Juvenile flounder, *Platichthys flesus* (L.), under hypoxia: effects on tolerance, ventilation rate and predation efficiency

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Received 9 March 1999; received in revised form 23 June 1999; accepted 2 July 1999

Abstract

The flounder, *Platichthys flesus*, is the most abundant flatfish species in the northern Baltic Sea. Juvenile *P. flesus* are important epibenthic predators on shallow sandy bottoms in this area, and play an important role in structuring benthic infaunal communities by both lethal and sublethal predation. These areas are affected by periodic oxygen deficiency during summer and autumn when drifting algal mats are abundant, which causes changes in both structure and function of the zoobenthic community. Experiments were performed to test for the effects of hypoxia on: (1) tolerance; (2) ventilation rate; (3) lethal predation efficiency; and (4) sublethal predation efficiency (siphon cropping) of *P. flesus*. In the lethal predation experiment, the amphipod *Bathyporeia pilosa* was used as prey, and in the siphon cropping experiment, the bivalve *Macoma balthica* was used. Recorded \(LT_{50}\) values of *P. flesus* exposed to hypoxia were 1835±87 (mean±SE) min at 20%, 149±8 min at 10% and 23±2 min at 5% oxygen saturation (13°C, \(S\,5\%\)). In hypoxia, ventilation rate of *P. flesus* increased at 30% and 20%, and decreased at 10% and 5% oxygen saturation compared with normoxia. The predation efficiency of juvenile *P. flesus* was significantly lower at 20% and 30% compared with 40% and 100% oxygen saturation. In the sublethal predation experiment, the flounders cropped siphons most efficiently at moderate hypoxia (40% oxygen saturation) compared with normoxia. Our results indicate that sublethal oxygen saturations (30–40%) cause changes in physiology and predation behaviour of juvenile *P. flesus*. Such functional changes in benthic communities, at sublethal oxygen saturations, may influence the energy flow of benthic food webs in areas that are affected by periodic oxygen deficiency. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Benthos; Flatfish; Hypoxia; *Platichthys flesus*; Predation; Siphon cropping

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PII: S0022-0981(99)00096-9
1. Introduction

Organic enrichment, due to increased nutrient input, has caused an increase in coastal benthic macrofauna biomass in the Baltic Sea during the last decades (Cederwall and Elmgren, 1980; Bonsdorff et al., 1991, 1997; Mattila, 1993). The increased benthic biomass, as well as the increase in organic matter, enhance oxygen consumption in the bottom waters, causing permanent or temporary anoxic conditions (Larsson et al., 1985; Cederwall and Elmgren, 1990). Oxygen deficiency can reduce the diversity of benthic communities (Rosenberg, 1977; Josefson and Widbom, 1988; Baden et al., 1990; Diaz and Rosenberg, 1995), and living conditions for bottom feeding fish (cod, flounder) are deteriorated (Baden et al., 1990; Hansson and Rudstam, 1990). Worldwide, hypoxia and/or anoxia is considered a major threat to coastal benthic ecosystems (Diaz and Rosenberg, 1995).

In the coastal areas of the northern Baltic Sea, conditions with low oxygen saturation in bottom waters are, at least partly, induced by drifting algal mats varying in abundance and distribution throughout the year (Bonsdorff, 1992; Holmström, 1998). The occurrence of drifting algae in the Archipelago Sea and around the Åland Islands, SW Finland, is seasonal and the highest densities are recorded in summer and autumn, i.e. when juvenile fish are most abundant (Aarnio and Bonsdorff, 1997). Drifting algal mats are known to induce severe oxygen deficiency with consequences for both population and community levels of benthic macrofauna (Norkko and Bonsdorff, 1996a,b). Many demersal fish species use shallow coastal areas as nursery sites and the juveniles are dependent on these habitats as foraging grounds. Benthic fish can use sites affected by periodic hypoxia as foraging grounds, by preying on infauna forced up to the sediment surface by oxygen-poor conditions (Jørgensen, 1980; Pihl et al., 1992, 1994).

In the northern Baltic Sea, the flounder, Platichthys flesus (L.), is the most abundant flatfish while the other flatfish species present, the turbot, Scophthalmus maximus, is less abundant. Juveniles of *P. flesus* spend the summer season on shallow sandy bottoms despite variations in temperature, while the adults migrate to deeper areas when the surface water is warmed up (Suuronen, 1979). *P. flesus* prey on a variety of infaunal groups including bivalves, polychaetes and crustaceans (Pihl, 1982; Westberg, 1997). Harpacticoids, amphipods, chironomid larvae and ostracods are the most common food sources for juvenile *P. flesus* (Aarnio et al., 1996). Juvenile flounders are also known to crop bivalve siphons (Ansell and Trevallion, 1967; de Vlas, 1979; Summers, 1980).

Hypoxia has been reported to affect the distribution and biomass, as well as the diet of different flatfish species (Pihl et al., 1991, 1992; Pihl, 1994; Petersen and Pihl, 1995). Moderate hypoxia can also affect mean length and growth of flatfish populations (Petersen and Pihl, 1995). Several studies report metabolic depression in flatfish at oxygen saturations under 20% (Van den Thillart et al., 1994; Dalla Via et al., 1994, 1997; Moser et al., 1996), and changes in activity at 40% oxygen saturation (Van den Thillart et al., 1994). High concentrations of haemoglobin in the blood of *P. flesus* suggest that the ability of this species to adjust to hypoxic conditions is higher than for some other flatfish species, such as plaice, Pleuronectes platessa (Weber and Wilde, 1975). Furthermore, the flounder heart has a high aerobic and anaerobic capacity, suggesting that flounders can tolerate oxygen-poor conditions (Jørgensen and Mustafa, 1980a,b).
In aquatic systems, oxygen deficiency can cause changes in predator–prey interactions, inducing changes at different trophic levels (Kolar and Rahel, 1993; Norkko and Bonsdorff, 1996c; Sandberg et al., 1996; Breitburg et al., 1997; Johansson, 1997; Sandberg, 1994, 1997). Physiology, behaviour, and predatory capacity of benthic feeding species can change at moderate oxygen saturations, revealing the importance of studying these processes. In this study we examine:

1. Effects of hypoxia on the survival of *P. flesus*
2. Effects of hypoxia on the ventilation rate of *P. flesus*
3. Effects of hypoxia on predation efficiency of *P. flesus* on a benthic invertebrate species (the amphipod *Bathyporeia pilosa* Lindström)
4. Effects of hypoxia on siphon cropping efficiency of *P. flesus* on the Baltic clam *Macoma balthica* (L.)

2. Methods

2.1. General experimental conditions

Organisms used in the experiments were collected on two shallow sandy bottoms in the Åland archipelago, northern Baltic Sea (60°N, 20°E). Juvenile flounders were caught with a push-net, and the amphipod *Bathyporeia pilosa* and the bivalve *Macoma balthica* were collected with an Ekman-Birge grab or with a shovel. In the laboratory, animals were carefully picked out and stored in tanks under normoxic conditions until the start of experiments. The prey specimens were collected 2 days, and the flounders 2 weeks before being used in experiments. In all experiments, the oxygen saturation was lowered by bubbling nitrogen gas into each aquarium (replicate) and the required oxygen saturation was maintained by adding nitrogen gas or air during the experiment. In the predation and siphon cropping experiments, oxygen diffusion into the water was prevented by a transparent plastic sheet on the water surface. Oxygen saturation was measured 2–3 times a day with calibrated portable oxygen meters (YSI model 57 with a 5739 field probe, YSI 95 with a MicroElectrode Array dissolved oxygen sensor or WTW Oxi 91). A WTW pH 91 probe was used for pH measurements and salinity was measured using an YSI 30 probe. Air-dried and sieved (0.5 mm) sand was used as sediment in all experiments. To avoid outside disturbance, black plastic was wrapped around the aquaria.

2.2. Tolerance experiment

To test for the tolerance of *P. flesus* to hypoxia, an experiment with juvenile flounders was conducted. Four treatments were used: 100% (control), 20%, 10% and 5% oxygen saturation (Table 1). The experiment time was 48 h, with 18 h/6 h light/dark conditions. Each treatment consisted of three replicates with 12 juvenile flounders of different sizes in each replicate aquarium (13 l), corresponding to and density of 200 ind./m². Before the start of the experiment, the fish were allowed to acclimatize in the
Table 1
Experimental set-up and hydrographical conditions in the tolerance experiment (mean±SE)

<table>
<thead>
<tr>
<th></th>
<th>100% O2</th>
<th>20% O2</th>
<th>10% O2</th>
<th>5% O2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of replicates</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No. of P. flesus/replicate</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>P. flesus length (mm)</td>
<td>44±1.6</td>
<td>44±1.9</td>
<td>45±2.0</td>
<td>44±1.7</td>
</tr>
<tr>
<td>Range</td>
<td>29–74</td>
<td>31–79</td>
<td>28–84</td>
<td>28–68</td>
</tr>
<tr>
<td>Oxygen (mg l⁻¹)</td>
<td>9.9±0.05</td>
<td>2.0±0.02</td>
<td>1.0±0.01</td>
<td>0.6±0.01</td>
</tr>
<tr>
<td>Range</td>
<td>9.7–10.2</td>
<td>1.6–2.2</td>
<td>1.0–1.1</td>
<td>0.5–0.6</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>98±0.5</td>
<td>19±0.2</td>
<td>10±0.1</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>Range</td>
<td>97–100</td>
<td>16–21</td>
<td>9–10</td>
<td>5–6</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>13.5±0.04</td>
<td>13.5±0.02</td>
<td>13.4±0.02</td>
<td>13.4±0.01</td>
</tr>
<tr>
<td>pH</td>
<td>8.0</td>
<td>7.8</td>
<td>7.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

experimental aquaria for 3 days and they were not fed during the experiment. During storage and acclimatization, the fish were fed with bivalve meat. The fish were considered dead when no opercular movements could be seen and the fish didn’t respond to disturbance by a stick. The time of death was registered for each flounder and their length was measured to the nearest mm. Sediment depth was 4 cm and the salinity was 5.2‰ in all treatments.

2.3. Ventilation rate experiment

To test for changes in ventilation rate as a measure of changes in activity and stress due to decreasing oxygen saturation, a 22 h experiment was performed. Two treatments were used: a control (normoxic conditions throughout the experiment) and a hypoxia-treatment, and each treatment was represented by four replicates. In the hypoxia-treatment, oxygen was lowered every second hour by 10%, and the ventilation rate was counted after 1 h of exposure at each oxygen saturation. Ventilation rate was counted as the mean number of 3 subsequent 15 s counts of opercular strokes for each fish (Pihl et al., 1991; Moser et al., 1996). Six juvenile flounders, with a mean size of 40±1 mm (range 35–49 mm) in normoxia and 41±1 (range 36–50 mm) in hypoxia treatment, were used in each replicate aquarium (3 l, 166 ind./m³). The ventilation rate was counted for fish buried in the sediment and also for specimens on the sediment surface. It was not possible to count ventilation rate for all the individuals at every counting event since some specimens were buried too deep into the sediment, therefore the number of specimens counted varies for each oxygen saturation and time. Sediment depth was 4 cm, temperature was 13°C and the salinity was 5.0‰ in both treatments. The experiment was performed under constant light conditions. Before the start, the flounders were acclimatized in the experimental aquaria for 1 week to get them used to possible disturbance by the investigator. The fish were fed with bivalve meat during storage and acclimatization but were not fed for 1 day before and during the experiment.
2.4. Predation experiment

The predation rate of juvenile flounders was examined in an experiment with eight treatments (with six replicates of each treatment), four treatments with the amphipod *Bathyporeia pilosa* at four different oxygen saturations (100%, 40%, 30% and 20% oxygen saturation), and four predation treatments with *B. pilosa* as prey and juvenile *Platichthys flesus* as predators, at the same four oxygen saturations (Table 2). The treatments without predators served as controls of survival of *B. pilosa* at each oxygen level. On shallow sandy bottoms in the Baltic Sea, the amphipod *B. pilosa* is one of the most abundant macrofauna species (Persson, 1982; Blomqvist and Bonsdorff, 1986). *B. pilosa* is known to be an important prey for juvenile flounders and other epibenthic predators (Kohn and von Sammour, 1990; Aarnio et al., 1996; Mattila and Bonsdorff, 1998). It is considered stress tolerant (Mettam, 1989) and can tolerate an oxygen saturation of 30% (13°C, 5%e) for more than 10 days (Sandberg et al., 1996).

In the experiment, each replicate aquarium (13 l) contained 4 cm sediment and 100 *B. pilosa* individuals, corresponding to 1642 ind./m², which equals natural densities (Blomqvist and Bonsdorff, 1986). *B. pilosa* were kept in the experimental aquaria for 48 h before the start. In the predation treatments, one juvenile flounder was added to each aquarium. The experiment time was 24 h under 12 h/12 h light/dark conditions. Until the required oxygen saturation was reached, the predators were kept separate from prey. At the end of the experiment, live and dead *B. pilosa* were counted and the length of the flounders was measured to the nearest mm. Salinity was 5.0%e during the experiment.

2.5. Siphon cropping experiment

The effect of moderate hypoxia on the siphon cropping efficiency of juvenile *Platichthys flesus* was studied in four treatments: two treatments in normoxia (95% oxygen saturation with and without predator), and two in moderate hypoxia (40% oxygen saturation with and without predator, Table 3). The aquarium (24 l) contained 6 cm of sediment (to allow appropriate burial depth for the clams) with 30 *M. balthica* individuals in each. The experiment time was 3 weeks under 12 h/12 h light/dark conditions. In the predation treatments, one predator was added to each replicate and predators were kept separate from prey until the required oxygen saturation was reached. To avoid high concentrations of ammonia, half the water volume was changed every third or fourth day. Before adding new water, the oxygen saturation was adjusted to the appropriate level. At the end of the experiment, bivalves were placed in filtered sea-water for 24 h prior to length and weight measurements. From each replicate, 20 individuals were used for condition index measurements, 5 for siphon index measurements and 3 for determination of glycogen content. Condition index was measured to detect possible changes in body weight due to stress from hypoxia, predation, or both combined. The siphon index measurements were made in order to get a measure of siphon cropping effects and glycogen was measured in order to detect possible changes in energy allocation. For the condition index measurements, the bivalves were boiled for about 2–3 s to open the shells, and the soft parts were separated from the shells. Soft body parts and shells were dried at 100°C for 24 h and weighted separately. Shell
Table 2
Experimental set-up and hydrographical conditions in the predation experiment (mean±SE)

<table>
<thead>
<tr>
<th></th>
<th>100% O₂</th>
<th>100% O₂</th>
<th>40% O₂</th>
<th>40% O₂</th>
<th>30% O₂</th>
<th>30% O₂</th>
<th>20% O₂</th>
<th>20% O₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
<td>+ predator</td>
</tr>
<tr>
<td>No. of replicates</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>No. of <em>P. flesus</em> / replicate</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>P. flesus</em> length (mm)</td>
<td>-</td>
<td>63±2.9</td>
<td>-</td>
<td>65±2.7</td>
<td>-</td>
<td>61±3</td>
<td>-</td>
<td>63±2.3</td>
</tr>
<tr>
<td>Range</td>
<td>-</td>
<td>55–74</td>
<td>-</td>
<td>55–72</td>
<td>-</td>
<td>52–69</td>
<td>-</td>
<td>54–69</td>
</tr>
<tr>
<td>No. of <em>B. pilosa</em> start</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>No. of <em>B. pilosa</em> end</td>
<td>97±0.6</td>
<td>44±5.0</td>
<td>95±1.6</td>
<td>52±6.6</td>
<td>97±1.3</td>
<td>81±2.7</td>
<td>92±1.6</td>
<td>86±2.7</td>
</tr>
<tr>
<td>Oxygen (mg l⁻¹)</td>
<td>10.3±0.07</td>
<td>10.3±0.08</td>
<td>4.4±0.06</td>
<td>4.3±0.05</td>
<td>3.0±0.03</td>
<td>3.0±0.02</td>
<td>2.0±0.02</td>
<td>2.0±0.03</td>
</tr>
<tr>
<td>Range</td>
<td>10.0–10.8</td>
<td>9.9–10.8</td>
<td>4.0–5.2</td>
<td>3.9–4.8</td>
<td>2.8–3.4</td>
<td>2.8–3.2</td>
<td>1.8–2.2</td>
<td>1.9–2.2</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>94±0.5</td>
<td>94±0.5</td>
<td>41±0.6</td>
<td>41±0.5</td>
<td>31±0.3</td>
<td>30±0.2</td>
<td>20±0.2</td>
<td>20±0.3</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>12.3±0.06</td>
<td>12.3±0.06</td>
<td>12.3±0.05</td>
<td>12.2±0.05</td>
<td>14.5±0.05</td>
<td>14.6±0.05</td>
<td>13.8±0.05</td>
<td>13.7±0.05</td>
</tr>
<tr>
<td>Range</td>
<td>11.8–12.8</td>
<td>11.9–12.8</td>
<td>11.9–12.7</td>
<td>11.7–12.7</td>
<td>14.3–15</td>
<td>14.2–14.9</td>
<td>13.4–14.1</td>
<td>13.4–14.1</td>
</tr>
<tr>
<td>pH</td>
<td>8.08±0.01</td>
<td>8.07±0.01</td>
<td>8.06±0.01</td>
<td>8.06±0.01</td>
<td>8.31±0.02</td>
<td>8.25±0.02</td>
<td>8.14±0.02</td>
<td>8.11±0.02</td>
</tr>
</tbody>
</table>
Table 3
Experimental set-up and hydrographical conditions in the siphon cropping experiment (mean±SE)

<table>
<thead>
<tr>
<th>Condition</th>
<th>100% O₂</th>
<th>100% O₂ + predator</th>
<th>40% O₂</th>
<th>40% O₂ + predator</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of replicates</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>No. of P. flesus/replicate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P. flesus length (mm)</td>
<td>83±5.6</td>
<td>83±5.6</td>
<td>81±5.2</td>
<td>81±5.2</td>
</tr>
<tr>
<td>Range</td>
<td>72–90</td>
<td>–</td>
<td>71–87</td>
<td>–</td>
</tr>
<tr>
<td>No. of M. balthica/replicate</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>M. balthica length (mm)</td>
<td>11.6±0.2</td>
<td>11.8±0.1</td>
<td>11.8±0.2</td>
<td>11.7±0.1</td>
</tr>
<tr>
<td>Range</td>
<td>10.8–13.2</td>
<td>11.2–12.6</td>
<td>11.2–13.5</td>
<td>10.9–12.7</td>
</tr>
<tr>
<td>Oxygen (mg l⁻¹)</td>
<td>10.0±0.04</td>
<td>9.9±0.04</td>
<td>4.2±0.04</td>
<td>4.0±0.03</td>
</tr>
<tr>
<td>Range</td>
<td>9.0–10.3</td>
<td>9.3–10.2</td>
<td>3.2–5.4</td>
<td>3.0–5.1</td>
</tr>
<tr>
<td>Oxygen (%)</td>
<td>97±0.4</td>
<td>97±0.3</td>
<td>41±0.4</td>
<td>40±0.3</td>
</tr>
<tr>
<td>Range</td>
<td>89–100</td>
<td>92–99</td>
<td>32–52</td>
<td>33–49</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>14.9±0.1</td>
<td>14.8±0.1</td>
<td>15.0±0.04</td>
<td>14.9±0.04</td>
</tr>
<tr>
<td>Range</td>
<td>13.9–15.5</td>
<td>13.7–15.6</td>
<td>13.8–16.3</td>
<td>13.7–16.1</td>
</tr>
<tr>
<td>pH</td>
<td>8.2±0.03</td>
<td>8.2±0.1</td>
<td>7.5±0.02</td>
<td>7.7±0.04</td>
</tr>
<tr>
<td>Range</td>
<td>8.1–8.3</td>
<td>8.1–8.3</td>
<td>7.8–7.9</td>
<td>7.6–7.8</td>
</tr>
</tbody>
</table>

lengths were measured to the nearest 0.05 mm with a caliper. Condition index was calculated according to Wenne and Styczynska-Jurewicz (1985): soft parts dwt (mg) × 1000/Shell length³ (mm). For the siphon condition measurements, the soft body parts were placed on a petri dish and both siphons were cut off using a scalpel. Siphons and the rest of the soft body parts were dried at 100°C for 24 h and weighted separately. Siphon condition was calculated according to de Vlas (1985), with the exception that we used both the inhalant and exhalant siphon weights as a measure of siphon dry weight: siphon dwt (mg)/[Body dwt (mg)]⅓. Glycogen analyses were done according to Sandberg et al. (1996).

2.6. Statistical analysis

All data, except for the ventilation rate data, were tested for normality and homogeneity of variances. According to these results, an appropriate parametric or non-parametric test was applied to compare treatments. When comparing means, a one-factor Anova was used if the requirements of normality and homogeneity of variances were met. If not, the non-parametric Kruskal-Wallis or Mann-Whitney U-test were used (Zar, 1996). A significance level of p < 0.05 was chosen for all statistical analyses and deviations from the mean are presented as standard errors in all experiments except for the ventilation rate experiment.

In the tolerance experiment, the $LT_{50}$ values were compared by one-factor Anova. In the ventilation rate experiment, results are presented as means with 95% confidence intervals. In the predation experiment, the results showed significant deviation from normality and the variances were heterogeneous. Results were analysed as follows: firstly, the effect of hypoxia on the prey species alone was analysed by comparing the treatments with Bathyporeia pilosa without predator at different oxygen saturations...
(Kruskal-Wallis nonparametric test and multiple comparison by the Tukey-type Nemenyi test; Zar, 1996). Secondly, a similar test procedure was performed on the four treatments with predators and finally, pairwise nonparametric testing (Mann-Whitney $U$-test) was performed to compare control and predation treatment at each oxygen saturation. For condition index, siphon condition and glycogen content of $Macoma balthica$ in the siphon cropping experiment, all treatments were normally distributed and the variances were homogenous. The linear regression for condition index plotted against shell length was tested for significance and since all treatments were significant, differences between slopes were tested (Fowler and Cohen, 1990). Since the slopes did not differ significantly, the lines were tested for differences in intercepts.

3. Results

3.1. Tolerance experiment

The $LT_{50}$ of juvenile $Platichthys flesus$ differed significantly between all treatments (Fig. 1). Mean $LT_{50}$ values were $1835\pm87$ min at 20%, $149\pm8$ min at 10% and $23\pm2$ min at 5% oxygen saturation. In the control treatment (100% oxygen saturation), all flounders survived (Fig. 1). At 20% oxygen saturation almost 40% of the flounders survived. This treatment was continued for a week, but only a few more flounders died. Since the fish were not fed during the experiment, it was impossible to know if the mortality in this treatment, after the original experiment time, was due to starvation or oxygen stress, hence the results are not presented here.

![Fig. 1](image_url)  
*Fig. 1. Mean survival rate of juvenile flounder, $Platichthys flesus$, at 100%, 20%, 10% and 5% oxygen saturation during a 48 h experiment (13°C).*
3.2. Ventilation rate experiment

The ventilation rate of juvenile *Platichthys flesus* (Fig. 2) did not differ between the control and hypoxia treatments at oxygen saturations down to 40%. At 30% and 20% oxygen saturations, the flounders had a higher ventilation rate ($105\pm 7$ and $104\pm 4$ opercular strokes/min, respectively) compared with control fish in normoxia ($79\pm 11$ and $87\pm 10$ opercular strokes/min). When oxygen saturation was lowered to 10% and 5%, the ventilation rate decreased until death of the fish. At 30% oxygen saturation, the flounders started to lift up their heads and show escape behaviour by swimming to the surface for air breathing, and at 20% oxygen saturations and lower, almost all the flounders were on the sediment surface.

Fig. 2. Mean ventilation rate (opercular strokes/min±95% confidence interval) of juvenile flounder, *Platichthys flesus*, in normoxia and at declining oxygen saturation.
3.3. Predation experiment

The survival of *Bathyporeia pilosa* was highest in the treatments without predators with a mean survival of 97 individuals in 100% and 30% oxygen, 95 in 40% and 92 in 20% oxygen saturation (Table 2). In the predation treatments, the mean survival of *B. pilosa* was 44 individuals in 100%, 52 in 40%, 81 in 30% and 86 in 20% oxygen saturation.

In the treatments without predators, a significant difference in survival rate of *B. pilosa* was recorded between treatments at 100% and 20% oxygen saturation (Fig. 3, filled bars). Significant differences between the predator treatments were recorded for 100% vs. 30%, 100% vs. 20%, 40% vs. 30%, and 40% vs. 20% oxygen saturation (Fig. 3, open bars). The survival rate of *B. pilosa* differed significantly between the treatments without a predator and predation-treatment at 100%, 40% and 30% oxygen saturation, but at 20% oxygen saturation the difference was non-significant. In the treatment with *P. flesus* at 20% oxygen saturation, 4 of 6 flounders died within 9 h.

3.4. Siphon cropping experiment

For the condition index of *Macoma balthica*, no significant difference could be detected between the treatments when comparing the slopes of the regression lines (Fig. 4). When comparing the intercept of the regression lines, a significant difference could
Fig. 4. Condition index of *Macoma balthica* in the siphon cropping experiment. The equation and significance of the regression line, and the regression coefficient $r$, are indicated for all treatments.
Fig. 5. Siphon condition of *Macoma balthica* from the siphon cropping experiment. The treatments without predators are represented by filled bars, and the treatments with *Platichthys flesus* as predator are represented by open bars. * = significant difference at *p* < 0.05 level.

be detected between the moderate hypoxia treatments with and without *P. flesus*. The treatment with *M. balthica* and *P. flesus* in moderate hypoxia showed a significant reduction in siphon condition compared with all the other treatments (Fig. 5). Siphon condition values were 0.20±0.02 for *M. balthica* at 100%, 0.20±0.02 for *M. balthica* at 40%, 0.19±0.01 for *M. balthica* + *P. flesus* at 100%, and 0.14±0.02 for *M. balthica* + *P. flesus* at 40% oxygen saturation. No significant differences were found in total glycogen content of *M. balthica* in the different treatments. The highest glycogen content of *M. balthica* was in the treatment with *M. balthica* + *P. flesus* at 40% (9.97±2.10% dwt), and the lowest in the treatment with *M. balthica* at 40% oxygen saturation (5.84±1.38). In the normoxia treatments, the glycogen content was 7.26±1.48% in the treatment with *M. balthica* + *P. flesus*, and 9.57±1.25% in the treatment with *M. balthica*.

4. Discussion

4.1. Tolerance to hypoxia

The tolerance experiment showed that an oxygen saturation of 20% can be lethal to juvenile *Platichthys flesus*. However, some of the specimens used in the experiment survived at that oxygen saturation without food for more than a week after the experiment, indicating that the initial condition of individual flounders may play an important role for survival. All flounders used in the experiment were caught at the same site, on the same day, and stored in the laboratory in the same tank which excludes the possibility of laboratory handling causing these individual differences. At 20% oxygen
satisfaction, most of the flounders were on the sediment surface and they seemed to have an increased ventilation activity which, however, was not measured in this experiment, but clearly demonstrated in the ventilation rate experiment. The flounders were inactive at the sediment surface, occasionally swimming vertically. At 10% oxygen saturation, vertical swimming behaviour of the flounders increased considerably. They swam to the surface, gulping for air, afterwards falling down to the sediment surface often turned upside down. This behaviour was repeated several times but between the surface ‘rushes’, the fish usually laid inactive on the sediment. This same behaviour was observed at 5% oxygen saturation but at this level the fish died much sooner, and all fish were registered dead within one hour. The same type of panic reaction or escape behaviour has been reported for other flatfish species like sole, *Solea solea* (Dalla Via et al., 1997), and winter flounder, *Pleuronectes americanus* (McEnroy and Krozlowitz, 1997). McEnroy and Krozlowitz (1997) recorded increased horizontal and vertical swimming activity for juvenile winter flounder already at moderate oxygen saturations.

In experimental situations, spot, *Leiostomus xanthurus*, has been recorded to die at an oxygen pressure of 24 mmHg (approx. 16%, 25°C, S 21%; Subrahmanyam, 1980), and Pihl et al. (1991) found that at oxygen saturations of 11–13% (25°C, S 18–20%), all specimens of this species died within 4 h. For sole, *S. solea*, an incipient lethal level has been suggested to lie between 12% and 20% (Van den Thillart et al., 1994). Moser et al. (1996) measured a lower lethal level of 15.9% oxygen saturation for witch flounder, *Glyptocephalus cynoglossus*, and a mean survival in hypoxia of <10% was recorded to 8 min for the same species. McEnroy and Krozlowitz (1997) found that juveniles of the winter flounder, *Pleuronectes americanus*, could survive for 1–2 h at oxygen concentrations of 1.0 mg/l (approx. 11%, 20–23°C, S 26%). Steffensen et al. (1982) described for the same species the same type of results were presented by Voyer and Morrison (1971), and for sole, *Solea solea*, by Van den Thillart et al. (1994). Positive correlation between respiration rate and oxygen saturation has been found for the winter flounder, *Pseudopleuronectes americanus*, but ventilation rate was constant down to an oxygen saturation of 15% after which it decreased (Moser et al., 1996). In our study, no significant difference in ventilation rate could be recorded between 100% and 40%

4.2. Effects of hypoxia on ventilation rate

The results of Jørgensen and Mustafa (1980a) show that the respiration rate of *P. flesus* is independent of the oxygen pressure between 55 and 150 mmHg (approx. 37–100%), and below 55 mmHg, the oxygen consumption declines. A constant oxygen consumption rate with declining oxygen saturation (until a certain level) has also been described for the same species by Steffensen et al. (1982). For the winter flounder, *Pseudopleuronectes americanus*, the same type of results were presented by Voyer and Morrison (1971), and for sole, *Solea solea*, by Van den Thillart et al. (1994). Positive correlation between respiration rate and oxygen saturation has been found for the winter flounder, *Glyptocephalus cynoglossus*, but ventilation rate was constant down to an oxygen saturation of 15% after which it decreased (Moser et al., 1996). In our study, no significant difference in ventilation rate could be recorded between 100% and 40%
oxygen saturation. In the hypoxia treatment, an increase in ventilation rate could be detected at 30% and 20%, and at lower oxygen saturations the ventilation rate declined rapidly until death of the fish. An increase in ventilation rate has also been recorded for spot, *Leiostomus xanthurus*, with linear increase in ventilation rate at oxygen saturations lower than 50% (Pihl et al., 1991). In the same study, spot almost doubled its ventilation rate in 30% and 40% oxygen saturation compared to the control in normoxia, which is a considerably higher increase than for *P. flesus* in our study. However, our study was performed with juveniles of another species and at lower temperatures, which may be the reasons for the difference. An increase in stroke volume due to hypoxia has been recorded for flatfish (Steffensen, 1982), but was not measured in our study.

Decreasing oxygen saturation cause elevated ventilation rates also among other fish taxa than flatfish, e.g. snake blenny, *Lumpenus lampretaeformis* (Pelster et al., 1988), striped bass, *Morone saxatilis*, and naked goby, *Gobiosoma bosc* (Breitburg et al., 1994), and also in crustaceans like the shrimp, *Squilla empusa* (Pihl et al., 1991), and young specimens of the lobster, *Homarus gammarus* (Hagerman, 1982). Elevated levels of lactate have been recorded in flatfish blood after exposure to hypoxia, which was assumed to be due to increased ventilation rate since no enhanced swimming activity was noted in the experiments (Jørgensen and Mustafa, 1980a; White and Fletcher, 1989).

4.3. Changes in feeding activity: lethal and sublethal predation

Oxygen deficiency can cause changes in predator–prey interactions by reducing or enhancing the efficiency of the predators or altering the vulnerability of prey (Kolar and Rahel, 1993; Rahel and Nutzman, 1994; Sandberg, 1994; Sandberg et al., 1996; Breitburg et al., 1994, 1997). The physiology and behaviour of benthic animals change under stress, with subsequent alternation in energy partitioning. Enhanced energy demand due to stress may increase the rate of predation, or the stressor can decrease the activity of the predator. The responses of predator and prey species to oxygen deficiency are species-specific. The changes in interspecific interactions can be short-term when the hypoxic events are short (days–weeks), or long-term when large areas of oxygen deficiency persist for longer periods (Pihl et al., 1992; Pihl, 1994).

In our predation experiment, the survival of *B. pilosa* was significantly higher in predator treatments at 20% and 30% oxygen saturation than at 40% and 100% oxygen saturation. This means that either the predator is less efficient at lower oxygen saturations (20% and 30%) or the prey is more available at higher oxygen saturations (40% and 100%). No activity measurements of *B. pilosa* were made, but visual observations indicated no marked differences in swimming behaviour between treatments. A decrease in predation rate due to short-term hypoxia has been suggested to be caused by a decrease in predator activity (Sandberg, 1996; Johansson, 1997). According to our study, the critical oxygen saturation causing a decrease in predation efficiency of *P. flesus* is at 30% oxygen saturation. For sole, *Solea solea*, a decrease in activity was recorded at an oxygen saturation of 40% and the lethal oxygen saturation for the same species was between 12% and 20% oxygen saturation (Van den Thillart et al., 1994). Juvenile white sturgeon, *Acipenser transmontanus*, show reduced growth rate, probably due to reduced feeding activity (Cech et al., 1984). A decrease in growth of plaice,
Pleuronectes platessa, and dab, Limanda limanda, is recorded at 50% and 30% oxygen saturation, and a reduced frequency of feeding for plaice at 30% oxygen saturation (Petersen and Pihl, 1995). A decrease in predation rate has also been recorded for invertebrate predators like the isopod, Saduria entomon (Sandberg, 1994), the brown shrimp, Crangon crangon (Sandberg et al., 1996), and the polychaete, Nereis diversicolor (Tallqvist et al., unpublished data). These species are considered tolerant to hypoxia (Dries and Theede, 1974; Hagerman and Szaniawska, 1986, 1988; Vismann, 1990), but reduce their predation rate at oxygen saturations that are considerably above lethal levels. Decreased swimming activity due to hypoxia has been found in Atlantic cod, Gadus morhua, (Schurmann and Steffensen, 1994), juvenile white sturgeon, A. transmontanus (Crocker and Cech, 1997), and eelpout, Zoarces viviparus (Fischer et al., 1992), which was explained as a method for reducing energy costs during hypoxia. For many other fish species, hypoxia increases swimming activity, probably due to escape responses (Petersen and Petersen, 1990; Tang and Boisclair, 1995). Mobile predators (like fish) can migrate between areas with normoxia and hypoxia and use areas affected by hypoxia as foraging grounds (Pihl et al., 1992; Rahel and Nutzman, 1994). This kind of behaviour can be supposed to increase the predation efficiency of the predators in contrast to situations where both predator and prey are affected by the same hypoxic conditions.

Siphon cropping by fish or other epibenthic predators can affect feeding behaviour (Kamermans and Huitema, 1994; Peterson and Skilleter, 1994; Skilleter and Peterson, 1994), condition (Bonsdorff et al., 1995; Sandberg et al., 1996) and growth (Trevallion, 1971; Peterson and Quammen, 1982; Coen and Heck, 1991; Irlandi and Mehlich, 1996) of bivalves. The siphon condition and body condition of Macoma balthica from the siphon cropping experiment indicate that flounders actively cropped siphons at 40% oxygen saturation. The flounders were more active in hypoxia than in normoxia, maybe because the bivalve siphons have been more exposed to predation in the hypoxia treatment. Juvenile plaice, Pleuronectes platessa, respond to visual cues and attack siphons that are visible on the sediment surface (de Vlas, 1985; Ansell, 1995). In the hypoxia treatment, the siphons could have been more extended and thus been more available for cropping by the flounders. This is, however, not confirmed by observations during the experiment. Other experiments show a clear increase in the portion of extended siphon at lower oxygen saturations (10% oxygen saturation and lower; Tallqvist, unpublished). However, the brown shrimp, Crangon crangon, has the highest cropping efficiency in normoxia compared to hypoxia (Sandberg et al., 1996), suggesting that siphon availability is not the only factor determining the cropping rate. Metabolic differences between the predator species and different energy demands under stress situations like hypoxia may be the reason for the differing results. The cost of maintenance might have been higher for flounders in hypoxia compared to normoxia, causing an enhanced cropping rate at this moderate oxygen saturation (40%). The results from the predation experiment are in contrast to this assumption, since there was no difference in predation rate at 100% and 40% oxygen saturation. However, it must be pointed out that the predation experiment was a short-term experiment (24 h), while the duration of the cropping experiment was three weeks. Flounders in normoxia lost weight during the cropping experiment, corresponding to the assumption that they did not crop
efficiently in this treatment. The decrease in weight of the flounders was $5.9 \pm 1.0$ to $5.0 \pm 1.0$ g in normoxia, and $5.2 \pm 1.0$ to $5.1 \pm 1.2$ in hypoxia.

In the cropping experiment, condition index, glycogen content and siphon condition of *M. balthica* did not differ significantly between the two treatments without predators. This suggests that a 40% oxygen saturation in a long-term exposure is not especially stressful for *M. balthica*, which is supported by other studies emphasizing *M. balthica* to be very tolerant to hypoxic conditions (Dries and Theede 1974; Jahn and Theede, 1997).

5. Conclusions

In order to evaluate the ability of different species to withstand hypoxic stress, studies on physiological effects are important. Equally important, but not as thoroughly investigated, are the functional changes in benthic communities that occur already at sublethal oxygen saturations. Our results, together with other studies, show that interactions between predator and prey species change already at intermediate oxygen saturations. Functional changes in benthic communities may occur before changes in community structure, revealing the importance of enhancing the knowledge of functional changes due to hypoxia.

Acknowledgements

All experiments were performed at Husö Biological Station. We are grateful to staff and students who helped collecting the organisms and to Katri Aarnio and Christoffer Bostöm who gave valuable comments on the manuscript. This work was financed by the Finnish Biodiversity Research Programme (FIBRE) and the Academy of Finland.

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