biomonitoring system demonstrated its ability to detect and record fish ventilation rates and changes in ventilation rates over extended periods and conditions.

Ventilation rates

Most studies involving fish ventilation rates did not note the variation in rates among specimens. This may be a reflection of the manner in which the data were collected and reported, with few specimens being used and rates determined for a relative few points (or short time interval) so most variation is not documented. Cairns et al. (1980) reported that ventilation rates varied for several light-acclimated fish species monitored by electrode chambers, including freshwater and estuarine specimens. Bluegill had ventilation rates reported in two places in the paper, Cairns et al. (1980) noted a mean ventilation rate of 25.8 cpm (Table 30) with a range depicted in the paper's Fig. 16 of about 20–30 cpm. The other part of the paper noted bluegill with an hourly mean rate (Table 2) ranging from 31.4 to 34.6 cpm with a range from Fig. 13 of $\approx$20–80 cpm. Rainbow trout (*Salmo gairdneri* Richardson) was reported to have a base ventilation rate of 69.9 cpm (Table 3) and, from Fig. 14 in Cairns et al. (1980), it appears that the rate varied from $\approx$60–80 cpm. Goldfish [*Carassius auratus* (Linnaeus)] had a mean base ventilation rate reported of 22.8 cpm (Table 3) with a range of $\approx$10–50 cpm, from Fig. 14. Fathead minnow had a mean rate noted of 62.9 cpm (Table 3) with an approximate range of 50–90 cpm (Fig. 14). Margined madtom [*Noturus insignis* (Richardson)] had a mean rate reported of 67.2 cpm (Table 3) with a range of $\approx$60–80 cpm (Fig. 15). Rio Grande perch [*Cichlasoma cyanoguttatum* (Baird and Girard)] had a mean rate of 58.7 cpm listed (Table 3) with a range of $\approx$50–70 cpm (Fig. 16). Sheepshead minnow (*Cyprinodon variegatus* Lacépède) had a reported mean ventilation rate of 38.9 cpm (Table 3) with a range of $\approx$30–45 cpm (Fig. 17). Mummichog [*Fundulus heteroclitus* (Linnaeus)] had a mean rate noted of 72.9 cpm (Table 3) with a range of $\approx$65–80 cpm (Fig. 17). However, as noted before, the variation among specimens of the same species was not reported, although this variation is indicated by the separate rates given for bluegill in Cairns et al. (1980).

This study found ventilation rates that varied greatly among individuals as well as among species (Tables 1–4). The mean baseline ventilation rates over the 24 h interval of the experiments, however, were fairly consistent for both species (Figs 3–5). Also, the ventilation rates reported in this study had greater ranges than shown in Cairns et al. (1980) which may be a reflection of species differences but also may be a result of the modifications made to the biomonitoring system. The baseline ventilation rates for light-acclimated white perch (Fig. 3a) and spot (Fig. 4a) had similar overall means (41 and 42 cpm) but the variation in the rates was higher for spot (Tables 1–4). Additionally, there were apparent differences between light-acclimated and dark-acclimated baseline ventilation rates for both species. White perch had the most striking change (Fig. 3a, c). The ventilation rates for dark-acclimated white perch were lower than for light-acclimated specimens. The overall mean decreased from 41 cpm (light-acclimated) to 35 cpm (dark-acclimated) and the range in values also decreased. Spot had a similar change (Fig. 4a, c), with the overall mean decreasing from 42 cpm (light) to 40 cpm (dark) and the range in values decreasing. This is probably a reflection of the fish being more active under light conditions.

The alteration in ventilation rates by estuarine fish in this

Table 1
Ventilation rates for each of the 10 experimental white perch acclimated to light conditions. Results are given as mean counts per minute for each hour of the two 24 h experiments for baseline and stressed conditions

Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 Base	54	50	36	30	62	55	55	49	48	27	28	46	33	47	24	31	38	38	29	10	35	44	49	27
Stressed	21	27	66	71	44	41	24	12	30	35	35	43	46	55	54	48	28	39	41	40	33	45	43	18
2 Base	19	2	1	1	1	2	7	4	21	33	7	13	12	6	5	15	3	2	6	1	1	7	2	10
Stressed	20	12	5	5	2	1	3	4	2	2	3	4	4	2	8	8	13	4	38	14	22	19	15	26
3 Base	25	25	24	20	18	10	10	14	15	12	15	9	20	19	24	20	16	20	29	20	25	24	17	20
Stressed	28	31	28	24	33	26	25	31	25	26	23	44	28	24	28	19	26	18	16	16	34	15	14	11
4 Base	36	32	32	34	35	38	36	39	39	38	38	38	37	40	40	39	41	41	41	42	42	41	40	40
Stressed	51	44	44	45	38	41	40	43	70	46	51	54	50	49	41	45	49	46	46	40	48	43	47	43
5 Base	45	50	55	38	40	37	47	42	39	43	41	51	48	50	58	50	47	45	43	43	42	45	51	52
Stressed	58	42	40	43	44	60	54	47	56	60	61	69	59	48	46	48	51	52	62	76	85	55	52	51
6 Base	23	24	24	21	22	29	28	26	27	25	26	29	27	25	24	46	44	45	45	49	44	41	44	44
Stressed	45	32	49	47	33	29	26	34	28	38	42	48	41	40	41	46	17	23	39	30	34	51	51	51
7 Base	9	39	23	19	19	15	17	63	58	56	34	27	26	22	25	21	20	22	20	19	18	18	18	16
Stressed	14	24	28	26	31	13	29	20	26	23	25	24	21	30	17	25	22	32	33	20	26	26	20	26
8 Base	90	88	88	87	88	88	88	90	91	88	89	90	90	91	92	91	91	91	92	92	91	92	91	93
Stressed	93	92	92	92	94	94	92	96	95	96	97	98	97	97	97	97	99	98	89	100	99	97	84	59
9 Base	91	61	62	59	57	58	57	57	55	82	73	75	72	78	72	65	64	74	93	97	59	79	82	80
Stressed	53	51	56	70	69	58	69	65	60	73	55	56	58	58	57	53	55	54	54	54	64	83	84	82
10 Base	42	41	39	37	39	35	46	38	49	41	40	42	35	36	38	39	43	53	60	51	54	52	53	68
Stressed	43	48	44	51	44	45	45	46	50	52	46	49	61	50	50	45	45	46	46	46	45	44	42	42

Table 2
Ventilation rates for each of the 10 experimental white perch acclimated to dark conditions. Results are given as mean counts per minute for each hour of the two 24 h experiments for baseline and stressed conditions

Specimen	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1 Base	68	71	47	36	28	28	27	25	47	52	68	37	33	32	28	33	29	31	40	30	37	45	32	31
Stressed	81	69	50	46	43	44	38	38	40	38	44	40	38	41	37	36	40	34	34	34	39	34	35	38
2 Base	34	35	35	35	34	35	37	35	39	36	37	35	35	36	35	33	36	35	34	34	34	32	33	35
Stressed	33	32	35	35	36	38	39	42	44	44	41	42	44	41	43	42	43	43	46	42	44	41	45	43
3 Base	27	33	24	26	22	23	26	25	25	24	25	22	26	25	25	23	21	21	21	18	20	19	19	18
Stressed	21	76	40	35	31	26	23	22	21	21	22	27	24	24	24	20	21	22	21	20	22	22	22	21
4 Base	41	36	33	33	29	31	21	27	24	24	17	15	16	14	13	35	36	90	13	24	24	24	26	22
Stressed	44	29	14	12	2	3	6	10	13	14	13	17	17	16	16	14	14	12	12	16	11	10	12	12
5 Base	42	39	39	37	37	39	69	61	49	47	46	45	45	40	39	37	37	37	36	34	33	50	54	53
Stressed	61	42	41	41	41	42	45	43	43	40	38	36	37	35	35	34	33	35	33	36	35	33	33	35
6 Base	25	25	26	27	27	29	27	28	27	27	27	39	44	39	46	45	30	29	28	27	26	27	26	26
Stressed	33	39	32	28	25	27	25	25	26	25	28	26	25	28	26	26	26	32	25	42	39	36	41	35
7 Base	23	24	28	23	21	22	21	21	21	20	22	19	20	19	19	22	19	20	20	17	19	18	19	19
Stressed	64	71	59	68	57	56	51	44	41	34	51	46	44	33	37	39	39	33	38	34	32	30	31	27
8 Base	65	79	79	64	60	41	38	36	37	35	37	38	37	36	34	35	33	32	32	33	30	33	33	33
Stressed	48	76	75	73	75	64	47	42	38	37	38	34	42	37	31	29	29	29	32	28	27	32	28	28
9 Base	26	36	33	31	30	26	18	14	1	1	1	3	14	4	5	2	1	9	12	25	5	3	1	1
Stressed	75	75	65	55	52	53	51	52	47	42	33	29	27	23	23	18	13	7	10	10	11	11	5	4
10 Base	28	26	31	24	26	25	25	26	24	24	36	37	31	27	29	26	28	24	25	23	26	22	21	23
Stressed	66	30	25	24	22	27	13	24	41	43	44	37	33	36	36	33	45	42	37	37	36	32	31	36

study when stressed by strobe light (Tables 1–4) was similar to alterations in ventilation rates obtained from fish exposed to sublethal concentrations of toxicants in electrode monitoring chambers. As an example, Cairns et al. (1970) noted either a decrease in ventilation rates (below control rates) followed by a sharp rise in rates (above control rates) or a continuous rate above control rates when bluegill were exposed to low concentrations of zinc in electrode chamber waters. Lang et al. (1987) found that rainbow trout ventilation rates increased dramatically when exposed to NH_3 compared with a control group, with mean ventilation rates increasing from a range of about 45–55 cpm to a range of 50–80 cpm.

This study found a range in reactions (Fig. 2) similar to that found by Cairns et al. (1970). Once strobe lights were initiated, specimens' ventilation rates decreased and stayed depressed, decreased followed by an increase to levels above the baseline rates, or increased and stayed above baseline rates (Tables 1–4). Also, the reaction to the strobe light stimulus in this study was normally found to be more consistent for dark-acclimated specimens as opposed to light-acclimated specimens. For white perch, light-acclimated specimens showed a varied reaction above and below the baseline rates which resulted in means that were often similar despite significant differences being exhibited by the individuals (Fig. 5a, Table 1). Dark-acclimated white perch, however, reacted to strobe light with more consistently elevated ventilation rates resulting in mean values exhibiting

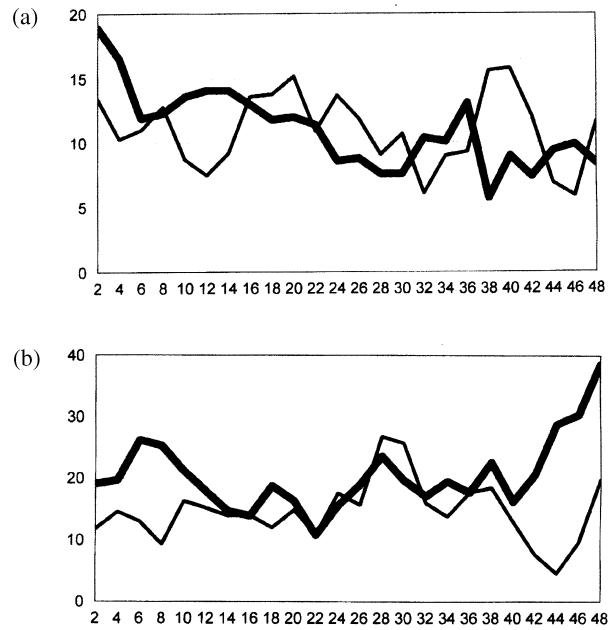

Fig. 6. Mean differences in ventilation rates (cpm) between baseline and stressed conditions (as absolute values) for (a) white perch and (b) spot for the 48 0.5 h intervals over the 24 h experiments. Light-acclimated conditions (thin line) and dark-acclimated conditions (thick line) are represented for each species

a relationship (Fig. 5b, Table 2) with a large initial difference between base and stressed mean rates with a gradual decline in the difference over time (Fig. 6a).

Spot had similar patterns, but with a greater difference between base and stressed conditions for both light conditions than that found for white perch. For light-acclimated spot, mean stressed ventilation rates were steadily below base rates for 16 h (Fig. 5c, Table 3) after which the rates varied around each other. Dark-acclimated spot had stressed ventilation rates that decreased below base rates and remained well below those rates for the entire 24 h period (Fig. 5d, Table 4). These results support findings of Patrick et al. (1985) and Sager et al. (1987) that specimens of these species exhibited greater avoidance reactions during low light level experiments, unlike other behavioral systems that were ineffective under dark conditions (Hocutt 1980).

Accommodation to strobe light

Previous studies on fish accommodation to strobe light have involved long-term experiments or re-exposing previously tested freshwater fish [gizzard shad, *Dorosoma cepedianum* (Lesueur)] to see if a reaction would be repeated (Patrick 1979a, b). These visual observation experiments on fish schools were used to detect gross changes in avoidance behavior or 'whole animal' physiological responses, but could not establish the influence of strobe light stimuli on individual specimens. In this study, the estuarine species tested (spot and white perch) did not statistically accommodate to strobe light over the time period (24 h) tested, based on chi-square analysis. There was a large variation in ventilation rates between individuals and species (Tables 1–4).

Total accommodation to xenon strobe light at a flash rate of 300/min did not take place across all specimens for either white perch or spot. There were visual indications that differences between base and stressed ventilation rates decreased over time and some accommodation may have been occurring by the end of the 24 h experiment (Fig. 5). Lang et al. (1987) conducted a 28 day experiment exposing rainbow trout to NH_3, noting a significant increase in ventilation rate on exposure. The exposed specimens' breathing rate decreased for 7 days before leveling off, but remaining above the breathing rate of control fish. This pattern is similar to that observed in some of the 24 h experiments in this study. Dark-acclimated white perch had a decrease in the differences between baseline and 'stressed' ventilation rates over the 24 h experiments (Figs 5b, 6a). Light-acclimated spot (Fig. 5c) and white perch (Fig. 5a) had a more confused pattern compared with the dark-acclimated specimens

Table 3
Ventilation rates for each of the 10 experimental spot acclimated to light conditions. Results are given as mean counts per minute for each hour of the two 24 h experiments for baseline and stressed conditions

Specimen	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
1 Base	13	9	13	12	18	13	12	12	7	13	8	13	8	10	12	11	14	20	18	14	9	9	9	13
Stressed	7	11	10	14	8	17	15	10	7	10	15	9	11	15	14	11	15	12	13	12	13	13	9	12
2 Base	88	82	87	89	92	88	94	92	94	97	102	115	107	124	136	138	127	123	127	130	134	132	128	127
Stressed	113	108	111	105	105	109	107	101	107	100	87	58	58	55	35	67	110	124	133	128	132	132	130	53
3 Base	79	82	72	52	29	57	52	69	62	69	60	85	61	57	36	34	36	38	27	29	32	37	36	32
Stressed	55	49	51	52	46	51	46	45	35	46	47	41	39	37	54	54	52	55	59	60	42	44	50	51
4 Base	27	33	44	27	26	24	29	28	32	33	36	33	18	29	28	16	13	22	17	22	29	22	25	14
Stressed	12	15	21	16	11	17	6	12	13	10	11	17	11	11	13	21	19	22	17	15	23	24	17	20
5 Base	88	94	92	97	104	99	92	95	104	98	88	97	84	90	92	93	91	92	103	97	96	92	89	91
Stressed	86	85	73	79	80	84	82	87	88	91	91	95	93	93	92	103	97	92	97	93	92	92	92	94
6 Base	29	29	36	34	31	33	29	26	27	29	28	28	30	29	27	25	26	22	22	22	23	25	14	22
Stressed	18	16	30	18	24	21	26	22	31	29	26	25	39	21	19	37	36	37	17	27	15	23	22	35
7 Base	11	19	16	38	26	42	34	58	72	74	42	20	21	13	10	10	7	6	10	38	37	46	36	32
Stressed	24	26	30	51	42	34	40	61	51	47	47	38	32	53	60	37	56	43	57	62	40	40	66	69
8 Base	37	28	24	19	62	77	79	69	5	38	6	4	5	73	36	6	17	77	68	45	13	11	17	30
Stressed	31	12	19	17	11	14	16	9	8	8	7	6	4	5	4	3	2	2	3	5	4	3	3	5
9 Base	14	17	13	13	12	19	15	14	13	13	16	15	15	15	17	13	14	14	12	14	15	13	18	15
Stressed	3	2	2	3	2	3	3	2	2	2	3	7	5	4	2	2	2	2	2	5	2	3	4	3
10 Base	6	17	8	25	14	14	15	17	24	36	39	42	45	41	36	15	13	8	9	21	11	9	14	13
Stressed	12	10	12	20	14	14	15	15	18	14	14	20	9	15	20	16	19	20	18	15	28	16	17	21

Table 4
Ventilation rates for each of the 10 experimental spot acclimated to dark conditions. Results are given as mean counts per minute for each hour of the two 24 h experiments for baseline and stressed conditions

Specimen	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48
1 Base	10	4	4	13	10	4	6	5	4	3	4	2	3	4	3	3	2	2	2	3	4	2	4	3
Stressed	1	2	2	2	3	2	5	5	6	9	9	18	8	27	13	21	12	10	17	16	16	22	24	31
2 Base	32	25	36	29	32	37	58	78	74	73	15	74	71	68	80	68	68	70	72	70	63	62	48	58
Stressed	5	11	13	16	31	23	18	18	18	11	21	20	13	16	13	20	9	12	16	47	22	18	16	12
3 Base	5	6	8	10	12	13	8	11	11	11	16	11	12	16	10	10	15	23	12	4	7	9	5	5
Stressed	19	5	8	14	5	6	3	3	10	9	6	4	3	5	3	2	3	5	3	2	3	4	3	5
4 Base	33	38	60	82	66	61	62	66	69	67	70	76	80	76	73	70	75	85	78	80	82	81	94	90
Stressed	45	34	31	37	47	48	33	53	56	66	62	57	61	55	67	66	58	61	50	51	57	52	54	50
5 Base	5	6	9	13	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1	6	2	11	16	62
Stressed	8	46	47	59	45	17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6 Base	42	56	62	67	71	68	58	56	68	67	74	62	66	75	61	57	41	32	72	53	63	62	75	60
Stressed	25	25	16	22	17	31	30	35	32	26	32	28	15	7	12	12	10	20	7	14	12	6	12	6
7 Base	75	79	72	66	65	65	58	60	69	68	58	58	66	64	68	65	66	62	63	61	50	63	66	69
Stressed	22	27	30	27	36	29	41	46	51	51	47	55	41	31	40	34	37	39	38	55	46	36	34	34
8 Base	45	43	54	53	64	61	62	67	73	62	66	66	66	56	59	65	63	62	61	68	76	85	81	74
Stressed	44	35	20	12	18	26	37	55	39	39	60	72	61	63	49	59	30	43	49	43	28	15	35	6
9 Base	64	58	60	59	58	65	50	64	65	57	73	61	63	65	61	58	54	48	46	44	68	37	34	45
Stressed	12	15	15	54	53	46	50	55	47	49	56	48	49	47	45	51	53	58	56	58	55	57	56	64
10 Base	3	3	4	4	5	5	4	5	6	4	5	4	4	5	5	4	5	6	6	6	4	7	10	7
Stressed	6	5	7	8	7	6	6	7	6	8	8	8	8	8	9	8	8	11	12	12	12	14	42	42

(Fig. 5b, d). The base and stressed lines seemed to be closer, or cross more, towards the end of the experimental period in the light-acclimated results. The dark-acclimated spot results never really indicated any consistent decrease in differences between base and stressed ventilation rates for the 24 h period (Fig. 5d).

A clearer representation of the change in ventilation rates is given by examining the absolute value of the difference between the ventilation rates (Fig. 6). This examination of the data indicates that the overall mean change over 24 h for light-acclimated white perch was 11.01 cpm (ranged from 6 to 16 cpm; Fig. 6a) which is several times higher than the change (from 41 to 44 cpm; baseline vs. stressed) indicated by the difference between the overall means of the ventilation rates. The dark-acclimated white perch had a slightly higher overall mean of 11.13 cpm for the differences between base and stressed rates (ranged from 6 to 19 cpm; Fig. 6a), which is also over twice the change in mean ventilation rates (35 cpm baseline and 30 cpm stressed). The light- and dark-acclimated white perch results indicated a general decrease in the difference between base and stressed ventilation rates over time (Fig. 6a), but the pattern is clearer in the dark-acclimated results. Generally, greater differences between baseline and stressed ventilation rates were found for spot than for white perch (Fig. 6). Light-acclimated spot had an overall mean difference of 14.68 cpm (5–26.8 cpm range; Fig. 6b), while dark-acclimated spot had an overall mean of 20.56 cpm (10.8–38.6 cpm range; Fig. 6b). The overall mean differences for spot were also greater than the difference between mean base and stressed ventilation rates for light- (42 cpm and 36 cpm, respectively) and dark-acclimated specimens (40 cpm and 25 cpm, respectively). These results reflect the conclusions noted for avoidance experiments, i.e. spot exhibited greater avoidance reactions to strobe lights than white perch (Patrick et al. 1985; Sager et al. 1987). Spot are more bottom oriented in feeding habits than white perch (Hildebrand and Schroeder 1972) and may prefer (or need) less light, which may contribute to its greater reaction to strobe lights.

Figure 5 illustrates that definite accommodation is not indicated for several hours after strobe light initiation, even from a strictly visual examination of the data. Figure 6 illustrates that while white perch (Fig. 6a) may indicate some accommodation with a decrease in differences over time, spot maintain a fairly consistent reaction over time (Fig. 6b). These results are similar to indications given in long-term experiments on freshwater species. Patrick (1979a) found only weak accommodation (called acclimation in Patrick's paper) by gizzard shad over a 12 h period from direct visual observation. Patrick (1979b) reported that gizzard shad could be repeatedly exposed to strobe light, after an unexposed period of several days, and still elicit avoidance reactions.

These data indicate that migratory schools of the species tested could move through a strobe lit area prior to accommodation taking place. The species react to strobe light over several hours (over 24 h statistically) and react well to the stimuli under darkened conditions, unlike other behavioral guidance systems (Sager et al. 1987). The use of strobe light as a guidance system for estuarine species continues to be promising.

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Author's address: Dr David R. Sager, Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, TX 78744-3218, USA.
E-mail: David.Sager@tpwd.state.tx.us