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Oxygen dynamics around buried lesser sandeels *Ammodytes tobianus* (Linnaeus 1785): mode of ventilation and oxygen requirements

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Summary

The oxygen environment around buried sandeels (*Ammodytes tobianus*) was monitored by planar optodes. The oxygen penetration depth at the sediment interface was only a few mm. Thus fish, typically buried at 1–4 cm depth, were generally in anoxic sediment. However, they induced an advective transport through the permeable interstice and formed an inverted cone of porewater with 93% air saturation in front of the mouth. From dye experiments the mean ventilatory flow rate was estimated at 0.26±0.02 ml min^{-1} (86.9±7.3 ml min^{-1} kg^{-1}) (*N*=3). Expelled water from the gills induced a 1 cm circular plume with <15% air saturation around the gills. During this quasi-steady ventilation mode, fish extracted 86.2±4.8% (*N*=7) of the oxygen from the inspired water. However, 13% of the investigated fish (2 of 15) occasionally wriggled their bodies and thereby transported almost fully air-saturated water down along the body, referred to as 'plume ventilation'. Yet, within ~30 min the oxic plume was replenished by oxygen-depleted water from the gills. The potential for cutaneous respiration by the buried fish was thus of no quantitative importance. Calculations derived by three independent methods (each with *N*=3) revealed that the oxygen uptake of sandeel buried for 6–7 h was 40–50% of previous estimates on resting respirometry of non-buried fish, indicating lower O_2 requirements during burial on a diurnal timescale. Buried fish exposed to decreasing oxygen tensions gradually approached the sediment surface, but remained in the sediment until the inspired water reached 5–10% air saturation.

Key words: sandeel, *Ammodytes tobianus*, oxygen imaging, sediment, oxygen uptake, ventilation, hypoxia.

Introduction

Sandeels, a collective term for a number of species in the Ammodytidae family, are key prey organisms within coastal ecosystems. Their behaviour is peculiar among fish. They swim in the open waters in well-formed schools during daytime, while feeding, but at night, or when frightened, they bury in the sediment. During winter they also spend most of their time in the sediment where they remain dormant and rarely emerge (Winslade, 1974a; Winslade, 1974b; Winslade, 1974c; Freeman et al., 2004). Sandeels prefer sandy sediments to those with more gravel, silt or mud, as evident from both laboratory choice experiments and field observations (Meyer et al., 1979; Pinto et al., 1984; Wright et al., 2000; Holland et al., 2005). This might be due to high energy requirements for burying in gravel and the oxygen depleted environment of silt and mud (Glud et al., 2003). Sandy sediments preclude the option of having a permanent opening to the overlying water and the hypothesis that sandeels obtain oxygen from the porewater was based on early presumptions that sandy interstices contain plenty of oxygen (Pinto et al., 1984; Quinn and Schneider, 1991; Holland et al., 2005). Studies have indeed documented that advective porewater transport facilitates solute transport through sand and that oxygen occasionally can extend several cm into permeable sandy sediment (Huettel and Webster, 2001; de Beer et al., 2005; Cook et al., 2007). However, this is not generally the case, and changes in wave actions, flow characteristics and bottom topography affect oxygen penetration and such sediments are characterized by a dynamic mosaic of oxic and anoxic compartments (Ziebis et al., 1996; de Beer et al., 2005). During calm periods oxygen penetration in sand will be constrained to only a few mm (de Beer et al., 2005; Cook et al., 2007).

With sediment characteristics like those described above it is questionable whether buried sandeels can rely on porewater to provide sufficient oxygen to sustain their requirements, not least considering that average winter densities are 60 m^{-2}, with up to 200 m^{-2}, in the central North Sea (Høines and Bergstad, 2001). Despite the enormous abundance of sandeels in near-shore waters and their importance for commercial fisheries (Gislason and Kirkegaard, 1998), only sparse information is available on the behaviour and physiology related to their peculiar lifestyle. This is presumably because technical

difficulties have limited our insight into oxygen dynamics around living and mobile creatures buried in the sediment. The introduction of planar oxygen optodes to aquatic biology has now made it possible to obtain fine-scale, two-dimensional oxygen distributions in benthic communities and around buried structures and animals (Glud et al., 1996; Wenzhöfer and Glud, 2004; Precht et al., 2004; Franke et al., 2006; Frederiksen and Glud, 2006).

The standard metabolic rate (SMR), which is the minimum oxygen requirements for maintenance of a resting, post-absorptive fish, and the S_{crit} (the 'critical oxygen saturation', below which the basal O_2 requirements can no longer be met) are key parameters affecting the ability of fish to cope with oxygen limitation. A recent study employing conventional respirometry showed that despite their small size, the lesser sandeel *Ammodytes tobianus* has a SMR of ~72 mg O_2 kg^{-1} h^{-1} at 10°C for fish weighing 3 g. However, their tolerance to hypoxia appears to be no better than that of many other fish with a S_{crit} of ~20% at 10°C and a salinity of 30‰ (Behrens and Steffensen, 2006). It has been speculated that their burrowing behaviour lowers predation pressure, but also represents a strategy for conservation of energy (Reay, 1973; Quinn and Schneider, 1991; Wright et al., 2000). The idea of reduced oxygen requirements during burial lacks experimental evidence, however, but has been based on the observation that fish do survive on limited fat deposits for extended periods of dormancy during winter (Robards et al., 1999).

Through the use of planar oxygen optodes, the present study investigated the mechanism by which sandeels buried in sandy sediment obtain oxygen and estimate their oxygen requirements on a diurnal basis. Furthermore, behavioural responses to declining water oxygen levels, e.g. emergence from or relocation in the sediment, were investigated.

Materials and methods

Specimens of the lesser sandeel *Ammodytes tobianus* (L.) were caught by seine in 1–2 m deep water in Øresund, Denmark. These waters are frequently visited by large shoals of sandeels during summer and autumn months. The fish were transferred to the Marine Biological Laboratory, Helsingør, where they were kept in a 1660 l circular tank, at 10±0.2°C and 30‰ salinity in recirculating, fully oxygenated seawater. The light regime followed a 12 h:12 h light:dark cycle. The bottom of the tank was covered by a ~20 cm layer of medium fine sand, allowing the fish to maintain their burying behaviour. The fish were fed with frozen *Artemia* or live *Mysis* shrimps and allowed to acclimate to the laboratory conditions for approximately 2 months before the experiments were initiated. Fish were starved for approximately 48 h before the experiments began to exclude any increased oxygen consumption associated with digestion and defecation. Prior to the experiments fish were transferred to glass chambers (height 21 cm, width 14 cm, depth 3 cm) equipped with a planar O_2 optode along one of the sides and filled with sediment from the sampling site. The water column, of fresh seawater, was maintained at a height of 9–14 cm (Fig. 1). The chambers were kept in a 12 h:12 h light:dark cycle and the fish typically buried in the sand 10–20 min after the transfer (Fig. 1). The chambers were then placed in a flow-though aquarium filled with 40 l of seawater at constant temperature and salinity (10±0.2°C and 30‰, respectively), and the fish were left to acclimatize before any measurements were performed (Fig. 2). Details of the acclimation procedures for each experiment are described below.

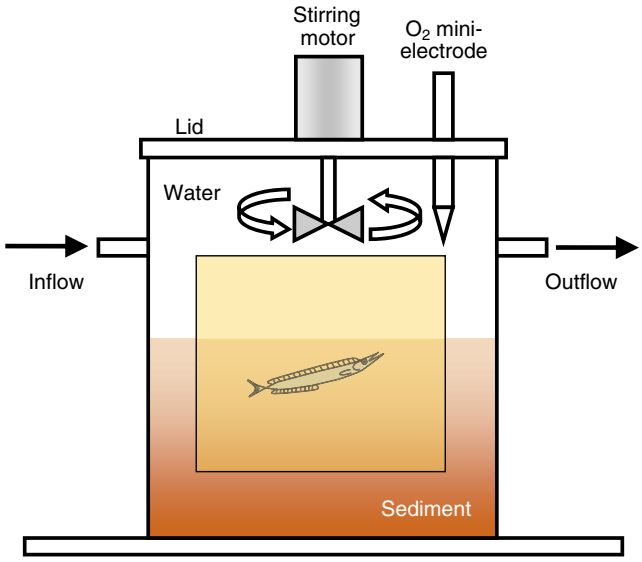

Fig. 1. The experimental chamber (height 21 cm, width 14 cm, depth 3 cm) filled with sediment (height 7–12 cm) and overlying water (height 9–14 cm). The yellow square indicates the position of the transparent planar optode sensor.

Planar optodes

The measuring principle of planar oxygen optodes has previously been described in detail (Glud et al., 1996; Holst et al., 1998) and is therefore only briefly presented below. The planar optode sensor consisted of an oxygen quenchable fluorophore, Ruthenium(II)-tris-4,7-diphenyl-1,10-phenatholine perchlorate, that was immobilized in plasticized PVC on a transparent oxygen-impermeable polyester support foil (total thickness ~175 μm). The luminophore was excited by blue LEDs (Luxeon, Calgary, Alberta, Canada), equipped with short-pass excitation filters (C-54, Linos, Garches, France). The emitted, oxygen-sensitive, red luminescent light-signals were imaged with a 12-bit digital CCD camera (PCO Computer Optics, Kelheim, Germany). The camera was equipped with a 17 mm/f1.4 lens covered by a long-pass emission filter (OG 570, Schott, Warwick, UK) to remove any reflected blue light from the excitation source (Fig. 2). The Peletier cooled camera chip consisted of 1280×1024 pixels which, at the given optical configuration (pixel binning of 2), gave a pixel resolution of ~150 μm $pixel^{-1}$. To quantify the oxygen distribution in front of the planar optode we applied a

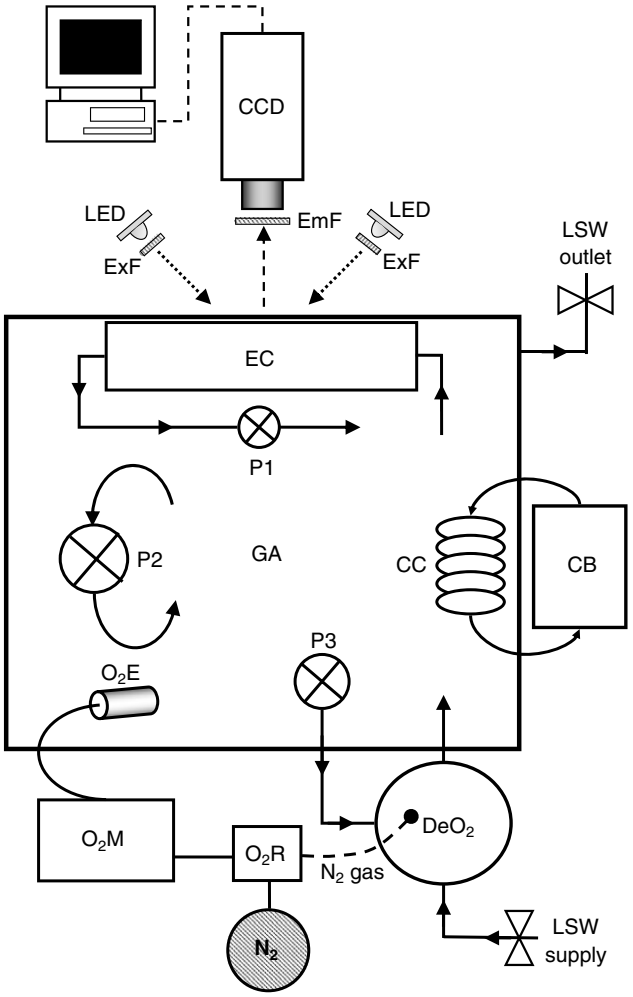

Fig. 2. A schematic illustration of the experimental set-up. GA, glass aquarium; EC, experimental chamber; P1, flow pump; P2, central mixing pump; P3, recirculation pump; DeO_2, deoxygenation tower; LSW, laboratory seawater supply/outlet; O_2M, oxygen meter; O_2R, oxygen regulating meter; N_2, nitrogen gas supply; O_2E, oxygen electrode; CB, thermostated cooling bath; CC, cooling coils; CCD, digital camera; LED, excitation light source; ExF, excitation filter; EmF, emission filter; TD, trigger device.

lifetime-based sensing scheme (Holst et al., 1998). The luminescent light was measured after the eclipse of the excitation light in two well-defined time windows (4 μs), separated by a short interval (0.2 μs). Window one was opened 0.2 μs after the first excitation cycle and window two was opened 4.2 μs after the second excitation cycle. The luminescent lifetime was calculated from these images assuming a mono-exponential decay curve. The lifetime images were calibrated into oxygen images by a two-point calibration procedure using a modified Stern–Volmer equation:

$$C = (\tau_0 - \tau) / K_{sv}(\tau - \alpha\tau_0) ,$$

where τ and τ_0 represent the luminescent lifetime at a given oxygen concentration (C) and at anoxia, respectively. K_{sv} is a constant expressing the quenching efficiency of the immobilized luminophore and a represents the non-quenchable fraction of the luminescence, which was set to the empirically derived image constant of 0.2.

The lifetime approach made it possible to use transparent optodes (Holst and Grünwald, 2001), facilitating the alignment between oxygen images, the position of the fish and the sediment surface. The CCD camera and LED light source were positioned perpendicular to the side of the aquarium hosting the experimental chamber (Fig. 2). Data acquisition and image analysis were performed with custom-made software (Molliview v 1.85 and CalMolli v 0.93). All planar optode images were taken in darkness to avoid any potential artefacts induced by ambient light.

Sandeel-mediated flow patterns

To visualize any flow patterns in the interstice around the buried sandeel, and to estimate the ventilatory flow rate of the fish, an isotonic dye solution (Rhodamine) was added to the sediment surface of the experimental chamber and digital colour films were obtained as the tracer percolated through the sand (Fig. 3). The ventilatory flow rate (ml min^{-1}) was estimated from the tracer percolation rate, the volume of the dyed sediment, and the sediment porosity.

To follow the oxygen dynamics around buried fish, oxygen images were obtained in experimental chambers equipped with planar optodes. During time laps recordings a flow-pump (P1) ensured proper water exchange between the ambient aquarium and the experimental chamber (Fig. 1). Oxygen images and dye experiments confirmed that no advection of the interstice was induced by water exchange. Fish were placed in the experimental chamber during late morning and left to acclimate until late afternoon (5–6 h). A series of 15 overnight (10–12 h), time-lapse oxygen imaging sequences of sediments containing buried fish were obtained over a period of 3 weeks. From such series, the percentage oxygen extraction was calculated as $[(S_{O2in}–S_{O2out}/S_{O2in})\times100]$, where S_{O2in} is the oxygen saturation right in front of the fish, and S_{O2out} is the oxygen saturation in the small sphere of oxygen depleted water leaving the gills.

Hypoxia experiment

Buried fish were acclimatised overnight in normoxic water in the open experimental chamber (Fig. 1). The following morning, water exchange with the ambient aquarium was stopped and the overlying water recirculated with an adjacent deoxygenation-tower kept at a lower, constant air-saturation level by regulated nitrogen bubbling. The deoxygenation-tower ensured rapid and precise adjustment of air-saturation level without disturbing the fish, and water exchange with the experimental chamber was ensured by a small flow-pump (Fig. 2). The oxygen saturation was monitored continuously with a galvanic oxygen probe (Oxyguard, Tjele, Denmark), located in the ambient aquarium. The signal of the probe was used to automatically regulate the nitrogen injection in the deoxygenation-tower.

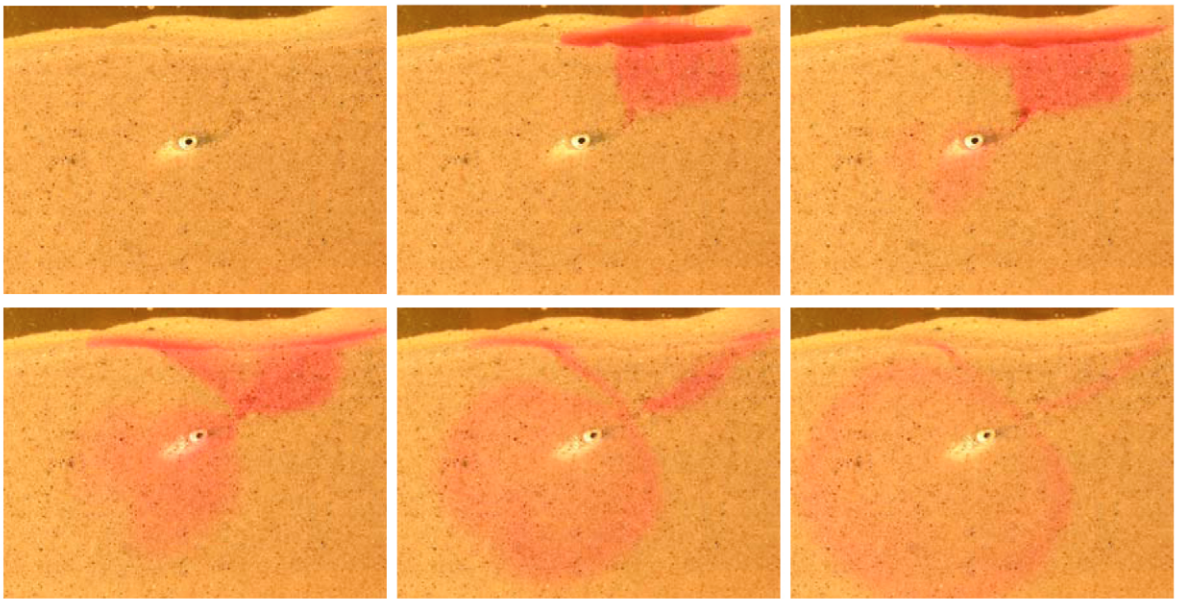

Fig. 3. Digital images showing the sandeel-mediated movement of Rhodamine-coloured water deposited at the sediment surface. During ventilation, the fish dragged the coloured water through the interstice and into its mouth. The exhalent water formed a lightly coloured plume around the gills that gradually became diluted.

Oxygen uptake measurements

Three different, independent procedures were used to determine the oxygen uptake $\dot{V}_{O_2}$ of the buried fish. The methods are described below and in the following text referred to as 'method 1', 'method 2' and 'method 3'.

Method 1

Sediment was ignited overnight at 680°C and subsequently autoclaved for 1 h at 120°C. After cooling, the sediment was bubbled with compressed air and then added to the experimental chamber. A fish was added and soon buried in the sterilized fully aerated, oxygenated sand. After 1 h of acclimation, the incubation was initiated by stopping the recirculating flow and closing the chamber lid (Fig. 1). During incubations the overlying water was continuously mixed by the central stirrer (Fig. 1). The oxygen uptake rate of the fish was calculated from the concentration decrease in oxygenation of overlying water in the experimental chamber, as monitored by the section of the calibrated planar oxygen optode exposed to the overlying water phase. To minimize the likelihood of cutaneous oxygen uptake from the sediment, only images obtained after the fish had been buried for 4–5 h were used for calculations. At this stage, local anoxia had evolved around the fish as the oxic porewater was replenished by oxygen-depleted water leaving the gills. In parallel, a conventional oxygen polarographic mini-electrode, connected to a picoampere-meter and a strip-chart-recorder, followed the oxygen concentration in the water phase of the closed chamber; the two independent determinations of the oxygen decline rate never deviated by more than ~3%. Incubations of the sterile, aerated sediment without fish confirmed a low oxygen uptake probably induced by microbial biofilms establishing on chamber walls. This background value was always less than ~15% of values from incubations including fish and was corrected for during the oxygen uptake calculations. Individual rates of oxygen uptake for 3 g fish ($\dot{V}_{O_2(3g)}$) were calculated as $\dot{V}_{O_2(3g)}=\dot{V}_{O_2(measured)}\times(M_b/0.003)^{(1-A)}$, where $\dot{V}_{O_2(measured)}$ is the measured rate of oxygen consumption, M_b is the mass of the fish in kg, and A is a scaling exponent of 0.8 (Clarke and Johnston, 1999), i.e. the relationship between metabolic rate and size.

Method 2

Method 2 is based on measurements of fish buried in natural sediments. Here we subtract the calculated sediment related O_2 uptake (see below) from the total O_2 uptake of incubated experimental chambers hosting sediment and buried fish. The diffusive oxygen uptake (*DOU*) of the sediment was calculated from one-dimensional microprofiles extracted from the oxygen images in areas not affected by the fish using Fick's first law of diffusion: $DOU=-\Phi\times D_{sed}\times dC/dz$, where Φ is the sediment porosity, D_{sed} is the porosity corrected, oxygen sediment diffusion coefficient at the given temperature and salinity (Li and Gregory, 1974; Boudreau, 1997) and $\Delta C/\Delta z$ is the steepest linear concentration gradient right below the sediment surface (see also Fig. 4C). The average volume-specific sediment respiration rate of the aerobic sediment (R_{vol}) along the sediment surface was calculated by dividing the *DOU* with the oxygen penetration depth, assuming 0-order kinetics for the oxygen consumption rate (Glud et al., 2003). The total oxygen uptake related to the oxic sediment was subsequently calculated by multiplying R_{vol} with the oxygenated volume of the sediment, i.e. the volume along the primary interface plus the volume induced by the advection of the fish. The latter volume

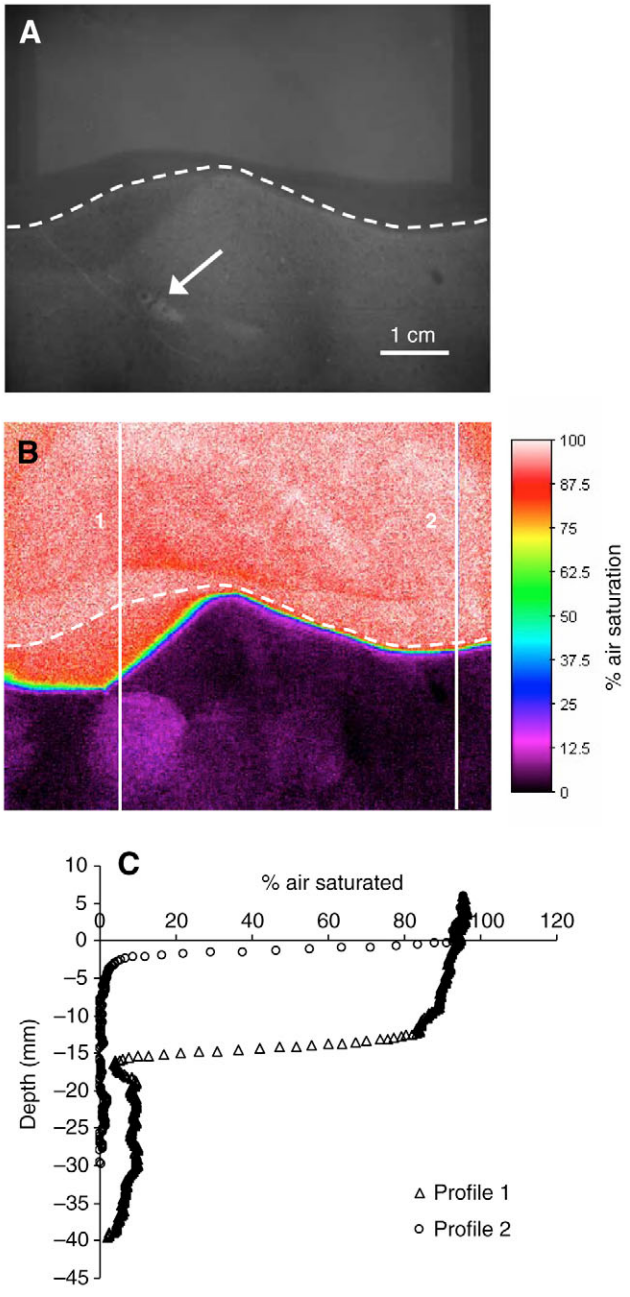

Fig. 4. (A) A black and white image showing the position of the sandeel (arrow) in the sediment. Broken line indicates the sediment water interface. (B) The corresponding oxygen image, showing the O_2 distribution in the sediment around the buried sandeel. The fish sustained its oxygen requirements by advection of normoxic water from above the surface and into the mouth by gill ventilation, thus creating a 'funnel' of oxygenated sediment. During this mode of ventilation a mean of 86.2±4.8% (*N*=7) of the oxygen in the inspired water was extracted, and a strongly O_2-depleted (average of 11.4±6.0%, *N*=7) plume surrounded the gill area. 1 and 2 refer to profiles in C. (C) Extracted oxygen profiles from the oxygen image showing the vertical O_2 distribution and penetration depths at two different positions indicated by the vertical lines in B. The oxygen penetration in profile 1 is clearly affected by the actively ventilating fish whereas profile 2 is only shaped by the diffusive mediated microbial O_2 consumption of the sediment.

was estimated from the oxygenated volume in front of the fish (see above) and the spherical plume around the gills. The respective values for the O_2 concentration in the two compartments could be directly inferred from the O_2 images. On average, 69±4% (*N*=3) of the sediment uptake was related to the primary interface while 26±5% (*N*=3) was related to the fraction of sediment oxygenated by the fish activities. To calculate the oxygen uptake related to the metabolism of the fish uptake, the sediment related uptake was subtracted from the total oxygen decline in the overlying water during subsequent incubations of experimental chambers. The weight-normalized metabolism was calculated as described for 'method 1'.

Method 3

In method 3, the O_2 consumption of the fish was approached by simple Fick'ian calculations, multiplying the ventilatory flow rate with the O_2 extraction efficiency as reflected in the O_2 images.

All three methods are independent and rely on different measurements. One-way ANOVA was used to compare the estimated oxygen uptake rates obtained from the three different calculation procedures described above. All values are mean ± s.d. and significance was accepted at $P<0.05$.

Results

Water flow and ventilation

Sediment was collected from the sub-tidal zone off the beach at Helsingør that is frequently visited by shoals of sandeels. The sand was medium coarse with a grain size of 200–500 μm, a porosity of 0.39 and ~0.5% (dry mass) of organic matter. When sandeel were added to the experimental chamber they initially moved around, pausing in different positions, but after less than 5 min they buried 1–4 cm below the sediment surface orienting the snout upward. The tracer showed that buried fish induced a water flow from the sediment surface, through the interstice and into the mouth. Subsequently, water was ejected from the gills leading to a diffuse coloured sphere evolving around the gill area. Later the sphere developed into a gradually growing ring as the tracer was replenished by uncoloured water ejected from the gills (Fig. 3). The ventilatory flow rate was estimated to be 0.26±0.02 ml min^{-1} (*N*=3), corresponding to 86.9± 7.3 ml min^{-1} kg^{-1}.

Oxygen images

The ventilatory activity of the gills advected oxygen from above the sediment towards the mouth of the fish and created a funnel of oxygenated sediment (Fig. 4A,B). Microbial respiration consumed 5–10% of the available oxygen during the passage of the interstice and before the water entered the mouth of the fish, as shown (profile 1 in Fig. 4C). This was also directly evident from the O_2 recordings in the ventilated funnel created by the fish. During this quasi-steady ventilation mode the fish extracted an average of 86.2±4.8% (*N*=7) of the oxygen from the inspired water, as evident from the low

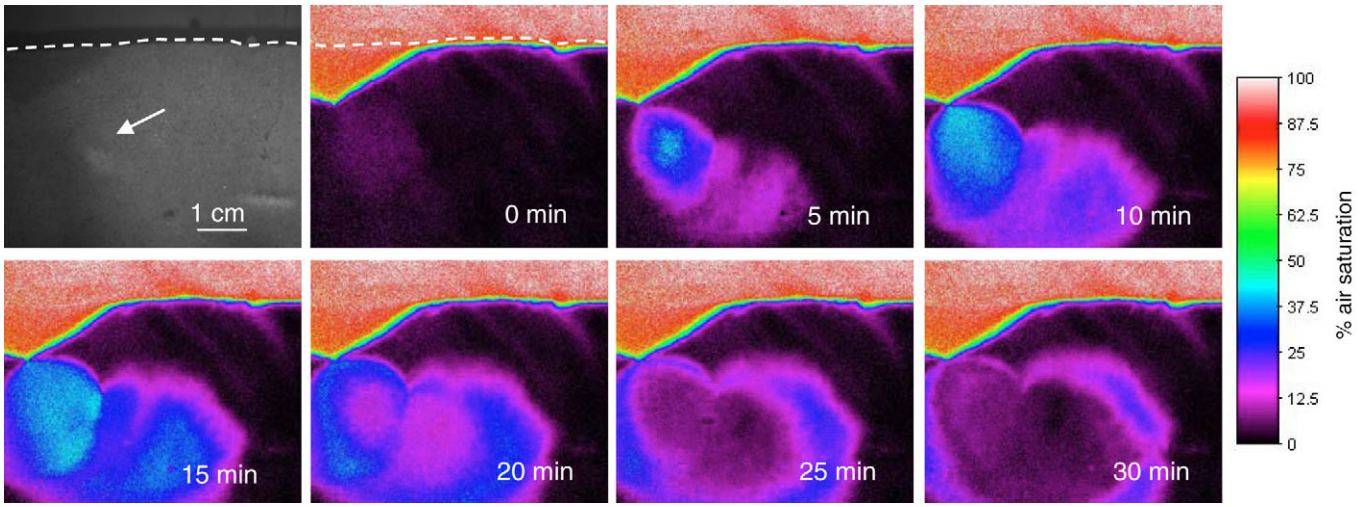

Fig. 5. On rare occasions a phenomenon termed 'plume ventilation' was seen, which caused significant temporal and horizontal variations in the sediment oxygen distributions. Here the fish (arrow) made a wriggling body movement, which channelled oxygenated water down along the body creating a 'pocket' of oxygenated sediment around the fish. The plume, which typically lasted 20–30 min (the time before anoxic conditions were re-established around the fish), penetrated into the interstice as the oxygenated water was replenished by oxygen-depleted water leaving the gills. The oxygen was gradually consumed by microbes and chemical oxidation processes during sediment percolation.

oxygen saturations in the water, leaving the gills percolating into the surrounded anoxic porewater (Fig. 4B,C). However, occasionally some fish made a wriggling body movement and this channelled a pocket of oxygenated water down along the body, a phenomenon here referred to as 'plume ventilation'. With time the plume dispersed away from the fish as the water was replenished and diluted by oxygen-depleted water leaving the gills. During sediment percolation the oxygen was gradually consumed by the interstitial microbes and chemical oxidation processes. The effect of the undulatory movements generally lasted 20–30 min and during this period there were significant horizontal and temporal variations in the sediment oxygen distributions (Fig. 5). These events were nevertheless unusual. Only 13% of fish (2 out of 15) displayed undulatory movements and then only 3 or 4 times during a 12 h period. Thus, these fish experienced an oxygenated environment in the sediment for 8–17% of the time they were buried. In conclusion, ignoring the minor oxygen depleted plume around the gills, fish were completely surrounded by anoxic sediment for the vast majority of time.

Fish behaviour during decreasing water oxygen saturations

When buried fish were exposed to a steadily decreasing oxygen saturation in the overlying water, from normoxic water down to 5–8% air-saturation within 2 h, they gradually moved closer to the sediment surface and most stuck their head up well in advance of 'whole body emergence' (N=8) (Fig. 6). There was, however, no specific oxygen threshold value prompting an escape from the sediment. A couple of fish emerged when oxygen levels fell to 45–50% air-saturation, while the

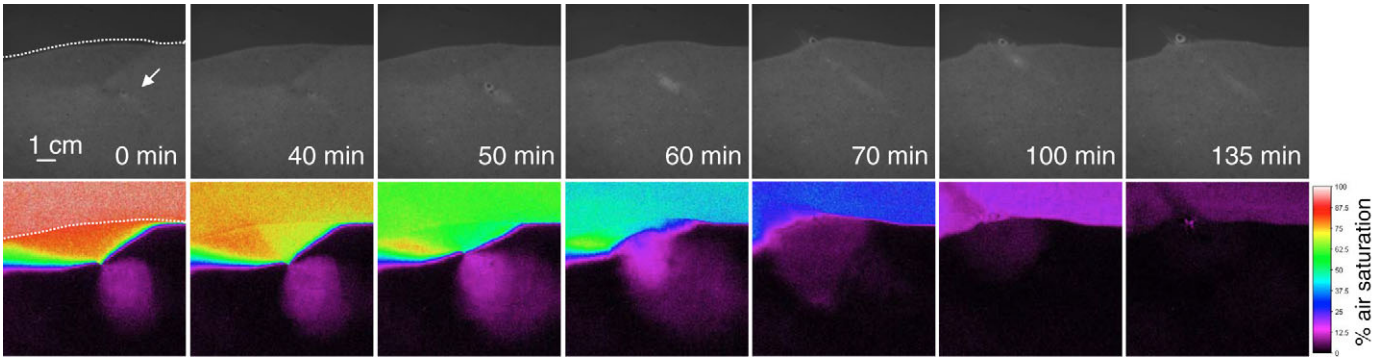

Fig. 6. Planar optode images illustrating the behavioural response of a buried sandeel (arrow) to a progressive decrease of the oxygen saturation in the overlying water. The upper row of greyscale photos shows sandeel position and the lower row the corresponding oxygen images. In the illustrated case, the fish stayed completely submerged until oxygen saturation in the above water had declined to ~40%, whereafter its head emerged from the sediment. Despite increasingly lower levels of oxygen in the above water, the fish stayed in this position until it finally fully emerged after approximately 140 min at 5–8% air saturation. The time (min) is time elapsed from the onset of the experiment.

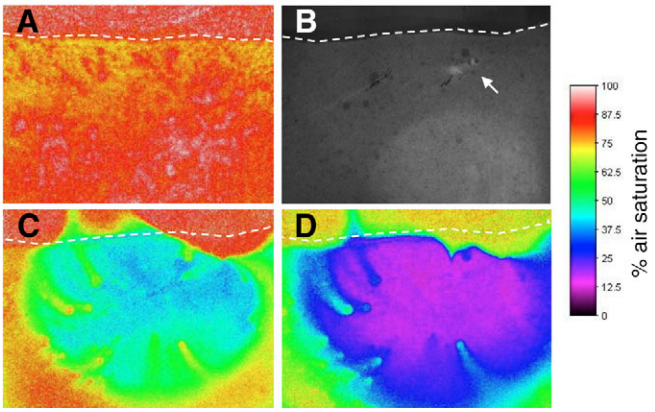

Fig. 7. An example of the sterile, aerated sediment used for determinations of the oxygen uptake rates of buried fish by method 1. (A) Before burial of the fish, (B) alignment of the fish (arrow) in the sediment, (C,D) progressive deoxygenation of the sediment due to oxygen-depleted water leaving the gills, percolating into the adjacent interstice and replenishing the oxic porewater. C is approximately 1 h after the fish buried, while (D) is taken about 4 h later. To minimize the likelihood of cutaneous oxygen uptake from the sediment, only images obtained after the fish had been buried for 4–5 h were used for calculations of oxygen uptake rates, i.e. when local anoxia had evolved around the fish, as illustrated by D.

remaining six stayed buried much longer, until oxygen conditions were limited with 5–8% air-saturation in the overlying water. The period endured by these six fish being buried with almost anoxic conditions in the overlying water varied from 10 to 140 min. Even though a general pattern was apparent there was thus significant individual variability in the specimen response.

Oxygen uptake rates of buried sandeel

Mass-balance calculations done with method 1, i.e. on oxygen images taken 4–5 h after the fish had buried in sterile and fully oxygenated sediments (Fig. 7D), resulted in a mean (± s.d.) oxygen uptake rate of 28.8±7.8 mg O_2 kg^{-1} h^{-1} for fish weighing 3 g (N=3). In comparison, method 2 resulted in an oxygen uptake rate of 34.5±2.1 mg O_2 kg^{-1} h^{-1} for fish weighing 3 g (N=3) (Table 1). When applying Fick's approach (method 3) an oxygen uptake rate (N=3) of 36.0±3.0 mg O_2 kg^{-1} h^{-1} for fish weighing 3 g was obtained. There were no significant differences between the oxygen uptake rates obtained by the independent methods (P>0.05). Combined, the methods indicated an oxygen uptake rate of buried fish of 33.1±4.4 mg O_2 kg^{-1} h^{-1} for fish of 3 g body mass (N=9).

Table 1. *Comparison of oxygen uptake rates obtained on buried sandeels versus those obtained from a conventional resting respirometer*

	N	Oxygen uptake rate (mg O_2 kg^{-1} h^{-1})
Present study		
Method 1	3	28.8±7.8
Method 2	3	34.5±2.1
Method 3	3	36.0±3.0
Behrens and Steffensen*	7	71.7±12.2

All values are mean ± s.d. for fish of 3 g body mass.
*(Behrens and Steffensen, 2006).

Discussion

Modes for sustaining the oxygen requirements during burial

With no passive ventilation of the interstice, oxygen only penetrated a few mm into the surface sediment. Thus the interstitial water did not hold an oxygen reservoir for buried sandeels. Buried sandeels were nonetheless capable of sustaining their oxygen requirements by advecting oxygen-rich water towards the mouth by gill ventilation. During gill passage, an average of 86.2±4.8% (N=7) of the oxygen in the water was extracted, which is at the higher end of the range determined for other teleost species (Kerstens et al., 1979; Lomholt and Johansen, 1979; Steffensen et al., 1982; Randall and Daxboeck, 1984). Being confined by the sediment, sandeels must employ constant gill movements to maintain water flow into the mouth and this could involve high costs apparently compensated by the efficient oxygen extraction. The mean ventilatory flow rate of 86.9±7.3 ml min^{-1} kg^{-1} (N=3) for sandeel is very comparable to values estimated for partially buried flounder and plaice (87–108 ml min^{-1} kg^{-1}) at equivalent temperature (Kerstens et al., 1979; Steffensen et al., 1982). The sandeel were largely embedded in anoxic sediment, and thus the possibility of oxygen uptake across the skin is generally excluded, but the plume events presumably enable the skin to act as an additional respiratory surface when oxygen-rich water surrounded the fish's body, as known from non-burying fish (Kirsch and Nonnotte, 1977; Steffensen et al., 1981; Steffensen and Lomholt, 1985). These events were, however, only observed for 13% of the fish and lasted for a maximum of 17% of the time for these fish. This suggests that cutaneous respiration generally is of minor importance for buried sandeels and the contribution to O_2 requirements of the fish can presumably be ignored.

Both *in situ* and laboratory based measurements have shown that the oxygen distribution in sandy sediments can be extremely dynamic (de Beer et al., 2005; Precht et al., 2004; Cook et al., 2007). Hydrodynamic forcing, interrelating with the topographic relief of the sediment surface, can induce extensive oxygen oscillations in the top 10 cm of the sediment, and it remains to be proven if sandeels benefit from this intermittent oxygenation of the interstitial water when taking refugee in sand during night or during winter dormancy. In intertidal areas, pockets of air trapped in the sediment could act as an oxygen reservoir for buried sandeels. In deeper waters, high densities of buried sandeel are often seen in sand banks characterised by ripples, intense wave actions and strong bottom currents (Wright et al., 2000; Freeman et al., 2004). In these areas, interactions between flow velocity gradients and

topographic structures enhance advective water exchange, augmenting the sediment oxygenation (Forster et al., 1996; Ziebis et al., 1996), which may explain fish aggregation in such areas. The fish will, however, experience periods where hydrodynamics of the overlying water and ripple movement will leave the sediment anoxic hence excluding the option of exploiting oxygen in the interstitial water during such periods.

Behavioural strategies towards hypoxia

The majority of buried sandeels (6 of 8) showed no fleeing response when exposed to a gradual decline in the ambient oxygen levels down to 5–8% air-saturation, but they remained buried despite ventilating severely oxygen-depleted water. With the ultimate aim to survive under such unfavourable conditions, the fish balances between two strategies; the need to reach more oxygenated waters or to save energy and hence avoid major physiological stress. Different species vary in their behavioural response when exposed to low ambient oxygen levels; some increase swimming speed as an escape strategy (Bejda et al., 1987; Van Raaij et al., 1996; Domenici et al., 2000; Johansen et al., 2006) whereas others exhibit a more quiescent behaviour presumable to maintain physiological homeostasis (Metcalfe and Butler, 1984; Fischer et al., 1992; Nilsson et al., 1993; Dalla Via et al., 1998; Cech and Crocker, 2002). Apparently, sandeels generally employ a 'sit and wait for better times' strategy, relying on endurance until oxygen conditions improve. Maybe this is a consequence of their lifestyle, being very stationary after settling in a sandy area, to which they will return after feeding in deeper waters during daytime (Meyer et al., 1979; Pinto et al., 1984; Wright et al., 2000; Holland et al., 2005). This strategy may, however, be maladaptive during extensive and prolonged oxygen depletion events, as has been observed during recent years in the inner Danish waters (HELCOM, 2003; Conley et al., 2007), where, for periods of weeks, water oxygen levels reached values critical for sandeels (S_{crit}=20% at 10°C).

Comparative oxygen uptake rates

The present results provide evidence that sandeels indeed have a reduced oxygen uptake while buried. This was confirmed by three independent approaches for estimating the oxygen consumption. Combining all three approaches, the average O_2 uptake of the buried fish amounted to 33.1±4.4 mg O_2 kg^{-1} h^{-1} (N=9), corresponding to ~46% of the value obtained for similar sized sandeels by conventional resting respirometry at equivalent temperature (Behrens and Steffensen, 2006). Diffusion of oxygen into epidermal cells can occur despite low ambient oxygen levels (Steffensen et al., 1981), but care was taken in the calculations in method 1 only to use oxygen images after deoxygenated sediment had developed around the fish in the otherwise sterile and aerated sediment (see Fig. 7D). In addition, considering that there was no significant difference between the results from the present three methods (Table 1), where the two latter exclude the possibility of cutaneous oxygen uptake (ignoring the small circle of oxygenated water around the gills), we conclude that the present study provides evidence that buried sandeels have lower oxygen uptake than fish enclosed in a respiration chamber.

There are three factors that, alone or in combination, can explain this observation. (1) Buried fish may express lower oxygen requirements due to metabolic depression. If so, this supports earlier speculations that burying represents a strategy for energy conservation (Quinn and Schneider, 1991; Wright et al., 2000). (2) The oxygen uptake of buried fish may not balance its energy requirements. If this is the case and the fish supplements its energy requirements with anaerobic metabolism, it will 'push the problem ahead' by developing an oxygen debt, which can then be repaid when the fish leaves the sediment. In case of such a strategy the preset estimates of oxygen uptake will underestimate the actual energy use of buried fish. The routine metabolism of swimming sandeels has a considerable anaerobic component, which could favour this idea (Behrens and Steffensen, 2006). Being buried has the obvious advantage of being hidden but despite that, an anaerobic metabolic component could be a necessary consequence, or adaptation, of this behaviour. However, we find it unlikely that a 50% decrease in the O_2 requirements can be explained by anaerobic metabolism, as this would require massive accumulation of lactate, well above previous measurements for other fish (Milligan and Wood, 1987; Baker et al., 2005). (3) We cannot exclude elevated oxygen uptake of specimens confined in a small respirometer where they cannot bury. Visual inspection of the fish concluded, however, that enhanced stress-induced O_2 uptake of this magnitude was unlikely as no obvious signs of stress were observed. We therefore conclude that the reduced oxygen uptake during burial is caused, at least in part, by lower energy requirements through metabolic depression, potentially in combination with a gradual accumulation of lactate.

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