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Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*

Christel Lefrançois[1,2,*], Guy Claireaux[1]

[1]**Centre de Recherche sur les Ecosystèmes Marins et Aquacoles (CNRS-IFREMER), BP 5, L'Houmeau 17137, France**

[2]***Present address:*** **IMC—International Marine Centre, Localita Sa Mardini 09072 Torregrande-Oristano, Italy**

ABSTRACT: The objective of this study was to quantify the constraints exerted by temperature and oxygenation on both metabolic scope and scope for heart rate of the common sole *Solea solea*. We exposed sole to a large range of temperature and oxygenation conditions and a modelling procedure was implemented to describe metabolic and cardiac responses. Standard metabolic rate (*SMR*) rose exponentially from 4 to 24°C, whereas active metabolic rate (*AMR*) increased from 55.4 to 159.2 mg O_2 kg^{-1} h^{-1} between 4 and 19.7°C, and then dropped to 129.9 mg O_2 kg^{-1} h^{-1} at 24°C. In parallel, optimal temperature for maximising metabolic scope, defined as the difference between *AMR* and *SMR*, was estimated to 18.8°C. Active heart rates increased linearly with temperature and attained 15, 79 and 97 beats min^{-1} at 4, 20 and 24°C, respectively. Hyperoxia had no influence on maximal heart rate nor on maximal metabolic rate. In hypoxic conditions, on the other hand, a significant decrease of the maximal metabolic rate was recorded when oxygen dropped below 75% air saturation. A significant decrease in maximal heart rate was also detected below 50% at 16 and 20°C, and below 25% air saturation at 8°C. The potential ecological consequences of the variation in sole metabolic and cardiac performances are discussed.

KEY WORDS: Metabolic scope · Heart rate · Biological performance · Temperature · Oxygen · Common sole

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 environmental fluctuations. For instance, Petersen & Pihl (1995) noted that during a hypoxic episode in the Kattegat (between Denmark and Sweden), dominant demersal roundfishes (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*

*Email: christel.lefrancois@tiscali.it

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 & Dutil (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 thermoregulated 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-

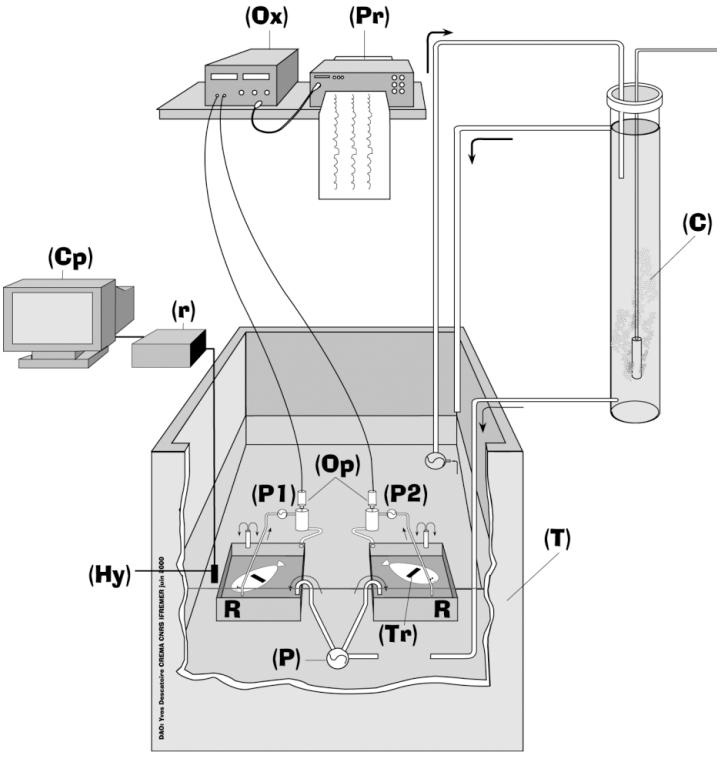

Fig. 1. Experimental set-up (for explanations see subsections 'Oxygen consumption measurement' and 'Heart rate measurement' in 'Materials and methods')

sphere Laboratories 2610, Ox) and a chart recorder (Pr). Oxygen probes were inserted in thermoregulated cuvettes connected to the respirometer via 2 pumps (P_1 and P_2). 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 respirometers was turned off and the decrease in the chambers' water oxygen content monitored during the next 15 min. Oxygen consumption (MO_2 in mg O_2 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 (mg O_2 l^{-1}) during Δt 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 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 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 accoustic 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 (January), 16 (March) and 20°C (April to May). Values of 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 MO_{2max} and HR_{max} was analysed using a randomised-block ANOVA (Von Ende 1993). In order to analyse the effect of supersaturation, only 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. MO_{2max} and HR_{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 (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 MO_2 is oxygen independent until the corresponding *LOC* is reached. Below that point, MO_2 becomes oxygen dependent and declines with water oxygen content. The relationships between ambient oxygenation and maximum sustainable oxygen consumption (MO_{2max}) was described in sea bass *Dicentrarchus labrax* using an asymptotic curve, known as the LOC_{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 MO_2 departs from *AMR* and drops along the LOC_{MO_2} curve. At the critical oxygen saturation, i.e. when the maximal sustainable 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 HR_{max} was carried out using the same type of curve as described for MO_{2max}, i.e. the LOC_{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-

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 (%)

Variable	Definition	Units	Equation	Equation no.
MS	Metabolic scope	mg O_2 kg^{-1} h^{-1}	$MO_{2max} - SMR$	(1)
MO_{2max}	Maximal metabolic rate	mg O_2 kg^{-1} h^{-1}	$AMR \times [1 - \exp(\alpha_1 \times C_wO_2^{\beta 1})]$	(2)
AMR	Active metabolic rate	mg O_2 kg^{-1} h^{-1}	$\delta \times \left(\frac{T_m - T}{T_m - T_{opt}}\right)^{\omega} \times \exp\left[-\omega \times \left(\frac{T_m - T}{T_m - T_{opt}}\right)\right]$	(3)
SMR	Standard metabolic rate	mg O_2 kg^{-1} h^{-1}	$a \times \exp(b \times T)$	(4)

Parameter	Definition	Mean ± SE	No. of observations
a	Constant	15.450 ± 1.833	6
b	Constant	0.052 ± 0.006	6
α_1	Constant	−0.005 ± 0.001	484
β_1	Constant	1.320 ± 0.076	484
δ	Constant	1038.410 ± 154.724	484
ω	Constant	1.870 ± 1.509	484
T_m	Maximal temperature	30.480 ± 4.837	484
T_{opt}	Optimal temperature	19.740 ± 0.405	484

ation. This relationship was described using Eq. (2) (Table 1), where α_1 and β_1 are constants. At a given temperature, this equation defines the LOC_{MO2} curve, illustrating the relationship between the maximal metabolic rate and the ambient oxygenation. In Eq. (2), the asymptote corresponds to the 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_{opt} is the optimal temperature, i.e. at T_{opt} AMR is maximised, and T_m is the maximal temperature for AMR, i.e. at T_m, $AMR = 0$. δ and ω are constants.

Standard metabolic rate: 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_{max}) were described using Eq. (5) (Table 2), where α_2 and β_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 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 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 (%)

Variable	Definition	Units	Equation	Equation no.
HR_{max}	Maximal heart rate	beat min^{-1}	$AHR \times [1 - \exp(\alpha_2 \times C_wO_2^{\beta 2})]$	(5)
AHR	Active heart rate	beat min^{-1}	$c \times T + d$	(6)

Parameter	Definition	Mean ± SE	No. of observations
α_2	Constant	−0.077 ± 0.028	178
β_2	Constant	0.83 ± 0.11	178
c	Slope	4.10 ± 0.13	178
d	Intercept	−1.71 ± 0.35	178

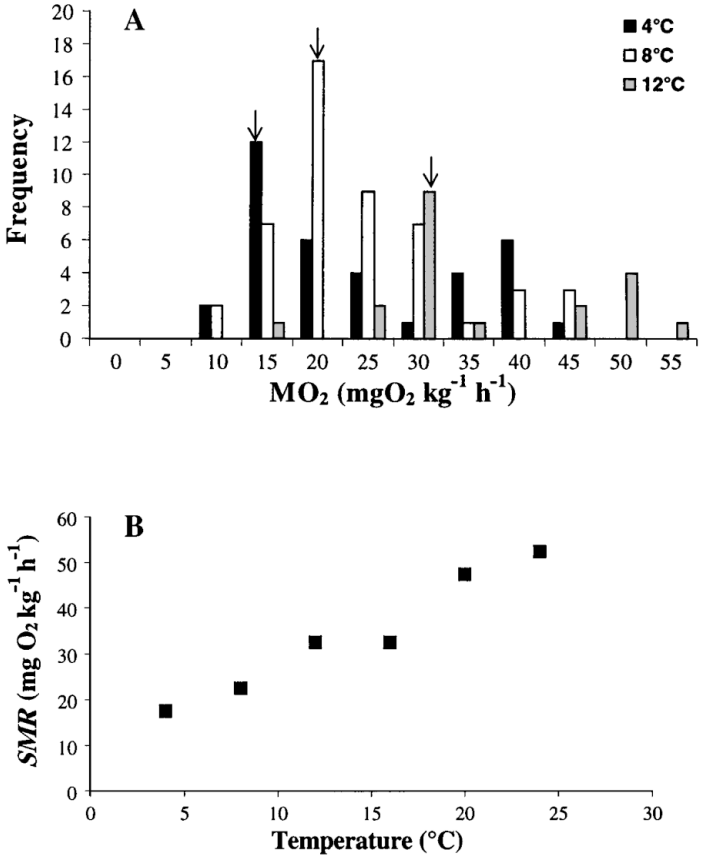

Fig. 2. *Solea solea*. (A) Examples of frequency distribution of oxygen consumption (MO_2 mgO_2 kg^{-1} h^{-1}) at 4, 8 and 12°C during resting conditions. Arrows indicate peak of distribution at each of these 3 temperatures, which is considered the estimation of standard metabolic rate (*SMR*). (B) Estimation of *SMR* at the 6 experimental temperatures. Only MO_2 measurements carried out on Group 1 individuals were considered

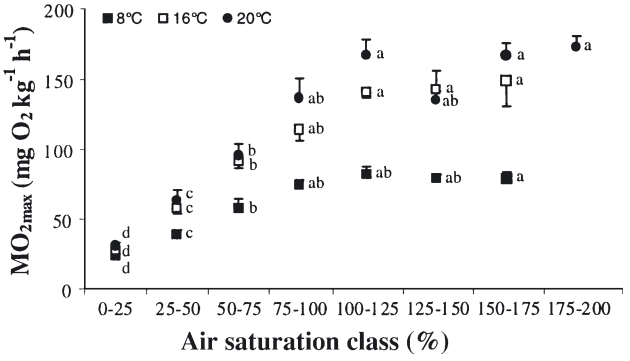

Fig. 3. *Solea solea*. Oxygen effect on maximal oxygen consumption (MO_2) at 8,16 and 20°C. Values are mean MO_{2max} ± SE in mgO_2 kg^{-1} h^{-1}. At 1 experimental temperature, means not sharing a common superscript are significantly different ($p < 0.05$, ANOVA)

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 MO_{2max} was also found (ANOVA; $p < 0.001$). *A posteriori* multiple range tests (SNK) showed that as long as water saturation remained above 75%, oxygenation had no significant influence on MO_{2max} (Fig. 3). Below this limit, MO_{2max} in each class of air saturation were significantly different.

The general model describing the combined effect of ambient oxygenation and temperature on MO_{2max} 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)

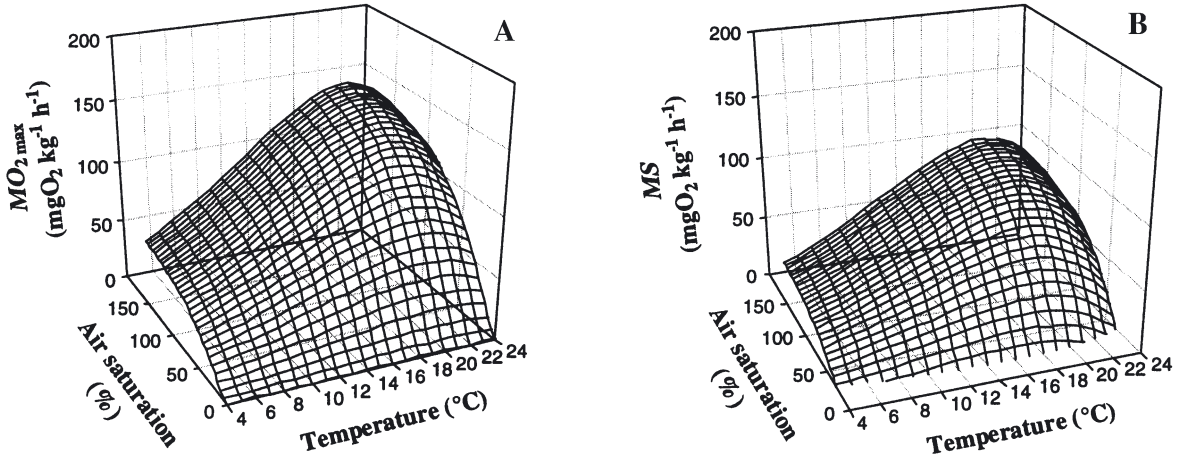

Fig. 4. *Solea solea*. Effect of oxygen and temperature on (A) maximal oxygen consumption (MO_{2max}) and (B) metabolic scope (*MS*)

and a slight underestimation at 12°C (Fig. 5C). However, an overall good fit between the observed and predicted values of MO_{2max} was found (Fig. 5G, $r^2 = 0.79$, n = 484). Furthermore, the slope and intercept of the relationship between observed and predicted values were not significantly different from 1 and 0 respectively ($p > 0.05$).

AMR attained under normoxia quickly increased from 55.4 at 4°C to a maximum of 159.2 mg O_2 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 mg O_2 kg^{-1} h^{-1}.

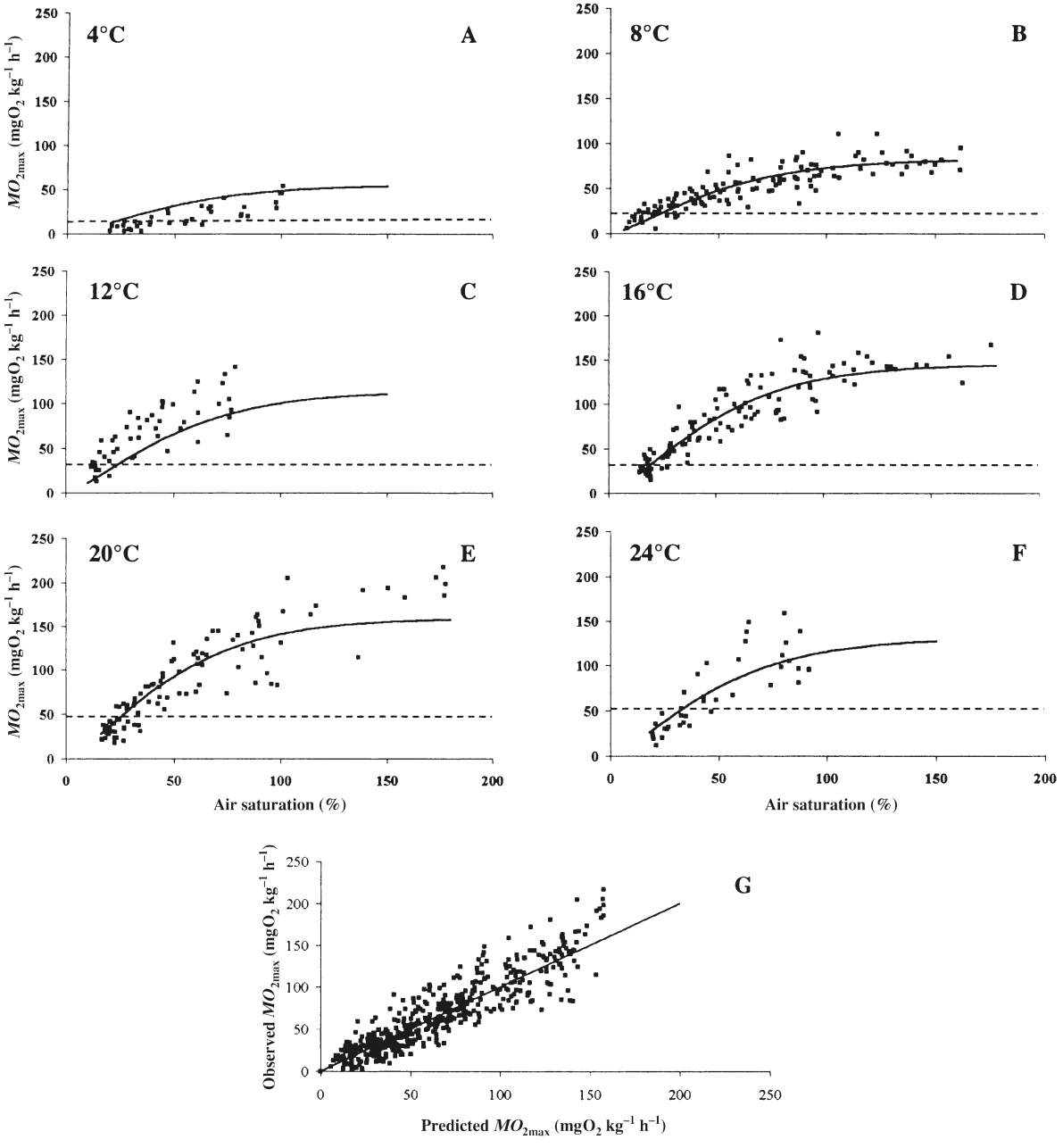

Fig. 5. (A–F). *Solea solea*. Effect of water temperature (A: 4, B: 8, C: 12, D: 16, E: 20 and F: 24°C) and oxygenation on maximal oxygen consumption (MO_{2max}). Solid lines indicate LOC_{MO_2} curves (Eqs. 2 & 3, Table 1) fitted to MO_{2max} recorded at the different level of oxygenation (closed squares). 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, $r^2 = 0.79$; ANOVA, $F = 1895.6$, $p < 0.0001$

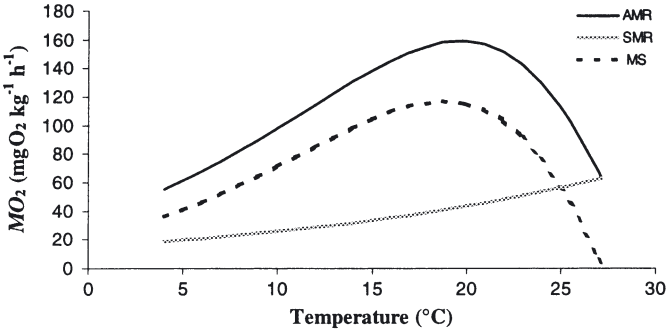

Fig. 6. *Solea solea*. Influence of ambient temperature on active metabolic rate (*AMR*, Eq. 3, Table 1), standard metabolic rate (*SMR*, Eq. 4, Table 1) and metabolic scope (*MS*, Eq. 1 with C_wO_2 = 100%, Table 1)

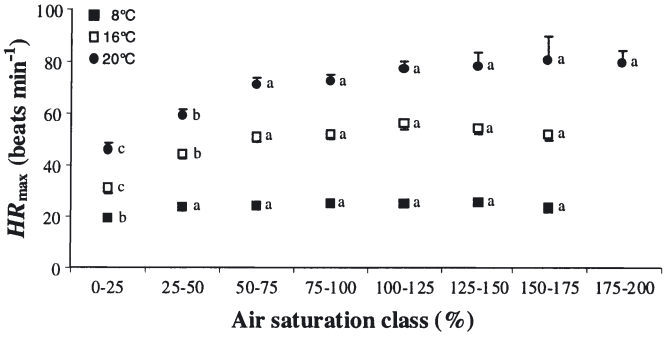

Fig. 7. *Solea solea*. Oxygen effect on maximal heart rate (HR_{max}) sustainable at 8, 16 and 20°C. Values are mean HR_{max} ± SE in beats min^{-1}. At each temperature, means not sharing a common superscript are significantly different ($p < 0.05$, ANOVA)

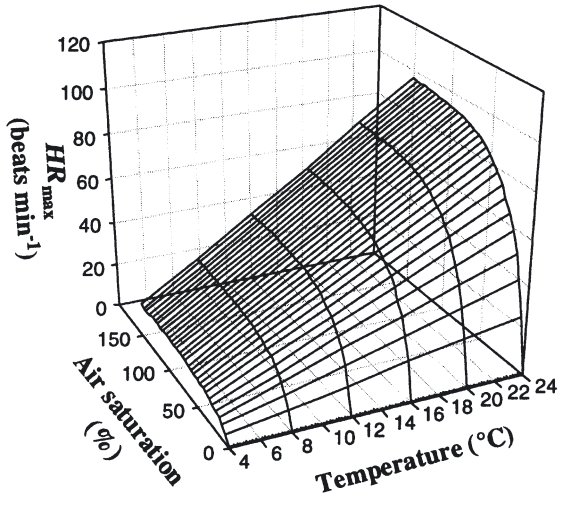

Fig. 8. *Solea solea*. Effect of oxygen and temperature on maximal heart rate (HR_{max}, Eqs. 5 & 6, Table 2)

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 inter-individual differences in HR_{max} were found (ANOVA, $p < 0.001$). The effect of the ambient oxygenation on HR_{max} 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 HR_{max} (Fig. 7). Below that threshold, however, HR_{max} was significantly different in each air saturation class at 16 and 20°C. At 8°C, however, a significant decrease of HR_{max} was recorded only for the 0 to 25% air saturation class.

The model describing the combined effect of oxygenation and temperature on HR_{max} was established according to Eq. (5) (Table 2, Fig. 8). An overall good fit between the observed and the predicted HR_{max} (LOC_{HR} curves) was observed at 20°C (Fig. 9C). At 8°C, the LOC_{HR} curve showed a slight overestimation of HR_{max} above 50% air saturation and a slight underestimation at the lower levels (Fig. 9A). At 16°C, an overestimation of HR_{max} can be observed (Fig. 9B). Globally, however, observed and predicted HR_{max} exhibited a linear relationship (Fig. 8D, n = 178, $r^2 = 0.91$) 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 mg O_2 kg^{-1} h^{-1}) and 24°C (52.5 mg O_2 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 mg O_2 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

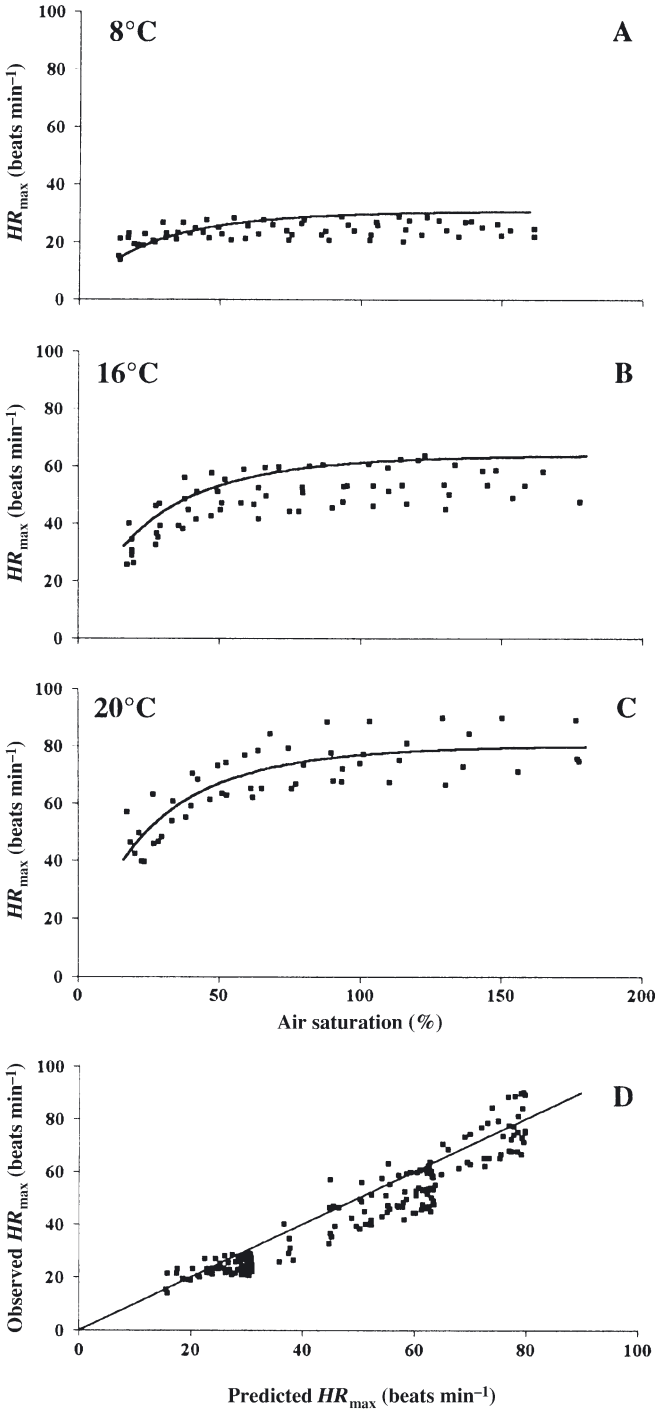

Fig. 9. *Solea solea*. (A–C) Effect of water temperature (A: 8, B: 16 and C: 20°C) and oxygenation on maximal heart rate (HR_{max}). Solid lines indicate LOC_{HR} curves fitted to HR_{max} recorded at the different level of oxygenation (closed squares). LOC_{HR} curves were calculated using Eqs. (5) & (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, $p < 0.0001$, $n = 178$, $r^2 = 0.91$; ANOVA, $F = 1685.0$, $p < 0.0001$

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 O_2 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 O_2 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 O_2 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 O_2 kg^{-1} h^{-1} at 6°C and 218 mg O_2 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 O_2 kg^{-1} h^{-1} at 5, 10 and 15°C respectively, when *AMR* in sole was estimated at 61, 97 and 138 mg O_2 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 O_2 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).

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 (Mallekh & 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_{2max} gradually dropped down the LOC_{MO_2} curve, bringing about a reduction of sole *MS*. On the other hand, hyperoxic conditions did not influence sole MO_{2max}. This corroborates similar observations made on European sea bass *Dicentrarchus labrax* (Lefrançois 2001) and turbot *Scophthalmus maximus* (Mallekh & Lagardère 2002). In the latter species, neither food intake (Mallekh 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_{crit}) are not raised by hyperoxia either (Duthie & Hughes 1987).

The present study also shows that in sole HR_{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_{max} in hyperoxic conditions (Lefrançois 2001). In that study, we showed that neither in sole nor in sea bass is HR_{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 *Scyliorhincus 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_{max}, MO_{2max}, *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.

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