Abstract.—Atlantic menhaden *Brevoortia tyrannus* larvae inflate their swimbladders at night and deflate them during the day. The present study considered the relationship of inflation to light intensity, the time-course of inflation, and the presence of an endogenous rhythm in inflation. The percentage of laboratory-reared larvae that inflate their swimbladders increased upon sudden exposure to a decrease in light intensity. Percentage inflation was maximal at an intensity of 10^{13} photons cm^{-2} s^{-1} and lower. For any specific size of larvae, the inflation volume did not vary significantly with light intensity, but volume increased with total larval length. Inflation began within 5 min of introduction into darkness, and maximum percent inflation was evident by 20 min. There was a rhythm in which darkness induced a low percent inflation during the day phase and a high percentage at the beginning of the dark-phase. This dramatic increase in inflation at sunset may function for predator avoidance.

Swimbladder inflation of the Atlantic menhaden *Brevoortia tyrannus*

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Diel inflation and deflation of the swimbladder are common to larval clupeoid fishes (Uotani 1973, Hunter & Sanchez 1976, Blaxter & Hunter 1982). Both Atlantic (*Brevoortia tyrannus*; Hoss et al. 1989) and gulf (*Brevoortia patronus*; Hoss & Phonlor 1984) menhaden larvae inflate their swimbladders during the night and deflate them during the day. Inflation occurs by moving to the surface, swallowing air into the alimentary canal, and moving it to the swimbladder through the pneumatic duct (Hoss & Phonlor 1984). Deflation is presumed to take place by diffusion, because menhaden have no open connection between the anal opening and swimbladder (Tracy 1920).

Inflation/deflation by menhaden larvae is clearly related to the light: dark cycle. Field studies of both Atlantic and gulf menhaden found most larvae had deflated swimbladders and low swimbladder volumes during the day, with the reverse conditions at night (Hoss & Phonlor 1984, Hoss et al. 1989). Since inflation is rapid, occurring within 1 h after sunset, Hoss et al. (1989) suggested that change in light intensity may cause the inflation response.

The present study was undertaken to determine (1) the relationship between inflation and light intensity for Atlantic menhaden larvae, (2) the time-course for inflation, and (3) the presence or absence of an endogenous rhythm in inflation.

Materials and methods

Atlantic menhaden *Brevoortia tyrannus* were spawned and reared in the laboratory using methods described by Hettler (1983). After egg hatching, the larvae were held in circular tanks (100 L) at about 20°C on a 12:12 h LD cycle. The dark phase began at 1800 h. Lighting during the light phase was provided by daylight fluorescent tubes at a surface intensity of 1.6×10^{15} photons cm^{-2} s^{-1} (400–700 nm) as measured with a scalar irradiance meter with a 4ii collector (Biospherical Instruments, Inc.). Young larvae were fed rotifers *Brachionus plicatilus* cultured in the laboratory. Older fish (13–15 mm) were fed brine shrimp (*Artemia* sp.) nauplii. Larvae measuring 9–20 mm were used in the experiments.

Inflation was quantified by measuring light-refractive bubbles in the swimbladder and alimentary canal to the nearest 0.02 mm under a microscope. It was assumed that any bubbles in the alimentary canal were being transported to the swimbladder. Gas bubble volume was calculated using the equation of Hunter

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& Sanchez (1976): $V = 4/3\ 4ii\ ab^2$, where b = half the bubble width and a = half the bubble length. The total volume of all bubbles was used as the swimbladder volume.

There were three sets of experiments. The first was designed to determine the relationship between light intensity and swimbladder inflation. Larvae were removed from the rearing tanks just before the beginning of the dark phase and maintained under approximately the same light intensity (cool white fluorescent lamps: intensity = 1.7×10^{15} photons cm^{-2} s^{-1}). A control group of the larvae was measured for total length (TL), presence of gas bubbles in the swimbladder and alimentary canal, and size of the bubbles. Similar standard measurements were made on the remaining larvae after being exposed to the test light intensity for 3 h. The light-stimulus source was a 300 W incandescent lamp filtered to the blue region with a Corning 4-96 filter. The transmitted wavelengths encompassed the major spectral-sensitivity maxima of most fish (e.g., Munz 1958, McFarland & Munz 1975). All intensities below the maximum level were controlled by neutral density filters. Each larva was only measured once.

Since the total-length range of larvae was 9–20 mm, an important question is whether all sizes were equally responsive to lighting changes that induce swimbladder inflation. An answer to this question was provided by considering (1) the percentage of different-sized larvae that inflate their swimbladder before and after exposure to new lighting conditions, and (2) the percentage of the total number of larvae sampled in each size-category.

The second set of experiments was designed to determine the time-course for swimbladder inflation. Larvae were again removed from the rearing tank near the end of the light-phase and illuminated at about the intensity used for rearing. Standard measurements were made on a control sample of larvae. The remaining larvae were put into darkness at the beginning of the dark-phase, and standard measurements made on subsamples at various time-intervals (5, 15, 30, 60, 90, 120 min).

The third experiment tested for the presence of an endogenous rhythm in swimbladder inflation. A large group of larvae was removed from the rearing tank at the beginning of the light phase and placed in constant temperature (22°C) and light (cool-white fluorescent lamps: intensity = 1.7×10^{15} photons cm^{-2} s^{-1}) both of which were similar to the light intensity and water temperature of the rearing tanks. Standard measurements were made at 3 h intervals on a subsample of larvae. At the beginning of each of these measurements, a similar subsample was placed in darkness and standard measurements made 2 h later. A change in the percent inflating over the day would suggest the presence of an endogenous rhythm.

The percentage of larvae with inflated swimbladders and the total volume of bubbles in each larva were calculated for each observation within each experiment. Means, standard deviations, and standard errors of percent data were calculated after the data were arcsin-transformed. A Z statistic was used to test differences between two proportions, while a Student's *t*-test was used to compare mean values (Walpole 1974).

Results

Relation of swimbladder inflation to light intensity

The percentage of larvae inflating their swimbladder under all experimental conditions changed with size (Fig. 1). Prior to exposure to new light conditions, the percent of larvae with inflated swimbladders remained relatively low for all sizes of larvae. In contrast, when subjected to a decreased light intensity, the percent response changed dramatically with size. None of the 9 mmTL larvae inflated their swimbladder, and only a low percentage of 10 mmTL larvae showed inflation (Fig 1). Percent inflation increased as size increased, reaching 100% for larvae >17 mmTL. The following study will focus on larvae 11–16 mm in length, because the percent inflation can vary with lighting condition.

Equal numbers of larvae were not sampled in each size-category (Fig. 2), as most larvae were 11–12 mmTL and there were few >15 mmTL. This size distribution

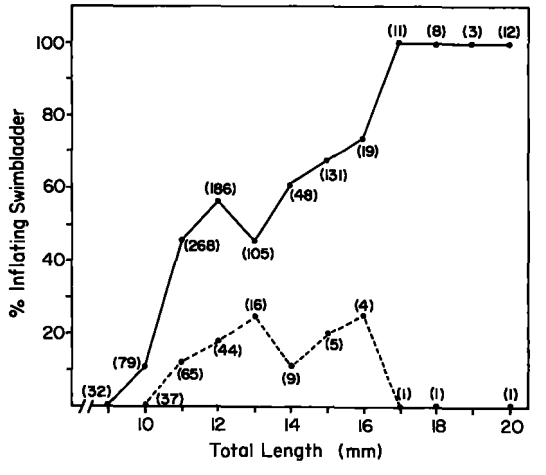

Figure 1

Percentage of larvae inflating their swimbladder versus size for all larvae in the swimbladder inflation versus light intensity experiment (Fig. 3). The dashed line shows the percentages before exposure to different light conditions, and the solid line is the experimental percentages.

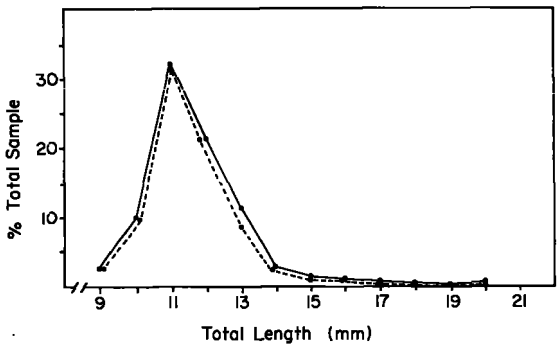

Figure 2

Percentage of the total number of larvae of the different total length sampled for the swimbladder inflation vs. light intensities experiment (Fig 3). Dashed line shows larvae sampled before exposed to different light levels (control; total n=193), whereas the solid line is larvae exposed to different light conditions (experimental; n=834).

was consistent for all experiments. Since the probability of swimbladder inflation was not equal for each size-category (Fig. 1), results will be biased to responses of the most-abundant size if all size-classes are grouped together. Thus, detailed analyses should only consider individual size-classes. The most-abundant size (11 mmTL; Fig. 2) will be used for this purpose.

The percentage of fish inflating their swimbladders increased as they were exposed to lower light intensities (Fig. 3). The highest light intensity to induce a significant increase in the proportion of fish with inflated swimbladders (threshold intensity) varied slightly with fish size. For 11 mmTL larvae (Fig. 3A) the threshold intensity was ~6×10^{13} photons cm^{-2} s^{-1}, whereas for 12–16 mmTL larvae, it was 1 log unit higher (Fig. 3B). For both size-groups, the proportion filling their swimbladders at ~10^{13} photons cm^{-2} s^{-1} and lower light intensities was not significantly different from the proportion in darkness.

The variation in swimbladder volume with light intensity was considered in detail for 11 mmTL larvae (Fig. 4). Mean volume increased as light intensity decreased, but the difference was not significant between the initial mean volume and that in darkness due to large variances (Fig. 4). Similar results were also obtained for 12 and 13 mmTL larvae.

In contrast, swimbladder volume increased proportionately with larvae size (Fig. 5). When the relationship between mean volume (V) in darkness and total length (L) was expressed as the allometric equation ($V=aL^b$), the slope of the regression (b) equaled 5.31 (r^2=0.99, p<0.0001). Means were calculated for larvae of each size in all conditions, because volume did not change with lighting condition (Fig. 4). Since volume changed with larval length, volumes could not be averaged for larvae of different lengths.

Timing of swimbladder inflation

The timing of swimbladder inflation was measured upon transfer from rearing-light intensity to darkness. By producing the maximum rate of intensity change, we assumed the maximum rate of inflation should be evoked. Results were combined for larvae 11–16 mmTL because the proportion inflating in darkness for 11 mm larvae was not statistically different from the proportion of 12–16 mmTL larvae (Fig. 3). A significant increase in the proportion with inflated swimbladders was evident after 5 min in darkness (Fig. 6). The maximum percent inflation was reached within 20 min. The proportion of fish with inflated swimbladders then remained relatively constant for about the next 1.5 h.

Endogenous rhythm in swimbladder inflation

The percent inflating prior to placement in darkness remained low throughout the 24h sampling interval, which

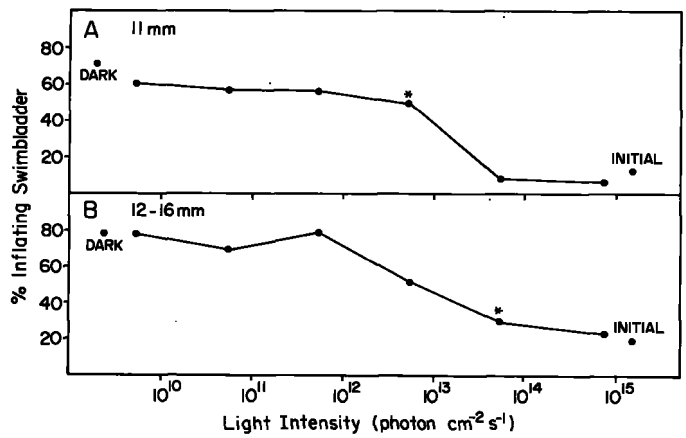

Figure 3

Percentage of larvae 11 mmTL (**A**) and 12–16 mmTL (**B**) inflating their swimbladder when exposed to different light intensities and darkness (dark). "Initial" is the percentage sampled shortly after removal from the rearing tank. Average sample sizes for each condition in A and B are 44 and 58, respectively. Asterisk indicates the highest light intensity to evoke a response that was significantly (p<0.05) greater than the initial response.

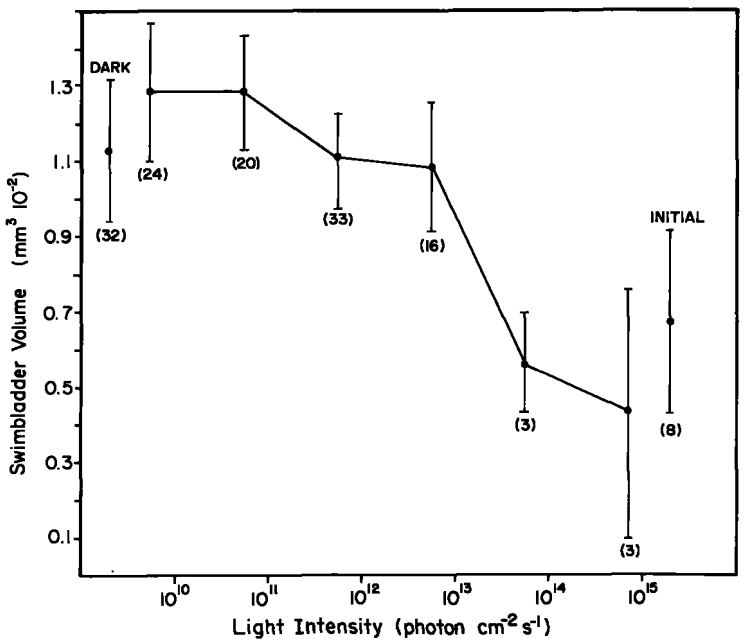

Figure 4

Swimbladder volume of 11 mmTL larvae shortly after removal from the rearing tank (initial) and when exposed to different light levels and darkness (dark). Means and standard errors are shown. Number below each plot is the sample size.

indicates there was no endogenous rhythm in inflation in constant high light conditions (Fig. 7A). Placement in darkness induced inflation in ~40% of the larvae during the normal light-phase. This percentage increased dramatically to 70% at the normal time for the beginning of the dark-phase (Fig. 7A). This response level continued into the time of the next light-phase. Mean swimbladder volume of 11 mmTL larvae varied over time, but the maximum and minimum means over the first solar day were not significantly different due to the large variances (Fig. 7B).

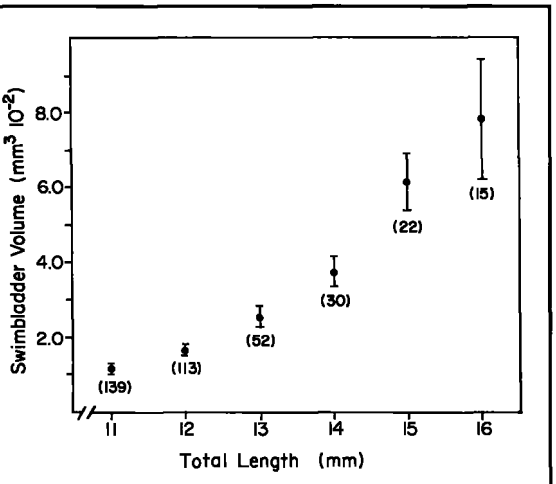

Figure 5

Swimbladder volume vs. total length of larvae from all lighting conditions. Means and standard errors are plotted. Number under each plot is the sample size.

Discussion

Atlantic menhaden inflate their swimbladders in response to a decrease in light intensity. The smallest size observed with an inflated swimbladder was 10 mmTL, which is smaller than the minimum size of 13 mmTL found by Hoss & Blaxter (1982). This difference may result from the large sample size used in the present experiment, since the percentage of 10 mmTL larvae with an inflated swimbladder was ~10%. The percentage of larvae inflating their swimbladder in response to a decrease in light intensity increased with size and reached 100% at 17 mmTL and greater.

Swimbladder volume increased with size, which is not surprising, since larger larvae have larger swimbladders. However, within any fish size, the mean volume did not vary significantly with lighting condition. This result disagrees with the qualitative conclusion of Hoss et al. (1989) and is likely due to the wide variation in volume. Hoss et al. (1989) also found high variances, but failed to compare mean values statistically.

The percentage of larvae inflating their swimbladders increased as the light intensity decreased, which clearly indicates that the decrease in light intensity cued the response. However, a step function was observed, in that once light was below a particular absolute level, maximum inflation occurred. Future experiments are needed to determine whether inflation is cued by exposure to light intensity below an absolute level or to the rate of change in intensity. This information will allow predictions of the time of inflation in the field.

Hoss et al. (1989) failed to find an endogenous rhythm in swimbladder inflation for larvae held under conditions similar to the present experiment. In contrast, our study showed a clear rhythm during the first day for larvae held under constant light. The percent inflation was low in fish introduced to the dark during the time of the light-phase, and nearly doubled at the time the dark-phase began. This high percent response did not return to a low level at the time of the next light-phase. Hoss et al. (1989) did not begin measuring swimbladder inflation until after ~24 h in constant light, which may be why they failed to detect an endog-

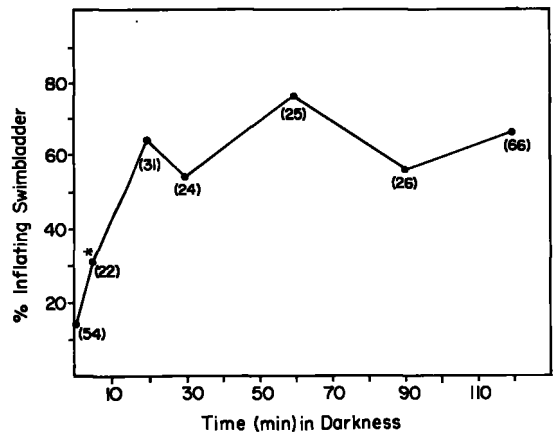

Figure 6

Percentage of larvae 11–16 mmTL filling their swimbladders after different times in darkness. Number under each point is the sample size, and asterisk is the first time that a proportion was significantly greater ($p<0.05$) than the proportion of larvae with inflated swimbladders initially in light, which is plotted at time zero.

enous rhythm. After this time in constant light, variation in inflation after introduction to dark was not evident in the present study. There are two possible explanations for this result. First, the rhythm could fail to continue because larvae were kept in constant light, a condition that frequently suppresses an endogenous rhythm (Hastings et al. 1991). Second, the rhythm could consist of one cycle in which inflation is suppressed during the light-phase and larvae become "ready" to inflate at the beginning of the dark-phase. Readiness then continues until a dark cue is received, which resets the endogenous clock.

Clearly, Atlantic menhaden larvae are adapted for swimbladder inflation at sunset. Their rhythm indicates they are most responsive to a light-intensity decrease at this time and most inflation occurs within 20 min. Such a dramatic response suggests swimbladder inflation has an important functional advantage.

Menhaden larvae are negatively buoyant even with a fully inflated swimbladder. Nevertheless, inflation reduces their sinking rate (Hoss et al. 1989). Past investigators have suggested that swimbladder inflation acts as an energy-saving mechanism, allowing larvae to expend less energy for maintaining their position in the water column at night when they are not feeding (Hunter & Sanchez 1976). During the day, a fully inflated swimbladder may reduce the speed of movement and, thereby, the effectiveness of prey capture and predator avoidance.

In addition, Uotani (1973) proposed that inflation allows larvae to decrease their movement at night, which serves to reduce detection by predators that hunt by vibrations, such as chaetognaths. Field studies show some indication that menhaden larvae undergo reverse diel vertical migration (DVM) in which they descend in the water column near sunset and ascend near sunrise (Hoss et al. 1989). Chaetognaths exhibit the opposite pattern of nocturnal DVM (Pearre 1973, Sweatt & Forward 1985). Reverse DVM is proposed as a mechanism for avoiding zooplankton predators that undergo nocturnal DVM (Ohman et al. 1981, Neill 1990). A slower descent rate at sunset by menhaden larvae due to inflated swimbladders may reduce detection by chaetognaths that are ascending toward the surface. Since the percentage of menhaden larvae with inflated swimbladders increases with size, the importance of reduced sinking rate for predator avoidance may increase with size. The threat of predation to menhaden larvae is probably reduced during their ascent at sunrise because the descending chaetognaths have been feeding all night.

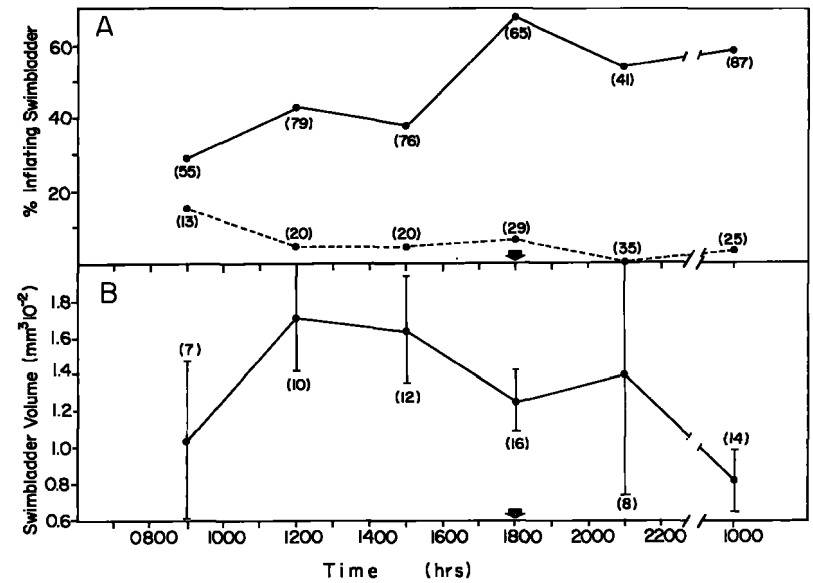

Figure 7

Percentage (**A**) of larvae 11–16 mmTL that filled their swimbladders before (– – –) and after (——) exposure to darkness for 2 h over the solar day. The swimbladder volume of 11 mmTL larvae (**B**) after exposure to darkness is also plotted against time in the solar day. Means and standard errors are plotted. Number near each plot is the sample size. Arrow indicates the times of the beginning of the dark phase of the rearing LD cycle.

Acknowledgments

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