INTRODUCTION

Temperature and oxygen are environmental variables that profoundly influence fish activities, with consequences for their biological performance, e.g. growth, reproduction or survival (Priede 1985, Huey 1991). Through appropriate behavioural strategies, fish can take advantage of the thermal heterogeneity of their environment (Rudstam & Magnuson 1985, Bryan et al. 1990, Huey 1991, Huntingford 1993). They are also able to alter their distribution pattern according to the amount of oxygen available in their surrounding milieu (Cech et al. 1990, Schurmann & Steffensen 1992, Schurmann et al. 1998). Because of their morphology and negative buoyancy, flatfish have limited behavioural means to respond to environmentally fluctuations. For instance, Petersen & Pihl (1995) noted that during a hypoxic episode in the Kattegat (between Denmark and Sweden), dominant demersal round-fishes (cod Gadus morhua and whiting Merlangius merlangus) rapidly escaped, whereas dab and plaice were still found in areas with less than 20% air saturation. Parallel laboratory experiments showed that in 30% air saturated water, both flatfish species were already constrained and displayed negative mean growth (Petersen & Pihl 1995). Because of this relatively restricted behavioural repertoire, flatfish are more liable to be exposed to environment-mediated changes in their biological performance (Duthie 1982, van den Thillart et al. 1994). The aim of the present study was to assess the influence of ambient temperature and oxygenation on the metabolism and cardiovascular physiology of a poorly studied but economically important flatfish species, the common sole Solea solea.
SOLEA. To do so we determined in parallel the metabolic scope and scope for heart rate of sole exposed to combinations of oxygen and temperature conditions.

Metabolic scope (MS) is a suitable gauge for assessing the environmental influence on fish biological performance (Fry 1971). It is defined as the difference between active metabolic rate (AMR) and standard metabolic rate (SMR) measured in temperature-acclimated animals. SMR supports maintenance activities such as ventilation or osmoregulation. It corresponds to the oxygen consumption of a resting, fasted and non-maturing fish (Fry 1971). AMR is the maximum sustained aerobic metabolic rate measured in non-limiting conditions, i.e. those that do not interfere with oxygen supply (hypoxia, hypercapnia, ammonia or heavy metals, Fry 1971, Priede 1985, Neill et al. 1994). MS therefore represents the confines within which aerobic activities must be accommodated (Fry 1971, Evans 1990). According to Fry (1971), these activities must be viewed in a broad sense and include all physiological work such as swimming, feeding or growth. Any environmental condition causing a reduction of metabolic scope is thus liable to lead to energy budgeting conflicts between these activities (Priede 1985, Evans 1990). Temperature and oxygen are, respectively, potent controlling and limiting factors of fish metabolism, and they interact through their joint effects on MS (Fry 1971, Neill & Bryan 1991, Claireaux & Lagardère 1999). Using data from Chabot & Dutill (1999), Claireaux et al. (2000) examined the consequences of reduced MS in Atlantic cod Gadus morhua exposed to hypoxia. These authors showed that a linear relationship could be established between the MS of cod and its growth. They also underlined the fact that the poor swimming ability of cod at low temperature (He 1991) could also be explained on the basis of reduced MS. More recently, Mallekh & Lagardère (2002) reported a linear relationship between a temperature-mediated decrease in MS, the food intake and the growth of the turbot Scophthalmus maximus. In Solea solea, van den Thillart et al. (1998) showed that progressive hypoxia (from 80 to 20% air saturation) was accompanied by a large reduction of MS and spontaneous swimming activity.

In fish, biological performances are intimately linked to the ability of the cardio-vascular system to provide tissues with the required amount of oxygen and nutrients. However, the level of work that the myocardium can sustain is strongly influenced by the physico-chemical characteristics of the environment (Farrell et al. 1996, Farrell 1997, Lefrançois et al. 1998). In rainbow trout Oncorhynchus mykiss, a drop in temperature from 20 to 15°C results in a ~25% reduction of the maximal heart rate (Farrell 1997). It has long been recognised that this decline accounts almost entirely for the temperature-induced decrease in cardiac output (Randall 1968). Oxygen depletion also results in reduced heart performances. For instance, the maximal heart rate of sea bass Dicentrarchus labrax exposed to progressive hypoxia is reduced from 65 to 40 beats min⁻¹ between ~90 and ~50% of air saturation (Lefrançois et al. 1998). The relationship between heart rate and metabolism is not straightforward in fish (Thorarensen et al. 1996). However, heart rate is liable to provide information about the environmental impact on fish physiological state, thereby widening our understanding of the general relationship between fish and their environment (Priede & Young 1977, Priede & Tytler 1977, Armstrong 1986, Lucas et al. 1991). Moreover, heart rate is one of the few correlates to metabolic demand that can be monitored in free-swimming fish using telemetry (Armstrong 1986, Bushnell & Brill 1991, Lucas et al. 1991, Sureau & Lagardère 1991, Claireaux et al. 1995). By examining the influence of ambient temperature and oxygenation on both MS and scope for heart rate, we aimed to design an analytical tool for investigating the ecological relevance of the environmental constraints on sole.

MATERIALS AND METHODS

Fish. Sole were allocated into 2 groups. Group 1 consisted of 8 fish (0.15 kg ± 0.08 SE) and Group 2 of 6 fish (0.82 kg ± 0.09 SE). Both groups of fish were collected in the Gironde estuary (France). Upon arrival at the laboratory, fish were transferred into 500 l indoor rearing tanks with a sand bottom, where the temperature followed natural seasonal changes. Tanks were supplied with recirculated and filtered natural seawater (water renewal rate: 30 to 50% per week). Fish were fed twice a week on frozen mussels or fresh oysters. Feeding was discontinued 24 h before fish were used in an experiment.

Oxygen consumption measurement. The complete experimental set-up (Fig. 1) was placed in a thermo-regulated room. To measure fish oxygen consumption, 2 stop-flow respirometers were used (R). Their volume was adapted to the size of the sole, 6.8 l for fish from Group 1 and 23.8 l for fish from Group 2. The respirometers were placed in a larger tank (T) where water oxygenation was regulated via a counter-current gas equilibration column (C) bubbled with (1) oxygen to create hyperoxia, (2) air to maintain normoxia or (3) nitrogen to establish hypoxic conditions. The respirometers were supplied with water from the outer tank at a flow rate of 10 l min⁻¹ via a pump (P). Oxygen concentration in the respirometers was monitored using oxygen probes (Orbisphere Laboratories 27141, Op) connected to a multichannel oxygen measuring system (Orbi-
sphere Laboratories 2610, Ox) and a chart recorder (Pr). Oxygen probes were inserted in thermoregulated cuvettes connected to the respirometer via 2 pumps (P1 and P2). These pumps ensured appropriate mixing in the measuring chambers. Oxygen probes were calibrated daily. To measure fish oxygen consumption, the pump (P) controlling the water supply to the respirometer was turned off and the decrease in the chambers’ water oxygen content monitored during the next 15 min. Oxygen consumption (\( MO_2 \) in mgO₂ kg⁻¹ h⁻¹) was calculated using the following formula:

\[
MO_2 = \Delta C_w O_2 \times \Delta t^{-1} \times VOL_{resp} \times M^{-1}
\]

where \( \Delta C_w O_2 \) is the change in water oxygen concentration (mgO₂ l⁻¹) during \( \Delta t \) the measuring time (h), \( VOL_{resp} \) is the volume of the respirometer minus the volume of the fish (l), and \( M \) the mass of the fish (kg).

Routine oxygen consumption was standardised to a body mass of 100 g using the following formula (Schurmann & Steffensen 1997):

\[
MO_{2cor} = MO_{2meas} \times (M_{meas} \times M_{cor}^{-1})^{1-A}
\]

where \( MO_{2cor} \) is the mass specific \( MO_2 \) of a fish weighing \( M_{cor} \), \( MO_{2meas} \) the mass specific \( MO_2 \) measured, and \( M_{meas} \) the mass of the fish. \( A \) is the allometric exponent describing the relationship between metabolic rate and body mass (0.8: van den Thillart et al. 1994). Background oxygen consumption by micro-organisms was routinely assessed before the fish was transferred into the chamber by recording the oxygen consumption of an empty respirometer. In some cases, only 1 of the 2 chambers contained a fish, the other being left empty to assess the changes in background \( MO_2 \) over time. Oxygen consumption by micro-organisms never exceeded 10% of fish \( MO_2 \), but was accounted for in the calculations.

**Heart rate measurement.** Heart rate (HR) was monitored in fish from Group 2 by recording the acoustic pulse rate of an electrocardiogram transmitter (Tr). The tag and the tagging procedure are described in Claireaux et al. (1995). Acoustic signals from the tag were detected by a hydrophone (Vemco VH65, Hy) placed in the outer tank and connected to a receiver (Vemco VR20, r). The receiver was interfaced with a computer (Cp) that processed and stored the data. HR (beat min⁻¹) were averaged over the 15 min period corresponding to the oxygen consumption measurement period.

**Protocol for Group 1.** All fish from Group 1 were tested at each of the 6 experimental temperatures (4, 8, 12, 16, 20 and 24°C). Ten days before each experimental trial, Group 1 fish were transferred into a tank situated in the thermoregulated room where they were acclimated to the experimental temperature. The fish acclimation procedure was designed in such a way that experimental temperatures were always within 2°C of the temperature prevailing in the rearing tanks (20 and 24°C in summer, 12 and 16°C in autumn, 4 and 8°C in winter). In the thermoregulated room the photoperiod matched the natural photoperiod cycle. Following acclimation, each fish was individually tested in hypoxia. The fish was first chased until exhaustion in order to raise its metabolic rate towards \( AMR \). It was then rapidly transferred into the respirometer chamber and the monitoring of the water oxygen content immediately started. After measuring oxygen consumption under normoxic conditions, water oxygenation was progressively reduced in a stepwise manner (step: 20% air saturation) down to 20% air saturation. In order to improve the modelling of the relationship between \( MO_{2max} \) and ambient oxygenation, fish were chased between hypoxic steps. Following exhaustion (~5 min), the respirometer was washed with hypoxic water until the desired oxygen level was reached (about 1 min). The respirometers were then closed and the water oxygenation level in the chambers monitored during the next 30 min. Only the last half of the monitoring periods was used to calculate oxygen consumption. After the last measurement, normoxic conditions were restored.
During the next 48 h, measurements of oxygen consumption were carried out to estimate SMR. Oxygen consumption was automatically recorded on the undisturbed animals. The pump controlling the water supply to the respirometers was turned off for 15 min every hour using a timer. The $\text{MO}_2$ values obtained were used to construct frequency distribution histograms. According to the method proposed by Steffensen et al. (1994), the peak of the distribution was considered as an estimator of the SMR. Since sole are more active during the night, only diurnal $\text{MO}_2$ values were considered. To improve this estimation further, we only considered the diurnal values recorded during the last day of each experimental trial, i.e. the third day. We assumed that by this time, stress due to handling and hypoxia had disappeared. After each experiment, fish were anaesthetised (0.3 ml l$^{-1}$ 2-phenoxyethanol), weighed and placed back into the rearing tanks.

**Protocol for Group 2.** The experimental protocol followed for this group was identical to the previous one with 2 exceptions:

- Firstly, 48 h before being transferred into the respirometer, each fish was anaesthetised (0.5 ml l$^{-1}$ 2-phenoxyethanol), weighed and equipped with a cardiac transmitter (10 cm long, 1.6 cm in diameter, 37.5 g in air). Sole were placed on an operating table and gills were continuously perfused with oxygenated water containing anaesthetic (0.3 ml l$^{-1}$ of 2-phenoxyethanol). Two electrodes were inserted under the skin (pigmented face) near the pericardial cavity and connected to an acoustic tag, which was sutured externally and transversally to the principal axis of the body. Tagging procedure took less than 15 min and fish resumed ventilatory activity within 5 min of being ventilated with anaesthetic free water.

- Secondly, the step decrease in ambient oxygenation started from hyperoxia (175 to 200%) instead of normoxia. Preliminary studies had shown that estimation of the maximal heart rate was considerably more accurate when the oxygenation range was extended to hyperoxia. Hyperoxic conditions were set 15 to 20 min prior to the start of the experiment by bubbling pure oxygen in the gas equilibration column. Each Group 2 fish was successively exposed to this experimental protocol, which was repeated after acclimation at 8°C (Janu-ary), 16 (March) and 20°C (April to May). Values of $\text{MO}_2$ measured from resting tagged fish were not included in the modelling procedure of the SMR, as the tag is likely to cause increased energy expenditure notably due to additional drag (Lewis & Muntz 1984, Mellas & Haynes 1985, Lefrançois et al. 2001).

**Statistical analysis.** Prior to analysis, data were log$_{10}$ transformed to ensure the normality of the data set and to improve homogeneity of variances (Sokal & Rohlf 1981). At each experimental temperature, the effect of ambient oxygenation on $\text{MO}_{2\text{max}}$ and $\text{HR}_{\text{max}}$ was analysed using a randomised-block ANOVA (Von Ende 1993). In order to analyse the effect of supersaturation, only $\text{MO}_2$ recorded from Group 2 fish (at 8, 16 and 20°C) were considered. Each fish was considered as a different block within which the oxygen treatment was applied. $\text{MO}_{2\text{max}}$ and $\text{HR}_{\text{max}}$ values were pooled among 7 oxygen saturation classes (from 0 to 175%) using a class interval of 25. The null hypothesis was rejected at $p < 0.05$. When necessary, an *a posteriori* Student-Newman-Keuls (SNK) test was applied ($p < 0.05$). Throughout the text, data are routinely expressed as mean ± SE (standard error).

**Concept.** The modelling of the MS as a function of water oxygenation is based on the fact that, for any given routine oxygen consumption ($\text{MO}_2$), we considered a limiting oxygen concentration (LOC), below which that particular oxygen demand cannot be satisfied (Beamish 1964, Fry 1971, Gehrke 1988,Neill & Bryan 1991, Claireaux & Lagardère 1999). It implies that during progressive hypoxia, routine $\text{MO}_2$ is oxygen independent until the corresponding LOC is reached. Below that point, $\text{MO}_2$ becomes oxygen dependent and declines with water oxygen content. The relationships between ambient oxygenation and maximum sustainable oxygen consumption ($\text{MO}_{2\text{max}}$) was described in sea bass *Dicentrarchus labrax* using an asymptotic curve, known as the LOC$_{\text{MO}_2}$ curve (Claireaux & Lagardère 1999). According to these authors, the asymptote was attained at 100% air saturation and represented the maximal metabolic rate that bass could sustain in normoxic conditions, i.e. AMR. In the present study, we also considered this asymptote as an estimator of AMR. As a consequence, we hypothesized that sole MS (i.e. AMR minus SMR) was maximal under normoxic conditions. When the ambient oxygenation level decreases, MS gradually shrinks as the maximal sustainable $\text{MO}_2$ departs from AMR and drops along the LOC$_{\text{MO}_2}$ curve. At the critical oxygen saturation, i.e. when the maximal sustainable $\text{MO}_2$ is equal to SMR, the MS is nil.

We extended the concept of the LOC curve to HR. For any given HR, we considered a limiting oxygen concentration (LOC), below which HR switches from being oxygen-independent to being oxygen-dependent. The description of the restriction imposed by ambient oxygenation on $\text{HR}_{\text{max}}$ was carried out using the same type of curve as described for $\text{MO}_{2\text{max}}$ i.e. the LOC$_{\text{HR}}$ curve. **Metabolic scope. Mathematical equations:** This was estimated as the difference between the maximal metabolic rate and the SMR (Eq. 1 in Table 1).

**Maximal and active metabolic rate:** In the present experimental conditions, the maximal metabolic rate was related to the ambient temperature and oxygen-
This relationship was described using Eq. (2) (Table 1), where \( \alpha_1 \) and \( \beta_1 \) are constants. At a given temperature, this equation defines the \( \text{LOCMO} \) curve, illustrating the relationship between the maximal metabolic rate and the ambient oxygenation. In Eq. (2), the asymptote corresponds to the \( \text{AMR} \), the value of which is temperature dependent (Eq. 3). Eq. (3) was proposed by Strasbaka & Gnauck (1985) to describe biological temperature-dependent processes showing a thermal optimum. In this equation, \( T \) is the experimental temperature, \( T_{\text{opt}} \) is the optimal temperature, i.e. at \( T_{\text{opt}} \) \( \text{AMR} \) is maximised, and \( T_m \) is the maximal temperature for \( \text{AMR} \), i.e. at \( T_m \) \( \text{AMR} = 0 \). \( \delta \) and \( \omega \) are constants.

**Standard metabolic rate:** \( \text{SMR} \) was described as a function of temperature using an exponential relationships (Eq. 4 in Table 1), where \( a \) and \( b \) are 2 constants.

**Active heart rate:** The relationships between temperature, oxygen and \( (HR_{\text{max}}) \) were described using Eq. (5) (Table 2), where \( \alpha_2 \) and \( \beta_2 \) are 2 constants and \( AHR \) the active heart rate. The relationship between the latter and the temperature was established using a linear equation (Eq. 6 in Table 2), where \( c \) is the slope and \( d \) the intercept.

## RESULTS

**Standard metabolic rate**

The method used to determine \( \text{SMR} \) is illustrated in Fig. 2A and the results obtained at each of the 6 experimental temperatures are presented in Fig. 2B. These data were used to fit Eq. (4) (Table 1).

---

**Table 1.** Definition and formulae of variables and parameters used in the metabolic scope model where \( T \) is temperature (°C) and \( C_wO_2 \) is air saturation (%)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Units</th>
<th>Equation</th>
<th>Equation no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( MS )</td>
<td>Metabolic scope</td>
<td>mg O_2 kg(^{-1}) h(^{-1})</td>
<td>( MO_{\text{max}} - \text{SMR} )</td>
<td>(1)</td>
</tr>
<tr>
<td>( MO_{\text{max}} )</td>
<td>Maximal metabolic rate</td>
<td>mg O_2 kg(^{-1}) h(^{-1})</td>
<td>( \text{AMR} \times [1 - \exp(\alpha_1 \times C_wO_2^{\beta_1})] )</td>
<td>(2)</td>
</tr>
<tr>
<td>( \text{AMR} )</td>
<td>Active metabolic rate</td>
<td>mg O_2 kg(^{-1}) h(^{-1})</td>
<td>( \delta \times \left( \frac{T_m - T}{T_m - T_{\text{opt}}} \right)^\omega \times \exp \left[ -\omega \times \left( \frac{T_m - T}{T_m - T_{\text{opt}}} \right) \right] )</td>
<td>(3)</td>
</tr>
<tr>
<td>( \text{SMR} )</td>
<td>Standard metabolic rate</td>
<td>mg O_2 kg(^{-1}) h(^{-1})</td>
<td>( a \times \exp(b \times T) )</td>
<td>(4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Mean ± SE</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>( a )</td>
<td>Constant</td>
<td>15.450 ± 1.833</td>
<td>6</td>
</tr>
<tr>
<td>( b )</td>
<td>Constant</td>
<td>0.052 ± 0.006</td>
<td>6</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>Constant</td>
<td>-0.005 ± 0.001</td>
<td>484</td>
</tr>
<tr>
<td>( \beta_1 )</td>
<td>Constant</td>
<td>1.320 ± 0.076</td>
<td>484</td>
</tr>
<tr>
<td>( \delta )</td>
<td>Constant</td>
<td>1038.410 ± 154.724</td>
<td>484</td>
</tr>
<tr>
<td>( \omega )</td>
<td>Constant</td>
<td>1.870 ± 1.509</td>
<td>484</td>
</tr>
<tr>
<td>( T_m )</td>
<td>Maximal temperature</td>
<td>30.480 ± 4.837</td>
<td>484</td>
</tr>
<tr>
<td>( T_{\text{opt}} )</td>
<td>Optimal temperature</td>
<td>19.740 ± 0.405</td>
<td>484</td>
</tr>
</tbody>
</table>

---

**Table 2.** Definition and formulae of variables and parameters used in the model of the scope for increasing heart rate \( (HR) \) where \( T \) is temperature (°C) and \( C_wO_2 \) is air saturation (%)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Definition</th>
<th>Units</th>
<th>Equation</th>
<th>Equation no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( HR_{\text{max}} )</td>
<td>Maximal heart rate</td>
<td>beat min(^{-1})</td>
<td>( AHR \times [1 - \exp(\alpha_2 \times C_wO_2^{\beta_2})] )</td>
<td>(5)</td>
</tr>
<tr>
<td>( AHR )</td>
<td>Active heart rate</td>
<td>beat min(^{-1})</td>
<td>( c \times T + d )</td>
<td>(6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Mean ± SE</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_2 )</td>
<td>Constant</td>
<td>-0.077 ± 0.028</td>
<td>178</td>
</tr>
<tr>
<td>( \beta_2 )</td>
<td>Constant</td>
<td>0.83 ± 0.11</td>
<td>178</td>
</tr>
<tr>
<td>( c )</td>
<td>Slope</td>
<td>4.10 ± 0.13</td>
<td>178</td>
</tr>
<tr>
<td>( d )</td>
<td>Intercept</td>
<td>-1.71 ± 0.35</td>
<td>178</td>
</tr>
</tbody>
</table>
AMR and maximal metabolic rate

At each experimental temperature, maximal oxygen consumption differed significantly among individuals (ANOVA; p < 0.01). A significant effect of the oxygen level on $\text{MO}_{2\text{max}}$ was also found (ANOVA; p < 0.001). Post-hoc multiple range tests (SNK) showed that as long as water saturation remained above 75%, oxygenation had no significant influence on $\text{MO}_{2\text{max}}$ (Fig. 3). Below this limit, $\text{MO}_{2\text{max}}$ in each class of air saturation were significantly different.

The general model describing the combined effect of ambient oxygenation and temperature on $\text{MO}_{2\text{max}}$ was fitted using the whole data set (Eqs. 2 & 3 in Table 1 and Fig. 4A). When compared with the experimental data, the maximal oxygen consumption predicted by this model showed a slight overestimation at 4°C (Fig. 5A).
AMR attained under normoxia quickly increased from 55.4 at 4°C to a maximum of 159.2 mgO₂ kg⁻¹ h⁻¹ at the optimal temperature, i.e. 19.7°C (Eq. 3 in Table 1, Fig. 6). Above 19.7°C, however, a downward trend was observed and, at 24°C, AMR was 129.9 mgO₂ kg⁻¹ h⁻¹.

**Fig. 5.** (A–F). *Solea solea*. Effect of water temperature (A: 4, B: 8, C: 12, D: 16, E: 20 and F: 24°C) and oxygenation on maximal oxygen consumption (*MO*₂₉₉₉). Solid lines indicate *LOC*ₙ₉₉₉ curves (Eqs. 2 & 3, Table 1) fitted to *MO*₂₉₉₉ recorded at the different level of oxygenation (closed squares). Dotted lines represent standard metabolic rate (SMR). All oxygen consumptions are corrected for a sole weighing 100 g. (G) Predicted and observed maximal oxygen consumption (solid line represents observations = predictions). Model testing: linear regression analysis; intercept –1.03 ± 1.82, p > 0.5; slope 1.03 ± 0.02, p < 0.0001, n = 484, r² = 0.79; ANOVA, F = 1895.6, p < 0.0001
Metabolic scope

The influence of water temperature and oxygenation on MS was modelled by subtracting Eq. (4) from Eq. (2) (Table 1 and Fig. 4B). Due to the exponential increase of SMR with temperature, MS was found to be maximal at 18.8°C and to be zero at 27.2°C (Fig. 6).

Heart rate

At each experimental temperature, significant interindividual differences in HRmax were found (ANOVA, p < 0.001). The effect of the ambient oxygenation on HRmax was also significant (ANOVA, p < 0.001). Multiple range tests (SNK) showed that down to 50% air saturation, ambient oxygenation had no significant effect on HRmax (Fig. 7). Below that threshold, however, HRmax was significantly different in each air saturation class at 16 and 20°C. At 8°C, however, a significant decrease of HRmax was recorded only for the 0 to 25% air saturation class.

The model describing the combined effect of oxygenation and temperature on HRmax was established according to Eq. (5) (Table 2, Fig. 8). An overall good fit between the observed and the predicted HRmax (LOCHR curves) was observed at 20°C (Fig. 9C). At 8°C, the LOCHR curve showed a slight overestimation of HRmax above 50% air saturation and a slight underestimation at the lower levels (Fig. 9A). At 16°C, an overestimation of HRmax can be observed (Fig. 9B). Globally, however, observed and predicted HRmax exhibited a linear relationship (Fig. 8D, n = 178, r² = 0.91) and the slope and intercept were not significantly different from 1 and 0 respectively (p > 0.05).

DISCUSSION

Effect of the temperature

The thermal dependence of sole metabolism is illustrated by the exponential increase of SMR (Fig. 6) between 4°C (17.5 mgO2 kg⁻¹ h⁻¹) and 24°C (52.5 mgO2 kg⁻¹ h⁻¹). Our values for SMR in sole are generally lower than the average values reported for flatfish e.g. 26.6 and 139.8 mgO2 kg⁻¹ h⁻¹ at 4 and 24°C respectively (Duthie 1982). This discrepancy is particularly significant at high temperatures. It is worth noticing that Duthie (1982) established a general relationship between temperature and SMR based on a data-set collected on 14 flatfish species, among which 4 are from the genus Platichthys, 7 from the genus Pleuronectes and none from the genus Solea. Therefore, the relationship established by Duthie may not be
Lefrançois & Claireaux: Physiological effects of O₂ and temperature in *Solea solea*

fully representative of the common sole. On the other hand, our results are in accordance with those of van den Thillart et al. (1994), who estimated SMR of a 100 g common sole to be 41 mg O₂ kg⁻¹ h⁻¹ at 20°C.

The influence of thermal conditions on sole metabolism is also illustrated by the bell-shaped curve describing the changes in AMR with temperature (Fig. 6). The maximal value attained at the optimal temperature (159.2 mg O₂ kg⁻¹ h⁻¹ at 19.7°C) is in accordance with that measured by van den Thillart et al. (1994) in 20°C acclimated sole (152.2 mg O₂ kg⁻¹ h⁻¹). The scarcity of published data on active metabolism in flatfish prevents thorough inter-specific comparison. Most of the available data indeed relate to resting or routine metabolism and only cover a narrow range of the species’ thermal range. To our knowledge, only Mallekh & Lagardère (2002) have examined the influence of a large range of acclimation temperature on AMR in a flatfish, the turbot *Scophthalmus maximus*. According to their study, AMR was 107 mg O₂ kg⁻¹ h⁻¹ at 6°C and 218 mg O₂ kg⁻¹ h⁻¹ at 18°C, i.e. higher than the AMR values we measured in sole. Comparison of our results with those of Duthie (1982) reveals that AMR in flounder *Platichthys flesus*, common dab *Limanda limanda* and lemon sole *Microstomus kitt* also exceed AMR values found in the common sole. For instance, AMR of a 100 g dab attained 141, 181 and 226 mg O₂ kg⁻¹ h⁻¹ at 5, 10 and 15°C respectively, when AMR in sole was estimated at 61, 97 and 138 mg O₂ kg⁻¹ h⁻¹.

The AMR of the European sea bass, which generally shares the same environment as sole, is approximately 4 times higher, i.e. 586 mg O₂ kg⁻¹ h⁻¹ at 22.5°C (Claireaux & Lagardère 1999). Bass and sole can also be distinguished on the basis of their level of thermal specialisation (Huey & Stevenson 1979). For instance, we can compare the temperature range over which each species can sustain a metabolic rate corresponding to 80% of their maximal MS. In sole, this range is 10°C (13.3 to 22.9°C), whereas in bass it is only 5°C (19.5 to 24.8°C, Claireaux & Lagardère 1999). A parallel can be drawn between the relatively low susceptibility of sole metabolism to temperature changes and its poor locomotive ability. Physiology, morphology and behaviour are closely related (Huey 1991). It can be hypothesised that the reduced behavioural repertoire of sole connects with a greater subordination of thermoregulation to the physiological/biochemical mechanisms involved in the environmental adaptation. Greater mobility, on the contrary, is generally associated with broader behavioural abilities. This allows a faster and possibly more temperature-sensitive metabolism, providing such species with an efficient means for tuning their metabolism according to their physiological needs, e.g. feeding/starving (Sogard & Olla 1996) or to the environmental conditions encountered, e.g. hypoxia (Schurmann & Steffensen 1992).

Fig. 9. *Solea solea*. (A–C) Effect of water temperature (A: 8, B: 16 and C: 20°C) and oxygenation on maximal heart rate (*HR* max). Solid lines indicate *LOCIR* curves fitted to *HR* max recorded at the different level of oxygenation (closed squares). *LOCIR* curves were calculated using Eqs. (5) & (6) with the adequate experimental temperature (*T*). (D) Predicted and observed maximal heart rate (solid line represents observations = predictions). Model testing: linear regression analysis; intercept –3.18 ± 1.62, p > 0.05; slope 0.97 ± 0.02, p < 0.0001; n = 178, r² = 0.91; ANOVA, *F* = 1685.0, p < 0.0001.
In the present study, we quantified the variations in aerobic MS that sole are liable to experience when exposed to given changes in ambient temperature and oxygenation (Eq. 1). The MS represents the energetic framework within which all physiological functions must be accommodated. Its maximisation reduces the occurrence of situations requiring energetic prioritisation between conflicting physiological processes and thereby has positive repercussions on fish biological performance (Priede 1985, Evans 1990). In this regard, the temperature where MS is maximised also yields to maximised growth performance in sole (Anonymous 1983), in turbot (Mallek & Lagardère 2002) and bass (Lefrançois 2001). On the contrary, fish with reduced MS are likely to experience impaired performance (Neill & Bryan 1991). For instance, the temperature where MS is nil (27.2°C) corresponds to the incipient lethal temperature of sole (Anonymous 1983).

As noticed in other fish species (Randall 1968, Cech et al. 1976, Farrell et al. 1996, Farrell 1997), the maximal heart beat frequency of the sole increased linearly with temperature (Eq. 6). The calculated $Q_{10}$ of 2.3 is within the range of values classically reported in teleosts (1.3 to 3.0, Farrell 1997). Furthermore, our measures of $AHR$ agree with the observations reported on free-swimming sole by Sureau & Lagardère (1991). The highest $HR$ they telemetered was 50 beats min$^{-1}$ (temperature between 8 and 12°C), while our model predicts $AHR$ between 31 and 47.5 beats min$^{-1}$ in the same thermal range.

The linear increase of $AHR$ with temperature contrasts with the concomitant bell-shaped relationship observed with AMR. A parallel can be drawn between this observation and the hypothesis made by Farrell (1997) concerning the influence of temperature on cardiac output. It was reported that, in salmonids, cardiac output followed a bell-shaped relationship with temperature. Farrell (1997) hypothesised that the ascending portion of that relationship resulted from a temperature-driven increase in both $HR$ and stroke volume. Farrell (1997) also proposed that above a certain thermal threshold, the still increasing $HR$ gradually resulted in reduced cardiac filling and/or cardiac contractility, bringing about a progressive decrease in stroke volume and cardiac output. On the same basis, it can be hypothesised that in sole, the observed divergence in the course followed by AMR and $AHR$ at temperatures above 20°C originates from a stroke volume-related decrease in cardiac output.

**Effect of oxygen**

Together with temperature, oxygen is an important determinant of MS. During progressive hypoxia, $MO_{2\text{max}}$ gradually dropped down the $LOC_{MO_{2\text{max}}}$ curve, bringing about a reduction of sole MS. On the other hand, hyperoxic conditions did not influence sole $MO_{2\text{max}}$. This corroborates similar observations made on European sea bass *Dicentrarchus labrax* (Lefrançois 2001) and turbot *Scophthalmus maximus* (Mallek & Lagardère 2002). In the latter species, neither food intake (Mallek et al. 1998) nor growth (Person-Le Ruyet et al. 2001) was improved when fish were raised under hyperoxic conditions. In rainbow trout *Oncorhynchus mykiss*, the maximal oxygen consumption measured in forced-swum fish and the critical swimming speed ($U_{\text{crit}}$) are not raised by hyperoxia either (Duthie & Hughes 1987).

The present study also shows that in sole $HR_{\text{max}}$ is not influenced by hyperoxic conditions (ca. 150 to 175 % air saturation, Fig. 6). To our knowledge, only 1 study reports values of $HR_{\text{max}}$ in hyperoxic conditions (Lefrançois 2001). In that study, we showed that neither in sole nor in sea bass is $HR_{\text{max}}$ affected by oxygen supersaturation.

The concept of the $LOC_{HR}$ curve is based on the assumption that for any given $HR$ we considered a limiting oxygen concentration ($LOC$), below which $HR$ switches from being oxygen-independent to being oxygen-dependent. Bradycardia is a common cardiovascular response in teleosts facing hypoxia (Satchell 1971, Randall 1982). Satchell (1971) pointed out that the ambient oxygen level that triggered bradycardia was species specific (see also Marvin & Burton 1973, Cech et al. 1977, Fritsche & Nilsson 1989). The present results (Figs. 8 & 9) suggest that the oxygen level at which bradycardia occurs, i.e. the limiting oxygen concentration ($LOC_{HR}$), also depends on the cardiovascular work load at the heart at the onset of hypoxia. Lefrançois et al. (1998) observed that sea bass fed to satiation displayed $HR$ near the $LOC_{HR}$ curve defined for this species. In these conditions every subsequent mild (90 % sat) and short (less than 5 min) hypoxic episode was associated with bradycardia. Reciprocally, in *Scyliorhinchus canicula* presenting a low $HR$, bradycardia was observed only under severe hypoxia (Taylor 1985). In *Torpedo marmorata* (Hughes 1978) or *Gobius cobiti* (Berschick et al. 1987) which both exhibited low $HR$, bradycardia was never observed. Our results suggest that in fully active sole a reduction of ambient oxygen to 50 % air saturation is sufficient to trigger bradycardia.

Based on the measures of $HR_{\text{max}}$, $MO_{2\text{max}}$, SMR and MS, the present study aimed at gaining an holistic view of the combined effect of temperature and oxygen on the metabolic and cardiovascular performances of a poorly studied flatfish species. The connections that we have tried to establish between physiology, morphology and behaviour are clearly ten-
tative. However, the proposed approach highlights possible avenues of future research to disentangle, understand and predict the ecological repercussions of environmental forcing on fish performance at large.

Acknowledgements. This study was financially supported by IFREMER, the Institut Français pour la Recherche et l’Exploitation de la Mer. The authors are indebted to Marcel Guillaut and Michel Prineau for their technical help, and thank P. Domenici, S. Lefebvre and the referees for helpful comments on the manuscript. All experimental procedures in this work comply with the current laws in France.

LITERATURE CITED

Anonymous (1983) Fiches biotechniques d’aquaculture. La sole. CNEXO, Brest
Armstrong JD (1986) Heart rate as an indicator of activity, metabolic rate, food intake and digestion in pike, Esox lucius. J Fish Biol 29:207–221
Bushnell PG, Brill RW (1991) Responses of swimming skipjack (Katsuwonus pelamis) and yellowfin (Thunnus albacares) tunas to acute hypoxia, and a model of their cardiorespiratory function. Physiol Zool 64:787–811


Evans DO (1990) Metabolic thermal compensation by rainbow trout: effects on standard metabolic rate and potential usable power. Trans Am Fish Soc 119:585–600


Marvin DE, Burton DT (1973) Cardiac and respiratory re-
sponses of rainbow trout, bluegills and brown bullhead catfish during rapid hypoxia and recovery under normoxic conditions. Comp Biochem Physiol 46A:515–524
Randall DJ (1968) Functional morphology of the heart in fishes. Am Zool 8:179–189

Schurmann H, Steffensen JF (1992) Lethal oxygen levels at different temperatures and the preferred temperature during hypoxia of the Atlantic cod, Gadus morhua. J Fish Biol 41:927–934
Sureau D, Lagardère JP (1991) Coupling of heart rate and locomotor activity in sole, Solea solea (L.), and bass, Dicentrarchus labrax (L.), in their natural environment by using ultrasonic telemetry. J Fish Biol 38:399–405

Submitted: January 29, 2001; Accepted: April 29, 2003
Proofs received from author(s): September 2, 2003

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany