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AVOIDANCE REACTION OF YOLK-SAC LARVAE OF THE INLAND SILVERSIDE *MENIDIA BERYLLINA* (ATHERINIDAE) TO HYPOXIA

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Summary

Larvae of the inland silverside *Menidia beryllina* (Atherinidae) were observed in a chamber with two horizontal water layers to study their aversive response to hypoxia. When larvae descended from an upper normoxic layer into a lower hypoxic layer, they displayed a characteristic avoidance reaction consisting of a burst of fast swimming that always ended in an upward direction leading the larva away from the hypoxic region. Each swimming burst lasted for approximately 2–3 s, with a maximum speed of approximately 25 mm s^{-1}, which is approximately four times the maximum swimming speed observed in the normoxic layer. The avoidance reaction was present in larvae from 6 h until 64 h post hatching. At 72 h we observed 50 % mortality as a result of starvation. The avoidance reaction was correlated with the O_2 concentration but not with the N_2 concentration nor with salinity or with salinity gradients. The avoidance response was observed at O_2 levels ranging from 4.7 to 0.8 mg O_2 l^{-1}. For all concentrations below 3.8 mg O_2 l^{-1}, the distribution was significantly different from that observed in normoxic control layers of the same salinity. No avoidance responses were observed at 6.6 mg O_2 l^{-1}. We propose that the avoidance reaction of larvae of *M. beryllina* to hypoxic water is an innate behavioural adaptation to the hypoxic conditions present in the estuaries that are the spawning, hatching and nursery grounds of this species.

Key words: *Menidia beryllina*, silverside, avoidance reaction, innate behaviour, oxygen, swimming burst, teleost larva.

Introduction

Hypoxic conditions can occur in many aquatic environments as a result of the combined effects of geomorphology, physical processes in the water and organic and nutrient loading. In some cases, hypoxic regions are permanent and extensive, while in others they occur sporadically and locally. In either case, only fish that tolerate hypoxia will survive. Because hypoxic regions are lethal to most fish, it would be advantageous for the fish if they could be detected and avoided. The present paper describes a distinctive avoidance reaction that occurs when larval fish encounter a hypoxic environment. In previous attempts to study the responses of adult fish to hypoxic environments, horizontal gradients of oxygen have usually been used. In one of the first studies on fish responses to hypoxia, Shelford and Alle (1913) concluded that fishes are clearly affected by a lack of oxygen. Høglund (1951) showed that roach (*Leuciscus rutilus*) and minnows (*Phoxinus phoxinus*) tended to avoid low oxygen concentrations. Jones (1952) exposed sticklebacks (*Gasterosteus aculeatus*), minnows (*P. phoxinus*) and trout fry (*Salmo trutta*) to low oxygen levels and concluded that: ‘fish do not have any instinctive ability to recognise immediately and avoid water of abnormal low oxygen content’. However, Petersen and Petersen (1990) studied the behaviour of the sand goby (*Pomatoschistus minutus*) in a hypoxic environment and showed that at oxygen saturations below 40 % the fish became restless and performed random activity that would eventually lead them to normoxic water. Young red hake (*Urophycis chuss*) were shown to move upwards in the water if the oxygen concentration fell below 4.2 mg O_2 l^{-1} (Bejda et al., 1987), and walleyes (*Stizostedion vitreum vitreum*) lost their innate negative phototactic response under conditions of oxygen depletion (Scherer, 1971). Also, Petrosky and Magnuson (1973) and Magnuson et al. (1985) reported upward swimming behaviour in adults of several species of freshwater fish during extreme hypoxia (0.25 mg O_2 l^{-1}) in ice-covered lakes.

In addition to the responses of adult fish described above, larval and juvenile fish also respond to hypoxic conditions. For example, postlarval flounders (*Paralicthys lethostigma*) showed non-random ‘withdrawal’ from waters with oxygen concentrations below 5.3 mg O_2 l^{-1} (Deubler and Posner, 1963). Ammocoetes of *Ichthyomyzon hubbsi* responded to low oxygen levels with an increase in the rate and amplitude of

beating of the branchial basket (Potter et al., 1970), while another ammocoete, *Geotria australis*, emerged from their substratum when exposed to hypoxic conditions (Galloway et al., 1987). Larval and early postlarval sole (*Solea solea*) showed no avoidance response in a horizontal gradient of dissolved oxygen. However, in vertical oxygen gradients, they migrated upwards at oxygen concentrations of less than 2.5 mg O_2 l^{-1} (Macquart-Mouling, 1997). Despite the results described above, characteristic avoidance responses of fish larvae to hypoxic conditions have not been adequately documented (Spoor, 1984; Kramer, 1987).

The purpose of this study was to evaluate the responses to hypoxia of larvae of the inland silverside (*Menidia beryllina*). We developed a method of creating a distinct hypoxic region by using two horizontal water layers, which allowed us to observe the larval distribution. This enabled a detailed description of larval behaviour and a quantification of their avoidance reaction.

Materials and methods

Incubation of eggs and larvae

In a commercial hatchery, eggs of the inland silverside *Menidia beryllina* (Cope, 1869) (Atherinidae) were spawned onto floating polyester floss mats in large tanks containing 15‰ salinity sea water at 26 °C, and maintained on a 16 h:8 h L:D photoperiod. Sea water was recirculated through a subsoil gravel filtration system. Fertilized eggs were transferred to other tanks and maintained at 20‰ salinity under otherwise similar conditions. One day before hatching (approximately 5 days after fertilization), eggs were transported to the laboratory and kept in plastic beakers containing aerated 24‰ sea water at 26 °C under continuous light (250–330 μW). Eggs were induced to hatch by placing them in the dark for 1 h in the early morning (05:00 h) of the day they were expected to hatch. This synchronised hatching took place at approximately 06:00 h. Larvae were not fed and died when the yolk-sac was reabsorbed approximately 3 days post hatch.

Preparation of artificial sea water

Artificial sea water (ASW) was used in all experiments. ASW at 35‰ was prepared as follows: 24.7 g of NaCl, 0.66 g of KCl, 1.9 g of $CaCl_2$, 4.7 g of $MgCl_2$, 6.3 g of $MgSO_4$ and 0.18 g of $NaHCO_3$ were dissolved in 1000 ml of distilled water, and the pH was adjusted to 8.0 using 1 mol l^{-1} NaOH (Cavanaugh, 1964). ASW with a lower salinity was obtained by dilution with distilled water. Salinities were monitored with an American Optical special scale refractometer (American Optical, Buffalo, NY, USA).

When used in trials involving hypoxic water, the ASW was depleted of oxygen by bubbling with N_2 for more than 2 h. To ensure low oxygen levels, 25 % pyrogallol in a 10 % solution of sodium bicarbonate was bubbled through the N_2 gas (Gustafsson et al., 1966), before the N_2 gas was bubbled into the experimental solution. The different oxygen concentrations used in the experimental trials (see below) were obtained by mixing various amounts of oxygen-rich and oxygen-poor ASW under an atmosphere of nitrogen. Oxygen concentrations were measured using a YSI 50B oxygen meter equipped with a YSI 5739 probe (Yellow Springs Instrument Co., Yellow Springs, Ohio, USA).

Establishing saltwater layers in the observation chamber

Larval swimming was observed in acrylic chambers, 9 cm×15 cm×6 cm (width, height, depth), containing two distinct water layers of different salinities and therefore of different densities. The slow diffusion of ions and oxygen in water makes it possible to create horizontal layers with different properties so that the layers remained stable on the time scale of the present study (maximum 15 min). The acrylic chamber was partially filled with 150 ml of ASW of 28‰ salinity; thereafter, 150 ml of ASW of 31‰ salinity was introduced slowly through a tube opening at the bottom. This simple procedure created two clearly distinct layers, and the horizontal border could be observed under appropriate illumination. In a pilot test using different-coloured water in a chamber containing 10 *M. beryllina* larvae, the distinction between the two layers was evident for more than 24 h. In all experiments, except those concerning preferred salinity or inversion of hypoxic layers (see Table 1), the water used for the bottom layer (31‰) was made hypoxic before introduction into the chamber, whereas the upper layer (28‰) was close to saturation with oxygen (approximately 6.5 mg O_2 l^{-1}; 100 % saturation is 7.3 mg O_2 l^{-1} at 25 °C, 28‰ salinity and 100 kPa).

Observations of larvae

The swimming behaviour of *M. beryllina* larvae was recorded from the side for 5 min using a Sony CCD-VX3 Hi8 video camcorder at a distance of approximately 80 cm. Each 5 min observation period with 10 larvae in the tank is referred to as one trial. At least 30 min before a trial, larvae were acclimated to ASW of the same salinity, temperature and O_2 concentration (normally 28‰) as those of the upper layer in the acrylic chambers. In each trial, 10 individuals that had not been used in previous trials were carefully transferred into the upper layer using a polyethylene Pasteur pipette with a wide, fire-polished tip to avoid injury. Because no change in salinity or temperature occurred when the larvae were transferred, distortion of layers was minimal. Each trial started 2–3 min after transfer of larvae to the acrylic chamber. Because *M. beryllina* larvae show positive phototaxis (F.-A. Weltzien, K. B. Døving and W. E. S. Carr, personal observation), we used the minimum light level necessary to make larvae clearly visible on the video screen during trials, and no phototactic responses were observed. Light intensity, measured immediately in front of the chamber using a light intensity meter (Sekonic L-398, Hamburg, Germany), was kept between 0.5 and 1.5 μW. The oxygen concentration in the two water layers was measured within 30 s after completion of filming. To avoid mixing of water layers during measurements, water at the tip of the probe was continuously renewed by gentle

Table 1. *List of trials performed with* Menidia beryllina *larvae to evaluate their avoidance reaction to hypoxia*

Type of trial	Time post hatch (h)	Upper layer Salinity (‰)	Upper layer [O_2] (mg l^{-1})	Lower layer Salinity (‰)	Lower layer [O_2] (mg l^{-1})	Significance
Dose–response	38–44	28	6.3	31	0.8	***
	38–44	28	6.4	31	1.1	**
	38–44	28	6.2	31	1.9	***
	38–44	28	6.4	31	2.0	**
	38–44	28	6.5	31	3.0	*
	38–44	28	6.4	31	3.2	*
	38–44	28	6.4	31	3.8	*
	38–44	28	6.3	31	3.9	NS
	38–44	28	6.4	31	4.6	NS
	38–44	28	6.5	31	4.7	NS
	38–44	28	6.7	31	6.6	NS
	38–44	28	6.7	31	6.6	NS
	38–44	28	6.9	31	6.6	NS
Ontogeny	6	28	5.9	31	0.9	*
	7	28	6.0	31	0.9	*
	10	28	5.7	31	1.2	*
	11	28	5.6	31	0.9	*
	32	28	6.1	31	1.2	*
	33	28	4.9	31	1.1	*
	38	28	5.7	31	0.9	*
	39	28	6.0	31	1.0	*
	54	28	4.9	31	1.2	*
	55	28	5.5	31	1.1	*
	63	28	6.4	31	0.8	*
	64	28	4.6	31	1.0	**
Inverted layers	61	31	2.4	34	6.1	**
Hypoxia by vacuum	52	28	6.1	31	1.6	*
	53	28	5.8	31	1.7	*
Preferred salinity	65	16	7.1	19	6.9	NS
	50	22	7.1	25	6.9	NS
	6	31	6.9	34	6.4	NS

Each trial is listed together with the corresponding larval age (hours post hatch), salinity and oxygen concentration in the two water layers, type of trial, and level of significance for each individual trial.

Levels of significance: *P<0.05; **P<0.01; ***P<0.001; NS, not significant.

stirring without disturbing the water layers. Two experimental trials, one a replicate of the original trial (2×10 larvae observed for 5 min), were performed for each oxygen concentration, each larval age and each experimental condition.

Test design

After having established that larvae of *M. beryllina* showed an avoidance reaction to hypoxia, we performed a series of experiments to describe the properties of this avoidance reaction under different conditions. The experiments, all involving two water layers of different salinities, are summarized in Table 1 and were as follows. (1) Nine control trials established the distribution of larvae in the experimental chamber during normoxic conditions. (2) A dose–response relationship was established in an experiment involving 13 individual trials, each with a level of oxygen ranging from 0.8 to 6.6 mg O_2 l^{-1} in the lower 31 ‰ layer. Two or three trials (i.e. one or two replicates) were performed for each oxygen concentration. (3) The ontogeny of the avoidance reaction was revealed by using 12 individual trials of larvae ranging in age from 6 to 64 h post hatch. In this experiment, we used oxygen concentrations of approximately 6 mg O_2 l^{-1} in the upper water layer and approximately 1 mg O_2 l^{-1} in the lower water layer, and two trials (i.e. one replicate) were performed for each larval age. (4) To obtain a better impression as to whether the avoidance reaction was a random approach to high oxygen levels or an innate fixed reaction pattern occurring when larvae were exposed to low oxygen levels, larvae were tested in a situation that is probably uncommon in their natural environment: one trial measured larval response to a low oxygen concentration in the upper 31 ‰ layer of water and a normoxic lower layer of 34 ‰ salinity. (5) Two trials (i.e. one replicate) measured larval responses to a low oxygen concentration in the lower layer obtained by vacuum using

water suction instead of nitrogen bubbling: these trials determined whether the avoidance reaction was the result of a high nitrogen content rather than of low oxygen concentration. (6) Finally, three trials measuring larval responses to normoxic conditions in layers of 16 and 19‰, 22 and 25‰, and 31 and 34‰ salinity in the upper and lower layers, respectively (instead of the usual 28 and 31‰ salinity) determined whether the larvae had a preferred salinity that could have affected the interpretation of the results obtained for the avoidance reaction. The larval distribution in all these trials was compared with that of normoxic controls using salinities of 28 and 31‰.

Data analysis

Video recordings were used to make counts, every 30 s, of the number of larvae in the upper and lower water layers. During each 5 min trial, these counts gave 10 determinations of the vertical distribution of the 10 larvae used in the trial. The larval distribution for each of these 10 distributions in an experimental trial was compared with distributions observed in corresponding control experiments. The data were fitted to a 2×2 contingency table and analysed using Fisher's exact probability test (N=10 larvae) using tables from Statistica 5.0 for Windows (Sokal and Rohlf, 1995). If at least eight of the ten observations within one experimental trial gave probability values that differed significantly from their corresponding control observations, then the experimental trial was interpreted as being significantly different from the control. Because individual control trials did not differ significantly (P>0.05), all nine control trials were collapsed to give one average set of distribution of larvae.

Vertical movements of larvae (total swimming distance and corresponding velocities) were analysed using the NIH image program version 1.61 (National Institutes of Health, 1997). The 10 individual larvae from one trial were followed for 5 min with a cursor on the video screen, and the x (horizontal) and z (height position) coordinates of the cursor position were registered every 0.28 s. Larval movements during the 5 min trial period were calculated from the x,z cursor positions and displayed in the figures either as the vertical position *versus* time (see Fig. 1A,B) or as the x,z position in the chamber within one defined time interval during the 5 min period (see Fig. 2).

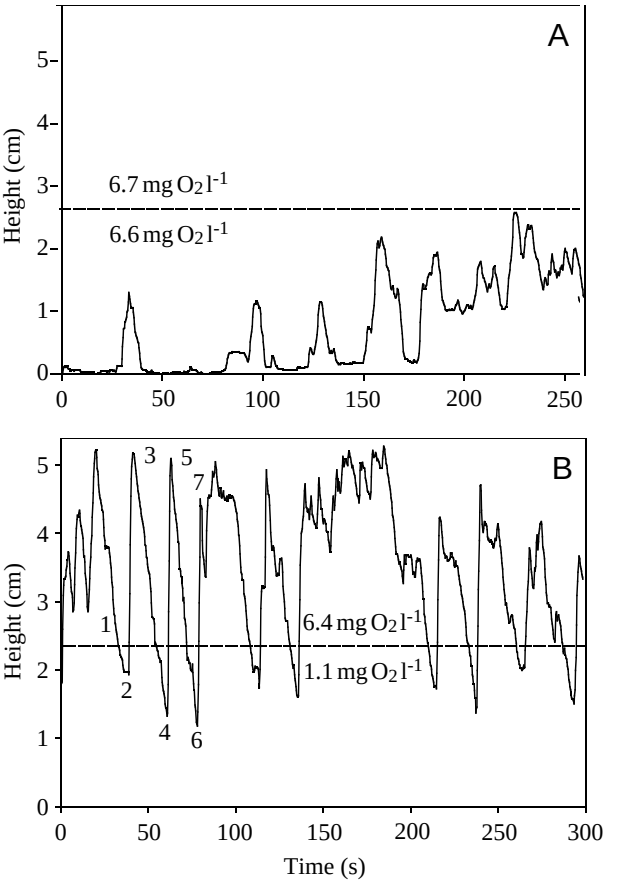

Fig. 1. The vertical position of a single *Menidia beryllina* larva in the experimental chamber plotted as a function of time during (A) control conditions with water layers of 28 and 31‰ salinity, with oxygen concentrations of 6.7 and 6.6 mg l^{-1}, respectively, and (B) an experimental trial with a hypoxic lower layer, with oxygen concentrations of 6.4 mg l^{-1} (28‰) in the upper and 1.1 mg l^{-1} (31‰) in the lower layer. Numbers from 1 to 7 in B indicate the larval positions illustrated in Fig. 2. The broken line indicates the border between the two salinity layers.

Results

Behaviour at high oxygen concentration

Under control conditions, where the oxygen concentration was approximately 6.5 mg O_2 l^{-1} in both water layers, *M. beryllina* larvae (at all stages and in all salinities tested) were negatively buoyant; i.e. they sank slowly to the bottom of the chamber. Thus, 70–80% of the larvae were at or near the bottom during most of the 5 min observation periods. The larvae performed occasional bursts of swimming leading to a higher vertical position, and then sank slowly towards the bottom. A typical example of the vertical movements of a larva is illustrated in Fig. 1A. The swimming bursts lasted approximately 1–2 s with an observed maximum swimming speed of 6.3 mm s^{-1}. With a larval standard length of 3.9 mm, consistent with the results of Gleason and Bengtson (1996), the maximum swimming speed corresponds to approximately 1.6 body lengths s^{-1}. The mean total distance covered by one larva during the 5 min test period was 805±312 mm (mean ± S.D., N=20), a swimming speed of 2.7 mm s^{-1}.

Dose–response trials in low oxygen concentration

The distribution of larvae in chambers with a hypoxic lower layer was conspicuously different from that seen in chambers with two normoxic layers (Fig. 1A,B). After being placed in the upper layer (28‰ salinity) of high oxygen concentration (6.4 mg O_2 l^{-1}), larvae slowly sank into the lower layer (31‰ salinity) of reduced oxygen concentration (1.1 mg O_2 l^{-1}) (Fig. 1B). Shortly after a larva entered the hypoxic layer, it started swimming at high speed. The initial direction of

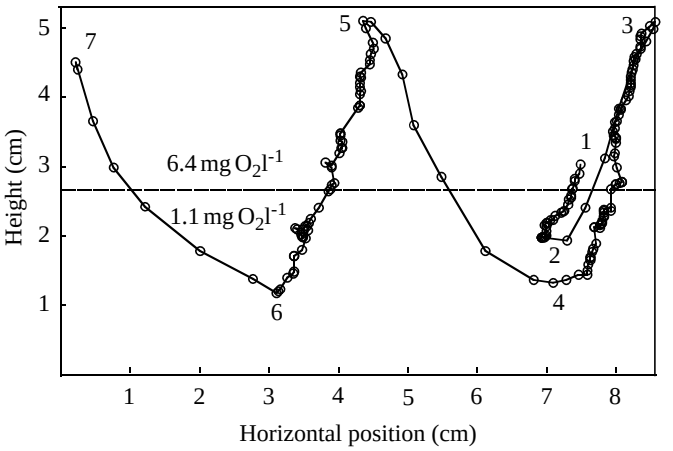

Fig. 2. Horizontal (*x*) and vertical (*z*) positions of the *Menidia beryllina* larva shown in Fig. 1B over a 1 min period. The distance between any two circles is equal to a period of 0.28 s. Tracking of the larva begins at position 1 and ends at position 7. Numbers 1–7 refer to numbers in Fig. 1B.

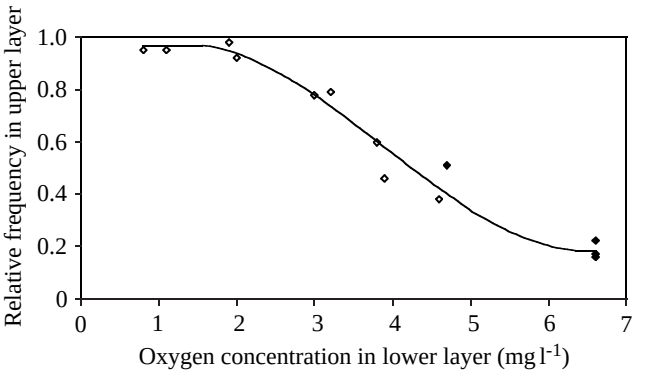

Fig. 3. Relationship between oxygen concentration and the avoidance reaction of *Menidia beryllina* larvae. The curve gives the relative frequencies of larvae present in the upper normoxic layer, during the 5 min trial period, compared with the total number of larvae in the experimental chamber. Each data point represents one experimental trial in which 10 larvae were observed for 5 min. The data were fitted to a third-order polynomial equation: $y=0.0116x^3-0.1357x^2+0.2993x+0.7875$ (N=13; r^2=0.97).

movement seemed to be random, but after a short interval (approximately 0.5 s), movement was directed upwards for 2–3 s. The mean swimming speed was 24.8 mm s^{-1} or approximately 6.4 body lengths s^{-1}, which is four times the maximum swimming speed observed in the normoxic layer. This behavioural response to a hypoxic layer will be referred to as an avoidance reaction.

A short subsample from Fig. 1B is given in Fig. 2 to illustrate the slow larval sinking and the burst-like upward swimming after the larva enters the hypoxic layer. As a result of the avoidance reaction, few if any larvae were in the lower hypoxic layer at any particular time. The mean total distance covered by one larva during the test period of 5 min was 1117±290 mm (N=20), a swimming speed of 3.7 mm s^{-1}.

Trials with decreasing levels of dissolved oxygen in the lower water layer showed that larvae displayed an avoidance reaction and that their distribution differed significantly from that of the controls (P<0.01) at all oxygen concentrations below 2 mg O_2 l^{-1}. Larvae responded in a concentration-dependent manner to oxygen concentrations of between approximately 0.8 and 4.7 mg O_2 l^{-1}. Within this concentration range, avoidance of the hypoxic layer remained significant (P<0.05) but decreased with increasing oxygen concentration in the hypoxic layer (Fig. 3). For all concentrations below 3.8 mg O_2 l^{-1}, the distribution in the two layers differed significantly from that of the controls with normoxic concentrations. Thus, the threshold for the avoidance reaction occurred at approximately 3.8 mg O_2 l^{-1}. No avoidance occurred at 6.6 mg O_2 l^{-1} (Table 1).

Ontogeny of avoidance reaction

Larvae of *M. beryllina* lived for only 72 h in the present experiments. The distribution of larvae at oxygen concentrations between 0.8 and 1.2 mg l^{-1} differed significantly from the distribution in control conditions for all ages between 6 and 64 h post hatch (P<0.05, Table 1). No significant effect of age was observed.

Low oxygen concentration in the upper ASW layer

When the upper layer (31‰ salinity) was made hypoxic (2.4 mg O_2 l^{-1}) and the lower layer (34‰ salinity) contained a normal high oxygen concentration of 6 mg O_2 l^{-1}, the larvae started an upward swimming burst soon after being placed in the upper hypoxic layer. Some larvae became 'trapped' at the upper surface film, making continued attempts to swim upwards. Larvae sinking through the upper hypoxic layer settled at the bottom and seldom moved towards the upper layer again. However, if a larva reached the upper hypoxic layer, it again performed a swimming burst moving it towards the water surface and it then became trapped at the upper surface. The average total distance covered by one larva during the test period of 5 min was 419 mm (N=2). Although some larvae (approximately 5%) became trapped at the upper surface, the distribution of larvae in the upper and lower layers did not differ significantly (P>0.5) from normal control experiments such as that shown in Fig. 1A. The larval distribution, however, differed significantly from that of trials in which the lower layer was made hypoxic (P<0.01) (Table 1).

Low oxygen concentration without nitrogen

The distribution of larvae at an oxygen concentration of 1.7 mg O_2 l^{-1} differed significantly from that observed in controls (P<0.05), but not from that observed in water through which nitrogen had been bubbled (P>0.5) (Table 1).

Water layers of different salinity

Larvae showed no significant difference in distribution between the two layers compared with the controls when

salinities of 16 and 19‰, 22 and 25‰, and 31 and 34‰ were used (Table 1).

Discussion

To the best of our knowledge, this is the first description of specific avoidance of water with a low oxygen concentration by teleost larvae. The reaction pattern is stereotypical and characteristic. After exposure to a low oxygen level for a short time, larvae of *Menidia beryllina* began swimming at high speeds that reached a maximum of approximately 25 mm s^{-1}, which is four times faster than the maximum swimming speed observed in fully oxygenated water. Swimming always ended with the larvae moving in an upward direction, even if their initial direction was horizontal or slightly downwards. The initial swimming direction was apparently related to the body position of the larva at the time of its detection of the low oxygen level. This behaviour pattern brought the larva out from the hypoxic region. Since the avoidance reaction was present from soon after hatching (6 h post hatch) until the larvae died from starvation, we considered it to be innate for unfed *M. beryllina* yolk-sac larvae.

Our chosen method of using horizontal layers with different salinities takes advantage of the different densities and layering caused by gravitation. This is, in fact, a small-scale model of a common natural property of the aquatic environment, wherein different water layers will have different origins. The stratification of the water masses is therefore a major source of information for aquatic animals, information that these animals can collect by moving in the vertical instead of the horizontal plane. For example, salmon use this feature of their environment in their migration to their home river (Døving et al., 1985). Teleost larvae in estuaries also use this feature, and the tidal cycle, to maintain their position and to avoid seaward tidal drift (Fortier and Leggett, 1983; de Lafontaine, 1990). However, few observations have been reported on the avoidance of low oxygen concentrations by aquatic animals. This could be because of the infrequent use of horizontal layers of differing salinities in the experimental design for studies on aquatic animal behaviour, which thereby ignores an important feature of the natural aquatic environment.

Menidia beryllina*: life and environment*

The natural habitats of *M. beryllina* include shallow regions of estuaries, coastal rivers and lakes from Massachusetts to the Mississippi Basin and southward to Vera Cruz, Mexico (Middaugh et al., 1993; Sherrill and Middaugh, 1993). This species is euryhaline and eurythermal and occurs at salinities ranging from a few parts per thousand to greater than 35‰ and at temperatures ranging from 9.8 to 30 °C (Middaugh et al., 1993).

The ability to detect and respond promptly to broad variations in ambient oxygen level is a valuable adaptation in a species such as *M. beryllina* whose life cycle is spent in a highly variable environment. Large differences in available oxygen would be expected in habitats characterised by wide ranges of salinity and temperature and with a high biochemical oxygen demand imposed by the presence of large amounts of decomposing detrital material. Oxygen levels ranging from 10.8 mg l^{-1} in winter to 3.0 mg l^{-1} in late summer have been recorded in the shallow habitats of *M. beryllina* in north-west Florida (Middaugh and Hemmer, 1992). The oxygen levels reported above were recorded in the morning and would be expected to be even lower at night when only respiration is occurring. For example, during night-time low tides, Wenner et al. (1999) recorded values below 2 mg O_2 l^{-1} in most estuarine tidal creeks monitored in South Carolina. However, when monitored during daytime low tides, the same tidal creeks had oxygen levels of approximately 4.0–5.5 mg O_2 l^{-1}.

Even during the daytime, increased respiratory activity by fish can contribute to a major decrease in dissolved oxygen levels in an estuarine environment similar to that occupied by both adults and larvae of the inland silverside *M. beryllina*. For example, Middaugh et al. (1981) showed that dissolved oxygen levels in the intertidal zone decreased from an ambient concentration of approximately 6 mg O_2 l^{-1} to below 1.0 mg O_2 l^{-1} during a single spawning run of the Atlantic silverside *Menidia menidia*. After spawning, spent fish moved to the air–water interface and then swam offshore to a position beyond the hypoxic zone. Other fish species that normally prey upon *M. menidia* were excluded from the hypoxic zone where the most intense spawning occurred (Middaugh et al., 1981).

A 24 h exposure to oxygen levels of less than 1.5 mg O_2 l^{-1} was lethal to 95 % of *M. beryllina* larvae ranging in age from less than 1 day to 12 days old (D. C. Miller, S. Poucher and L. Coiro, manuscript submitted for publication). These lethal levels correlate well with those in the current study, in which the most highly significant avoidance responses in *M. beryllina* larvae occurred at oxygen concentrations below 2 mg O_2 l^{-1} (Table 1). However, because the larvae had a distribution that differed significantly from that of controls at all oxygen concentrations below 3.8 mg O_2 l^{-1}, the hypoxic conditions were detected and avoidance was initiated well above lethal levels.

Regions of hypoxia

Regions of hypoxia are frequently encountered in the aquatic environment, and they often show temporal fluctuations. Examples include the occasional periods of hypoxia during low tide at night in water covering coral reefs (Kinsey and Kinsey, 1967; Wise et al., 1998) and at night in intertidal pools containing algae (Stephenson et al., 1934; Congleton, 1980; Davenport and Woolmington, 1981). Oxygen concentrations also decline in ice-covered lakes (Breitburg et al., 1994) and in the river Ob, where water can be close to anoxic during winter (Nikolsky, 1963). Examples of environments with local regions of hypoxia include the following: bottom waters of stratified lakes and seas (Wetzel, 1983; Swanson and Parker, 1988; O'Hara, 1993; Rahel and Nutzman, 1994), tropical floodplain forests (Courtenay and Keenleyside, 1983; Gehrke et al., 1993), stagnant eutrophic regions of tropical lakes (Payne, 1986; Breitburg et al., 1994; Wenner et al., 1999), the

deeper, more saline layers in fjords and brackish waters (Nissling, 1994) and estuaries (Swanson and Parker, 1988; Breitburg et al., 1994). In addition, the great oceans have regions with a low oxygen concentration. For example, a large body of water, with oxygen concentrations as low as $0.4\,mg\,O_2\,l^{-1}$, occurs at depths between 200 and 1200 m in the North Pacific off the American coast between the equator and 28°N (Sverdrup et al., 1946).

In summary, the present observations of larvae of *M. beryllina* demonstrate a consistent avoidance reaction to environments with a low oxygen concentration. When larvae encounter hypoxic conditions, they swim rapidly upwards away from the hypoxic region. This avoidance reaction occurs even at oxygen concentrations greater than those reported by others to be lethal to larvae of this species. The initial avoidance reaction is directed upwards, regardless of the oxygen content of the water above. Because larvae reacted to low oxygen levels at 6 h post hatch, we consider the behaviour to be innate for unfed yolk-sac larvae of *M. beryllina* and to be an example of a fixed reaction pattern. This response can be considered to be an adaptive advantage, because in most natural environments a high oxygen content will be encountered near the surface. Considering the widespread distribution of hypoxic environments in the aquatic biosphere, many species of fish may encounter hypoxic conditions during their life cycle. Thus, an innate behavioural pattern, such as that exhibited by unfed yolk-sac larvae of *M. beryllina*, is probably present in and important for both the larval and adult stages of a large number of teleost species.

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