PRESSURE SENSITIVITY OF ATLANTIC HERRING, *CLUPEA HARENGUS* L., LARVAE[1]

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ABSTRACT

Larval Atlantic herring, *Clupea harengus harengus* L., are known to change their vertical distribution by day and night, but it is not clear if they can sense their depth by perception of hydrostatic pressure. Two experiments were designed to test whether herring larvae would respond to imposed pressure changes by making appropriate compensatory vertical movements and whether such ability could be related to the development of the bulla system (stage I, bulla absent; stage II, bulla liquid-filled; stage III, bulla gas-filled). In the first experiment, pairs of larvae were exposed to a fixed sequence of pressure changes (ΔP) from ±13 cm H_2O to ±66 cm H_2O. Members of simultaneously tested pairs tended to be influenced by one another when responding to pressure change. The response of stage-I larvae tended to first increase and then decrease over a 20-min test period for a given ΔP. Stage-II and stage-III larvae showed better performances in compensating for imposed pressure changes than did stage I. Larvae exposed to a sudden pressure increase of 5 atm (atmospheres) (5,000 cm H_2O) before the experiment did not perform as well as those not so exposed, but the differences were not statistically significant. A second experiment tested the response of individual larvae to randomized sequences of pressure changes. Stage-III larvae moved most frequently to compensate for the pressure changes, but stage-I and stage-II larvae also responded to changes in pressure. Both experiments show that herring larvae of all three stages compensate for applied pressure changes by moving up when pressure is increased and down when it is decreased, but that they rarely move sufficiently far in the vertical plane to fully compensate.

Larval fish are known to change their vertical distribution diurnally. Although this behavior is probably controlled by changes in light intensity, it is not clear whether hydrostatic pressure perception is important in limiting or controlling the depths reached at different stages of the vertical migration cycle. A few workers (e.g., Qasim et al. 1963) have shown that fish larvae can respond to pressure changes; in particular, Bishai (1961) and Blaxter and Denton (1976) have shown that Atlantic herring, *Clupea harengus harengus* L., larvae are pressure sensitive.

The most likely site for a pressure receptor is a gas-filled structure, such as a swim bladder, which, if compliant, undergoes large changes in volume during vertical movements (10 m change of depth being equivalent to 1 atmospheric pressure). However, clupeoids, together with some other groups such as mormyrids, have gas-filled bulla. In herring the bulla appears to be sensitive to pressure changes (Allen et al. 1976; Denton and Blaxter 1976). In herring the prootic bulla has two parts: one filled with gas, the other with perilymph. The two parts are separated by an elastic membrane. This membrane responds to pressure changes, driving the perilymph in or out of a fenestra, which is situated close to the utriculus of the inner ear. The gas-filled part of the bulla is also connected to the swim bladder by a very narrow gas duct. This connection allows the prootic membrane to adapt to slow changes of pressure. If the pressure increases, the membrane bows in and being elastic tends to return to its resting position. The swim bladder wall is compliant and the pressure differential created along the gas duct causes gas to flow into the bulla from the swim bladder. If the pressure decreases, the membrane bows outward (into the perilymph space) and gas flows from the bulla back to the swim bladder.

In the fully functional system described above the bulla may respond to hydrostatic pressure changes, but because the system adapts in 15-30 s, there will be no perception of absolute pressure. In the very early larval stages of herring (from hatching to 18 mm TL) no bulla is present; the bulla appears at about 18 mm and usually is filled with gas by 26 mm. The swim bladder is not fully formed until 35 mm or more (Blaxter

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and Denton 1976). One would predict that herring larvae up to 18 mm would have little or no pressure sensitivity. As the bulla becomes filled with gas but before the swim bladder develops, we would expect very high sensitivity to absolute pressure (no adaptation being possible) and herring larvae from 26 to 35 mm would be in this category. Larger larvae would retain sensitivity to pressure change, but the development of the adaptation mechanism would prevent its being an absolute sense. One also would predict that herring larvae with gas-filled bullas but no swim bladders would be especially vulnerable to large pressure changes that could cause the membrane to burst. Hoss and Blaxter (1979) have shown that herring larvae do appear to be especially vulnerable to large, rapid pressure changes at about this stage of the life history. Blaxter and Hoss (1979) followed the development of the adaptation mechanism, measured its time constant, and have shown that adaptation usually does not develop until a length of >30 mm.

This paper describes a detailed analysis of pressure sensitivity in herring larvae, using the hypothesis that a larva will swim up to compensate for increasing pressure and down to compensate for decreasing pressure and that this is due in part to the development of the bulla system. In the two experiments to be described, particular attention was paid to measuring changes in sensitivity during the development of the bulla-swim bladder system. In addition, the effect of a large, rapid pressure change on subsequent pressure sensitivity also was investigated in one experiment.

MATERIALS AND GENERAL METHODS

Herring were reared from fertilized eggs, using the techniques of Blaxter (1968). The temperature during development increased from about 7°C near hatching to 12°C, 4 or 5 mo later. The pressure sensitivity experiments were conducted in a constant temperature room at 9°-10°C, using the apparatus of Blaxter and Denton (1976). This apparatus consisted of a Plexiglas[4] cylinder 80 cm high and 7 cm in diameter, the transparent wall being marked on the outside to give 16 equal sections numbered 1-16. The surface was designated 0, the bottom as 17. This allowed an observer to record the position of a larva in the cylinder at any given instant with a number from 0 to 17. The pressure in the cylinder could be changed by a preset amount by opening a two-way tap at the top, which exposed the water surface to atmospheric pressure or to positive or negative pressures in a gas reservoir.

Each larval herring was anesthetized after it was tested and the developmental stage of its bulla (stage I, no bulla; stage II, bulla liquid-filled; stage III, bulla gas-filled) was ascertained. A complication arose that the bulla does not become instantaneously filled with gas and may contain only a few or many bubbles. Pressure sensitivity is more likely to be high if the bulla is full of gas. At least 10 larvae of each developmental stage were used.

EXPERIMENT I

Design

Pairs of larvae of approximately equal length and stage of development were tested simultaneously. After a 2-3 min acclimation period at atmospheric pressure, 10 observations on the position of each fish were made at 15-s intervals. The pressure was then changed and the observations were repeated at the new pressure.

The pressure sequence selected was based upon prior research (Blaxter and Denton 1976) and involved changing the pressure from atmospheric to each of the following pressures four times: ±13, ±39, and ±66 cm H_2O (1 cm H_2O = 0.001 atm), for a total of 480 observations and 47 changes of pressure (Fig. 1). This fixed sequence of increasing pressure differentials was chosen to avoid the potential danger of larvae becoming overstimulated initially at the higher pressures. Earlier evidence (Blaxter and Denton 1976) indicated that larvae moved upwards to compensate for increased pressure and downwards to compensate for decreased pressure, and the extent of vertical movement was correlated with the extent of pressure change (ΔP) applied. Large pressure changes early in the sequence might not only block responses to smaller subsequent pressures but might also cause earlier fatigue. Therefore, pressure changes were not randomized and an experiment commenced regardless of larval distribution in the water column. Approximately half the larvae used in Experiment I were preexposed for 1 min to an abrupt pressure increase

[4]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

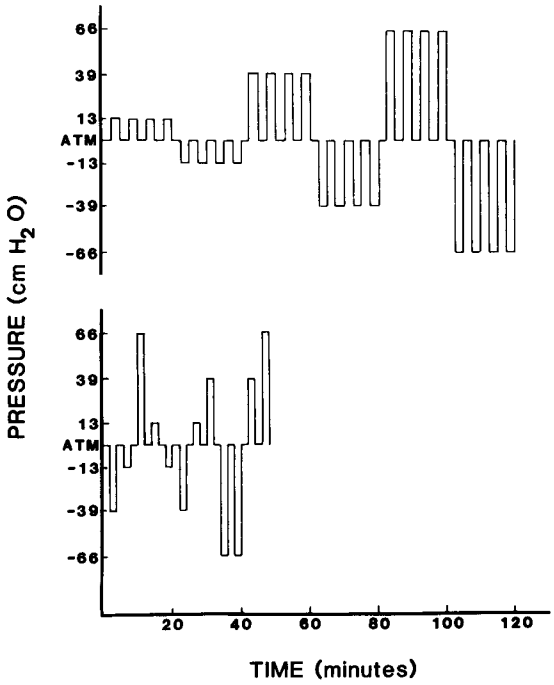

FIGURE 1.—The upper figure depicts the fixed pattern of pressure changes to which pairs of larval herring were exposed in Experiment I. The lower figure shows an example of one of the randomized sequences of pressure changes to which a larval herring was exposed in Experiment II. An independently randomized sequence was drawn for each larval herring tested.

of 5 atm (5,000 cm H_2O) before the onset of the regular pressure series to determine if this abrupt ΔP would impair subsequent pressure sensitivity differently in the different developmental stages. These are referred to as treated larvae, whereas those not preexposed are referred to as control larvae.

Results

Although pairs of larvae selected for simultaneous testing were judged visually to be of equal length, the developmental status of their bulla systems was evaluated only after they were subjected to the pressure tests and was sometimes found to differ (Table 1).

A total of 480 locations within the cylinder were recorded for each herring tested. We averaged 10 locations of a larva during a 2.5-min test at a given pressure to obtain a more concise summary of the response pattern. We then assigned a score to the larva for each 2.5-min series: +, if its average position indicated it had moved in the vertical direction to compensate for the change in pressure; −, if it moved in the opposite direction; and nr, if it showed no net change in its vertical position. We have used these assigned scores in the analyses to follow. Larvae not responding were treated as if they had moved in the noncompensatory direction, so the analyses are conservative. The scoring technique allows one to evaluate the frequency with which a fish, contending with a dynamic pressure regime, moved correctly, i.e., moved in the appropriate vertical direction to compensate for the imposed pressure change.

A separate analysis of variance of the number of compensatory responses was calculated for each treatment group, using only the data for members of homogeneous pairs to determine whether the paired larvae tend to respond together. The intraclass correlation coefficients ranged from 0.91 to 0.92 for the two stage-I groups to 0.20 and 0.32 for the two stage-III groups, respectively (Table 1). Thus for the least, well-developed fish (stage I) the variation among members of a pair was only one-ninth as great as the variation between average values for pairs of fish, i.e., the two members of a pair tended to respond together as a unit. The stage-II control group was an exception to this general pattern. For that group the variation among members of a pair was greater than the variation among pairs, suggesting that the members of a pair tended to move away from one another as pressure within the cylinder was changed.

The lack of independence in the responses of fish tested simultaneously invalidates use of the data for mixed pairs (i.e., pairs of fish of different developmental stages) and requires that we consider pairs of larvae as the experimental unit in testing hypotheses about the average performances. An analysis of variance of data for homo-

TABLE 1.—Numbers of herring larvae, numbers of pairs of larvae of the same developmental stage, and intraclass correlation coefficients for fish tested in Experiment I.

	Control	Preexposed to 5 atm (treated)
Stage I		
Number of individuals	9	8
Number of homogeneous pairs	4	4
Intraclass correlation coefficient	0.92	0.91
Stage II		
Number of individuals	15	11
Number of homogeneous pairs	4	4
Intraclass correlation coefficient	−0.42	0.41
Stage III		
Number of individuals	10	11
Number of homogeneous pairs	2	4
Intraclass correlation coefficient	0.20	0.32

geneous pairs disclosed that stage-I fish moved vertically to compensate for the imposed pressure changes less often than those possessing a more developed bulla system (Table 2). No advantage for fish possessing gas-filled bullas rather than liquid-filled bullas was detected. Although there was a consistent tendency for herring previously exposed to 5 atm to move vertically to compensate less frequently than those not so exposed, this tendency was not statistically significant. The overall intraclass correlation coefficient was 0.67, implying that the average variation among pairs within a treatment group was twice that between members of a pair.

The hypothesis testing reported above ignores the fact that a larval herring could respond in three ways to the 47 changes in pressure; it could move vertically to compensate, it could move vertically in the opposite direction, or it could simply maintain its current position within the cylinder. A plot of the data for pairs of larvae shows that nonresponse to changing pressure was more frequent for stage-I larvae than for the more developed fish (Fig. 2). Two pairs of larvae in particular, one for the stage-I control group and one from the stage-I treated group, are clearly outliers, showing no response during 49% and 86% of the trials, respectively. If these two pairs are dropped from the analysis, then the differences reported above are no longer significant and the means are 27.7 and 25.2, respectively (instead of 23.8 and 19.8) and are close to those for the corresponding stage-II groups (Table 2).

A plot of moving averages of the percentage of compensatory responses against the sequential series of pressure changes reveals that the stage-I herring exhibited a rather consistent pattern of

TABLE 2.—Analysis of variance of number of compensatory moves of fish during pressure sensitivity tests, and table of means: I = bulla absent, II = bulla liquid filled, III = bulla gas filled.

Source	df	Mean square	F
Preexposure	1	45.47	0.49
Developmental stage	2	314.26	3.40
II versus III	1	88.05	0.95
I versus II and III	1	540.47	5.85*
Interaction	2	28.48	0.31
Experimental error	16	92.44	5.08**
Sampling error	22	18.20	

Intraclass correlation coefficient = 0.67

Average number of compensatory moves per fish:

	Control	Preexposed to 5 atm
Stage I	23.8 (51%)	19.8 (42%)
Stage II	28.9 (61%)	26.1 (56%)
Stage III	32.2 (68%)	30.5 (65%)

*$P<0.05$; **$P<0.001$.

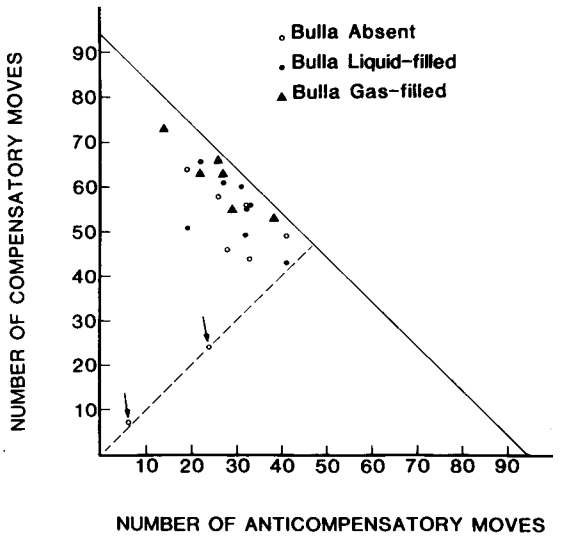

FIGURE 2.—Total numbers of compensatory vertical movements of a pair of herring plotted against the corresponding number of anticompensatory movements for that pair. The dashed line indicates the evenly divided response expected under the null hypothesis. Nonresponse is indicated by the vertical (or horizontal) distance from the hypotenuse. Control and preexposed pairs are not plotted separately. Arrows indicate two outliers.

response over the 20 min test period for a given ΔP, especially for the larger ΔP's (Fig. 3). Performance improved at the onset of a new increment or decrement of pressure and then fell off as the test continued, only to improve when the next increment or decrement was used. The other two developmental stages showed a relatively high initial frequency of compensation that rapidly decreased, then subsequently increased until the test of the final pressure change.

Discussion

The results in Figures 2 and 3 clearly show that some larvae are responsive to pressure. However, the relatively small sample size, the correlation in the behavior between members of a pair simultaneously tested, and the relatively high variation in response among experimental units within a given treatment group reduced our ability to distinguish differences in the response to changes in pressure of herring of different developmental stages. Additional problems in interpreting the first experiment arose from the evidence that tests of a given pressure

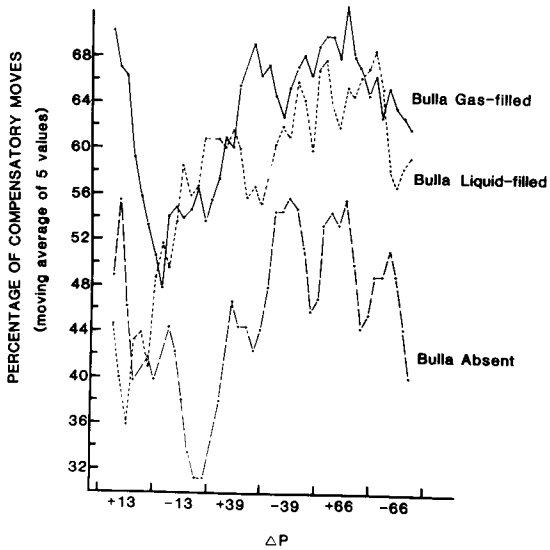

FIGURE 3.—Moving averages of the percentage of correct vertical movements for the three developmental stages of herring in the first experiment. Data for control and preexposed herring are not plotted separately.

change were too long and that the test series as a whole was too protracted as well. The fixed nature of the pressure series, while reducing the chance that larvae would be overstimulated if initially exposed to higher pressure changes, confounded possible differences in response to different pressure changes with habituation to the stimulus or possible learning effects. A second series of experiments was designed therefore to reduce or eliminate some of these problems.

EXPERIMENT II

Design

The major features of the second design were:

TABLE 3.—Analysis of variance of the number of compensatory responses of herring in the second experiment and table of means: I = bulla absent, II = bulla liquid filled, III = bulla gas filled.

Source	df	Mean square	F
Developmental stage	2	62.9394	5.86**
I versus II	1	0.4091	0.030
I and II versus III	1	125.4697	11.68**
Experimental error	30	10.7394	
Average number of compensatory responses per fish:			
Stage I	14.18 (62%)		
Stage II	13.91 (60%)		
Stage III	18.18 (79%)		

**$P<0.01$.

1) a single factor, developmental stage of the bulla system; 2) all herring tested individually; 3) an independently randomized test sequence for each fish; 4) each pressure change tested twice; 5) shorter duration of the test series (Fig. 1b) (the larvae were subjected to 23 changes of pressure rather than 47 as in the first experiment); and 6) experiments started when herring were at the center of the test column. Eleven herring of each developmental stage were tested.

Results

As in the first experiment, average positions within the test cylinder were calculated for each pressure change. Vertical movements, as indicated by successive differences in these averages, were then scored as compensatory, anticompensatory, or no response. Analysis of variance of the number of scores indicated that herring with gas-filled bullas compensated more frequently than herring having either liquid-filled bullas or no bullas at all (Table 3; Fig. 4). The stage-III herring on the average moved vertically to compensate 79% of the time compared with 60% and 62% for stage-II and stage-I herring, respectively. However, in Figure 4 we show that even several stage-I larvae achieved relatively high scores, suggesting that a different test of the hypothesis might be based upon classifying larvae into two

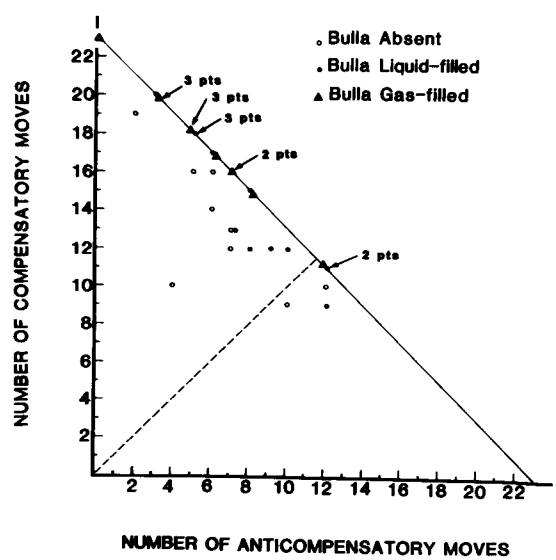

FIGURE 4.—Total numbers of compensatory vertical movements by individual herring in Experiment II plotted against the number of anticompensatory moves. (See legend for Fig. 2.)

categories: 1) those that made more compensatory movements than one would expect by chance, and 2) those that did not.

In Experiment II, each herring was exposed to a random sequence of 23 changes in pressure. If we regard nonresponse as noncompensatory, then the binomial distribution provides a basis for classifying the larvae into two groups, those that moved in the compensatory direction more frequently than one would expect by chance, and those that did not. Under the null hypothesis the probability that a herring would make 16 or more compensatory shifts in vertical position is <0.05. Using this criterion, we classified 5 of the stage-I larvae, 4 of the stage-II larvae, and 10 of the stage-III larvae as having made more compensatory vertical movements than one would expect by chance. A chi-square test showed that the stage-III larvae more frequently compensated for the imposed pressure change than the two earlier stages (chi-square = 7.69, df = 2, $P<0.03$). This implies that the bulla system contributes to the larval herring's hydrostatic pressure perception only after it contains gas.

Because the average position of a larval herring was determined for each pressure level, we could calculate the average vertical distance it moved for each change in pressure. The average distances moved for the 19 successful fish were regressed against the corresponding change in pressure (Fig. 5). The lines for stage-I and stage-II larvae nearly coincide and their slopes are about half that of the regression for stage-III herring. The greatest departure from these lines, fitted through the origin, is for the stage-III herring at the −66 cm $H_2O\,\Delta P$. They failed to move downward in the column as much as their performance at other pressure changes would predict. We note that even the stage-III herring moved only about 17% of the distance required to compensate fully for an imposed pressure change.

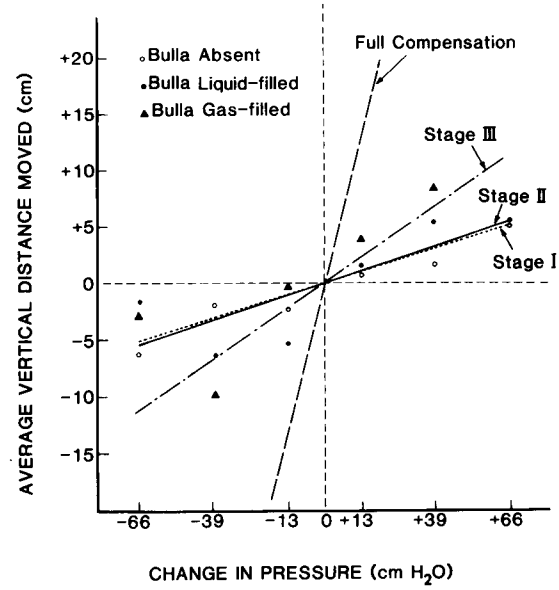

FIGURE 5.—Average vertical distances moved by herring larvae to compensate for changes in pressure.

Discussion

The average proportion of tests in which a larval herring moved vertically to compensate was higher in the second experiment than in the first. This was probably due to the shorter duration of the trials and the test series which should have reduced any effects of habituation or fatigue. However, the random nature of the pressure changes in the second experiment may also have contributed to the enhancement of the response.

Both of the experimental designs employed in this investigation yielded information. The first design revealed that when two herring are tested simultaneously, the response of each is influenced by the presence of the other. It also revealed that the response of a herring to a repeated pressure change tends to increase and then decrease over a 20-min period. The second design provided a more satisfactory test of the null hypothesis that a herring's response to pressure change is independent of the developmental stage of the bulla system and also confirmed an implication from the first experiment: namely, that even before the full development of the bulla system, herring are capable of detecting changes in pressure of the magnitude used in this investigation. Finally, the second experiment demonstrated that herring possessing a gas-filled bulla system will exhibit a markedly improved performance when compared with less mature larvae.

In very few instances did the larvae move a sufficient vertical distance to fully compensate for the imposed pressure change—a similar finding to that of Blaxter and Denton (1976). Even the stage-III larvae, on the average, only moved 17% of the distance to compensate fully. This is partly a statistical artifact of the manner in which we measured a larva's response. We re-

corded its position at 15-s intervals over the 2.5-min period of a given ΔP and then averaged those 10 values. Thus, unless a larva either overcompensated or fully compensated during the first 15 s, its average position during the 2.5-min test would necessarily not be at the level of compensation.

The vertical and horizontal limits of the apparatus also probably impeded vertical progress of larvae in some instances, because the position of the larva within the apparatus at the initiation of a change in pressure determined the potential vertical distance the larva could move to compensate; this would be most important as a source of bias at the larger ΔP's.

Still another possible explanation for the incomplete nature of the compensation may lie in the artificiality of the experiment. Fish are not normally subjected to abrupt hydrostatic pressure changes as they swim with a vertical component. It is difficult to design an experiment to show a hydrostatic pressure sense in a free swimming vertically moving fish larva. Gibson[5] has shown, however, that the activity of juvenile plaice (which lack a swim bladder) varies regularly during sinusoidal changes of hydrostatic pressure of amplitudes of about 50 cm H_2O repeated over a 4-h period, thus demonstrating sensitivity to slow changes of pressure in a fish without a swim bladder.

The site of pressure sensitivity in the herring larvae has not been identified, but it seems to be related to the bulla because sensitivity is enhanced when the bulla is full of gas. It is possible that abrupt changes of pressure applied to the top of a column of water might generate particle displacements in the water that could be perceived by neuromast organs. We do not believe this is a likely explanation of the observed pressure sensitivity in stage-I and stage-II larvae, however, because in some experiments the pressure change was applied over about 5 s, which reduced any resonant effects in the apparatus, but was equally successful in causing correct responses.

Because the swim bladder serves as a gas reservoir for the bulla, the bulla cannot provide perception of absolute pressure for a juvenile or an adult herring. However, in the larva the development of the gas-filled bulla precedes that of the swim bladder and therefore the bulla may temporarily serve as a depth indicator (Blaxter et al. 1981), permitting a larva to limit the maximum depth reached during vertical movements initiated by changes in light intensity. Having a mechanism to limit the maximum depth of vertical migration may enable a larva to maintain its position in the water column. This could be of adaptive value similar to that described for anchovy by Hunter and Sanchez (1976), in that it may serve to keep larvae together and facilitate the development of schooling. A depth indicator might also serve as an energy-saving mechanism if it enables a larva to maintain its position in that portion of the water column where food is most abundant.

In conclusion, we have found that herring larvae display pressure sensitivity both before and after the bulla system has developed, although it is enhanced in larvae with a gas-filled bulla. The threshold of sensitivity was not determined but lies below 13 cm H_2O (1 cm Hg). For a herring larva near the sea surface this observation implies that pressure sensitivity is <1.3% of the ambient pressure. Prior treatment of larvae to 5 atm pressure did not significantly impair sensitivity.

[5]R. N. Gibson, Principal Scientific Officer, Dunstaffnage Marine Research Laboratory, P.O. Box No. 3, Oban, PA34 4AD, Argyll, Scotland, pers. commun. March 1979.

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