Swimbladder deflation in the Atlantic menhaden, *Brevoortia tyrannus*

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Larval clupeoid fishes usually have a pronounced cycle of swimbladder inflation and deflation (Uotani, 1973; Hunter and Sanchez, 1976; Blaxter and Hunter, 1982). Field and laboratory studies of both Atlantic (*Brevoortia tyrannus*; Hoss et al., 1989) and gulf (*Brevoortia patronus*; Hoss and Phonlor, 1984) menhaden found that larvae inflated their swimbladders during the night and deflated them during the day.

Our past studies of Atlantic menhaden larvae found that the cue for inflation is a decrease in light intensity at sunset (Hoss et al., 1989; Forward et al., 1993). Inflation occurs rapidly and begins within 5 minutes of onset of darkness. The process involves moving to the surface, swallowing air into the alimentary canal, and moving this air into the swimbladder through the pneumatic duct (Hoss et al., 1989). Menhaden have no connection (pneumatic duct) between the swimbladder and anus as do some clupeiods (Tracy, 1920). Deflation is less studied. It is hypothesized to occur by diffusion of gas from the swimbladder throughout the night and perhaps by active movement of gas to the alimentary canal and then out through the mouth and anus (Hoss and Phonlor, 1984).

The present study was undertaken to determine 1) the manner in which swimbladder deflation occurs (by diffusion or active gas movement) in Atlantic menhaden larvae, 2) the relationship between deflation and light intensity, 3) the time-course for deflation, and 4) the presence or absence of an endogenous rhythm in deflation.

Materials and methods

Atlantic menhaden, *Brevoortia tyrannus*, were spawned and reared in the laboratory (Hettler, 1983) on a 12:12 hour light-dark cycle with the dark phase beginning at 1900 hours. 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 4π collector (Biospherical Instruments, Inc.). Our previous study found that swimbladder inflation began when larvae were 10 mm total length (TL) but the percentage with inflated swimbladders was low (Forward et al., 1993). Between 11 and 16 mm TL, swimbladders were inflated during the night and deflated during the day. Above 16 mm TL, most fish always had some gas in their swimbladders. Since the percentage of larvae with deflated swimbladders varies between day and night for 11–16 mm TL larvae, they were used in the present experiments.

Deflation was quantified by determining the proportion of larvae with deflated swimbladders. In addition, inflation was quantified by measuring the size of the light-refractive bubbles in the swimbladder and alimentary canal to the nearest 0.02 mm under a microscope. It was assumed that bubbles in the alimentary canal were transported either toward or away from the swimbladder and thereby contributed to the swimbladder volume. Gas bubble volume (v) was calculated by using the equation of Hunter and Sanchez (1976): $V=(4/3)\pi\ ab^2$, where b=half the bubble width and a=half the bubble length. Swimbladder volume was the total volume of all bubbles. Since swimbladder volume increases with larval length (Forward et al., 1993), this relationship is presented when volume is considered. Only larvae with inflated swimbladders were used to calculate the mean volume at each larval length. In contrast, the percentage of larvae with deflated swimbladders was calculated for all larvae (11–16 mm TL), because the previous study found that the proportion of larvae inflating swimbladders did not vary significantly with larval length (Forward et al., 1993).

Four sets of experiments were conducted, all of which began by removing larvae from rearing tanks and by placing them in darkness at the time of the beginning of the dark phase. Our previous study showed that darkness cued initial

Manuscript accepted 22 February 1994.
Fishery Bulletin 92:641–646 (1994).

swimbladder inflation at the beginning of the night (Forward et al., 1993). In all experiments each larva was used only once. It was assumed that all test larvae could potentially inflate their swimbladders in darkness. However, the maximum percent inflation was around 92% (Fig. 1), which suggests that about 8% of the larvae were developmentally incapable of inflation. This low percentage would not alter the overall result of any experiment.

The first experiment measured swimbladder deflation in larvae kept in continuous darkness with and without access to air. Deflation was defined as the absence of gas bubbles in the swimbladder and alimentary canal. Three hours after the beginning of night (2200 hours), a subsample of the larvae was removed from the dark and measured for total length, presence of gas bubbles in the swimbladder and alimentary canal, and for size of the bubbles (standard measurements). There was no evidence that larvae lost or took up gas during the measurement procedure. The remaining larvae were separated into two groups. The first group remained in finger bowls (19.3 cm diameter) in darkness with access to the air-water interface and was similarly sampled during the following day at times (0900, 1200, and 1700 hours) that should have occurred during the normal light phase. The bowls (19.3 cm diameter) containing the second group of larvae were sealed at 2200 hours, so that larvae did not have an air-water interface. The seal was accomplished by filling the bowl completely with water and by placing a Leucite plate over the top so that no air bubbles were present. Larvae were maintained in darkness and were similarly sampled at 0900 and 1700 hours the next day.

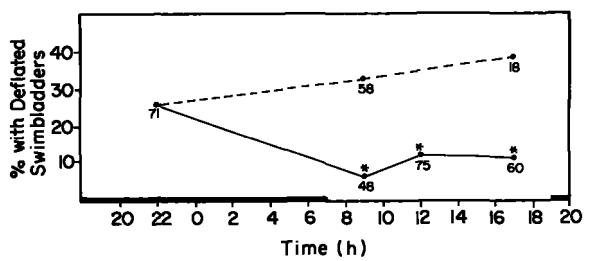

Figure 1

The percentage of larval Atlantic menhaden, *Brevoortia tyrannus*, with no gas in their swimbladder or alimentary canal (deflated) when maintained over time in continuous darkness with (solid line) and without (dashed line) access to the air-water interface. The dark bar indicates the time of the dark phase for the normal light-dark cycle. The number under each point is the sample size. An asterisk indicates the proportion with deflated swimbladders is significantly ($P<0.05$; Z-test for comparison of two proportions) different from that 3 hours after the beginning of the dark phase (initial point).

The second set of experiments was designed to determine the relationship between light intensity and swimbladder deflation. Larvae were placed in darkness at the beginning of the dark phase until two hours (0900) after the time for beginning the light phase. Larvae were then separated into two groups. For the control group standard measurements were made after three more hours of darkness. The second group was irradiated with a constant light intensity for three hours after which standard measurements were made. White light (cool white fluorescent tubes; 1.7×10^{15} photons$\cdot$cm$^{-2}\cdot$s^{-1}) was used in the initial experiment, because this was the normal daytime light during rearing. For determining the change in response with light intensity, the 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 and Munz, 1975). All intensities below the maximum level were controlled by neutral density filters.

The third set of experiments was designed to determine the time-course for swimbladder deflation. Larvae were maintained as above in darkness until 0900 hours when standard measurements were made on control groups of larvae. The remaining larvae were then exposed to light similar to that used in the normal light phase (cool white fluorescent lamps: intensity=1.7×10^{15} photons$\cdot$cm$^{-2}\cdot$s^{-1}) and standard measurements made on subsamples at various time intervals (5, 15, 30, 60, 90, 120 min).

The fourth experiment tested for the presence of an endogenous rhythm in swimbladder deflation. A large group of larvae was removed from the rearing tank at the beginning of the dark phase and placed in darkness at a constant temperature (22°C) that was similar to the rearing temperature. Standard measurements were then made at time intervals throughout the next 28 hours on subsamples of larvae. Each subsample was placed under blue light (intensity= 4.5×10^{15} photons) for one hour, after which standard measurements were made. Differences in the effect of light on percent deflation over the 28-hour interval would suggest the presence of an endogenous rhythm.

The percentage of larvae with deflated 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, and a Student's t-test was used to compare mean values of swimbladder volume (Walpole, 1974).

Results

To determine whether deflation was evident at the beginning of the light phase without the onset of light, percent deflation (Fig. 1) and swimbladder volume (Fig. 2) were measured in larvae kept in continuous darkness. When larvae had access to the air-water interface, there was a significant ($P<0.05$; Z-test) decrease in the percentage of fish with deflated swimbladders between three hours after beginning of the dark phase (23%) and two hours after the time for beginning of the light phase (8%). This significant decrease remained throughout the time for the light phase. In contrast, when fish had no access to the air-water interface, the percent deflation did not change significantly (Z-test) over these time intervals (Fig. 1). Thus, larvae do not sequentially inflate their swimbladders at sunset and then deflate them by gas diffusion or active processes by sunrise. Because the percentage of larvae with a deflated swimbladder decreased through the night when larvae had access to the air-water interface, larvae appear to continue to inflate their swimbladders when in darkness. Measurements of swimbladder volume (Fig. 2) support this suggestion.

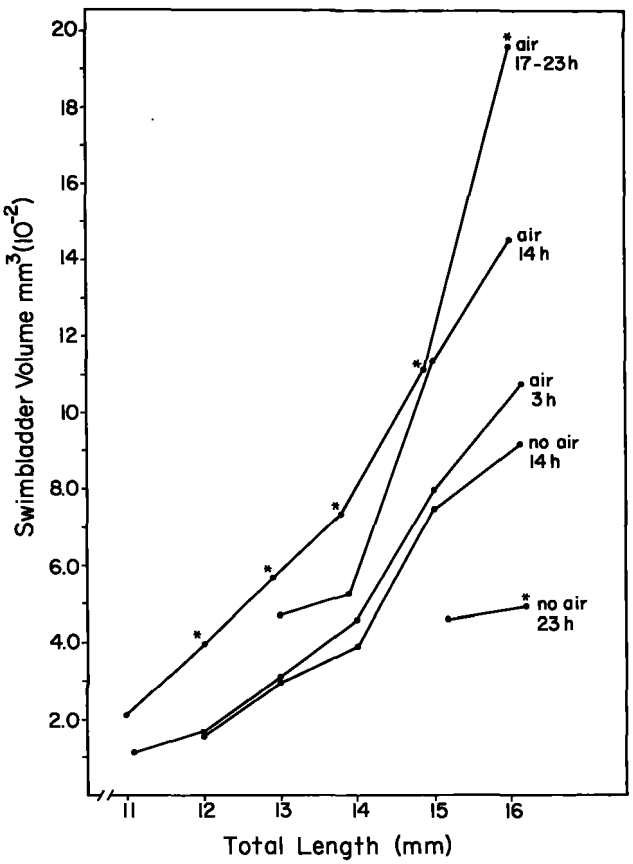

Figure 2

Swimbladder volume for different size larval Atlantic menhaden, *Brevoortia tyrannus*, with (air) and without (no air) access to the air-water interface. The times (e.g. 3 hours) indicate the time in darkness after the beginning of the dark phase when larvae were sampled. Means are plotted and the average sample sizes for calculating the means in each experiment are as follows: air for 17–23 hours=19; air for 14 hours=9; air for 3 hours=19; no air for 14 hours=7; no air for 23 hours=6. The asterisks indicate the mean volume was significantly different ($P<0.05$; Student's t-test) from the mean volume 3 hours after the beginning of the dark phase (air, 3 hours).

The swimbladder volumes over time in darkness with and without access to the air-water interface (Fig. 2) were compared to volumes after inflation at the beginning of the night phase (air, 3 hours) to indicate volume changes due to bubble ingestion and removal, and gas diffusion. Swimbladder volume increased in darkness when fish had access to the air-water interface and decreased when they lacked access. These changes were apparent after 14 hours in darkness but only become statistically significant ($P<0.05$; Student's t-test) after 17–23 hours (Fig. 2).

Thus, larvae with access to the air-water interface continued to actively inflate their swimbladder, whereas swimbladder volume slowly decreased in larvae that lacked this access. Nevertheless, shortly after the beginning of the light phase (Fig. 2; no air, 14 hours), the volumes in larvae without access to air were not significantly lower ($P>0.05$; Student's t-test) than levels after inflation at the beginning of the dark phase (air, 3 hours). Therefore, diffusion of gas from the swimbladder played a very small role in normal deflation at sunrise.

Relation of swimbladder deflation to light

Larvae deflated their swimbladders upon exposure to light. This response was demonstrated in the initial experiment, in which, at the end of the night, larvae were either maintained for three hours in darkness or exposed to white light for this time. The percentage of larvae with a deflated swimbladder in darkness was 12% ($n=75$), whereas the percentage in light was significantly ($P<0.001$; Z-test) greater at 84% ($n=25$). Further studies indicated percent deflation depended upon light inten-

sity (Fig. 3), as the percent deflation increased as light intensity increased. The lowest light intensity to evoke a significant ($P<0.05$; Z-test) increase in deflation (threshold) was 9×10^{12} photons·cm^{-2}·s^{-1}.

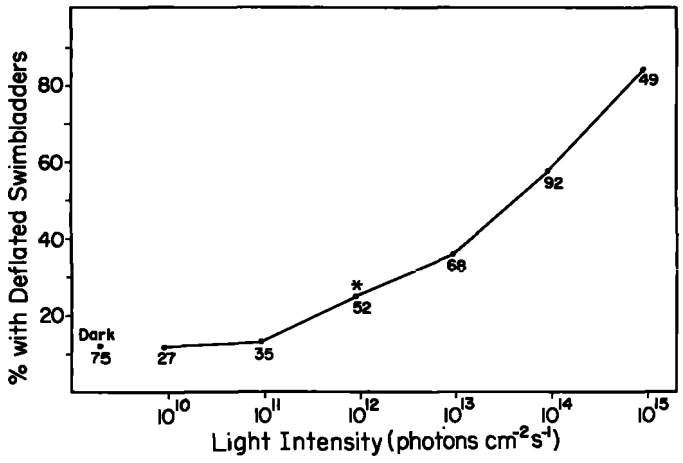

Figure 3

The percentage of larval Atlantic menhaden, *Brevoortia tyrannus*, with deflated swimbladders upon exposure to different light levels and darkness. The number below each point is the sample size. The asterisks indicate the lowest light level at which the proportion deflated was significantly ($P<0.05$; Z-test) greater than the level in darkness.

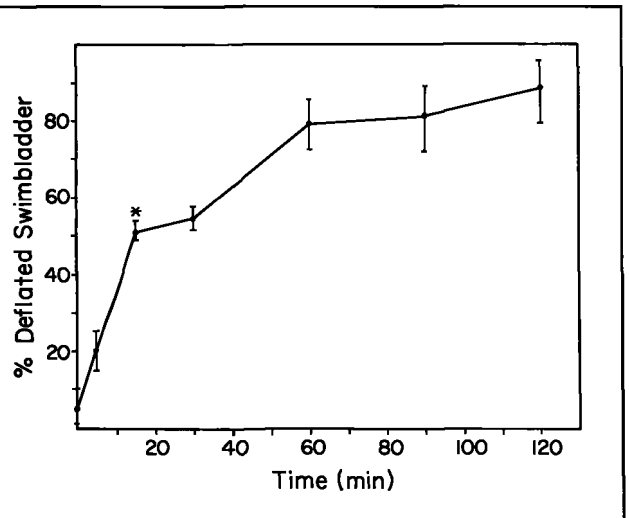

Figure 4

The percentage of larval Atlantic menhaden, *Brevoortia tyrannus*, with deflated swimbladders after different times in light. Means and standard errors are plotted. The average number of replicates was four. The asterisk indicates the first time at which the mean percent was significantly ($P<0.05$; t-test) greater than the initial level (time 0).

Timing of swimbladder deflation

The time course of swimbladder deflation in response to white light was measured upon transfer from darkness to 1.7×10^{15} photons·cm^{-2}·s^{-1}. By producing the maximum rate of light intensity change, it was assumed that the maximum rate of deflation would be evoked. An increase in the percent deflated was evident after 5 minutes and was significantly ($P<0.05$; t-test) greater than the initial level after 15 minutes (Fig. 4). Microscopic examination indicated this rapid deflation was accomplished by passing bubbles from the swimbladder into the gut and then out through both the anus and mouth.

Endogenous rhythm in swimbladder deflation

The percent deflation remained low if larvae were kept in continuous darkness (Fig. 1), which indicates there was no endogenous rhythm in deflation without exposure to light. However, light-cued deflation was not constant over time (Fig. 5). This experiment was conducted twice and involved maintaining larvae in darkness and measuring a subsample after exposure to light for one hour at different times during the solar day. The consistent cycle in both trials was that light-cued deflation was low during the normal dark phase and high during the normal day phase.

An interesting observation was made during these experiments. At the last sampling time, larvae had been in darkness for 27 hours, over which time they continued swimbladder inflation. Exposure to light at 2200 hours caused minimal deflation (Fig. 5). The water containing these larvae also had brine shrimp nauplii from the rearing tank. Larvae are visual predators and had, in effect, been starved for 27 hours in darkness. Under these conditions, once exposed to the light, they began to feed. While measuring swimbladder volumes, their digestive tracts were full of nauplii. Thus, larvae can feed with an inflated swimbladder.

Discussion

Swimbladder deflation by larval Atlantic menhaden is cued by an increase in light intensity. There was no cycle in which larvae inflated their swimbladders at sunset (Forward et al., 1993) and then deflated them gradually over time. In darkness at the end of the night, larvae, with and without air, had inflated swimbladders, and their volumes were not significantly reduced through the night. Thus, deflation

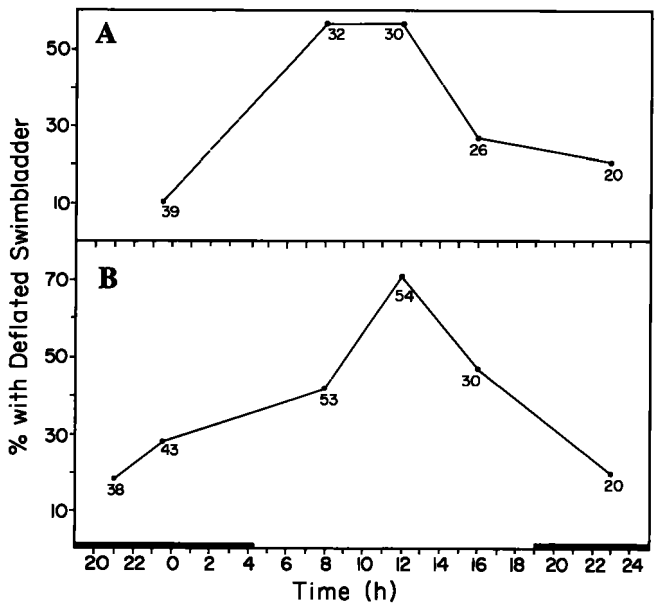

Figure 5

The percentage of larval Atlantic menhaden, *Brevoortia tyrannus*, with deflated swimbladders over the solar day. Larvae were maintained in continuous darkness and then exposed to light for one hour. The experiment was replicated twice (**A** and **B**). The dark bar indicates the time of the normal dark phase of the rearing light-dark cycle. The number under each point is the sample size.

through gas diffusion as suggested by Hoss and Phonlor (1984) did not occur.

If larvae were exposed to light near the time of the beginning of the light phase, deflation began within 5 minutes and was statistically apparent in 15 minutes. Rapid deflation occurred as the pneumatic duct opened between the gut and swimbladder and gas passed into the alimentary canal, where it was moved to both the mouth or anus for expulsion. The lowest light intensity that evoked deflation was about 10^{12} photons·cm^{-2}·s^{-1}. This threshold is below the lowest light intensity that inhibits inflation at sunset (12–16 mm larvae; 6×10^{13} photons·cm^{-2}·s^{-1}; Forward et al., 1993). Thus, larvae appear to be more sensitive to light at sunrise than at sunset. Maximum nighttime light intensity from the moon and star light is about 10^{11} photons·cm^{-2}·s^{-1} (McFarland and Munz, 1975; Lythgoe, 1979). Because this value is below the threshold intensity (10^{12} photons·cm^{-2}·s^{-1}) for deflation, moon and star light probably will not initiate deflation at night. Since surface light levels are about 10^{17} photons·cm^{-2}·s^{-1} at noon (Lythgoe, 1979), an intensity of 10^{12} photons·cm^{-2}·s^{-1} occurs earlier, probably near sunrise.

Larvae appear to have an endogenous rhythm in light-cued deflation. If they were maintained in constant darkness, light induced a low percent deflation during the night phase and a high percentage during the day phase. This rhythm is the reverse of the inflation rhythm, in which sudden darkness initiates inflation at night but rarely during the day (Forward et al., 1993). The functional significance of the deflation rhythm may be that 1) larvae do not deflate their swimbladder at night in response to any light and 2) they are "prepared" for rapid deflation at sunrise.

Field studies suggest Atlantic menhaden larvae undergo nocturnal diel vertical migration (DVM), in which they remain at moderate depths during the day and occur near the surface at night (Govoni and Pietrafesa, in press). Swimbladder inflation at sunset would increase buoyancy and reduce larval sinking rate (Hoss et al., 1989), which would maintain larvae closer to the surface. The present laboratory study supports a nocturnal DVM pattern by indicating that the percentage of larvae with inflated swimbladder and swimbladder volumes increased through the night, when larvae have access to the air-water interface. These increases are not predicted if larvae inflate their swimbladder only once at sunset and then sink. Thus, there is probably a cycle during the night, in which larvae sink while remaining motionless and then periodically return to the surface for additional gas. This pattern would retain larvae near the surface, which may be useful for transport from the offshore spawning area to the mouth of an estuary (Hoss et al., 1989).

A final consideration in the present study is why larvae deflate their swimbladders. Clearly, Atlantic menhaden larvae are adapted for deflation at sunrise. Their rhythm indicates they are most responsive to a light intensity increase at this time, and deflation occurs within 15 minutes. Such a dramatic response suggests deflation has an important functional advantage.

A fully inflated swimbladder may reduce the speed of movement and, thereby, the effectiveness of prey capture. Larvae feed during the day and use vision to find their prey (Blaxter and Hunter, 1982). Although a reduction in capture efficiency is possible, larvae with fully inflated swimbladders can still capture prey as observed in the rhythm experiment. Alternatively, an inflated swimbladder may increase detection of menhaden larvae by visual predators. Since larvae are relatively transparent, the difference in refractive index between air and water increases the contrast between an inflated swim-

bladder and the surrounding water. This increase in visibility could lead to increased predation. Thus, deflation at sunrise may be a predator avoidance response.

Acknowledgments

This research was funded by the National Oceanic and Atmospheric Administration Coastal Ocean Program Office through grant CF92-07 to the North Carolina Sea Grant Program.

Literature cited

Blaxter, J. H. S., and J. R. Hunter.
1982. The biology of clupeoid fishes. Adv. Mar. Biol. 20:1–223.

Forward, R. B., Jr., L. M. McKelvey, W. F. Hettler, and D. E. Hoss.
1993. Swimbladder inflation of the Atlantic menhaden *Brevoortia tyrannus*. Fish. Bull. 91:254–259.

Govoni, J. J., and L. J. Pietrafesa.
In press. Eulerian views of layered water currents, vertical distribution of some larval fishes and inferred advective transport over the continental shelf off North Carolina, USA, in winter. Fish. Oceanography.

Hettler, W. F.
1983. Transporting adult and larval gulf menhaden and techniques for spawning in the laboratory. Prog. Fish. Cult. 45:45–48.

Hoss, D. E., and G. Phonlor.
1984. Field and laboratory observations on diurnal swim bladder inflation-deflation in larvae of gulf menhaden, *Brevoortia patronus*. Fish. Bull. 82:513–517.

Hoss, D. E., D. M. Checklery Jr., and L. R. Settle.
1989. Diurnal buoyancy changes in larval Atlantic menhaden (*Brevoortia tyrannus*). Rap. P.-v. Reun. Cons. int. Explor. Mer. 191:105–111.

Hunter, J. R., and C. Sanchez.
1976. Diel changes in swim bladder inflation of the larvae of the northern anchovy, *Engraulis mordax*. Fish. Bull. 74:847–855.

Lythgoe, J. N.
1979. The ecology of vision. Clarendon Press, Oxford, 244 p.

McFarland, W. N., and F. W. Munz.
1975. The evolution of photopic visual pigments in fish. Vision Res. 15:1071–1080.

Munz, F. W.
1958. The photosensitive retinal pigments of fish from relatively turbid coastal waters. J. Gen. Physiol. 42:445–459.

Tracy, H. C.
1920. The membranous labyrinth and its relation to the precoelomic diverticulum of the swimbladder in clupeoids. J. Comp. Neurol. 31:219–257.

Uotani, L.
1973. Diurnal changes of gas bladder and behavior of postlarval anchovy and other related species. Bull. Jpn. Soc. Sci. Fish. 39:867–876.

Walpole, R. E.
1974. Introduction to statistics. Macmillan, New York.