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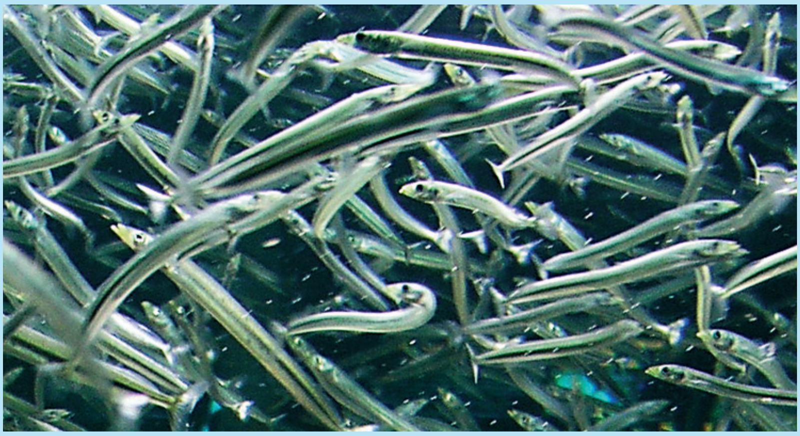
Effect of Global Change related oxygen depletion on fish; lesser sandeel (*Ammodytes tobianus*) as model organism

PhD thesis

Jane Windfeldt Behrens



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PhD thesis, 2007

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Abstract: Inner Danish waters have experienced seasonal oxygen depletion on an almost annual basis since the early 1980s. Oxygen is a key element in the metabolic processes of fish and the ecological effects of sub-lethal hypoxia can be profound. Sandeels are important in the food web, constituting a key prey item for many larger fish, marine mammals and sea birds. While sandeels school in the open water during daytime to feed, they bury into the sediment at night and during winter conditions. Thus, they are affected by pelagic and benthic conditions alike. The present study investigated basic metabolic and respiratory properties of lesser sandeel (*A. tobianus*), in particular how fish obtain oxygen when buried in anoxic sediment, and the effects of hypoxia (acute and chronic) on swimming speed and the fish's diurnal activity pattern. Based on the experimentally determined critical oxygen tension (P_{crit}) for sandeel, the coupling between low oxygen and habitat loss was predicted, both under present climate condition and expected future global warming. The results show that hypoxia indeed affects physiological and behavioral aspects of sandeel, though responses varied according to the rate and duration of hypoxic exposure. Sandeels have low minimum oxygen requirements but a hypoxia tolerance similar to other fish. While buried, the fish obtain oxygen by actively advecting water from above the sediment surface and across the gills. When exposed to acute hypoxia the fish remain buried, thus employing an energy saving strategy. Swimming fish on the contrary, exhibited neither reduced swimming speed nor showed an escape response when exposed to acute hypoxia. Prolonged moderate and severe hypoxia had a major influence on the diurnal activity pattern of the fish, affecting emergence and burying rates and the number of fish entering the water column during the day. Finally, up to one fourth of the suitable sandy seabed in which sandeels bury has been exposed to critical oxygen levels within recent years. Future global warning will exacerbate the situation considerably, leading to further possible habitat loss due to unfavorable oxygen conditions. In conclusion, hypoxia will likely exert negative effects on sandeels in the inner Danish waters. Further knowledge on any implications, whether direct or indirect, of climate change on sandeels population dynamics is of utmost importance, since a complete stock collapse is likely to have broad and severe effects on the entire ecosystem.

Keywords: Sandeel, *Ammodytes tobianus*, global warming, hypoxia, fish, oxygen, metabolism, behaviour, ventilation, physiology

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Contents

List of publications

The thesis consists of the following papers, referred to in the synthesis by their roman numerals

Paper I

Behrens, JW and Steffensen JF (2007) The effect of hypoxia on behavioural and physiological aspects of lesser sandeel, *Ammodytes tobianus* (Linnaeus, 1785). Mar Biol 150:1365-1377

Paper II

Behrens JW, Præbel K and Steffensen JF (2006) Swimming energetics of the Barents Sea capelin (*Mallotus villosus*) during the spawning migration period. J Exp Mar Biol Ecol 331(2):208-216

Paper III

Behrens JW, Stahl HJ, Steffensen JF and Glud RN (2007) Oxygen dynamics around buried lesser sandeels, *Ammodytes tobianus* (Linnaeus, 1785); mode of ventilation and oxygen requirements. J Exp Biol 210:1006-1014

Paper IV

Behrens JW, Ærtebjerg G, Petersen JK and Carstensen J. Oxygen deficiency impacts on burying habitats for lesser sandeel, *Ammodytes tobianus* (Linnaeus, 1785), in the inner Danish waters. Manuscript draft

Paper V

Behrens JW, Ærtebjerg G, Petersen JK and Steffensen JF. Influence of moderate and severe hypoxia on the diurnal activity pattern of lesser sandeel (*Ammodytes tobianus*). Manuscript draft

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Preface

First and foremost I would like to thank the Copenhagen Global Change Initiative (COGCI) for giving me the unique opportunity to realize this project. Special thanks goes to my supervisors, John Fleng Steffensen, whom with great ingenuity made it possible to solve even in advance doomed technical problems; Jens Kjerulf Petersen, who got me back on the beaten track when scientific writing evolved into never-ending stories; and Gunni Ærtebjerg who generously shared life-long aspired knowledge on the environment, not to mention his catching passion for the same.

The content of this thesis would however have been substantially more light-weighted was it not for fruitful collaboration with peers. Especially, I feel indebted to Kim Præbel (Norwegian College of Fishery Science) for opening my eyes to the splendor of fish biology by taking me cruising around North Cape and into pristine Norwegian fjords to catch capelin; Jacob Carstensen (National Environmental Research Institute) for the patience and time to make me realize that modeling actually can be fun and informative, and finally to Ronnie Glud and Henrik Staahl (Marine Biological Laboratory) for innovative and 'colorful' collaboration.

For a fish-novice like me it would have been impossible to carry out the present work without help from 'the fishy flock' at the Marine Biological Laboratory. My thanks are hereby given to them, in particular Maria Steinhausen, Anders Drud Jordan, Neill Herbert and Peter Skov. Thanks also go to the staff at the Øresundsakvarium for their caretaking of the sandeels, and to Egil Nielsen in the workshop for always having a spare 'handy hand' when needed.

Last, but far from least, a special thanks goes to my family. Being born and brought up in small fishing village on the west coast of Jutland must have left some impression even though no fishing traditions run within the family – thank you to my parents for that. My greatest regret is that you could only follow me halfway though this – but your spirits have been with me throughout. I am also greatly indebted to my two brothers who may not always understand why I am doing what I do, but nevertheless supports me blindly - the invaluable privilege of being a little-sister. Finally, the one to whom I owe my most sincere thanks is Claus. You have shown the greatest believe in me and without you this would not have been possible.

10th of April 2007

Jane

Summary

Seasonal hypoxia has since the beginning of the 1980s been observed on an almost yearly basis in inner Danish waters. Oxygen is a key element in the metabolic processes of fish and the ecological responses of sublethal hypoxia can be profound. This thesis has its main focus on how short-term and prolonged hypoxia impacts the physiology and behaviour of lesser sandeel (*Ammodytes tobianus*), one of five species of sandeels inhabiting the inner Danish waters. These small fish constitute an important link in the food web being key prey items for many larger fish, marine mammals and sea birds. Sandeels school in the open water during daytime while feeding but bury into the sediment at night, when frightened and during winter. The burying behavior is thus of importance for the fitness of the fish, but also makes them exposed to bottom water conditions where oxygen conditions are often less favorable. Estimates of basic respiratory parameters revealed that lesser sandeel have low minimum oxygen requirements but an ordinary hypoxia tolerance. Fish buried in anoxic sediment obtained oxygen by actively advecting oxygen-rich water from above the sediment surface and over their gills. When exposed to acute hypoxia sandeels remained buried, thus employing an energy saving strategy. Swimming fish, on the contrary, exhibited neither reduced swimming speed nor showed a fleeing response when exposed to acute hypoxia. Prolonged moderate and severe hypoxia had major influence on the diurnal activity pattern of the fish, where moderate hypoxia affected emergence and burying rates while severe hypoxia prompted an increased number of fish to stay in the water column during daytime. Finally, modeling of monitoring data revealed that within recent years up to one fourth of the sandy seabed areas which sandeels potentially use to bury in, have been exposed to oxygen levels critical for the fish. Future global warning may exacerbate the situation considerably, leading to further habitat loss due to unfavorable oxygen conditions. In conclusion, hypoxia may have negative impact on sandeel populations of the inner Danish waters. Further knowledge on any implications, whether direct or indirect, of climate change on sandeels population dynamics is of utmost importance, since a complete stock collapse is likely to have broad and severe effects on the entire ecosystem.

Sammenfatning (Danish summary)

Iltsvind (hypoxi) er ikke et nyt begreb, men har forekommet naturligt gennem geologisk tid. Den naturlige omsætning af organisk materiale ved havbunden kræver ilt, og specielt i afgrænsede, dybe områder med lille vandudskiftning kan iltsvind derfor opstå. Kort fortalt opstår iltsvind når der over en periode bruges mere ilt i bundvandet end der tilføres. Gennem de sidste par årtier er iltsvind blevet hyppigere og mere omfattende end tidligere på grund af øget eutrofiering. De indre danske farvande har således siden starten af 1980'erne været ramt af årligt tilbagevendende hypoxi i sommer- og efterårsmånederne. Ilt spiller en essentiel rolle i stofskifte-processerne hos fisk og begrænset ilt-tilgængelighed kan derfor have væsentlige økologiske konsekvenser.

Kysttobis (*Ammodytes tobianus*) er en af fem tobisarter, som lever i de indre danske farvande. Tobis er byttedyr for mange større fisk, marine pattedyr og havfugle og spiller således en vigtig rolle i økosystemet. Tobis har en speciel adfærd, idet de svømmer i stimer i de åbne vande om dagen og tager føde til sig, mens de om natten, eller hvis de bliver skræmt, graver de sig ned i sandet havbund. Her tilbringer de også hele vinteren i en slags dvaletilstand. Nedgravningsadfærden gør, at tobisen gennem en stor del af dens liv påvirkes af bundvandet, hvor iltforholdene ofte er dårligere end i de øvre vandlag. Disse forhold – dvs. tobis' vigtighed i fødenettet og det at den ofte vil opleve iltsvind i sine naturlige omgivelser – var grund til at kysttobis blev valgt som modelorganisme til denne Ph.d. afhandling. Hovedformålet med afhandlingen var at undersøge hvordan iltsvind kan påvirke fisk' fysiologi og adfærd.

Kysttobis har et hvilestofskifte, som er lavere end mange andre fiskearters (såsom lodde i Artikel II), hvorimod dens tolerance overfor hypoxi er mere sammenlignelig med de samme fiskearter's (Artikel I). Når fisken ligger gravet ned i anoxisk sediment, får den ilt ved at pumpe iltrigt vand fra sedimentoverfladen, ind i munden og over gællerne (Artikel III). Selvom iltindholdet sænkes akut i det overliggende vand, så vedbliver tobisen med at være nedgravet – en strategi som bruges for at spare på energien (Artikel III). Svømmende tobis' adfærd under akut hypoxi synes derimod hverken at pege på en strategi til at spare på energien eller til at prøve at komme væk fra de ugunstige iltforhold (Artikel I). Længerevarende moderat og svær hypoxi havde derimod stor effekt på tobisens døgnrytme (Artikel V). Moderat hypoxi øgede frekvensen hvormed tobis gravede sig op og ned i løbet af dagen, mens svær hypoxi gjorde at mange flere fisk opholdt sig i vandfasen, i stedet for at være gravet ned. Sådanne ændrede adfærdsmønstre vil medføre, at mindre energi er til rådighed til vækst og reproduktion samt gøre fiskene mere udsatte for predation. Modellering af moniteringsdata viste ydermere at op til en fjerdedel af de sandede havbundsområder, som tobis potentielt kan bruge til at grave sig ned i, har været udsat for kritiske iltforhold indenfor de sidste år (Artikel IV). Global opvarmning vil reducere ilts opløselighed i vandet og dermed forværre iltforholdene ved bunden, hvorfor yderligere habitat-tab forudsiges i fremtiden.

1 Introduction

1.1 Hypoxia and the inner Danish waters

Oxygen depletion, or hypoxia, is by its simplest definition an event where the balances between oxygen supply and oxygen demand is negatively skewed, resulting in conditions of low dissolved oxygen concentrations. Behind this basic description lie myriads of complex processes and events, leading to the multifaceted nature of hypoxia development. Hypoxia is not only a recent phenomenon that concurs with mankind's activities influencing the oxygen consumption rate in aquatic systems, but has existed through geological time. Especially deep, enclosed areas characterized by high supply of organic matter and limited water renewal are prone to either permanent or periodical depletion of dissolved oxygen. A well-know example is the Black Sea, which has been permanently anoxic at depths exceeding 150 to 200m for the last approximately 7000 years (Mee 1992). However, within the last couple of decades numerous reports from around the world clearly suggests that these naturally, although rarely occurring events, have escalated to become more severe, widespread and prolonged (see review by Diaz 2001). This escalation is related to excessive anthropogenic input of nutrients and organic matter from direct point sources, rivers, the atmosphere and from adjoining seas which increases the phytoplankton primary production. Increased primary production in turn augments the amount of organic matter to be decomposed in deeper water layers or in the sediment, thus comsuming more oxygen in the lower water layers. This will, together with physical factors, influence the timing and extent of hypoxia, and in several places around the world large areas are now affected by either permanent hypoxia/anoxia, e.g. the Baltic Sea (Conley et al. 2002), or seasonal hypoxia, such as the Chesapeake Bay (Hagy et al. 2004), the Gulf of Mexico (Rabalais et al. 2002) and the inner Danish waters (Baden et al. 1990; HELCOM 2003).

Kattegat, the Belt Sea (Northern-, Little- and Great Belt) and the Sound, all with connecting fjords and estuaries, compose a shallow transition area with estuarine character between the high saline Skagerrak towards the north and the more brackish Baltic Sea in the south (Figure 1.1A). Low saline water flow northward at the surface layers and high density Skagerrak water flow southward as a bottom current, creating an almost permanent halocline located at ~15 m depth which prevents vertical mixing of the water column (Figure 1.1B). During summer the halocline is additionally re-enforced by a thermocline (Anderson and Rydberg 1993; Stigebrandt and Gustafsson 2003). The straits are generally shallow with maximum depths in the north-eastern Kattegat of ~ 100 m and minimum depths in the western part of 10-20 m. Sandy sediment covers more than half of the bottom area, though with considerable regional differences. Pure sand dominates at shallow depths along the coastline down to about 15-20 m, but on deeper waters the seabed becomes increasingly covered with mud- and silt mixed sand. Below 40 m the sediments generally consists of mud and silt, as do extensive bottom areas in estuaries and coastal embayments, and along the Swedish Kattegat-coast rocks prevail in some areas (Paper IV). The water exchange between the Baltic Sea and the Kattegat through the straits is restricted by two shallow sills, Drodgen in the Sound where the water depth is 8 m, and the 18 m deep Darss in the southern Belt Sea (Anderson and Rydberg 1993; Stigebrandt and Gustafsson 2003). These differences in basin geometry, in addition to the stratification patterns, strongly influence regional residence times which can exceed one month (Gustafsson 2000a; 2000b). The shallow estuaries (mean depths less than 3m), which are prevalent in Denmark, experience only short episodes with stratification during periods with calm winds and high temperature (Conley et al. 2000). Altogether, the inherent hydrology and the physical characteristics of the seabed make these marine waters highly susceptible to nutrient loads and variations in climatic factors (Møhlenberg 1999; Rasmussen et al. 2003; Carstensen et al. 2006; Conley et al. in press).

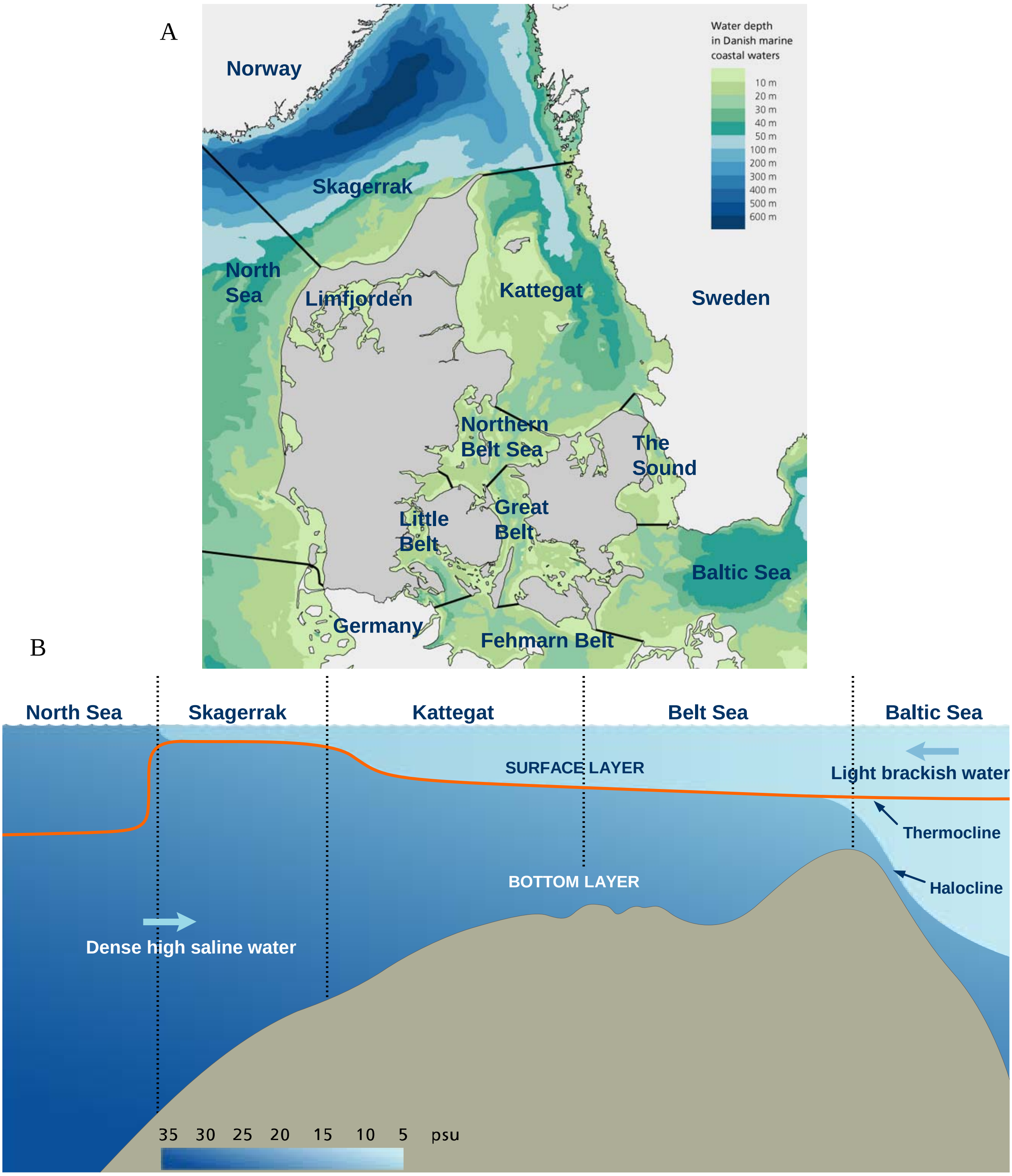

Figure 1.1 A: Study area with depth distributions. B: North-south cross section through the Danish waters (below) illustrating typical stratification patterns during summer. Modified after Aarup (1994) and Nielsen (2005), respectively.

In Denmark, as in several other parts of the world, a rapid increase in the use of fertilizer followed World War II (Richardson 1996). A manifest effect of this nutrient overenrichment was that primary production doubled in the Great Belt from the 1950s to the late 1970s (Rydberg et al. 2006) and more than doubled in the Kattegat from the 1950s to the late 1980s (Richardson and Heilmann 1995). This led to a gradual decrease in levels of oxygen in the bottom waters from the 1960s and onwards, which was especially pronounced during the 1980s, with sporadic and seasonal oxygen depletion events observed almost yearly. In 1986, massive hypoxia prompted the Danish government to introduce the National Action Plan for the Aquatic Environment with the agenda to reduce nitrogen and phosphorus discharged by 50% and 80%, respectively (Kronvang et al. 1993). However, the action plan was mainly successful regarding point-loadings from municipal wastewater, and a second action plan for sustainable agricultural production targeting primarily diffuse nitrogen loading followed in 1991. These significant and successful measures to reduce nitrogen and phosphorus discharges to the aquatic environment (Carstensen et al. 2006) should potentially have improved bottom water oxygen conditions. This has however not held true because the 1990s were characterised by very variable bottom water oxygen concentrations (Conley et al. in press; Søndergaard et al. 2006), culminating with the most severe hypoxic event ever reported in the open Danish waters in 2002 (HELCOM 2003). Disregarding the effects of nutrients and climate variations related to the North Atlantic Oscillation (NAO, a climatic phenomenon based on the difference between the low pressure at Iceland and the high pressure at the Azores) as explanation for the changing oxygen conditions, an increasing awareness has recently developed on the effects of global warning on hypoxia.

1.2 Climate change and oxygen dynamics

While the details of climate change and global warming remains subjects of intense debate, there is an increasing amount of evidence that the earth's temperature regime is changing. This has profound implications for marine ecosystems and is of great concern. An effect of higher temperatures can manifest itself in numerous ways, for example by decreasing the solubility of oxygen (Benson and Krause 1984). Consequently, because the oxygen concentration of the Skagerrak surface water in winter and early spring determines the oxygen pool available in the Kattegat and Belt Sea bottom water during summer and autumn, higher temperatures (i.e. lower oxygen content) may become the critical element in maintaining normoxic conditions during summer and autumn. Indeed, new evidence suggests that the decreasing oxygen solubility is likely to be the main temperature-related mechanism behind the recent worsening of the oxygen conditions in the inner Danish waters (Conley et al. in press). Moreover, higher temperature enhances oxygen consumption rates in the bottom water and sediments (Hansen and Bendtsen 2006). Finally, if the heating of the surface water is larger than that of the bottom water, stratification will be reinforced thus demanding stronger winds to induce mixing.

Until now, most climate-related research has focused on simplistic relationships between temperature and biota (Harley et al. 2006). However, besides temperature there exist a suite of potential interacting climatic variables that can drive future dynamics in marine systems. Rising temperatures will for example result in altered mean wind fields and extreme wind events, thus influencing water column stability. This again will affect up-welling intensities, a phenomenon which is of fundamental importance for nutrient availability in costal marine systems. It is difficult to predict complex oceanography and some controversy exist as to the exact nature of expected changes in upwelling. But because upwelled nutrients stimulate primary production any changes will have significant consequences on overall productivity (Harley et al. 2006; Hays et al. 2005). One could argue that intensified up-welling could increase primary production due to enhanced nutrient recycling from deep layers. Consequently, greater amounts of organic matter may sink to the bottom which thus requires more oxygen during decomposition, increasing the risk of oxygen deficiency. A comparable scenario which augments primary production has been proposed to apply for the inner Danish waters, based on the hypothesis that frequent hypoxia leads to a regime shift in the benthic community towards species which enhance the processing of organic matter in the sediment. This will increase the nutrient flux to the water phase which stimulates phytoplankton production. Subsequently more organic matter will be deposited on the sediment surface during the late phase of hypoxia, thus reinforcing and prolonging the process of oxygen depletion. This is a major concern because coastal marine systems which frequently experiences hypoxia therefore may become, in an irreversible manner, more susceptible to eutrophication and hypoxia (Hagy et al. 2004). However, intensified upwelling may alternatively lead more oxygen-rich surface water to the bottom layers, thus improving oxygen conditions in the bottom water. Changes in the timing of important event such as upwelling dynamics or increases in water temperature may also cause tropic mismatch if the relative response of primary and secondary producers is different, with subsequent effects on overall production (reviewed by Hays et al. 2005 and Harley et al. 2006). On the other hand, if precipitation increases, as predicted for Denmark (Stendel et al. 2001), upwelling intensity may decrease due to strengthened stratification as a result of augmented freshwater runoff from the Baltic Sea area. One of two alternative scenarios could then arise; bottom oxygen conditions may worsen because less oxygen-rich surface water is lead to the bottom, or, the seasonal increase in primary producers may become limited if nutrients are retained in the bottom layers, with subsequent lower oxygen demands to decompose the reduced amounts of organic matter sinking to the bottom. Finally, it should be noted that in case of increased future precipitation a subsequently more brackish surface layers will hold more oxygen as oxygen solubility increases with decreasing salinity (Benson and Krause 1984) – this however only improve bottom oxygen conditions if wind stress is sufficient to induce mixing of the water column. In conclusion, although the exact directions of these changes are difficult to predict, long-term changes may well occur in plankton abundance and composition. This may have significant impact on fish stocks, through so-called bottom-up responses. Recent evidence for example show that cod recruitment in the North Sea is related to the size composition of the copepod communities (Beaugrand et al. 2003), and that the return of salmon *Salmo salar* to homewaters in the Northeast Atlantic can be explained by availability of plankton suitable to support the juvenile fish (Beaugrand and Reid 2003). Also, most notably Arnott and Ruxton (2002) found that when spring conditions were warmer than usual in the North Sea it correlated with poorer recruitment of sandeel. As there was a positive association between specific stages of sandeel's preferred prey, the copepod *Calanus*, and sandeel recruitment, it was suggested that availability of this copepod is important for survival of young sandeels. It is apparent from the above examples that long-term climate-related changes in plankton can have significant impact on commercial fish stocks.

1.3 Fish and hypoxia: Ecological response

Oxygen is not only a key element in the metabolic processes of invertebrate benthic fauna, but also of fish. Beside fish-kills caused by severe hypoxia or anoxia, the ecological response of sub-lethal hypoxia can also be profound. The aerobic metabolic scope (MS) of a fish indicates the limits wherein growth, reproduction and locomotion must be accommodated (Figure 1.2). When limiting oxygen availability causes a reduction of the MS it is likely to lead to energy budgeting conflicts between these factors. Ultimately, this may limit the distribution of fish and consequently alter species compositions and numbers (Diaz 2001). Field studies have shown that the response to low oxygen saturation differs between species. In Kattegat, for example, cod and whiting left the area when oxygen saturation fell below 25-40% whereas dab, flounder and plaice were more resistant and stayed until the saturation dropped below 15% (Petersen and Phil 1995; Pihl 1989; Baden et al. 1990). These different reaction patterns are presumable governed by a complex interplay of factors such as tolerance towards low ambient oxygen availability, feeding habits and mobility. Nonetheless, trying to escape unfavorable oxygen conditions indicates subsequent risks of metabolic distress due to increased energy expenditure, a strategy which can be fatal unless favorable conditions are reached in time. When fish attempt to avoid hypoxia and migrate away from an area, reduced biomass will inevitably be the consequence (Pihl 1989; Baden et al. 1990; Pihl et al. 1991). However, limited oxygen concentrations also impose restrictions on feeding rates and digestion and, because somatic and reproductive investment can be viewed as being competitors for limited resources, also on the reproductive effort (Jobling 1994). Thereby the observed lower biomass may not only reflect escapes but also reduced growth in remaining individuals (e.g. Petersen and Pihl 1995; Chabot and Dutil 1999).

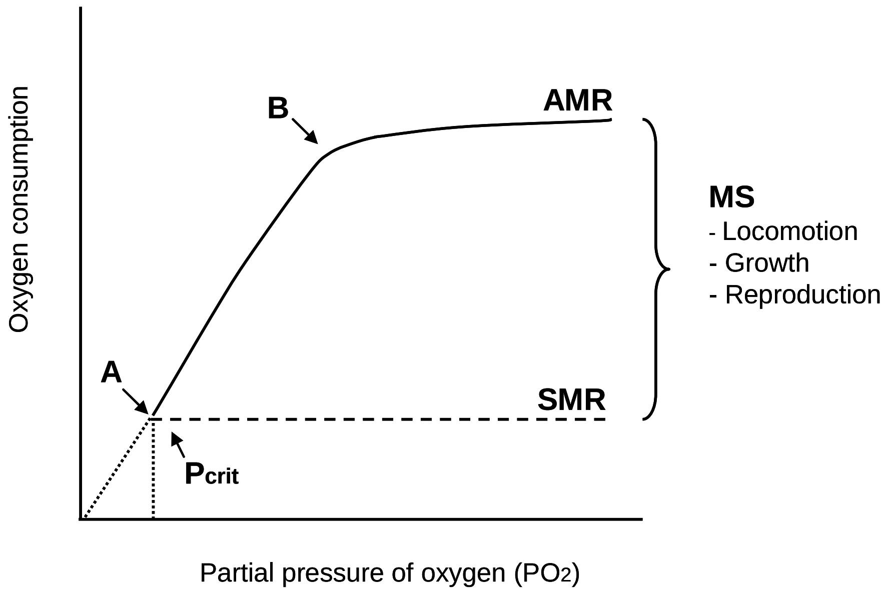

Figure 1.2 Schematic drawing of the relationship between ambient oxygen tension and oxygen consumption by the fish. For details please see the text. $P_{crit:}$:critical oxygen tension; SMR: Standard Metabolic Rate; AMR: Active Metabolic Rate; MS: Metabolic Scope. Redrawn after Jobling (1994).

1.4 Fish and hypoxia: Respiratory response

In general, water-breathing fish respond to hypoxia by first attempting to maintain oxygen uptake, then by conserving energy expenditure or reducing energy turnover, and finally by anaerobic energy metabolism. Fish are thus 'regulators' within a certain range of ambient oxygen levels, but will inevitable reach a level where they can no longer maintain their oxygen uptake and hence become 'conformers'. This species-specific value below which the oxygen requirements can no longer be met is often referred to as the critical oxygen tension, P_{crit}, (Figure 1.2). It represents a key parameter which, together with the fish' standard metabolic rate (SMR; the minimum oxygen requirements for maintenance for a resting, post-absorptive fish) can be used to evaluate the ability of the fish to cope with oxygen limitation. It should be noted that P_{crit} is not a constant value, but depends upon the rate of oxygen uptake exhibited by the fish. When rates of oxygen uptakes are high, e.g. in active fish, the P_{crit} is higher (denoted by point B in Figure 1.2) than when the rates of oxygen uptake are low, as denoted by point A in Figure 1.2. This latter point is also sometimes referred to as 'the point of no excess activity'. Note, however, that other factors such as temperature may also affect P_{crit} (see Paper IV). The maintenance of oxygen delivery may be achieved by increasing water flow over the gills via increases in ventilatory amplitude and/or frequency (Steffensen et al. 1982). Concomitantly, the respiratory area can be enlarged by recruitment of additional gill lamellae (Sundin 1995). At the cellular level, the oxygen transport capacity can be enhanced either by increasing the number of red blood cells or the oxygen-binding affinity of the hemoglobin (Wood et al. 1975). Finally, reduced energy expenditure can be achieved at the physiological/biochemical and/or behavioral level, through respectively 1) metabolic depression (Van Waversveld et al. 1989), which is a downregulation of aerobic metabolism presumably through suppression of ATP-producing and ATP-consuming pathways, 2) reduction in locomotor activity (Metcalfe and Butler 1984; Fisher et al. 1992; Schurmann and Steffensen 1994) or 3) selection of cooler waters (Schurmann and Steffensen 1992; Petersen and Steffensen 2003), also referred to as 'behavioral thermoregulation'. Decreased swimming activity is a commonly employed energy-conservation strategy during hypoxia and it is generally considered adaptive, especially if the exposure is prolonged or chronic (Metcalfe and Butler 1984; Herbert and Steffensen 2005).

2 Objective of the study

The overall objective of the present thesis was to investigate and evaluate how limited oxygen availability affects behavioural and physiological aspects of fish, with lesser sandeel, *Ammodytes tobianus* (Linnaeus, 1785), as a model organism.

2.1 The model organism

'Sandeel' is a collective term for a number of species in the family Ammodytidae. The family name derives from the Greek words 'ammo', which means sand, and 'dytes', meaning diver, words which relate to the peculiar lifestyle of these fish. They school 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; 1974b; 1974c; Freeman et al. 2004). Thus, they are not only affected by pelagic but also benthic conditions where oxygen depletion most often prevails. Sandeels prefer sandy sediments over 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). At sunrise, fish emerge from the shallow sand banks and swim to deeper waters to fed, returning later in the day. Site fidelity often leads to high abundances in the areas where the fish have settled, with average winter densities of 61 individuals per m^{-2} (up to 290 individuals per m^{-2}) in the central North Sea (Høines and Bergstad 2001). The small protein rich sandeels (Robards et al. 1999) are important prey items for a number of fish species (e.g. cod, saithe, haddock, whiting and flatfish, particularly plaice), marine mammals and sea birds (Scott 1968; Harwood and Croxall 1988; Monaghan 1992; Hammond et al. 1994; Høines and Bergstad 1999) and in the North Sea area the breeding success of several species of seabirds depends heavily on sandeel availability through the spring and summer months (Rindorf et al. 2000).

When referring to sandeels in the inner Danish waters five species are included. These can be distinguished by outer morphological features, but all exhibit the same characteristic lifestyle. By far, Raitt's sandeel (*Ammodytes marinus*) has received the most attention because this species comprise the vast majority of the commercial catches in the North Sea (Gislason and Kirkegaard 1998; Jensen et al. 2002). Commercial fishing for sandeel began in the early 1950s, and has, despite interannual variations, been high until 2002. In subsequent years, the North Sea landings decreased drastically (Figure 2.1) reflecting declining populations and this instigated real time monitoring. Real time monitoring is a close and instantaneous monitoring of a fish population for a limited period of time, to asses its size and age composition. In this way the fishing pressure can be regulated immediately to assure sustainable fishery. Such a system is the most appropriate way to regulate fishing pressures of short-lived species like sandeels, where the stock size can vary considerably between years. The burying behaviour of sandeels may however lead to misleading information on the spawning biomass, a problem which within recent years has been dealt with by counting buried sandeels in bottom dredging surveys during winter (according to the Danish Ministry of Fisheries, DIFRES 2007).

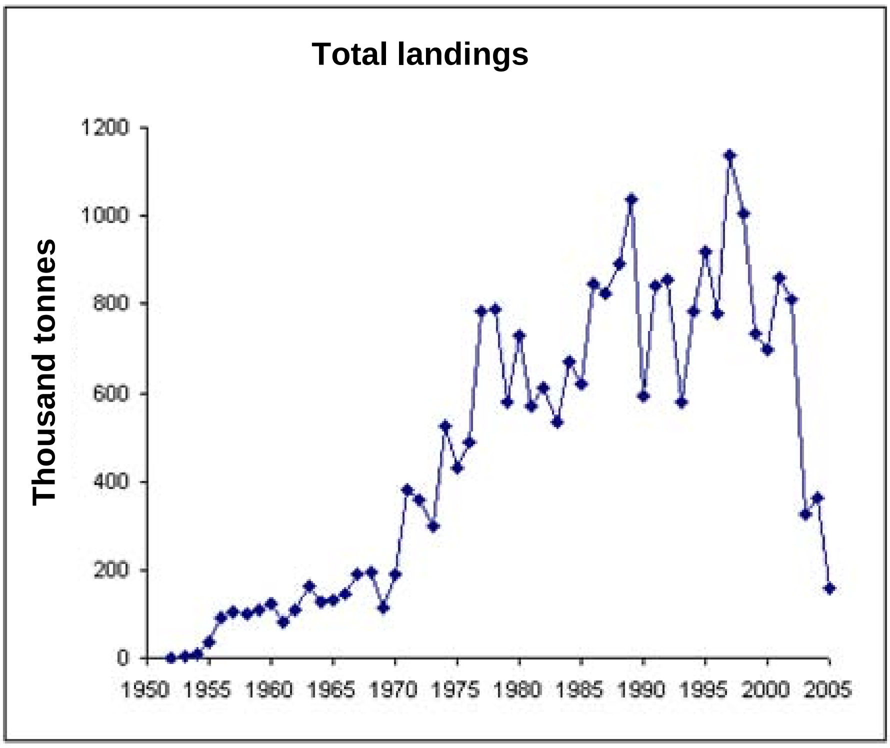

Figure 2.1 Yearly landings of Raitt's sandeel (*A. Marinus*) in the North Sea (1952-2005). Obtained from the homepage of the Danish Ministry of Fisheries (DIFRES).

Compared to Raitt's sandeel, the lesser sandeel (*Ammodytes tobianus*) inhabits shallower waters and is abundant in coastal areas of the inner Danish waters. No stock assessment information exists on *A. tobiamus*, presumably because there is no commercial fishery after sandeels in these waters. Nonetheless, large schools of *A. tobianus* frequently visit the shallow waters of the Sound (personal observations). The accessibility of this species, in addition to the fact that hypoxic conditions often prevail during summer and autumn months in its natural environment, was the main reasons for choosing it as model organism. Although the present results may possibly be specific to *A. tobianus* they can likely be applied to other sandeel species as well, because they exhibit similar life history traits. One exception is the Japanese sandeel (*A. personatus*) which has a somewhat different behaviour as it undergoes summer aestivation induced by rising water temperatures (Tomiyama and Yanagibashi 2004). Nevertheless, any new information is important to supplement the sparse knowledge which at current is available on the behaviour and physiology related to sandeel's peculiar lifestyle and the environments they encounter.

2.2 The sections of the present thesis

The first part of this thesis focuse on obtaining knowledge on basic respiratory features which can be used to describe the oxygen needs and hypoxia resistance of sandeels. Also, I investigated the aerobic energetic frame of these fish as this relates to their swimming performance (Paper I). For comparative purposes I examined the swimming energetics of a small salmonid fish, the capelin, which has much the same role in Arctic Ocean ecosystem as sandeel has in the Danish waters (Paper II). Finally, to elucidate what strategy sandeels employ during unfavourable oxygen conditions, I examined how the swimming activity of sandeel was affected by acute hypoxia (Paper I).

The second part of the project was instigated by the recognition, based on recent studies with marine sediments, that the previous assumption that buried sandeels obtain oxygen from the interstitial water is questionable. Using an innovative oxygen imaging technique, I investigated the mechanism by which sandeels obtain oxygen when buried in sandy sediment (Paper III). Besides the obvious advantage of being hidden, burying has also been hypothesised to be an energy saving strategy (e.g. Quinn and Schneider 1999). To test this theory, I estimated the oxygen requirements of buried fish (Paper III) and compared it to values obtained on fish in a conventional respirometer (Paper I). Furthermore, the behavioural responses to declining water oxygen levels, e.g. emergence from or relocation in the sediment were investigated (Paper III).

The third part of the work aimed at understanding to which extent low oxygen can influence on habitat availability for sandeels (Paper VI). This was done by coupling salinity, temperature and oxygen data from the Danish waters with information on bottom substrate type and the critical level of dissolved oxygen for sandeels (S_{crit}; Paper I). In this way the extent of habitat loss within recent years could be predicted. Also, future changes in habitat loss related to a global warming scenario was forecasted.

The fourth, and final, part of this thesis focus on the effects of prolonged hypoxia on the diurnal activity pattern of sandeels. Realizing that both time spend in the water column and buried in the sediment is important for the fitness of the fish (aiding in feeding or predatory avoidance and energy saving, respectively), I aimed to examine effects of moderate or severe hypoxia on the emergence and burying behavior, and number of fish entering the water column during a diurnal cycle.

3 Results and Discussion

3.1 Oxygen requirements

The standard metabolic rate (SMR) of fish not only relates strongly to temperature, but also to body mass, where larger fish have lower mass-specific resting oxygen uptake rates (Clarke and Johnston 1999). Small sandeels (typically 2-4 g) thus have low resting oxygen requirements averaging 69 mg O_2 kg^{-1} hr^{-1} for fish of 3.5-g at 10°C (Figure 3.1A), corresponding to 35 mgO_2 kg^{-1} hr^{-1} for a standard 100g fish (Paper I). In comparison, small specimens of the gadoids Atlantic cod, saithe and whiting and the salmonid capelin have SMR between 57 and 110 mg O_2 kg^{-1} hr^{-1} at 10ºC, respectively (Schurmann and Steffensen 1997; Steinhausen et al. 2005; Paper II). The metabolic demands of the benthic sole (*Solea solea*) is however less than sandeel' at equivalent temperature, with 25 mg O_2 kg^{-1} hr^{-1} for a 100g fish (Lefrançois and Claireaux 2003). This presumably relates to the less active nature of sole. Low minimum oxygen requirements are advantageous, especially when the fish are buried in sandy sediments without a permanent opening to the overlying water (Paper III). However, when sandeels are embedded in the sediment jaw movement is restricted. Most burying fish species rely on a forceful branchial pump to create the additional force needed to overcome the resistance to the exhalation current imposed by the sediment and the fish's body (Yazdani and Alexander 1967; Hughes and Morgan 1973). This may also apply to sandeels that additionally employ constant minute gill movements to create an advective transport of water towards the mouth (personal observations). It could consequently be speculated that the cost of ventilation, normally in the region of 10 to 15% of the oxygen uptake, excluding ram ventilators (Randall and Daxboeeck 1984; Steffensen 1985; Farrel and Steffensen 1987), would be higher for fish in sediment reflected in higher total oxygen uptake of buried sandeels. This could not be demonstrated in the present study. On the contrary, the total oxygen requirements of buried sandeels were considerable lower (~ 50%) than that of those in a conventional respirometer, i.e. fish with no limitation on branchial pumping activity (Paper III and I) (Figure 3.1B). This demonstrates that sandeels have a lower metabolism when buried, which, intuitively, is of great advantage especially during winter months where limited prey and light availability restrict feeding. Probably the fish compensate for high ventilatory costs by the efficient oxygen extraction where a high 86% of the oxygen is removed during gill passage (Paper III). Besides the metabolic advantage, an additional obvious gain of being buried is being hidden from predators. Clearly, the burying behavior is a central determinant of the fitness of these fish.

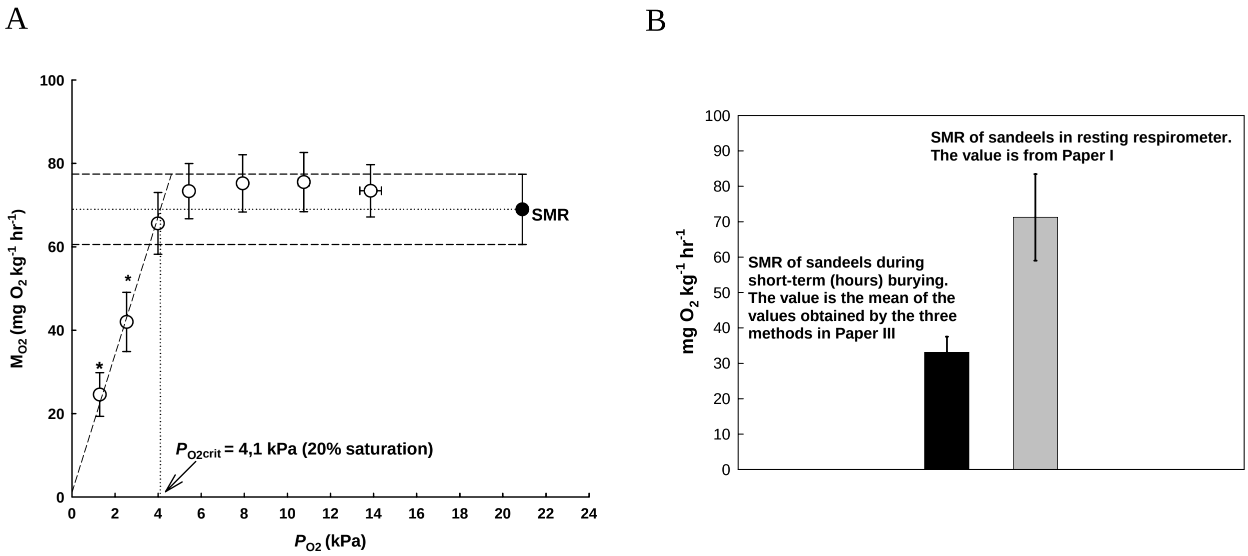

Figure 3.1 A: The standard metabolic rate (SMR) and critical oxygen tension (P_{crit}) of lesser sandeel (3.5-g fish at 10°C) in a resting respirometer. From Paper I. B: Comparison of the SMR of buried lesser sandeel (black bar) versus the value from fish in a conventional resting respirometer (grey bar, i.e. corresponding to the value in A). Both values are at 10°C and corrected to fish of 3-g. From Paper I and Paper III, respectively.

A sidetrack from the perspective of the fish leads to a note on bioturbation, that is, the ventilation and particle redistribution of sediment layers by macrofaunal digging activity, and bioirrigation, the process where macrofauna actively pump oxygenated bottom water through their burrow systems and deep into the otherwise anoxic sediment. These are significant processes on the ocean floor, mainly brought about by benthic invertebrate fauna such as brittle stars and polychaetes. In a typical costal area their activities in the sediment normally constitute around 50% of the total sediment oxygen uptake (Glud in progress), but may in extreme cases comprise up to 80 to 90% (Vopel et al. 2003; Wenzhöfer and Glud 2004). Considerations on how bottom-dwelling fish may influence oxygen fluxes to the sediment are, surprisingly enough, seemingly nonexiting. Mass-balance calculations nonetheless revealed that with average densities of 60 individuals per m^{-2}, the sandeels enhanced the benthic oxygen uptake by a factor two, of which approximately 50% was related to the respiration of the fish (personal data, unpublished). Without doubt, sandeels greatly influence the sediment oxygen dynamics and biogeochemistry in the areas they inhabit.

3.2 Mode of ventilation

Due to the difficulties of measuring sediment oxygen environments it has for many year been unclear from where sandeels obtain oxygen when buried. Consensus was that the fish obtain oxygen from the interstitial water. Indeed, the nature of sand will prevent maintenance of a burrow-like structure, which otherwise could ensure exchange with water above the sediment surface. Indeed, the interstitial spaces between grains of pure sand provide more space for water compared to silt or mud-rich sediments. And indeed, sandeels prefer rippled seabed or tidally active areas with strong bottom currents and intense wave actions (Meyer et al. 1979; Pinto et al. 1984; Wright et al. 2000), that is, areas where the driving force for advective oxygen transport into the sediment can be high. However, in natural environments wave actions, flow characteristics and bottom topography are constantly changing, resulting in extensive oxygen oscillations in the upper layers of the sediment (de Beer et al., 2005; Precht et al., 2004; Cook et al., in press). As a result, the fish will experience periods where anoxic sediment excludes the option of exploiting oxygen in the interstitial water. Under such circumstances oxygen only penetrates a few millimetres into the sediment and the fish rely on advecting oxygen-rich water from the sediment surface, through the interstice and into the mouth (Figure 3.2A, B and C). And indeed, the buried fish are capable of efficiently ventilating their gills, as evident from the high oxygen content of the water entering their mouth (mean saturation of 93.5±4.8%; Figure 3.2B and C) with a mean ventilatory flow rate of 86.9±7.3 ml min^{-1} kg^{-1} (Paper III) which is comparable to values estimated for partially buried flounder and plaice (87 to 108 ml min^{-1} kg^{-1}) at equivalent temperature (Kerstens et al., 1979; Steffensen et al., 1982). Still, with the many reports on sandeel's sediment and area preference it is likely that the fish benefits from an intermittent passive oxygenation of the interstitial water when dwelling in sand. It remains to be shown to what extent the fish benefit, and also if they keep relocating themselves to be in favourable conditions.

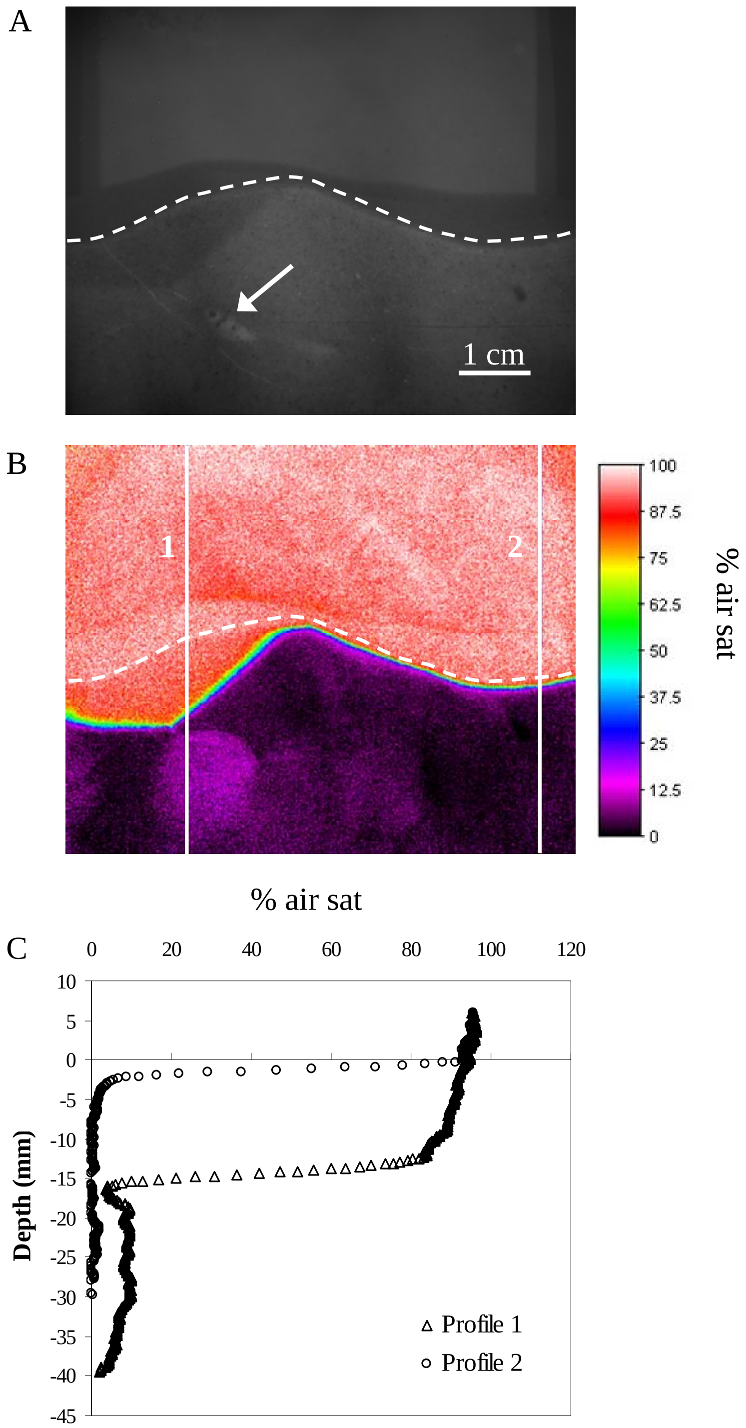

Figure 3.2 A: The position of the sandeel in the sediment (arrow points to the head and dotted line indicates sediment water interface). B: The corresponding oxygen image, showing how the fish sustain its oxygen requirements by advecting normoxic water from above the sediment surface and into the mouth, thus creating a 'funnel' of oxygenated sediment. 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. Modified from Paper III and reproduced with permission of the Company of Biologists.

3.3 Hypoxia tolerance

Species' hypoxia tolerance is generally believed to relate to their lifestyle and the environment they occupy. Besides the sand banks on deep waters, intertidal sandy beaches also host numerous sandeels (Quinn and Schneider 1991; Quinn 1999; personal observation). Intertidal flats experiences pronounced oxygen oscillations, with anoxic sand during low tide air exposure (Gordon 1960; de Beer et al. 2005). It has accordingly been expected that sandeels can maintain their oxygen uptake despite very low ambient oxygen (Quinn and Schneider 1991; Wright et al. 2000). In this sense the present result was a surprise; lesser sandeel has an ordinary hypoxia tolerance with a P_{crit} of 4.1 kPa at 10ºC (~ 20% sat.) (3.1A). However, two things, alone or in combination, may explain this; 1) a partial dependency on anaerobic energy production and 2) a lower P_{crit} of buried fish. Regarding the first point, despite ventilating well-oxygenated surface water the buried fish may, at least during periods with no passive penetration of oxygen into the interstice, be oxygen limited. If this is the case, then the oxygen uptake may not balance the energy requirements, which hence must be supplemented through anaerobic metabolism. Plausibly, this could be a necessary consequence or adaptation to the burying behaviour, an idea which is also supported by the routine metabolism of swimming sandeels which has a substantial anaerobic component of 2.2 ± 0.6 mmol l^{-1} blood lactate (Paper I). This is, however, a short-term solution where the fish postpone the problem by developing an oxygen debt, which can then be repaid when the fish leaves the sediment. How the fish cope during winter dormancy without severe acid-base disturbances is a puzzle. The Crucian carp provides a unique example of how this can be done; carp can at temperatures close to 0ºC survive anoxia for several months without massive build-up of lactate by producing ethanol as the major end product of anaerobic glycolysis. The process is catalysed by alcohol dehydrogenase (ADH) confined in the muscle tissue, and the ethanol subsequently diffuse into the ambient water when blood passes through the gills (Shoubridge and Hochachka 1980; Nilsson 2001). In this way tolerable steady-state levels of lactate and ethanol are maintained. It would be of utmost interests to investigate if sandeels are also capable of using this strategy to avoid self-polluting during prolonged burials. Finally, concerning the second point we speculated that the P_{crit} of buried fish may be lower than the suggested value (in Paper I) because the present P_{crit} estimate was obtained on sandeels with a higher apparent SMR (in the conventional respirometer; Paper I) than buried fish (Paper III). To further advance our knowledge on the hypoxia tolerance of sandeels future work should therefore aim to establish the P_{crit} of buried fish.

3.4 Effect of hypoxia on swimming sandeels

Swimming is for most fish a central determinant of their fitness, as it involves prey capture, escape from predators, migration and selection of a favorable environment. Sandeels can achieve a sevenfold increase in their metabolic rate, a scope which indicates good swimming abilities (Paper I), and which provides adequate aerobic capacity to spend hours feeding in high velocity frontal waters. Oxygen availability is one of the main factors affecting the swimming activity of fish. Although it is often difficult to theoretically predict how an affect may manifest, consensus is that fish which recognize and react to hypoxia balance between two strategies, placed at opposite ends of the 'behavioral spectrum'. The fish can either increase its activity (Bejda et al. 1987; van Raaij et al. 1996; Domenici et al. 2000; Johansen et al. 2006) and hence the probability of finding a more favorable environment, although this would also increase its risk of exhaustion, or it can employ a more quiescent behavior (Metcalfe and Butler 1984; Fischer et al. 1992; Nilsson et al. 1993; Dalla Via et al. 1998; Cech and Crocker 2002) to save energy and hence avoid major physiological stress. The latter option does on the other hand reduce the chances of reaching more suitable surroundings. Unexpectedly, when exposed to an acute decline in ambient oxygen small groups of sandels did not employ any of the two alternative strategies, nor did the fish exhibit a graded response (Paper I) as shown for schooling Atlantic herring (Domenici et al. 2000). Instead sandeels distinguish themselves by a noteworthy *lack of response* during oxygen decreasing from normoxia to severe hypoxia (oxygen levels below P_{crit}). However, eventually their swimming speed decreased (Figure 3.3), presumably due to physiological collapse as average blood lactate levels were very high with 7.5 ± 2.7 mmol l^{-1} (Paper I). It would seem that sandeels swimming capacity is not solely determined by the MS but rely also on anaerobic energy production with concurrent lactate accumulation. In an environment where hypoxic conditions are of short duration, being able to sustain swimming despite becoming metabolically distressed could be adaptive for a small prey to maintain its escape capacity. This strategy may be maladaptive during extensive and prolonged oxygen depletion events, as have been observed during recent years in the inner Danish waters (HELCOM, 2003; Conley et al., in press), where, for periods of weeks, water oxygen levels reached values critical for sandeels. Despite the above lines of reasoning it is still speculative what mechanism underlies the observed response. The rate of deoxygenation can also be a relevant factor influencing the strategy employed by the fish, as exemplified by the pioneer work of Moss and McFarland (1970) on schooling anchovies. They showed that this species did not respond to a slow reduction in oxygen levels, but increased speed as response to a rapid decrease in oxygen. Such context-dependent response has also recently been show for Atlantic cod (Herbert and Steffensen 2005; Johansen et al. 2006). Some debate exists as to whether fish actually recognise hypoxic waters or if a response merely is evoked by respiratory stress, and there may likely be species-specific differences in the recognition abilities. When swimming sandeels were exposed to acute hypoxia (Paper I) the exposure time to each level of oxygen may have been too short for the fish to perceive and integrate it aptly, leaving it in a 'state of confusion' and unable to make a beneficial choice. Maybe sandeels would react differently when exposed to a slow reduction in hypoxia. And maybe the reaction pattern would be different again if the fish swam in a large school and not in a small group. Fish may experience large reductions in oxygen availability when swimming in the centre-back region of a large school and these internal oxygen conditions may be worsened by ambient hypoxia (McFarland and Moss 1967). It has therefore been suggested that schooling fish may have a tendency to increase in speed as a response to hypoxia, in order to increase the reshuffling of individuals in the school, to avoid school break-up (Domenici et al. 2000).

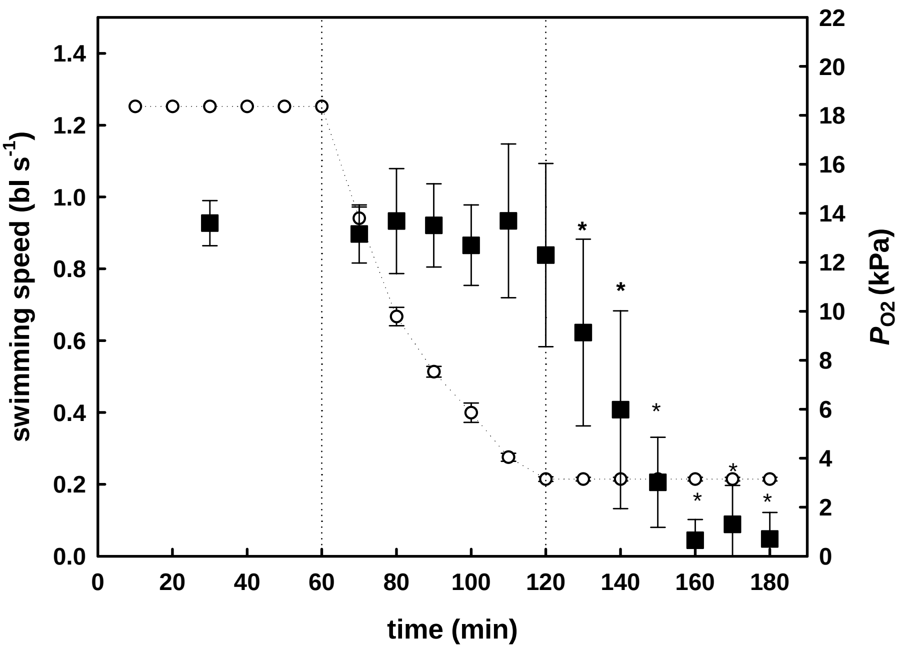

Figure 3.3 Swimming speed in body lengths per second (left scale and filled squares) of lesser sandeel during exposure to an acute decrease in water partial pressure of oxygen (P_{O2}, right scale and open circles). From Paper I.

Considering the special life-history of sandeels a continuous trade-off must exist between the need to swim in order to feed or stay in the sediment, to hide and save energy. Indeed, this appears to be the case, at least under laboratory conditions where only half or less of the sandeels were observed in the water phase at any time during the day despite plentiful daily feeding (Paper V). In nature, the outcome of this 'bioenergetic compromise' must intuitively relate strongly to food availability and presence of predators. But the present results show that also oxygen availability can be a decisive factor where the vast majority of the fish that had experienced prolonged severe hypoxia (~35% saturation for 1-2 weeks) swam throughout the day, compared to half or less of the fish in normoxia. Presumably, the need to ventilate the gills took precedence. Constant swimming is more costly than resting. Furthermore, most fish had ceased feeding, but still exhibited a normal schooling behavior, a trait which in the field will lessen the risk of predation for the individual. The latter is consistent with the behavior observed on herring where school structure prevailed until 12-25% oxygen saturation (Domenici et al. 2000). It was beyond the scope of the present study (Paper V) to include measurements of swimming speed under the different oxygen regimens. Establishing a relationship between prolonged hypoxia and swimming activity would indeed advance our understanding of possible ecological consequences of unfavorable oxygen conditions on sandeel populations.

3.5 Effect of hypoxia on buried sandeels

It was not clear-cut to foresee how buried sandeels would react when exposed to a gradual (within a couple of hours) decline in the oxygen level of the overlying water (Paper III). The fish balances between two, initially equally profitable, strategies; to escape and attempt to reach more oxygenated waters or to save energy by staying buried and in this way postpone major physiological stress. Planar optode imaging revealed that most fish moved closer to the sediment surface when exposed to moderately hypoxic water and stuck their head up well in advance of leaving the sediment. The fish did however not leave the sediment before they had endured a period of ventilating severely oxygen-depleted water. In a situation of acute hypoxic exposure sandeels apparently employ a 'sit and wait for better times' strategy, relying on endurance until oxygen conditions improve, presumably with substantial accumulation of lactate. This quiescent behaviour minimises their oxygen requirements and keeps them protected from predators. It could be argued that such acute exposure rarely is encountered in the field where hypoxia normally develops in a more gradual manner. Yet, when westerly winds blow the surface water away from east-facing coasts a special case can appear where the halocline tilt and a sudden flow of oxygen depleted water is lead towards the coast. An extreme example of this was observed along the western coast of Kattegat in autumn 2002 where dead fish numbering thousands washed onto the shore in numerous places (HELCOM 2003; Christensen et al. 2004). In conclusion, sandeels strategy of staying put when exposed to acute hypoxic exposure is beneficial in most cases and might increase their chances of survival, but can be fatal if they are too metabolically distressed to escape when the conditions becomes lethal.

Considering the subtle behavioral response to acute hypoxia described above, the diurnal activity pattern of sandeels was surprisingly sensitive towards more prolonged exposure. 1-2 weeks of moderate hypoxia (~60% saturation) disrupted the normal diurnal behavior where the fish emerged in the morning and gradually buried as the day progressed. Instead, fish acclimated to moderately hypoxic water continuously moved out from and into the sediment (Figure 3.4). Obviously, the fish recognized the altered oxygen regime, but their response seemed maladaptive due to the costs and time associated with repeatedly penetrating the sediment. The chaotic behavior additionally disrupted the normal schooling performance. As schooling primarily is considered an anti-predator or feeding adaptation (Pitcher and Parrish 1993), the individual will under such conditions be more vulnerable and also experience reduced feeding. This is germane as field measures suggests that sandeels in the inner Danish waters could be exposed to moderate hypoxia for weeks and up to several months during summer and autumn each year (HELCOM 2003; Conley et al. in press), which may influence their distribution and abundance through changes in growth or survival.

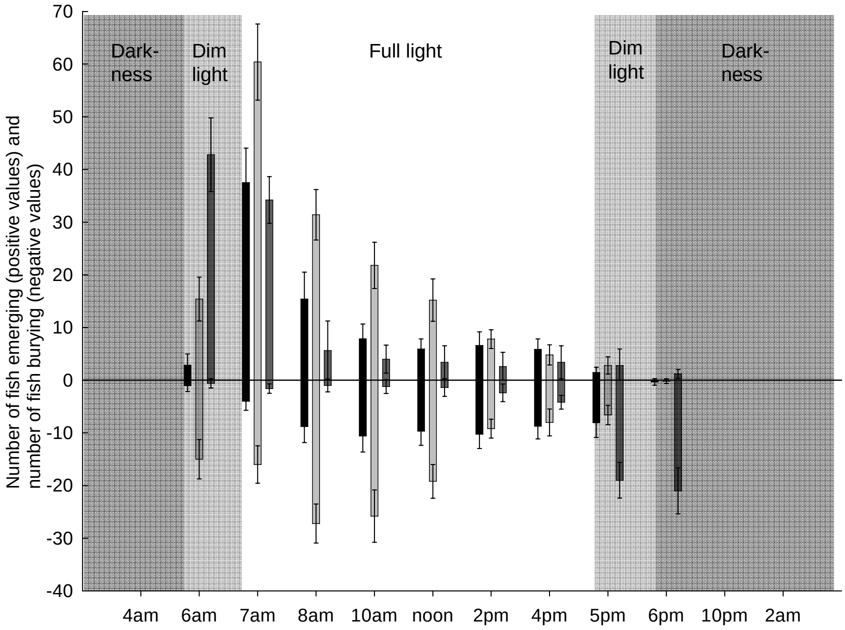

Figure 3.4 Diurnal activity pattern of sandeel. Black bars: fish in normoxia; Light grey bars: fish acclimated to moderate hypoxia; Dark grey bars: fish acclimated to severe hypoxia. From Paper V.

Prolonged exposure (1-2 weeks) to severe hypoxia (~35% saturation) evoked a completely different reaction pattern as many more fish left the sediment and stayed in the water column (Figure 3.5) (Paper V). This observation suggests that the buried fish were metabolically distressed under the prevailing conditions, despite that the oxygen level was above the species' P_{crit} (20% saturation at 10ºC). As stated previously, ventilatory costs are likely higher for buried fish and water-flow over the gills limited. Moreover, 10 to 15% of the oxygen in the inspired water is lost to microbial and bacterial respiration in the interstitial water (Paper III). This may well explain why the fish needs to swim when exposed to severe hypoxia. Concurrently, swimming enhances water-flow over the gills and hence facilitate oxygen uptake. The few fish that stayed put in the sediment had their head above the sediment surface, a behavior which was not observed in normoxia; in this way they are still semi protected from predators and spend less energy than when swimming. Remarkably, in spite of the poor oxygen conditions all fish buried during the dark period (Paper V), though consistently with their head above the sediment surface. This seemingly reflects a compromise between protection in darkness where the sandeels have poor vision and facilitated gill ventilation. In the field this may however prove fatal as some demersal feeders (like whiting, haddock, cod and flatfish) are known to concentrate their predatory effort to crepuscular hours and dawn where the sandeels move in and out of the sediment, or night time, by simply eating the sandeels out of the sediment (Girsa and Danilov 1976; Hobson 1986; Temming et al. 2004).

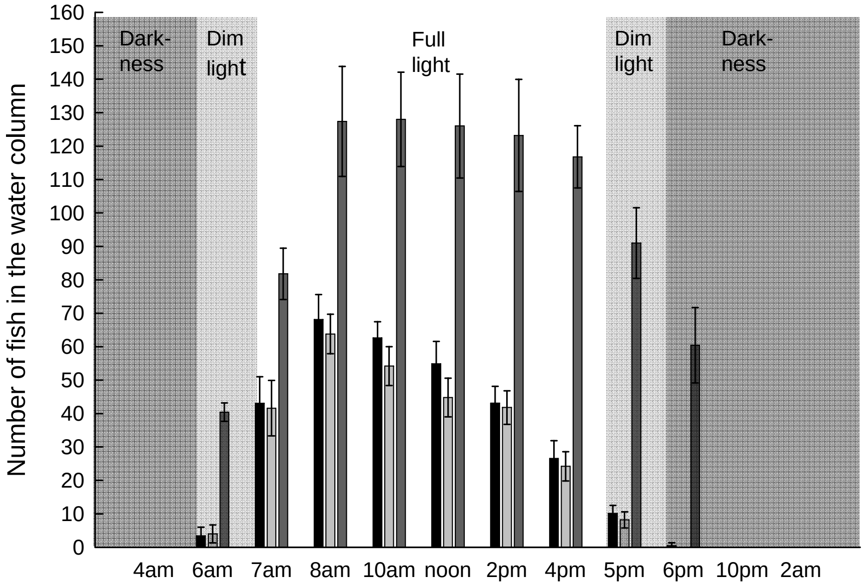

Figure 3.5 Diurnal variation in number of sandeels in the water column. Black bars: fish in normoxia; Light grey bars: fish acclimated to moderate hypoxia; Dark grey: fish acclimated to severe hypoxia.From Paper V.

Global warming is predicted to proceed rapidly this century (Easterling et al. 1997; Easterling et al. 2000), and it is imperative to improve predictive powers to mitigate the potentially harmful effects on coastal systems. In this way management and conservation of marine species and habitats may be successful. Access to long-term, regular time-series of monitoring data (temperature, salinity and oxygen) from the inner Danish waters provided a unique opportunity to model recent year's levels of oxygen at the bottom (Paper IV). A coupling of low oxygen saturation and habitat loss could thereafter be established by combining these results with information on sediment type and the P_{crit} of sandeel (Paper I). This demonstrated beyond doubt that critical oxygen levels will exclude sandeels from a fraction of the sandy seabed in which they prefer to bury. The extent of the unfavorable area varied between years, but in case of extreme climate events such as in 2002, nearly one fourth (~ 5800 km^2) of the areas with suitable sediment in the inner Danish waters were exposed to oxygen levels at or below the P_{crit} of sandeel (Figure 3.6). The more enclosed regions have a relatively constant coverage of oxygen deficiency, being most persistent in the Little Belt and Fehmarn Belt with >1000 km^2 affected every second year, whereas the open-water regions (Kattegat, the Sound, Northern Belt Sea and the Great Belt – see e.g. Figure 1.1A) are only affected in years where weak winds, high temperature and high run-off prevails. Such regional discrepancies in vulnerability will, assuming that the fish exhibit site fidelity, consequently influence the temporal and spatial prevalence of poor oxygen conditions that the fish will encounter. Some speculation is nevertheless unavoidable in a study like the present. Temperature plays a major role as it affects oxygen solubility of water and fish metabolism. In addition, fish are expected to be unable to meet their metabolic requirements starting at a higher level of dissolved oxygen at high than at low temperatures (i.e.

P_{crit} increase with temperature). Consequently, the current interpolation method (Paper IV) hinges on the relationship between temperature and P_{crit}, and, on reflection, an effort should have been made to experimentally establish this relationship for sandeel, instead of using data for cod. However, because the P_{crit} at 10° is very similar for cod and for sandeel (23 and 20% saturation, respectively; Schurmann and Steffensen 1997; Behrens and Steffensen 2007), and because reliable data exist on the relationship between temperature and P_{crit} for cod, it was assumed that the same correlation could be applied to sandeel. Also, it was assumed that the fish can not exploit sandy sediments which at any time during summer or autumn months have been exposed to oxygen saturations below P_{crit}. However, range shifts are often episodic rather than gradual (Pihl et al. 1991), and because hypoxia in the Danish waters is seasonal by nature sandeel may potentially return to the affected areas after oxygen conditions have improved. For that reason it could be argued that a time-threshold (days or weeks) of exposure to critical oxygen levels could be incorporated to rightly asses the likelihood of displacement or succumb of the sandeels. Although the study has its limitations and build on certain assumptions it is indisputable that the coupling of low oxygen and habitat loss by far is trivial, not least in the present situation where extensive and prolonged oxygen depletion events have been observed during recent years in the inner Danish waters, revealing periods of weeks with water oxygen levels critical for sandeels (HELCOM 2003; Conley et al. in press).

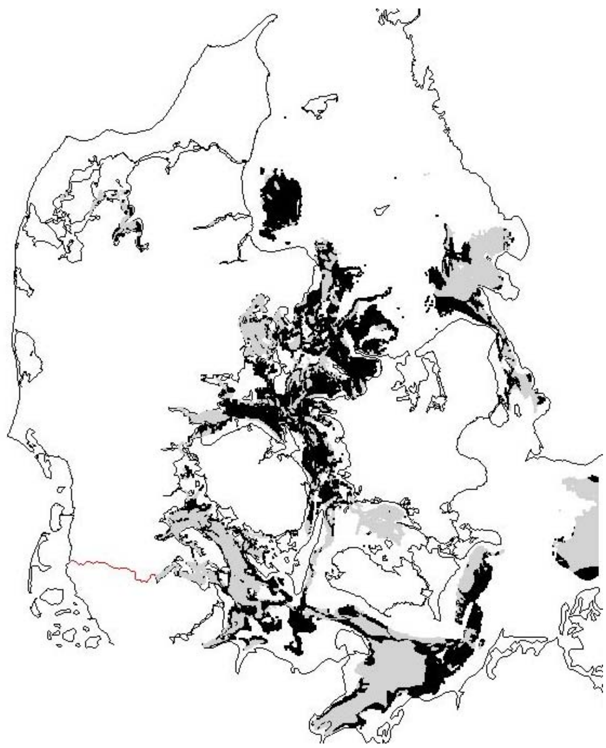

Figure 3.6 Areas with unsuitable sediments (grey) and suitable sediments (black) exposed to oxygen levels critical for sandeel (oxygen saturation ≤ S_{crit} of sandeel). Modified from Paper IV.

Future global warming may further enhance the risk for oxygen deficiency because the oxygen solubility will decrease, the stratification strengthen, while the degradation rate of organic matter increases hence augmenting oxygen consumption at the bottom. In this situation much depend on the balance between mixing and stratifying processes, as this determines nutrient recycling from deep layers on which the seasonal onset of primary production depends. Even though state-of-the art physical models allow for future prediction of the conditions in the Danish coastal waters due to climate change, such forecast are inescapably limited by certain assumptions, uncertainties and speculations. Such speculation should however not be discouraged, and consequently, an effort was made to predict the impact of global-warming related hypoxia on sandeel burying habitats (Paper IV). According to the scenario of the coupled atmosphere-ocean global change model ECHAM4/PYC3 regional temperatures will on average be 4.0º C higher during the first 4 months of the year in 2100 compared to present time, that is the months where the oxygen solubility and hence oxygen content of the incoming Skagerrak water is determined (pers. comm. Bendtsen and Hansen, National Environmental Research Institute, Denmark; Roeckner et al. 1999). Assuming that salinity and the supply of organic matter remain unchanged (which may be too simplistic an approach) such regional warming will increase the area exposed to critical oxygen levels by 2-4 times, with up to 10,000 km^2 (more than 40%) of the sandy seabed being affected in unfavourable years (Figure 3.7) (Paper IV). Notably, there is almost no suitable sediment below 15-20 m depth (Figure 3.8) and the fish must consequently bury on shallower water and swim higher in the water column. Several North Atlantic fishes have also undergone shifts in their mean depth distribution range towards shallower water in response to warming (Perry et al. 2005). For sandeel such a vertical shift could result in increased spatial overlap with marine predators and also make the fish easier to reach by avian predators. Furthermore, higher densities of buried sandeels will likely occur in the remaining suitable areas. This can lead to intensified aggregation of fish predators, thus escalating the predation pressure.

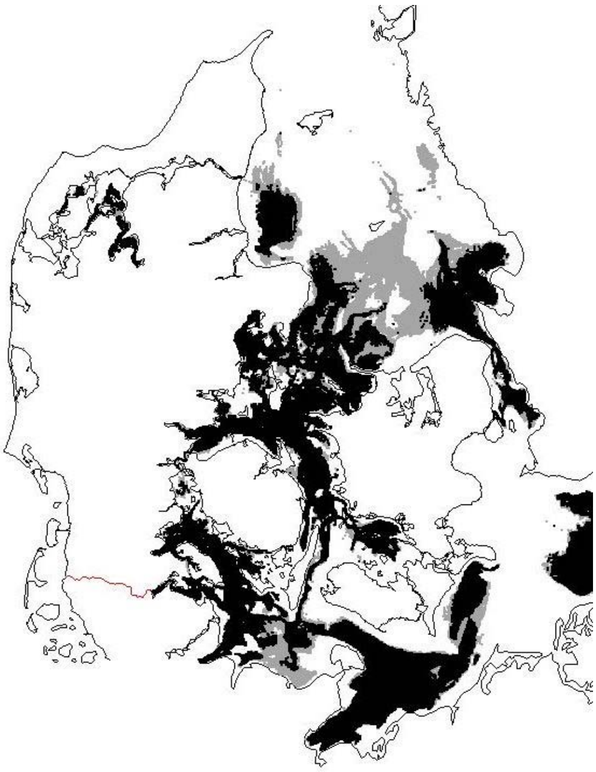

Figure 3.7 Spatial extent of oxygen deficient sandy sediments in 2002 (black) and the projected extent resulting from a 4 ºC temperature increase (grey). Modified from Paper IV.

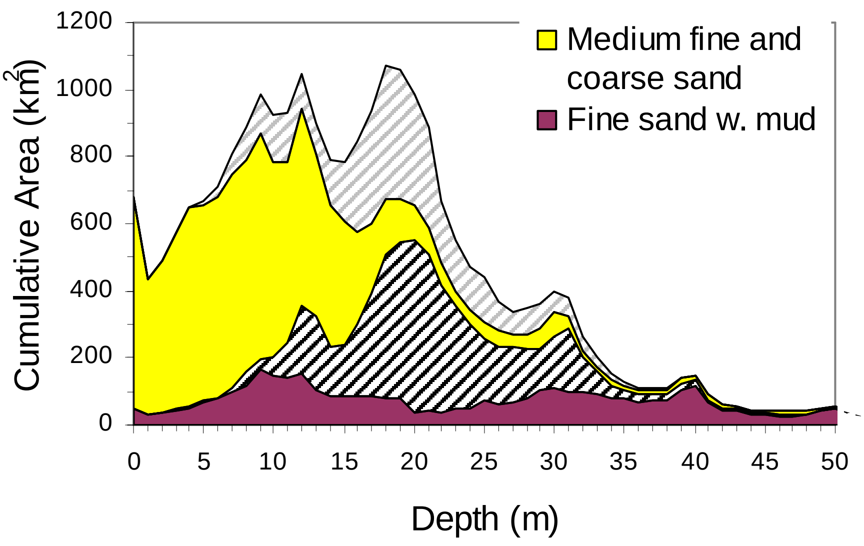

Figure 3.8 Depth distributions of sediments suitable for sandeels to bury in. The hatched parts indicate suitable areas which are predicated to become exposed to oxygen levels ≤ S_{crit} resulting from a 4º C temperature increase combined with unfavourable climatic conditions. Modified from Paper IV.

4 Summary and Perspectives

The present case study shows that the effects of hypoxia on fish can manifest itself in various ways. I aimed to elucidate the response to low oxygen of a single species and consequently, the various results, which at times may seem in contradiction, can not be explained by any interspecies discrepancies. Instead, when results of this thesis revealed alternative intraspecific strategies it is a result of complex context dependencies, such as rate and duration of hypoxic exposure, degree of respiratory distress, trade-off between life history traits and so forth. When prolonged severe oxygen levels prevail more sandeels are forced out of the sediment during daytime to swim in the pelagic water (Paper V). Parts of the sand banks will be exposed to bottom water too severely deprived of oxygen for the fish to cope with and they must hence concentrate in more favorable areas when seeking refuge. Future global warming is predicted to exacerbate the conditions, as oxygen deficiency may progress further into the water column and thus onto shallower water (Paper IV). Sandeels must as a result bury on shallower water and swim higher in the water column, creating vertical shifts which may increase their spatial overlap with predators. Intensified evening predation may also result as the fish have to keep their head above the sediment surface at night, to aid gill ventilation when ambient oxygen levels are low and to avoid oxygen loss to bacterial and microbial respiration in the interstice (Paper V and III, respectively). Feeding will cease due to loss of appetite and eventually energy allocation to growth and reproduction will decline. Ultimately, if conditions become severe enough, sandeels may disappear. Such a reduction in biomass, through migration and/or reduced growth, has generally been observed in hypoxic areas (Baden et al. 1990; Pihl et al. 1991; Petersen and Pihl 1995). Some fish species may however initially benefit. Hypoxia can induce buried benthos such as e.g. polychaetes and crustaceans to emerge and others, for example bivalves, to extent their siphons further into the water column where resistant opportunistic bottom-feeding fish can benefit and exploit this hypoxia-induced prey availability (Pihl et al. 1992). From comparison of marine ecosystems around the world it however appears that changes in fish species composition and abundance along a hypoxia gradient are very predictable and generally have followed the same path, in due course favoring smaller, short-lived pelagic species (Caddy 1993; Diaz 2001).

Fish live in a fluctuating thermal environment and because they are poikilotherms their body temperature oscillate accordingly. All fish species have a zone of temperatures they can tolerate and within which their optimum temperature lays. This is the temperature they prefer if given a choice and around which most physiological processes reach maxima. The limits of the zone of thermal tolerance however vary with species, life stage, abiotic factors and the previous thermal history of the fish (Jobling 1994). This indicates that the 'ecological relevant' tolerances is a graded and changing concept and the present geographical distribution of fish populations is a long-term response to environmental and climatic conditions in local areas. Is it then likely that any small chronic increments in temperature will have an effect when superimposed on natural regimes? This is a complex question to answer as forcing processes are rather unclear and actual latitudinal range shifts difficult to document. Recent work has nonetheless provided evidence for warming-associated distributional responses of North Sea fishes (Perry et al. 2005), showing that the centres of distribution of nearly half of 36 investigated demersal fish species shifted in relation to warming, and all but a couple northward, into cooler waters. For the species which had their southern or northern range limit in the North Sea, half of the boundaries moved significantly with warming, and all but one boundary shifted northward. The authors hypothesis that because climate change may influence fish distribution and abundances through changes in growth, survival, and reproduction, species with a more rapid turnover of generations may respond most rapidly to temperature changes. And indeed, the shifting species tended to have faster life histories than nonshifting species, significantly smaller body size, faster maturation and smaller size at maturation (Perry et al. 2005). An alternative hypothesis that encompasses both demersal and pelagic species is that especially species living on the edge of their thermal zone of tolerance may react by shifting their distributional range, where active, highly-migratory, pelagic species might react quickest compared to less active demersal fish with limited dispersal capabilities or specific habitat requirements.

It is tempting to speculate that long-term temperature increase may shift sandeels' distribution range northward, for two reasons. Firstly because the life history traits of sandeel (e.g. high population turnover) compares to the traits exhibited by the North Sea species which have shifted in response to warming (see above) (Perry et al. 2005), and secondly because the southern part of the North Sea and the Irish Sea constitute the southern boarders of their distribution range (Arnott and Ruxton 2002). Long-term distribution shifts may however not only depend on long-term average conditions, but also on future climatic variability related to different climatic phenomena. An example is the North Atlantic Oscillation index (NAO index) which has received increasing attention in recent years due to its major influence on the oceanography of the northern European marine ecosystems (Dickson et al. 2000; Ottersen et al. 2001). In years where unfavourable variations in the NAO index coincide with the increasing temperature it may have fatal influences on ecological and biological processes. Evidence for such relations has recently been provided for sandeels in the North Sea, where a negative relationship was detected between sandeel recruitment and the winter NAO index which affects sea temperature during the egg and larvae period. Thus, warmer sea temperatures (high NAO index) correlated with lower recruitment (Arnott and Ruxton 2002). These findings led the authors to suggest that climate change may impact upon sandeel populations in the North Sea, either through direct effects of higher temperature on larval development, growth or behaviour, or indirectly via altered feeding conditions and/or predation pressures experienced by the larvae. The cause of the recent year's recruitment failure of the North Sea sandeel populations remains unclear but apparently fisheries mortality does not play any significant role in the population dynamics of sandeel (ICES 2005a). Despite that a recent study provided a reason to believe that herring may exert some negative effect on recruitment of sandeel in the North Sea, predation by herring on sandeel larvae alone can not explain the recent years decline in the sandeel populations (van Deurs 2005). During the last couple of decades several reports have described the severely oxygen-deprived water overlying North Sea sandbanks (Ærtebjerg, personal com.). Such areas may be unsuitable for sandeels to bury in, and it is tempting to suggest that the poor oxygen conditions may have affected recruitment success, not least in years coinciding with warmer than average winter temperatures. Sandeels constitute one of the largest biomasses in the North Sea (Temming et al. 2004; ICES 2005b) and further knowledge on any implications, whether direct or indirect, of climate change on sandeels population dynamics is of utmost importance, since a complete stock collapse is likely to have broad and severe effects on the entire ecosystem of the North Sea and the inner Danish waters.

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Effect of Global Change related oxygen depletion on fish; lesser sandeel (***Ammodytes tobianus***) as model organism

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