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The effect of progressive hypoxia on school structure and dynamics in Atlantic herring *Clupea harengus*

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The effect of progressive hypoxia on the structure and dynamics of herring (*Clupea harengus*) schools in laboratory conditions was investigated. The length, width and depth of schools of about 20 individuals were measured from video recordings to test the hypothesis that during hypoxia fish schools change their shape and volume. School shape (calculated as the ratios of length/depth, width/depth and length/width) did not change significantly during hypoxia. School length, width, depth, area and volume were all significantly increased at 20% oxygen saturation. Volume, area and width were more sensitive to hypoxia; volume and width were also increased at 25% and area at 30% oxygen saturation.

The degree of position changing (shuffling) of individuals within the school was also analysed. Shuffling in normoxia was observed to occur largely through 'O-turn' manoeuvres, a 360° turn executed laterally to the school that allowed fishes in the front to move to the back. O-turn frequency during normoxia was 0.69 O-turns $\text{fish}^{-1}\ \text{min}^{-1}$ but significantly decreased with hypoxia to 0.37 O-turns $\text{fish}^{-1}\ \text{min}^{-1}$ at 30% oxygen saturation. Shuffling was also investigated by measuring the persistence time of individual herring in leading positions (i.e. the first half of the school). No significant changes occurred during hypoxia, indicating that the decrease in O-turn frequency does not affect shuffling rate during hypoxia, and that position shuffling in hypoxic conditions is mainly due to overtaking or falling back by individual fishes.

School integrity and positional dynamics are the outcome of trade-offs among a number of biotic factors, such as food, predator defence, mating behaviour and various physical factors that may impose certain limits. Among these, our results indicate that oxygen level modulates schooling behaviour. Oxygen alters whole-school parameters at oxygen saturation values that can be encountered by herring in the field, indicating that oxygen availability is an important factor in the trade-offs that determine school volume. An increase in school volume in the wild may increase the oxygen available to each individual. However, shuffling rate is not affected by hypoxia, indicating that the internal dynamics of positioning is the result of the balance of other factors, for example related to the nutritional state of each individual fish as suggested by previous studies.

Keywords: hypoxia; behaviour; swimming; schooling; herring; *Clupea harengus*

1. INTRODUCTION

Direct and indirect physiological responses to hypoxia in fishes are well documented and the effect of hypoxia may include species-specific impacts on fish distribution, behaviour, feeding rate, competition and vulnerability to predators (Burton *et al.* 1980; Coutant 1985; Kramer 1987; Pihl *et al.* 1991, 1992; Breitburg 1992; Breitburg *et al.* 1994). Hypoxic conditions are usually found in coastal areas, and both benthic and pelagic fishes may be subject to hypoxia at some time during their lives. Because the herring, *Clupea harengus* L. is a pelagic species, it can be assumed that oxygen is not normally a limiting factor for their schooling behaviour. However, herring can periodically experience hypoxia in Norwegian fjords (*ca.* 30% oxygen saturation reported by Dommasnes *et al.* (1994); *ca.* 22% oxygen saturation in Hognestad (1994)), as well as in other areas such as the Baltic Sea and the Kattegat (figure 1). According to observations made by Dommasnes *et al.* (1994) of northern Norwegian herring overwintering en masse in fjords at very low temperatures, the swimming speeds of individual fishes were estimated to be of the order of one body length per second, but the overall schools are almost stationary. Thus, there can be considerable shuffling of fishes within a school, even if the school itself has little ground speed.

Oxygen concentration in large schools may decrease towards the rear due to oxygen consumption by fishes further ahead. McFarland & Moss (1967) found a decrease in oxygen saturation of 30% in a 150 m long school of the grey mullet *Mugil cephalus* that were migrating along the coast. Green & McFarland (1994) reported a 10% decrease in oxygen saturation in schools estimated to contain more than 20 000 individuals of the blacksmith *Chromis punctipinnis* that were schooling whilst feeding against the current.

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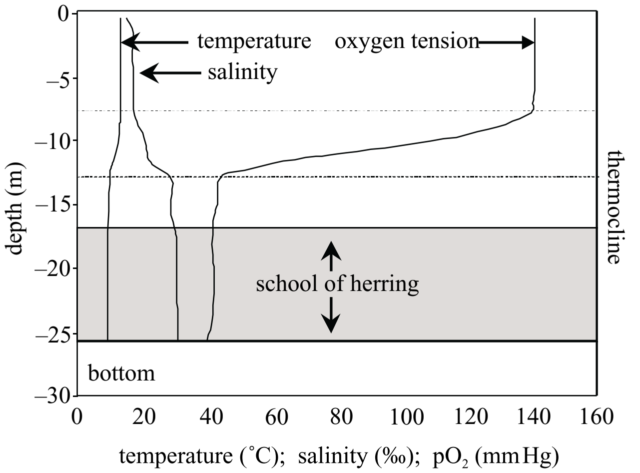

Figure 1. Field observation of herring schooling in hypoxic waters. Schools of herring are commonly observed in the hypoxic layer in the Kattegat during the autumn. This observation was made on 26 September 1991, in the position 55°58′819 N; 12°39′998 E. The school of herring was identified using a SIMRAD echosounder. Environmental variables were recorded using a CTD Seabird 911 plus. The vertical position of the school of herring is indicated by the shaded area. Oxygen level where the herring are located corresponds to *ca.* 25% oxygen saturation. The area between the two dotted lines indicates the thermocline.

Schooling behaviour can benefit fishes in a number of ways, such as improving predator defence (Godin 1986; Magurran 1990; Pitcher & Parrish 1993; Crook 1999), increasing feeding (Magurran *et al.* 1985; Pitcher *et al.* 1988) and reducing the energetic cost of swimming due to hydrodynamic advantages (Weihs 1973; Herskin & Steffensen 1998). Although the structure of a fish school can be influenced by a number of factors, McFarland & Moss (1967) suggested that many of the changes in internal dynamics, structure and shape could be a response to lowered ambient oxygen. A recent model based on fish oxygen consumption and nearest neighbour distance in a school showed that hypoxic conditions may limit school size (Steffensen 1995).

According to Krause *et al.* (2000), animal aggregations that show directional movement do not necessarily have leaders. There may be other factors, however, causing fishes to be in leading positions in a school, such as individual differences in nutritional state (Krause *et al.* 2000). The combination of advantages and disadvantages for individual fishes varies with position within a school and may be related to the species involved. With regard to predator–prey interactions, Bumann *et al.* (1997) suggested that the leading fishes of a school may incur a higher predation risk, but it has also been suggested that they will have preferential access to resources encountered by the group (Krause *et al.* 1992). However, work on the Atlantic silverside *Menidia menidia* (Parrish 1989) and the flat-iron herring *Harengula thrissima* (Parrish *et al.* 1989) showed that central locations were actually at higher risk than peripheral locations. There are also metabolic trade-offs associated with location within a school; fishes at the rear may experience physiological stress due to hypoxia (McFarland & Moss 1967; Green & McFarland 1994) but, conversely, they can experience the hydrodynamic advantage of reduced drag (Herskin & Steffensen 1998).

Earlier studies of northern anchovy *Engraulis mordax* schools (Moss & McFarland 1970) indicated that a slow decrease of dissolved oxygen concentration did not affect swimming speed but caused small changes in density and nearest neighbour orientation only at near-lethal oxygen concentrations. Recent work on the effect of progressive hypoxia in herring schools has shown that swimming speed is affected and that school disruption and variations in swimming speed during severe hypoxia (defined as less than 50% oxygen saturation) were modulated by the school's swimming speed prior to hypoxia (Domenici *et al.* 2000*b*). The same authors put forward the hypothesis that changes in speed observed in hypoxic conditions may result from changes in the shuffling of individual positions in the school. To test this hypothesis, we have investigated the effect of progressive hypoxia on school dynamics in laboratory conditions. Furthermore, we propose that hypoxia will increase the volume of fish schools so that a greater volume of water and therefore more oxygen is available to each individual. To test this hypothesis, we measured the size of schools in three dimensions at different levels of oxygen saturation.

2. MATERIAL AND METHODS

(a) *Experimental animals*

Atlantic herring *C. harengus* (total length (L) 12.3 ± 0.65 cm; mean ± s.e.m.; n of sub-sample = 20) were caught in Dunstaffnage Bay, near Oban, Scotland, using a beach seine. They were held in flowing seawater in large circular tanks (2 m in diameter; 90 cm depth) at the local ambient conditions of temperature and salinity for several months before the experiments. The experiments were performed during the month of August, at a water temperature of 15 ± 1 °C.

(b) *Experimental apparatus*

The experimental tank measured 2 m in diameter by 1 m high and the water depth was 60 cm. A video camera mounted 2 m above the tank was connected to a VCR and the entire experiment was recorded at 25 frames s^{-1}. Temperature and percentage oxygen saturation were recorded using a combined temperature sensor and oxygen electrode (WTW Oxi196 Microprocessor Oximeter) close to the tank wall, 20 cm above the bottom of the tank, and connected to a computer via an analogue data interface board (Data Translation DT2801). Details of this methodology are reported in Domenici *et al.* (2000*b*). During experiments, oxygen saturation was reduced by circulating water from the bottom of the tank through a cylinder (50 cm in diameter, 2 m high) where the water was exposed to a stream of nitrogen bubbles. Water was continuously pumped from the bottom of the experimental tank to the bottom of the deoxygenation cylinder and overflowed from an outlet near the top, back into the experimental tank. This resulted in a gradual decrease of the percentage of oxygen saturation within the experimental tank. Mixing due to water circulation through the tank and the deoxygenation cylinder ensured that the oxygen level was uniform throughout the tank. At the end of the experiments, differences in percentage of oxygen saturation between three horizontal positions (the centre of the tank, midway between centre and wall, and 5 cm from the wall) and at three depths (immediately below the surface, midwater and bottom of the tank) were less than 1%.

(c) *Experimental protocol*

Herring were transferred from the holding tank to the experimental tank containing normoxic water (between 96 and 100% oxygen saturation) 1 day prior to the experiment. All experiments were conducted in the afternoon. A total of 206 herring were used in 10 replicate experiments in which schools of 19–22 herring were exposed to a progressive reduction in oxygen concentration using the apparatus described above. Oxygen saturation decreased from 95% to 25% in 219 ± 9 min (mean ± s.e.m.; $n = 10$). Before and during the experiments, fish schools were swimming in a circular clockwise manner at a distance of about 10–30 cm from the wall, until schooling behaviour (*sensu stricto* Pitcher 1983) ceased, school disruption occurred and the experiments were terminated. School disruption was defined as the break-up of the school when fishes did not show uniform orientations and were swimming in different directions. In all experiments, the school was disrupted at low oxygen concentration but at a different, precise level of oxygen saturation (between 12 and 25%) in each experiment (Domenici *et al.* 2000*b*). Because only one school remained cohesive at 15% oxygen saturation, data for 15% saturation were excluded from the statistical analysis. No fishes died during the experiments or subsequently.

(d) *Measurement of schooling parameters*

All parameters were calculated at 5% intervals of oxygen saturation. Dimension and shape parameters were estimated in the same position of the tank and in no case were they measured during O-turn manoeuvres. We included only fishes that were less than two body lengths away from their nearest neighbour. This distance was chosen on the basis that within schools inter-individual distances are about one body length (Pitcher & Parrish 1993; Domenici *et al.* 2000*a*), and therefore individuals at distances much more than one body length from the nearest neighbour cannot be considered part of the school. Various parameters were used to describe the school dimension and shape, and the school shuffling behaviour, as described below.

(e) *School dimensions and shape parameters*

(i) *School length*

This was calculated as the distance between the fish in the first position and the fish in the last position of the school. Because the fishes were swimming along a curved path, school length was calculated using a curved line that approximated the swimming path. Schools, as described by Pitcher & Partridge (1979) can be considered as oblate spheroids, therefore the leading fish and the last fish tend to be in the central portion of the front or back end, respectively. As a consequence, the curved line used to determined school length passed through the centre of the school.

(ii) *School width*

School width was calculated as the largest transverse distance between the fish in the innermost position (i.e. towards the centre of the tank) and the fish in the outermost position (i.e. towards the tank wall) within the school.

(iii) *School area*

This was calculated, using Scion Image software, as the top-view area of the polygon resulting from those fishes in the outer positions within the school.

(iv) *School depth*

School depth was calculated as the distance on the vertical plane between the fishes closest to the water surface and the fishes closest to the bottom. The position of the fishes on the vertical dimension was estimated from the offset in the horizontal plane between the positions of the fishes and their shadows on the tank floor resulting from single-point oblique lighting. This method was calibrated by using the discrete shadows formed by five rectangular-shaped pieces of cardboard (length 10 cm, depth 1 cm, width 0.5 cm) aligned tangentially to the wall, at a distance of 10 cm from each other along a pole that was perpendicular to the tank bottom, at two known radial distances from the tank wall. The positions of the fishes on the vertical plane were then calculated by using linearly interpolated intermediate values from the calibration equations.

(v) *School volume*

School volume (V) was estimated following Pitcher & Partridge (1979), i.e. as an oblate spheroid using the formula $4/3\pi xyz$, where x, y and z are half the school length, width and depth as described above. School volume was expressed as the volume available to each fish in body lengths cubed (i.e. L^3), by dividing the total volume of the school by the number of fishes (Pitcher & Partridge 1979).

(vi) *Total school volume*

Total school volume (V_t) was estimated by considering the edge of the school as lying further out than the position of the individuals at the edge. As suggested by Pitcher & Partridge (1979), the true boundary of the school was considered to be half a nearest neighbour distance (NND) outside its remote member. Total school volume was therefore estimated for the purpose of comparison with previous work that considers the true boundary of the school. The NND value used was 0.82 body length, based on Pitcher & Partridge's (1979) data for 12 cm herring in schools of 20–30 individuals (Partridge *et al.* 1980) cruising at speeds between 20 and 29 cm s^{-1} (i.e. similar speeds to those observed in our experiments reported in Domenici *et al.* (2000*b*)). Like school volume, total school volume was expressed as the volume available to each fish, by dividing the volume of the school by the number of fishes (Pitcher & Partridge 1979).

(vi) *Shape parameters*

These were calculated as the ratios of school length/depth, width/depth and length/width.

(vi) *School shape index*

This was calculated as the position of the maximum width along the length of the school, relative to the length of the school (i.e. from 0 to 1; if the maximum width was close to the front of the school, the index was close to 0, while it was close to 1 if the maximum width was close to the back of the school).

(f) *School shuffling*

(i) *O-turn distributions*

These were calculated using the rank position of a given fish at the beginning of its O-turn manoeuvre. O-turn manoeuvres consisted of a 360° turn, mainly by fishes in the front executed on the outside of the school (laterally), at the end of which the fish joined the back of the school. The O-turns considered were those observed during a 2 min sample recording at each saturation level, i.e. 1 min before and 1 min after each 5% interval oxygen saturation level.

(ii) *O-turn frequency*

O-turn frequency (O-turns fish^{-1} min^{-1}) was calculated as the number of O-turn manoeuvres shown by fishes during the same 2 min period.

(iii) *O-turn index*

This was calculated as the number of fishes, out of the three first ranked fishes, that used an O-turn manoeuvre in order to reposition themselves from the first position to the second half of the school. This index, therefore, has values from zero to three. The three focal fishes were chosen as fishes that found themselves in the first position of the school, in succession starting from the time of the chosen saturation levels.

(iv) *Persistency index*

This was calculated as the average time the three focal fishes (chosen as for the O-turn index) stayed within the first half of the school, starting from the instance when each focal fish reached the first position. This was considered as an index of the amount of reshuffling that occurs in a school, i.e. a high persistency index corresponds to a low reshuffling rate.

(g) ***Statistical analysis***

Statistical analysis was based on repeated-measures ANOVA (using Proc Mixed in v. 8 of Sas), followed by a Dunnett test to compare the values at each oxygen saturation with the control value at normoxia (95% oxygen saturation). Because the shape parameters (length/width; length/depth; width/depth) are ratios, they were log transformed in order to apply the repeated-measures ANOVA. Because shuffle index is a meristic variable spanning from 0 to 3, shuffle index data were ranked using Sas Proc Rank in order to apply the repeated-measures ANOVA. The shape index, being a true proportion, was arcsine transformed before analysis.

3. RESULTS

(a) ***School dimensions and shape***

All school dimension parameters were significantly affected by hypoxia, although this effect commenced at different oxygen saturation levels for each parameter. None of the many variables measured changed significantly at 35% oxygen saturation or greater (figures 2–4), with the exception of a spurious observation of length/depth ratio at 65% oxygen saturation. Because three schools were disrupted at oxygen concentrations above 20% and these experiments terminated, any effect observed at 20% saturation is based on only 7 of the 10 experiments. Volume (both V and V_t), area and width were more sensitive to hypoxia than length or depth; both width and volume (V and V_t) increased significantly at 25% and below (figures 2*b* and 3*b*,*c*, respectively) and area at 30% oxygen saturation and below (figure 3*a*). School length and school depth were statistically different from the control only at 20% oxygen saturation (figure 2*a*,*c*, respectively). School shape parameters were not affected by hypoxia (figure 4).

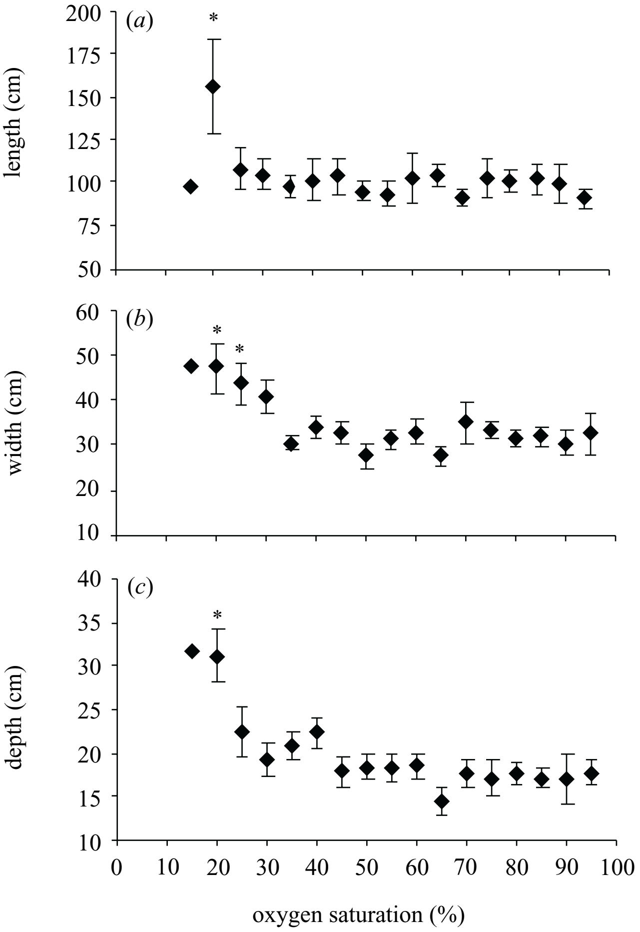

Figure 2. The average length (*a*), width (*b*) and depth (*c*) of herring schools at 5% intervals of oxygen saturation. Vertical bars indicate s.e. The asterisks indicate significant differences from normoxia.

Animals moving in a group at a high speed may need more inter-individual space to manoeuvre (i.e. a larger volume) than at low speed. Therefore, the possibility that the increase in school volume was an indirect effect of an increase in speed (measured for these runs in Domenici *et al.* (2000*b*)) was investigated by running a regression analysis of average V_t versus speed at each oxygen saturation level (figure 5*a*). The results show that V_t is not related to speed ($r^2 = 0.12$; $p > 0.1$; d.f. = 1,14), and that the highest V_t occurred at the lowest velocity, at 20% oxygen saturation (figure 5*a*). The pattern behind such a relationship is illustrated in figure 5*b*, which shows an example experiment in which speed increases at *ca.* 40% oxygen saturation, followed by a decrease at saturations below 30%. During these changes in speed, V_t increases steadily and does not reflect the pattern seen in swimming speed.

(b) ***School shuffling***

The distribution of the position at which O-turn manoeuvres are initiated by individual fishes shows that O-turns mainly occur in fishes in the leading positions of the school. The shape of this distribution does not appear to be affected by hypoxia (see absolute and relative frequency distribution; figure 6*a*,*b*, respectively). The occurrence of O-turn manoeuvres, however, decreases significantly with hypoxia at 30% oxygen saturation and lower (figure 7*a*). O-turn index, a measure of the use of O-turn manoeuvres for repositioning, also decreased with

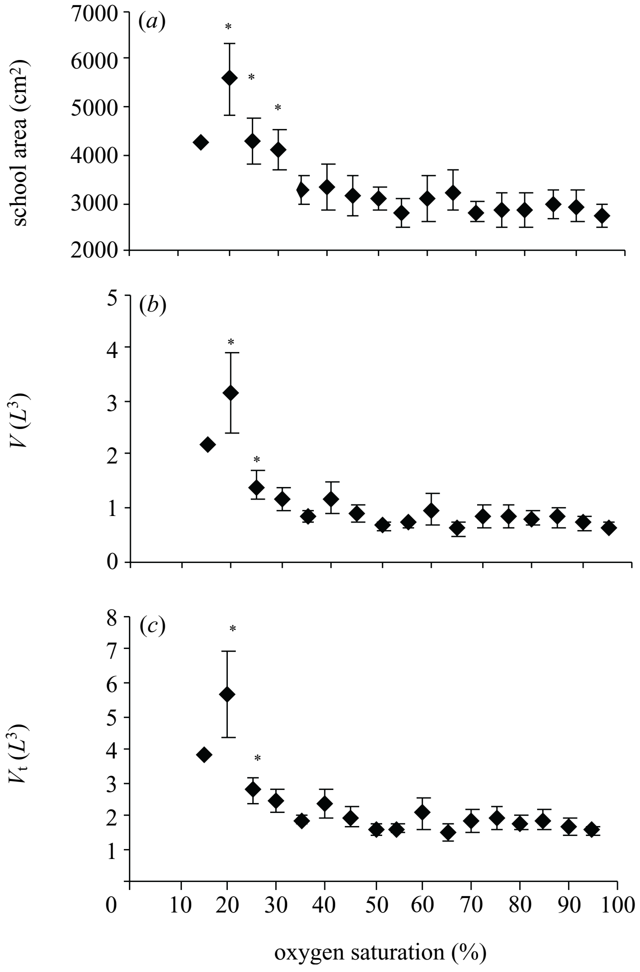

Figure 3. The average area (*a*), school volume per fish V (*b*), and total school volume per fish V_t (*c*) of herring schools at 5% intervals of oxygen saturation. Vertical bars indicate s.e. The asterisks indicate significant differences from normoxia.

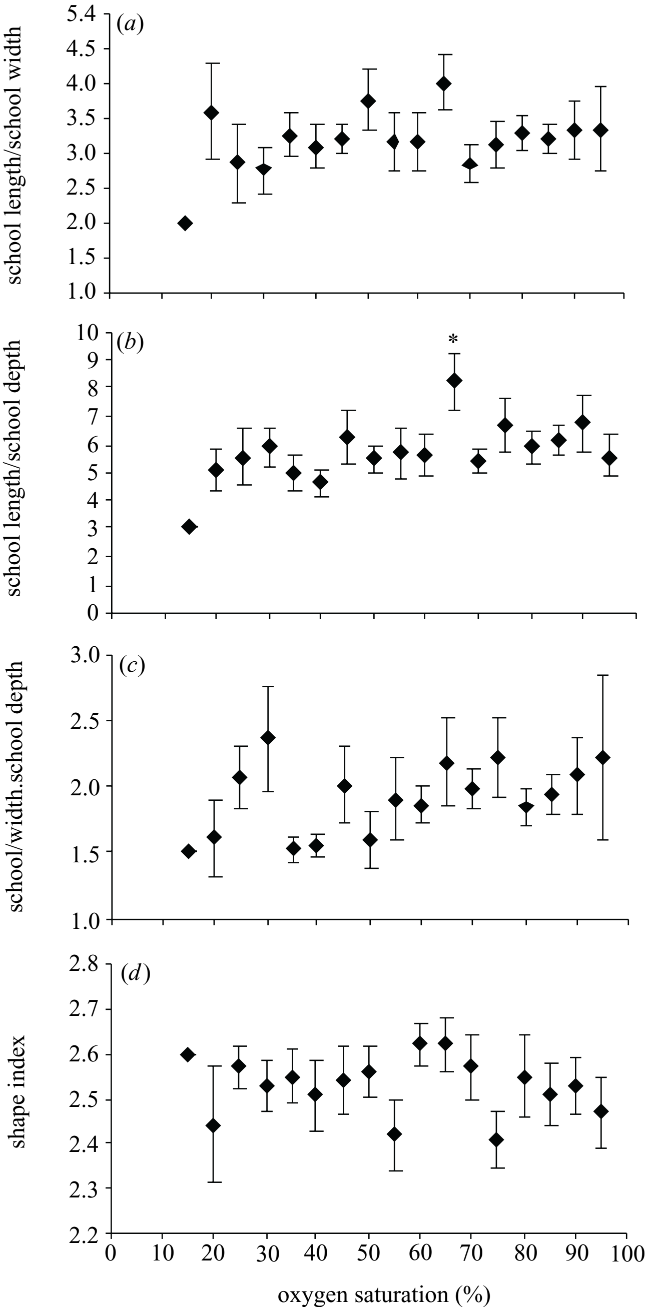

Figure 4. The average length/width ratio (*a*), length/depth ratio (*b*), width/depth ratio (*c*) and shape index (arcsine-transformed maximum width along school length) (*d*) of herring schools at 5% intervals of oxygen saturation. Vertical bars indicate s.e. The asterisks indicate significant differences from normoxia.

hypoxia, at an oxygen saturation of 25% and below (figure 7*b*). During normoxia, the O-turn index is around 1.5–2, indicating that about half or more of the repositioning occurs through O-turns (because the O-turn index can have values from 0 to 3). At low oxygen saturation levels, the O-turn index is less than 1, indicating that, during hypoxia, less than one-third of the reposition manoeuvres occur through O-turns. The persistency index (figure 7*c*) was not affected by hypoxia, indicating that the observed decrease in O-turn manoeuvres does not affect the total amount of reshuffling in hypoxic conditions.

4. DISCUSSION

Our results are, to our knowledge, the first demonstration that both school dynamics and dimensions, in addition to speed (Domenici *et al.* 2000*b*), can be affected by hypoxia. Earlier investigations on the effect of hypoxia on schooling density in the anchovy *E. mordax* (Moss & McFarland 1970) found significant changes during progressive hypoxia (at levels defined by the authors as 'near-lethal') and a temporary increase in speed under acute hypoxia exposure. Any differences in results between this earlier study and both our previous (Domenici *et al.* 2000*b*) and present study may be due to species-specific behaviours or to differences in the methods used for the analysis of schools in our studies compared with those used by Moss & McFarland (1970).

(a) *School dimensions and shape*

As predicted, a general increase of school dimensions during severe hypoxia was observed. This is in accordance with the hypothesis that increasing the volume of the school could counteract the limiting effects of hypoxia on schools. School length, width, depth, area and volume were all significantly increased at 20% oxygen saturation.

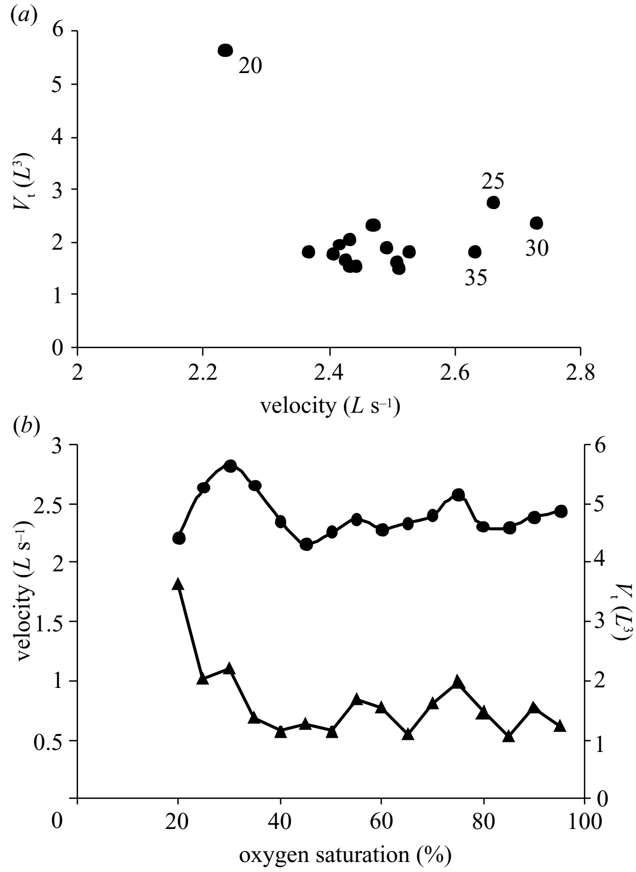

Figure 5. (*a*) The relationship between V_{t} and school velocity (data from Domenici *et al.* 2000*b*). Oxygen saturation percentage is indicated next to the data points for values of 35% or less. V_{t} is not related to speed ($r^2 = 0.12$; $p > 0.1$; d.f. = 1,14) and the highest V_{t} was observed at the lowest velocity, which occurred at 20% oxygen saturation. (*b*) The pattern of V_{t} and school velocity in an example experiment. As described by Domenici *et al.* (2000*b*), the increase in school velocity (line with circles) during hypoxia is followed by a decrease at the lowest oxygen saturation levels. This pattern is different from that of V_{t} (line with triangles) which increases steadily during hypoxia and reaches its highest values at the lowest oxygen saturation levels.

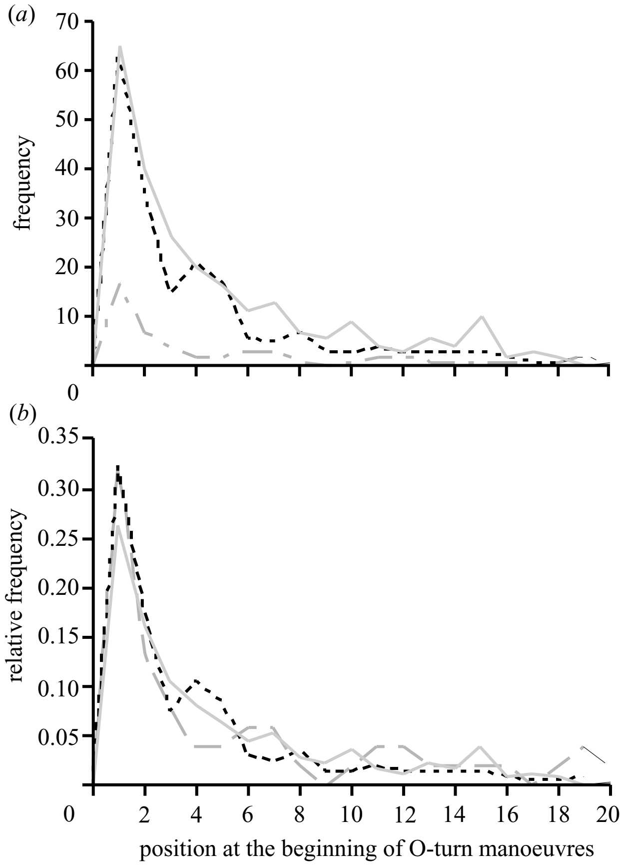

Figure 6. The O-turn absolute frequency distribution (*a*) and O-turn relative frequency distribution (*b*). Values of the abscissa indicate the position of the fish within the school at the beginning of its O-turn manoeuvre. (Grey solid line, 95% oxygen saturation; black dashed line, 60% oxygen saturation; grey long-dashed line, 25% oxygen saturation.)

Width, volume and area were more sensitive to hypoxia; width and volume were also increased at 25% and area at 30% oxygen saturation. The values of total school volume range from *ca.* 1.5 L^3 at normoxia, to over 5 L^3 at 20% oxygen saturation. Previous work on total school volume indicates that 1 L^3 is a good approximation for the volume of water surrounding each fish in a school, although greater volumes may be found in more loosely organized shoals (Pitcher & Partridge 1979). Our values for volume per fish at normoxia (95% oxygen saturation) are slightly higher than, but comparable with, the 1 L^3 reported by Pitcher & Partridge (1979), but our observations at 20% oxygen saturation are considerably higher, indicating that hypoxia causes school volume to increase above measurements under normal conditions that have been reported in the literature.

There are essentially two components to an increase in volume that will affect oxygen content towards the rear of a school in different ways: increase in cross-sectional area of the school normal to the swimming direction (i.e. related to increases in school width and depth, as observed). This will increase oxygen availability directly. An increase in school length will increase the spacing between individuals along the path followed by the school. This may result in an increase in oxygen availability, provided that sufficient mixing of water masses within and outside the school will also occur. This would not prevent hypoxia in the present study, because the individuals within the small schools we used were likely to experience similar oxygen conditions at any given time during the experiments. In nature, however, an increase in school volume may prevent the break-up of a large school by alleviating respiratory distress caused by hypoxia at the rear of the school.

School shape (i.e. school dimension ratios and school shape index) is not affected by hypoxia, indicating that the increase in school volume is a generalized three-dimensional phenomenon that is not specific for a given dimension. A significant increase in the length/depth ratio at 65% oxygen saturation is not easy to interpret, because no other phenomena occur at such oxygen saturation in school dimensions and dynamics (present results) or speed (Domenici *et al.* 2000*b*). The observed increase in school volume could result from either a behavioural mechanism that may have evolved as a response to hypoxia in schooling fishes, or from a reduction in sensory

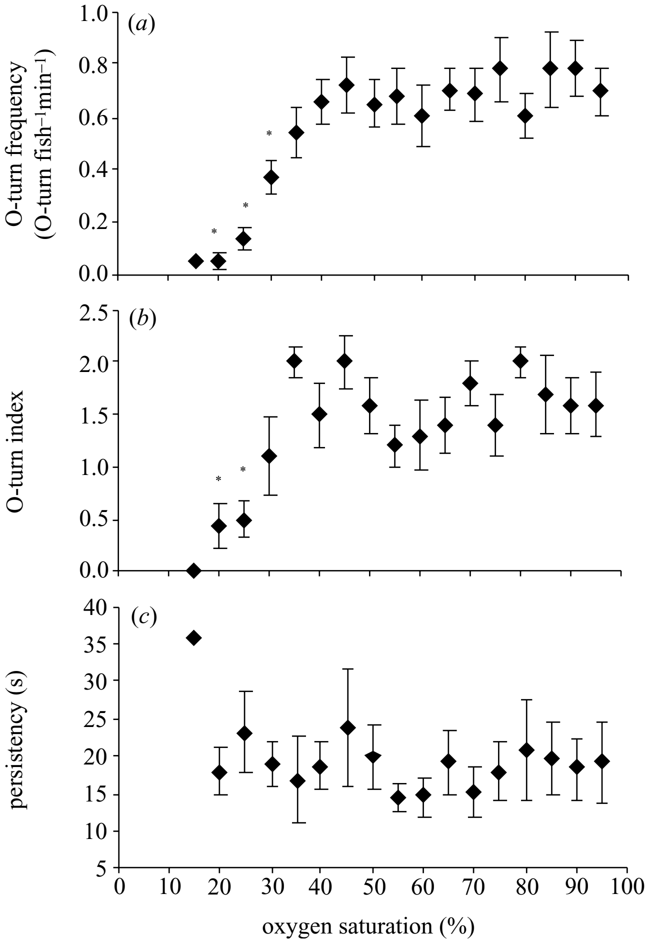

Figure 7. The average O-turn frequency (*a*), O-turn index (*b*) and persistency index (*c*) of herring schools at 5% intervals of oxygen saturation. Vertical bars indicate s.e. The asterisks indicate significant differences from normoxia.

or cognitive performance of the fishes and therefore their ability to maintain short nearest neighbour distances.

The observed increase in school volume is concomitant with an increase in swimming speed as observed by Domenici *et al.* (2000*b*). However, while speed increases with a peak at 15–34% oxygen and then declines (Domenici *et al.* 2000*b*), we have observed that school dimensions start increasing at *ca*. 30% saturation and do not show a subsequent reversal of trend (figure 5*b*). The changes observed here must, therefore, be independent of the changes in speed observed by Domenici *et al.* (2000*b*) although both phenomena are clearly a response to hypoxia.

(b) *School dynamics*

The turnover of individuals observed in many schools may serve to expose all members to the position-dependent advantages and disadvantages of the various physiological constraints imposed on the individual by the group. Previous work shows that the leading fishes of a school may be exposed to a higher predation risk (Bumann *et al.* 1997) but will have first access to any resources encountered by the group (Krause *et al.* 1992). Conversely, particularly in large schools in field conditions, fishes in the rear may experience reduced oxygenation due to oxygen consumption of the fishes ahead of them (McFarland & Moss 1967; Green & McFarland 1994). In hypoxic conditions, therefore, it could be advantageous for fishes at the rear to move to the front. We tested the hypothesis that hypoxia may increase the shuffling of individuals. This hypothesis was based on previous results that showed an increase in the speed of herring schools subjected to progressive hypoxia (Domenici *et al.* 2000*b*). This increase in speed may result from an increase in the shuffling rate of individuals' positions within the school. Contrary to our hypothesis, our results on persistency in the front positions show that shuffling is not affected by hypoxia. Hypoxia does, however, reduce the occurrence of O-turns (manoeuvres that allow mainly the front fishes to go to the rear of the school). This reduction may be related to the cost of relatively swift O-turn manoeuvres, which may not be supported during respiratory distress, and to a natural tendency to remain in the front during hypoxia because, in large schools, back positions may experience a further decrease in oxygen saturation (McFarland & Moss 1967; Green & McFarland 1994). Why fishes in normoxia use O-turn manoeuvres at all, instead of falling back passively, is not clear. A possible explanation is that, because it is executed on the outside of the school and does not cause manoeuvring by other school members, O-turning allows for sudden position changes without disturbing and disrupting the rest of the school. Because shuffling rate is maintained in hypoxia, position shuffling during hypoxia must, therefore, occur mainly through an increased rate of overtaking or falling back by individual fishes. If the hypothesis that a natural tendency to remain in the front during hypoxia is correct, then shuffling rate in hypoxia may be largely maintained by an increased tendency of fishes in the rear to overtake and reach the front.

(c) *Hypoxia and trade-offs in schooling behaviour*

Our results show that hypoxia affects schooling behaviour. While schooling is considered primarily as an anti-predator or feeding adaptation (Pitcher & Parrish 1993), it appears to be affected by a number of environmental factors in addition to the presence of predators and prey. The effect of light level on schooling behaviour and its sensory basis is widely documented (Glass *et al.* 1986; Batty *et al.* 1990). However, the effects of other environmental factors, such as oxygen saturation, temperature, turbidity and windspeed, on schooling behaviour have rarely been investigated (see Weetman *et al.* (1998, 1999) for the effect of temperature on schooling behaviour). The present results for one of these environmental factors, oxygen, the depletion of which along coastal areas is on the increase (Pihl *et al.* 1991; Breitburg 1992; Cloern 2001), indicate that schooling behaviour may be affected by factors other than those directly related to predator–prey relationships (e.g. the presence of a predator) or sensory systems (e.g. light intensity). The basis for this effect needs further investigation in order to distinguish any possible effect of hypoxia on sensory performance, such as lateral line and vision (Partridge & Pitcher 1980), from a behavioural response aimed at maximizing oxygen uptake in a school of fishes experiencing hypoxic conditions.

School integrity and positional dynamics are the outcome of trade-offs between a number of biotic factors such as foraging, predator defence, mating behaviour (for a review see Pitcher & Parrish (1993)) and also various physical factors that may impose certain limits; i.e. light intensity imposing sensory limits for school cohesion, and oxygen modulating metabolism and therefore school dynamics and integrity. While oxygen was expected to have an effect on schooling given its major effect on activity as revealed by previous studies on various species including herring (e.g. Bejda *et al.* 1987; Fisher *et al.* 1992; Schurmann & Steffensen 1994; Domenici *et al.* 2000*b*), it is necessary to consider which aspects of behaviour are affected and which remain unaffected.

As school volume increases, there may be a trade-off between increasing oxygen availability and reducing coordination of movement (given the larger inter-individual distances) and possibly increasing vulnerability to predator attack. It has been suggested that school compaction facilitates anti-predator manoeuvres by allowing the detection of the neighbours' pressure waves via the lateral line (Pitcher & Parrish 1993). The loosening of the school as a response to hypoxia may therefore increase inter-individual distances sufficiently to impair mechanoreception of signals from neighbouring individuals. In particular, although the fast sound pulses emitted by startled herring will be above the auditory threshold at nearest neighbour distances occurring under these conditions, their sound pressure will be below that required to trigger escape responses (Gray & Denton 1991).

Two key factors were not affected by hypoxia: school shape and persistency. An increase in school volume, as observed in our experiments, does not require a change in school shape. It is possible that there is an optimum shape for efficient swimming, for example a small school depth, as observed in our experiment, may confer a hydrodynamic advantage (Abrahams & Colgan 1985; Herskin & Steffensen 1998). However, as pointed out by Abrahams & Colgan (1985), while shallow school depth may be energetically advantageous, it trades off against the school's sensory alertness because it would cause considerable blockage of individual fishes' visual fields by their neighbours. In line with this hypothesis, Abrahams & Colgan (1985) have found that the presence of predators causes an increase in school depth.

The lack of an effect of hypoxia on shuffling rate implies that the internal dynamics of positioning is the result of the balance of other factors that include the nutritional state of each individual fish, protection from predators and hydrodynamic drag reduction. Krause *et al.* (2000) suggest that school positions are rotated by individuals according to their nutritional needs, with hungry fishes occupying front positions only for as long as necessary to regain their nutritional balance. This indicates that school members take turns at being leaders, as observed in our study. Our study also shows that while shuffling rate is a relatively stable parameter with respect to oxygen availability, the mechanisms (i.e. O-turn behaviour) that allow the maintenance of such a shuffling rate are modulated by hypoxia.

Schooling behaviour can be regarded as the balance of a number of trade-offs that act both at the level of the whole school (e.g. volume), and at the level of individuals (e.g. shuffling rate and individual positions). While whole-school parameters are relatively easy to measure, they are only the result of the sum of all the individual behaviours. Further research on schooling behaviour needs to focus on the individual level, in order to understand both how whole-school parameters are determined, and the internal dynamics of the school in terms of costs and benefits for the individuals. As argued by Krause *et al.* (2000), more theoretical and empirical work (tracking marked individuals whose positioning behaviour is monitored over extended time periods of hours or days) is necessary to reveal the factors controlling positioning behaviour within schools, as well as school shape and structure.

Finally, fieldwork is needed in order to ascertain the implications of this work for field situations. Oxygen has an effect on schooling at oxygen saturations (30%) that herring can tolerate for long periods of time in nature (Dommasnes *et al.* 1994; Hognestad 1994). Similar oxygen levels occur periodically (every autumn; J. F. Steffensen, personal observations) in the bottom layer of the Kattegat (figure 1) where herring are found. Effects of hypoxia on schooling are, therefore, likely to be observed in nature and need to be taken into account in field studies of fish aggregations. Various differences may be expected when extrapolating from a school of 20 fishes to a school size found in the wild, including edge effect and length of the school (hence reduction in oxygen at the rear of the school). We propose that the behavioural reaction to hypoxia that we observed will also occur in the wild in larger schools, although its magnitude and overall effect at the whole-school level may be different. Extrapolation to the situation in the wild is limited by both the size of the school and the laboratory situation. Because small schools have a larger proportion of fishes at the edge than large schools, this may imply that there are scale effects on volume change and school dynamics. For instance, because O-turns are initiated mainly by fishes in leading positions and in a large school a smaller proportion of fishes are in the leading positions, there may be a reduced frequency of O-turns. The observation of wild schools and their internal dynamics is challenging, but it will be necessary if we are to understand the mechanisms that regulate schooling behaviour in various environmental conditions.

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