

## RESEARCH ARTICLE

# Temperature and pressure dependency of oxygen consumption during long-term sustained swimming of European eels

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## ABSTRACT

Many aspects of the typically 5000–10,000 km spawning migration of the European eel (*Anguilla anguilla*) remain unknown. As part of this migration, eels undertake extensive diurnal vertical migrations to depths below 1000 m, being exposed to a wide range of temperatures and hydrostatic pressures. In this experimental study, we exposed eels to different combinations of temperature (12–20°C) and pressure (100–800 kPa) during long-term sustained swimming (32–47 days). Both temperature and pressure affected oxygen consumption rate, such that there was a significant increase of metabolic rate with temperature, whereas pressure reduced oxygen consumption, albeit only at higher temperatures. Average oxygen consumption rates ranged between 15 mg kg<sup>-1</sup> h<sup>-1</sup> (12°C, 100 kPa) and 30.2 mg kg<sup>-1</sup> h<sup>-1</sup> (20°C, 100 kPa), highlighting the remarkably high swimming efficiency of this species and, more importantly, indicating that past evaluations of the cost of transport are potentially overestimates as they are often based on experiments conducted at atmospheric pressure at higher temperatures.

**KEY WORDS:** *Anguilla anguilla*, Respirometry, Swim tunnel, Spawning migration, Energy demand

## INTRODUCTION

Great efforts have been made to elucidate the transatlantic spawning migration of the European eel (*Anguilla anguilla*) from its European and North African growth habitats to the Sargasso Sea in the Western Atlantic, but many aspects of this remarkable journey are still unknown. The missing information includes knowledge about the duration of the 5000–10,000 km long migration and the exact location of the migration route (Righton et al., 2016). The estimated duration of this journey, depending on the origin of departure, is between 4 months and more than a year (Palstra et al., 2008; Palstra and Van Den Thillart, 2010; Righton et al., 2016), even though these estimates are mainly based on a theoretical background because of the lack of direct observations of the entire migration. While these basic questions are still under investigation, a number of experimental swimming trials have aimed to assess the energetic demands of eels during long-term swimming activity (e.g. van Ginneken et al., 2005; Palstra et al., 2008; Tudorache et al., 2015) in

order to calculate the energy reserves that eels need for their spawning migration and successful reproduction. Given that the European eel stock is in a critical situation (ICES, 2022) and that limited energy reserves (i.e. low spawner quality) are considered a major threat for the stock (Belpaire et al., 2009, 2016; Clevestam et al., 2011), this information is also crucial for the management of this endangered species (IUCN). As eels do not feed during their spawning migration (Tesch, 1999; Palstra et al., 2008; Chow et al., 2010), they completely rely on energy reserves, that were built up during their continental growth phase, to fuel locomotion and to fully mature their reproductive organs (van Ginneken and Maes, 2005; Freese et al., 2019). Eels are considered to be very energy-efficient swimmers (van Ginneken et al., 2005; Palstra et al., 2008), yet a reduced swimming capacity could directly affect their long-range migration and jeopardize reproduction (Belpaire et al., 2009).

Calculations by Van Ginneken and Van den Thillart (2000) suggested that lipid reserves commonly found in emigrating European silver eels should be sufficient to fuel the energy demands for migration and gonadal maturation. Nevertheless, muscle lipid content in wild yellow and silver eels is highly variable (Pohlmann et al., 2018). Clevestam et al. (2011) argue that reduced energy reserves, disturbed fat metabolism due to accumulated contaminants (Belpaire et al., 2009), as well as an increased energy demand for swimming as a result of infestation with the invasive swim bladder nematode *Anguillicola crassus* in silver eels (Würtz et al., 1996; Palstra et al., 2007; Schneebeuer et al., 2020) or potentially prolonged migration periods (Righton et al., 2016) may further decrease the reproductive capacity of the stock.

Tagging studies with European eels revealed that they perform diel vertical depth changes during the course of their migration, swimming at a depth range of 200–300 m during the night, and at a depth range of 600–1000 m during the day (Aarestrup et al., 2009; Westerberg et al., 2014; Wysujack et al., 2015; Righton et al., 2016). This behaviour can result in depth differences of up to 800 m twice a day, causing large changes in hydrostatic pressure and water temperature within time periods of 1–2 h. It has been shown that the encountered diurnal changes in hydrostatic pressure cannot be compensated for by adjustment of swimbladder volume alone, and the resulting changes in the buoyancy status require compensation by hydrodynamic lift, which increases the energy demand (Pelster et al., 2015). Testing the effect of elevated hydrostatic pressure revealed that increasing hydrostatic pressure caused an increase in swimming activity and resulted in an elevated oxygen consumption (Sébert, 2002, 2009). But after this initial increase, oxygen uptake decreased again to a new steady state at levels below the initial oxygen uptake under low pressure conditions. The authors therefore concluded that eels swimming under constantly elevated hydrostatic pressure would have a reduced oxygen demand (Sébert et al., 2009). However, high hydrostatic pressures can eventually result in immobility of fish (Simon et al., 1989; Sébert et al., 1997, 2009; Sébert, 2002).

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The effect of temperature on metabolism and specifically oxygen consumption of fish is well studied, generally showing a positive correlation between temperature and  $O_2$  demand (Ege and Krogh, 1914; Fry, 1971; Beamish, 1964). While Degani et al. (1989) showed a clear positive, yet size-dependent, correlation of oxygen consumption with increasing temperature for young, resting individuals of *A. anguilla*, Palstra (2006) reported that European silver eels during a simulated 1000 km migration with daily temperature changes between 18 and 10°C showed no effect of temperature on oxygen consumption. Thus, potential effects of the temperature changes encountered during the diurnal migrations are not fully clear.

It has previously been demonstrated that eels are able to continuously swim at a speed of 0.5 body lengths per second ( $BL s^{-1}$ ) for a period of 6 months under experimental conditions (van Ginneken et al., 2005) with comparatively low energy requirements (van Ginneken et al., 2005; Palstra et al., 2008), but effects of pressure and temperature changes on their swimming efficiency during long-term migration have never been investigated in combination. In this study, a hyperbaric swim tunnel with respirometer function (Wysujack et al., 2022) was used for the first time to investigate the oxygen consumption of female European silver eels during long-term swimming at three different water temperatures (approximately 19, 16 and 13°C) and two hydrostatic pressures (100 and 800 kPa). While technical limitations of the swim tunnels did not allow for the application of the highest pressures or lowest temperatures experienced during the open ocean migration (>10,000 kPa and <2°C), depth and temperature ranges differ greatly between individuals on their oceanic migration (Aarestrup et al., 2009; Righton et al., 2016) and the aim in this study was to explore the general relationship and potential interactions between temperature and pressure. The obtained data provide new insights into the swimming physiology of migrating eels and facilitate a better understanding of their energy demand during the transoceanic migration, providing valuable information for stock managers regarding minimum energy requirements of escaping silver eels.

## MATERIALS AND METHODS

### Experimental animals

Female silver eels were caught with stow nets by commercial fishers in the rivers Ems and Rhine during downstream migration in late autumn 2018. Larger animals ( $n=9$ ) were deliberately selected to ensure sufficient oxygen consumption and allow more accurate measurements. Eels were quarantined for 1 week and subsequently transferred into rectangular 2500 l recirculation tanks equipped with plastic tubes for shelter. Light conditions were set to a 12 h:12 h

light:dark cycle and water temperature was kept at  $15\pm 1^\circ C$ , while salinity was increased from freshwater conditions to  $34.5\pm 1$  psu by a daily increase of approximately 7 psu. Eels were not fed during quarantine, acclimation and the experiment.

For individual identification, eels were equipped with passive integrated transponder tags (PIT tags; 8 mm×1.4 mm FDX-B ‘Skinny’ PIT Tag, Oregon RFID, Portland, OR, USA), inserted subcutaneously parallel to the dorsal fin after anaesthesia with 2-phenoxyethanol (approximately  $1 ml l^{-1}$ ; Carl Roth GmbH, Karlsruhe, Germany). Before and after the experiment, total length, body mass, eye diameter and pectoral fin length were measured, the muscle fat content was derived using microwave measurements (FM 692; Distell Inc., West Lothian, UK) and the silvering index (Durif et al., 2005) was calculated (Table 1). Before the experiment started, eels experienced at least 7 days without any handling.

### Experimental setup

The hyperbaric swim tunnel system with respirometer function used in this experiment was described in detail by Wysujack et al. (2022). Briefly, it consists of three identical swim tunnels (volume 205 l each) with transparent, horizontal cylindrical swimming chambers (length 140 cm, diameter 20 cm), which are connected to a common storage tank including a water treatment and aeration unit. Hydrostatic pressure (limited to a maximum of 800 kPa), velocity and light conditions in the swim chamber can be controlled separately for each swim chamber, whereas water temperature is regulated for the whole system in the storage tank.

In order to automatically adjust swimming speed, light barriers were installed at the front and rear end of each swimming chamber. If eels swim too fast and activate the light barrier at the front, the flow rate automatically increases by  $0.1 m s^{-1}$ . If the light barrier at the rear end is interrupted for more than 10 min, the system enters a ‘minimum mode’ where the flow rate is reduced to  $0.1 m s^{-1}$  in order to protect exhausted eels from harm. For measurement of oxygen consumption, each swim tunnel was equipped with an Oxygen Optode 4835 (Aanderaa Data Instruments AS, Bergen, Norway) that quantifies the decrease of oxygen saturation in each measuring chamber over the course of a measuring cycle. In the following, a ‘measurement’ is defined as the interval between full closure of the swim tunnel (i.e. no water exchange with the storage tank) and ‘flushing’ (i.e. exchange with water from the storage tank to restore oxygen levels near saturation). The following data were recorded during measurements: oxygen concentration ( $mg l^{-1}$ ; corrected for pressure and temperature) and saturation (%), temperature ( $^\circ C$ ), pressure (kPa), velocity ( $m s^{-1}$ ) and activation of front/rear light barriers by the fish.

**Table 1. Summary of biological and experimental parameters by individual eels**

ID	Length (cm)	SI	Biomass at start/end (g)	Fat at start/end (% wet mass)	Swim time (days)	Distance (km)	Pressure range (kPa)	Temperature range ( $^\circ C$ )	Velocity range ( $BL s^{-1}$ )
A	78	4	935/866	17.2/18.5	44.1	1727	99–827	12.3–20.2	0.37–0.81
B	74	5	732/701	20.9/21.6	44.1	1837	99–818	11.7–20.1	0.45–0.86
C	77	5	908/864	22.6/24.5	44.1	1814	99–822	11.6–20.7	0.43–0.84
D	78	5	973/928	19.2/17.6	32.6	1481	99–805	13.0–20.3	0.35–0.90
E	83	4	1045/1000	18.4/20	32.6	1573	98–804	12.2–19.7	0.43–0.88
F	90	4	1142/1079	21.6/20.8	32.2	1464	99–820	12.2–19.7	0.38–0.87
G	87	5	1345/1070	17/20.9	43.4	1935	99–805	12.9–20.5	0.47–0.81
H	81	4	987/947	19.1/19.1	45.8	2195	99–804	11.9–19.7	0.53–0.93
I	86	4	1345/1247	18.4/18.5	47.0	2169	98–814	11.9–19.5	0.34–1.07

Ranges refer to minima and maxima at any point in time (i.e. of single data points, not means per measurement). SI, silvering index according to Durif et al. (2005). Eels A and B were removed from analyses (see Materials and Methods).

### Experimental protocol

The effects of pressure and temperature on the oxygen consumption rates during long-term swimming were quantified for nine eels. Each individual was exposed to all possible combinations of three pre-defined temperatures (approximately 19, 16 and 13°C) and two pressures (100 and 800 kPa). Each individual was first exposed to the highest temperature, which was subsequently reduced. Within each temperature treatment, eels were first exposed to a pressure of 100 kPa, followed by a pressure of 800 kPa. Pressure change was set to a rate of 100 kPa min<sup>-1</sup> and flow rate was set to 0.6 BL s<sup>-1</sup>, corresponding to the optimal swimming speed ( $U_{opt}$ ) of eels, as reported by Methling et al. (2011) and Tesch (1978). If necessary, flow rate was adjusted according to the eels' swimming behaviour. Animals were acclimated for 30 h at the start of the experiment and following temperature changes, and for 24 h following pressure changes.

A complete set of measurements with all factor combinations (in the following referred to as a 'run') was conducted on three individual eels in parallel and in a series of three subsequent runs (i.e. nine individual datasets were derived). Each factor combination was maintained for 2.1–7.8 days (excluding acclimation; Table S1); swimming times were shorter in the second run, as the duration of this run had to be reduced because of outside influences. Throughout the experiment, oxygen consumption was measured in repeated 1 h intervals, each followed by flushing for 30 min.

While pressure generally remained constant throughout treatments (including flushing), it repeatedly dropped to <700 kPa for two individuals (A and B), which were completely removed from further analysis. For another individual (F), pressure dropped to <600 kPa, but only during two measurements, and the pressure was restored immediately – therefore, only these two measurements were removed for analyses. Additionally, eels occasionally swam in the front section of the tube, activating the light barrier and thus increasing the flow rate, with some (not all) seemingly using areas of lower flow or turbulence to conserve energy (see also Wysujack et al., 2022); this bias was accounted for in the modelling approach (see below).

For the remaining measurements, observed pressure was constant between 98 and 114 kPa as well as 781 and 822 kPa at the two pre-defined settings, temperature ranged from 12 to 14.6°C, 14.6 to 17.6°C and 17.8 to 20.6°C for the three pre-defined settings and velocity varied between 0.46 and 0.78 BL s<sup>-1</sup> (see also Table S1).

Note, that the priority in the experimental design was to minimize stress and allow constant swimming. Hence, animals were not handled within runs and temperatures were subsequently reduced, as temperature changes are a stress factor for most fish and this effect is more significant when ambient temperature increases. Accordingly, it was necessary to make assumptions about mass loss, and temperature partially co-varied with pressure and potential time effects (e.g. fasting), which cannot be fully separated. The entailed caveats are addressed in detail in the Discussion.

### Data processing and statistical analyses

Data processing, statistical analyses and visualizations were performed using R statistical software (version 4.1.2, <http://www.R-project.org/>). Total oxygen consumption rate was calculated by linear regression and corrected for background respiration (3–9.7 mg h<sup>-1</sup>; see Supplementary Materials and Methods) for each measurement. Assuming a mass scaling exponent (e.g. Clarke and Johnston, 1999; Moses et al., 2008; Jerde et al., 2019) equal or close to one for the European eel, as reported by Boldsen et al.

(2013), the individual oxygen consumption rate per measurement (in mg kg<sup>-1</sup> h<sup>-1</sup>) was calculated as:

$$\text{O}_2 \text{ consumption rate} = \frac{-s \times V}{m_i}, \quad (1)$$

where  $s$  is the slope of the regression after correction (in mg l<sup>-1</sup> h<sup>-1</sup>),  $V$  is the volume of the swim tunnel (in l) and  $m_i$  the individual body mass of the eel at the respective point in time (in kg). As mass loss during measurements could not be measured instantaneously, the assumption was made that body mass decreased linearly during each treatment. Differences in mass loss between treatments were weighted according to the mean individual oxygen consumption of the respective treatment (i.e. higher mass loss at higher oxygen consumption).

For quality assurance, several selection criteria were applied to the raw data: (i) obvious measurement errors (e.g. due to sensor malfunctions resulting in unrealistic temperatures or pressures) were excluded from raw data; (ii) measurements were only considered for analysis if an interval covered a period of at least 0.5 h, consisted of at least 60 data points, and distances between two data points were not greater than 60 s; (iii) the O<sub>2</sub> saturation at the start of a measurement exceeded 90%; (iv) the regression was sufficiently linear and with reasonable variation (defined as  $R^2 > 0.9$ ); and (v) temperature within a measurement did not vary by more than 1°C, and pressure did not deviate by more than 10 kPa from the nominal pressure.

Statistical testing was conducted by fitting a linear mixed effects model (nlme::lme; <https://CRAN.R-project.org/package=nlme>; Pinheiro and Bates, 2000), following the protocol from Zuur et al. (2009), predicting the ln of O<sub>2</sub> consumption rate as a function of temperature, pressure, velocity and the interaction between temperature and pressure as fixed effects (also see Supplementary Materials and Methods, 'Supplementary model selection', and Table S2). A random intercept was included for ID to account for differences in base metabolism and non-independence of repeated measures, hence allowing for predictions on both the individual and population level. Further, a random slope for velocity was added to account for individually different responses to velocity changes (see above), providing a significantly better fit as compared with the random intercept-only model ( $P < 0.001$ , likelihood-ratio test). For statistical testing, all predictor variables were mean centred (i.e. the global mean was subtracted from each measurement) to accurately reflect effect sizes and odds ratios of the main effects for values within the tested range of parameters (global means: 16.4°C, 400 kPa and 0.64 BL s<sup>-1</sup>).

### Ethical statement

The experiments with eels followed German legislation concerning the care and use of laboratory animals, and ethical permission for the experiments was given by the Authority of Science, Health and Consumer Protection of the Federal State Bremen (TV Nr. 142).

### RESULTS

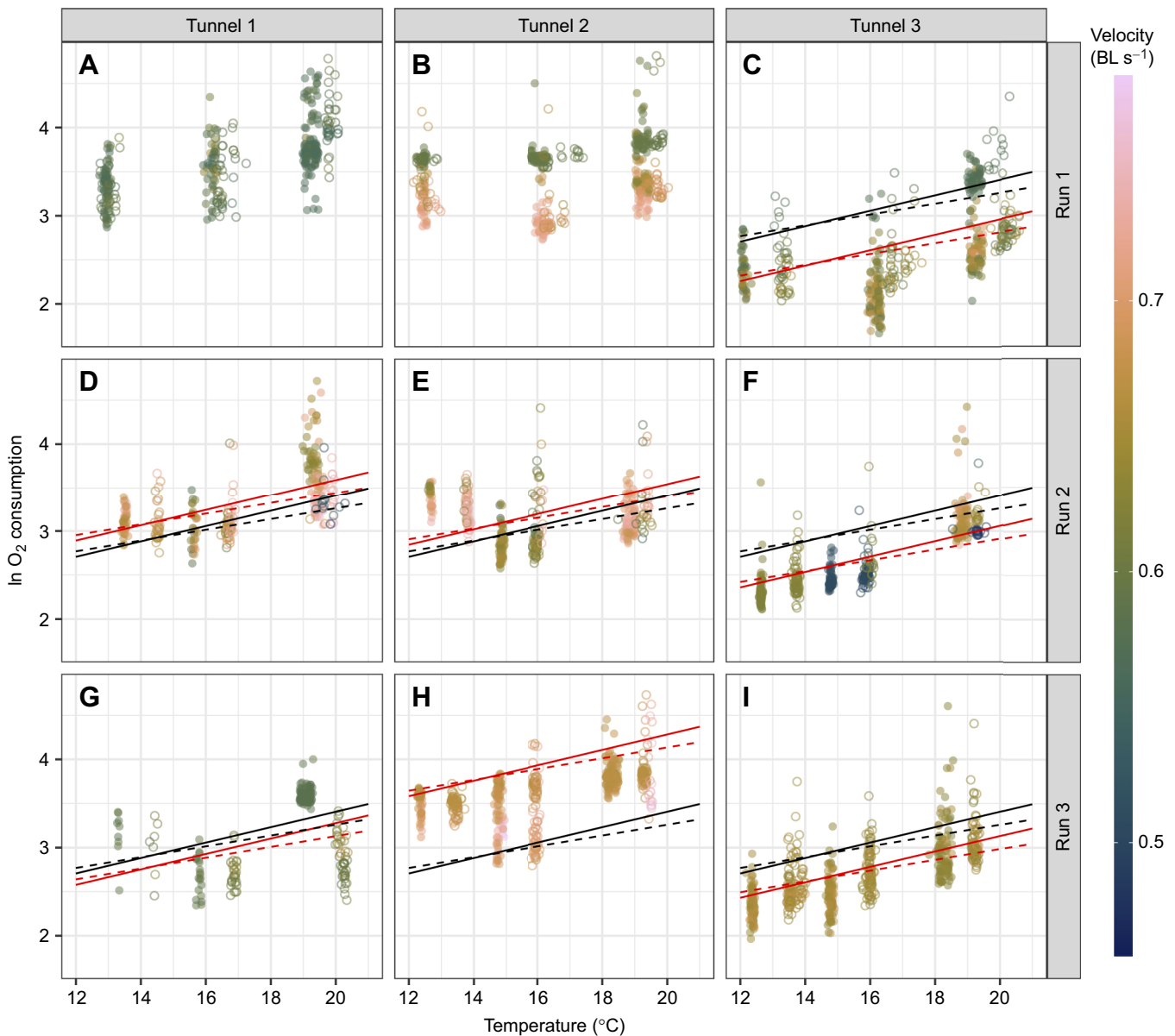
Silver eels were continuously swimming for 32–47 days, covering a total distance of 1464–2195 km. During swimming, eels lost between 31 and 98 g of biomass (4.1–7.4% of initial biomass) and muscle fat content in wet mass (WM) changed between –1.6 and +1.95 percentage points, with the exception of a single individual [individual G: lost 275 g biomass (20.4%) and increased fat WM by 3.85 percentage points] (Table S1). As the observed changes in fat content are below the measuring accuracy of the fat meter

(according to the manufacturer  $\pm 2\%$ ), fat content in WM was considered constant and the mean of both measurements was used for further calculations.

Mean observed oxygen consumption rates for each temperature and pressure combination varied between  $9 \pm 3.7 \text{ mg kg}^{-1} \text{ h}^{-1}$  (individual C,  $16.2^\circ\text{C}$ , 100 kPa and  $0.63 \text{ BL s}^{-1}$ ) and  $46.3 \pm 7.7 \text{ mg kg}^{-1} \text{ h}^{-1}$  (individual H,  $18.3^\circ\text{C}$ , 100 kPa and  $0.67 \text{ BL s}^{-1}$ ) (Table S2); individual measurements ranged from  $5.3 \text{ mg kg}^{-1} \text{ h}^{-1}$  (individual C,  $16.3^\circ\text{C}$ , 100 kPa and  $0.59 \text{ BL s}^{-1}$ ) to  $113.5 \text{ mg kg}^{-1} \text{ h}^{-1}$  (individual H,  $19.4^\circ\text{C}$ , 800 kPa,  $0.68 \text{ BL s}^{-1}$ ) (Fig. 1). Individual responses to temperature differed notably, with some individuals showing an increase in oxygen consumption at the lowest temperatures, towards the end of the run (Fig. 1; Table S1, E and G). At temperatures  $>18^\circ\text{C}$ , almost all eels showed largely increased variance in oxygen consumption rate, with values covering

almost the entire observed range ( $7.6\text{--}123 \text{ mg kg}^{-1} \text{ h}^{-1}$ ). At temperatures  $<18^\circ\text{C}$ , the variation was much less pronounced and oxygen consumption rate rarely exceeded  $50 \text{ mg kg}^{-1} \text{ h}^{-1}$  (range  $5.3\text{--}90 \text{ mg kg}^{-1} \text{ h}^{-1}$ ).

Generally, the model performed well in predicting the oxygen consumption rate and there was a notable difference between individuals, as indicated by the difference between individual and population predictions in Fig. 1 (s.d. of random intercept = 1.3 on ln-scale). No obvious pattern was observed for oxygen consumption in relation to body mass. Individual predictions of comparably small eels (Fig. 1A–C) were both above and below the population mean, whereas predictions for the largest eels were either also above (Fig. 1F,I) or below the population mean (Fig. 1G) or did not differ notably (Fig. 1E). An increase in velocity was occasionally associated with a decrease in oxygen consumption, but variation in



**Fig. 1. Oxygen consumption rate per individual as a function of temperature at 100 and 800 kPa.** Oxygen consumption ( $\text{mg kg}^{-1} \text{ h}^{-1}$ ) is given for individuals A–I. Solid circles and solid lines, 100 kPa; open circles and dashed lines, 800 kPa; circle colour indicates velocity in  $\text{BL s}^{-1}$ , representing the mean velocity per measurement. Modelled predictions are displayed at the population level (black lines) and for the individual (red lines), normalized to a swimming speed of  $0.6 \text{ BL s}^{-1}$ . Note, eels A and B were not included in statistical analyses (see Materials and Methods).

the individual response to changes in the flow rate was high (s.d. of random slope=1.8, as compared with a main effect of  $-1.32$ ), indicating a shift in the direction of the effect between individuals. This is highly unexpected and will be discussed in detail below.

Model results showed significant effects for both temperature ( $P<0.001$ ) and pressure ( $P<0.05$ ), as well as their interaction ( $P<0.01$ ), whereas the effect of velocity was not significant (Table 2). For the observed ranges of pressure and temperature, oxygen consumption rate increased exponentially with temperature, though the effect was less pronounced under elevated pressure ( $Q_{10}$  of 2.4 and 1.85 at 100 and 800 kPa, respectively; both values for a swimming speed of  $0.6 \text{ BL s}^{-1}$ ). Accordingly, pressure resulted in an increasingly lower oxygen consumption rate at higher temperatures. Note that the effect of pressure is theoretically nullified at, and reversed below, approximately  $14^\circ\text{C}$ . Similarly, the effect of temperature would be nullified at, and reversed above, approximately 2400 kPa. These values are, however, in the lower range (temperature) or well outside (pressure) those realized in this experiment and are therefore considered strictly mathematical and without biological meaning.

## DISCUSSION

The results of our study demonstrate that oxygen consumption of silver eels decreases at lower temperatures and increasing hydrostatic pressure (for temperatures above approximately  $14^\circ\text{C}$ ) during long-term sustained swimming. This result suggests that current estimates of the energy requirement for the oceanic migration of eels are probably too high, as they are often based on experiments conducted at temperatures higher than those experienced *in situ*.

### The effect of temperature and pressure

On their oceanic spawning migration, eels equipped with tags have been shown to dive as deep as  $\sim 1000 \text{ m}$ , which exposes the animals to more than 10,000 kPa of hydrostatic pressure and a wide range of temperatures (e.g.  $2\text{--}15^\circ\text{C}$ ; Righton et al., 2016), both of which affect the eels' metabolism and consequently their cost of transport (COT).

For the range of temperatures and pressures observed in the present experiment, oxygen consumption of swimming female silver eels increased with increasing temperature at atmospheric pressure as well as at a hydrostatic pressure of 800 kPa and the relationship was well described by an exponential function, whereas an increase in hydrostatic pressure caused a reduction in oxygen consumption at temperatures above approximately  $14^\circ\text{C}$ .

**Table 2. Short summary of the model statistics**

Parameter	Value	Lower CL	Upper CL	P
Intercept	3.004	2.683	3.325	<0.001
Temperature ( $^\circ\text{C}$ )	0.075	0.066	0.085	<0.001
Pressure (MPa)*	-0.007	-0.014	-0.001	0.035
Velocity ( $\text{BL s}^{-1}$ )	-1.332	-2.870	0.205	0.089
Temperature $\times$ pressure interaction*	-0.004	-0.006	-0.001	0.007

Model statistics are for fixed effects with mean centred predictors for statistical inference, i.e. significance as well as effect size and confidence limits of main effects, for a global mean temperature, pressure and swimming speed of  $16.4^\circ\text{C}$ , 400 kPa and  $0.64 \text{ BL s}^{-1}$ . CL, confidence limit; BL, body lengths. 'Value' is the change in the  $\ln$  of  $\text{O}_2$  consumption rate per change in one unit of the predictor. \*Note that pressure effects are given per 100 kPa rather than one unit of pressure.

Interestingly, the variability of metabolic rate was remarkably high at temperatures  $>18^\circ\text{C}$ , with peak values ( $123 \text{ mg kg}^{-1} \text{ h}^{-1}$ ) exceeding even the highest values reported in previous studies (e.g. Palstra et al., 2008; Tudorache et al., 2014; see below). While this aspect remains speculative, the high variation in oxygen consumption at higher temperature ranges could be a response to acclimation stress, as oxygen uptake increases with higher temperature while critical swimming speed ( $U_{\text{crit}}$ ) and the aerobic scope in general decrease at temperatures beyond the optimal temperature for swimming activity (Lee et al., 2003; Schulte, 2015).

These findings are well in line with those of previous studies: the relationship of temperature and metabolic rate in fish is well established, generally showing an increase in metabolic rate with temperature (e.g. Brett, 1971; Beamish, 1978; Lee et al., 2003; Yan et al., 2012; Trancart et al., 2015), while it has been shown that long-term exposure to hydrostatic pressure notably reduced oxygen consumption in resting and swimming eels (e.g. Sébert et al., 1997, 2009). It is an important finding in this context that the effects of temperature and pressure are not simply additive; that is, if oxygen consumption is reduced by temperature or pressure effects, the effect of the respective other factor will get smaller, as previously described by Sébert et al. (1997), who stated that the effect of hydrostatic pressure is expected to be higher the higher the oxygen consumption (e.g. due to higher temperature).

At the cellular level, a number of modifications have been identified after pressure acclimation which may contribute to the reduction in energy expenditure. Chronic exposure to high hydrostatic pressure caused an increase in membrane fluidity (Sébert et al., 1993; Theron et al., 2000). In addition to homeoviscous adaptation, cellular energy metabolism is of crucial importance and analysis of mitochondrial respiration indicated an increase in the efficiency of oxidative phosphorylation (Theron et al., 2000). A recent study revealed the presence of a remarkable fraction of mitochondria-independent respiration in isolated European eel gas gland cells, and it was concluded that respiratory coupling and mitochondrial function are not ideal (Drechsel et al., 2022). It therefore appears possible that under elevated hydrostatic pressure, mitochondrial function may be improved, which could contribute to a decrease in oxygen consumption under these conditions. Despite pressures in the present experiment being much smaller than those experienced during the oceanic migration (in fact, even the lower diving depths are often at pressures  $>800 \text{ kPa}$ ), a pressure effect, though small, was still detectable. Extrapolating from Sébert et al. (1997) – who found that oxygen consumption at 101 atm (standard atmosphere) was reduced by approximately 30% and 40% at  $15^\circ\text{C}$  and  $20^\circ\text{C}$ , respectively – a decrease of 2.4% and 3.3% at the respective temperatures would be expected at 800 kPa. Indeed, the reduction was 1.6% and 13.7%. Given the lack of direct comparability (e.g. freshwater versus saltwater, active swimming versus resting) and high variation in the upper temperature range, these values are somewhat consistent with previous findings and suggest a rather gradual (though not necessarily linear) decrease in oxygen consumption with increasing pressure.

In summary, the results of the present study provide strong evidence that temperatures in the lower range (well below  $18^\circ\text{C}$ ) are advantageous for the spawning migration of the European eel to the Sargasso Sea. The considerable variance between individuals makes the interpretation difficult, but oxygen consumption of several eels started increasing, or at least did not notably decrease further at temperatures below  $15^\circ\text{C}$ , while swimming speed was maintained. This suggests an optimum temperature below  $15^\circ\text{C}$ ,

potentially close to the ambient temperatures typically experienced *in situ* (11–13°C; Righton et al., 2016), which makes sense ecologically. Even at higher temperatures, oxygen consumption decreases, probably gradually, with diving depth as a result of pressure effects.

### Error discussion

Before discussing in more detail the relevance of these findings for the spawning migration and related science, some caveats of the present experiment should be considered.

Firstly, body mass is a highly relevant parameter in studies of metabolic rate; yet, as the priority was to minimize stress, only two values (before and after a run) were available per individual. Between-individual differences are of less concern as, given the comparably small individual changes in body mass, they are reasonably captured by the available measurements and statistically accounted for by the random effect for individual. The assumption of a mass scaling exponent of or close to one (Boldsen et al., 2013) could introduce bias as well, but our results do not indicate that the mass scaling component is different to 1 (as with data normalized per unit mass, a mass-related pattern of individual predictions being above or below the population mean is expected, which was not the case; also see Fig. 1). Though small, within-individual changes of body mass are of more concern, as both body mass and the parameters of interest (pressure and temperature) co-vary with time. That is, if for example the rate of loss in body mass is underestimated at 19°C, oxygen consumption per kilogram will be increasingly underestimated with time (i.e. greater underestimation at 800 kPa), thus directly affecting statistical results. By modelling the loss in body mass separately for each treatment (weighted by the average oxygen consumption for the respective treatment), this effect should be reasonably accounted for.

Secondly, it is a peculiar observation that, at least in some individuals (e.g. B, C and H; see Fig. S1), oxygen consumption was negatively correlated with velocity. Apart from the potential use of areas with lower flow or turbulence, we cannot offer a reasonable explanation, despite the swim tunnels being designed and tested for laminar flow. As oxygen consumption is expected to increase with swimming speed (Herskin and Steffensen, 1998; Pang et al., 2011, 2013; Yan et al., 2012; Trancart et al., 2015), this implies that the realized swimming speeds might be lower than the measured velocities. However, the respective individuals did not necessarily show lower oxygen consumption rates (e.g. H), the effect was highly variable between individuals, and the range of velocities was moderate with notable variation in oxygen consumption rates. Hence, this observation is not overly concerning and it is controlled for in the model by the implementation of a fixed effect and random slope for velocity. While relative changes in oxygen consumption should not be affected by a potential discrepancy between velocity and swimming speed, the derived absolute values should nonetheless be interpreted with care, more likely representing low estimates of oxygen consumption and high estimates for the calculated swimming distance.

Thirdly, the co-variation of time with both temperature and pressure treatments could introduce bias as, for example, any effect attributed to temperature could, in theory, be an effect of time (for example, due to mass loss or training effects). This potential bias is particularly relevant with regards to the effect of pressure as lower temperatures were experienced at the end of each run. If physiological adaptations to pressure exposure persist for a period of several days after decompression, as hypothesized by Simon et al. (1989), pressure would not have an effect towards the end of a run

(i.e. at the lower temperatures, as was the case for this experiment). However, no obvious temporal trends were observed within treatments and a similar interaction was described earlier by Sébert et al. (1997).

### Possible implications for the spawning migration

A number of studies have provided data describing the energy consumption or COT of swimming eels under different conditions. Under atmospheric pressure (~100 kPa), van Ginneken et al. (2005) reported a value of  $43.9 \pm 8.42 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  for smaller eels ( $43.2 \pm 3.2 \text{ cm}$ ) during short-term swimming (7 days) at  $0.5 \text{ BL s}^{-1}$  and a temperature of 18°C as well as an estimate of  $29.55 \text{ ml O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  (corresponding to approximately  $39 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ) for larger eels ( $73.1 \pm 3.8 \text{ cm}$ ) during long-term swimming (173 days) at 19°C. Tudorache et al. (2014) reported values of  $40.58 \pm 6.45$  and  $36.96 \pm 2.45 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  for farmed female silver eels ( $65.7 \pm 4.2 \text{ cm}$ ), recorded at a temperature of 18°C and swimming speed of  $0.68 \pm 0.03$  and  $0.7 \pm 0.08 \text{ m s}^{-1}$ , respectively (about 1.03 and 1.06  $\text{BL s}^{-1}$ , calculated based on average body length). Even higher values of 85–89  $\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  have been reported for eels swimming at  $0.5 \text{ m s}^{-1}$  (corresponding to approximately  $0.68 \text{ BL s}^{-1}$ ) and approximately 18°C (Palstra et al., 2008). These values are all considerably higher than the mean of  $27.6 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$  determined in the present experiments at atmospheric pressure, a temperature of 19°C and a swimming speed of  $0.6 \text{ BL s}^{-1}$ , which may be regarded as a low estimate (see above). However, direct comparisons are difficult because of the different experimental conditions (e.g. swimming speeds, duration of the experiments, size or maturation stage of eels and subsequent application of different treatments); yet, it is noteworthy that some of the lowest values previously reported were derived from long-term swimming trials (van Ginneken et al., 2005), suggesting that longer acclimation periods may contribute to a better settling of the animals, physiological acclimation to increased exercise and, potentially, a reduction in stress level, thus resulting in reduced oxygen consumption.

In conclusion, the results of the present study provide clear evidence that lower temperatures and increasing pressure at higher temperatures result in lower energy requirements for long-term swimming in European eels. Several studies calculated the energy requirements for the spawning migration of eels based on experiments performed at temperatures of 18°C and 19°C (van Ginneken et al., 2005; Palstra et al., 2008; Palstra and van den Thillart, 2010; Clevestam et al., 2011). Temperatures experienced *in situ* are much lower (typically 11–13°C) and pressures are much higher (>10,000 kPa). Oxygen consumption rates at 12°C are approximately 54% of the values derived at 19°C and while pressure had no notable effect at lower temperatures, at 19°C, oxygen consumption at 800 kPa was approximately 89% of the values derived at atmospheric pressure. Accordingly, current estimates of travel cost to the Sargasso Sea may be overestimated by as much as a factor of two, because of the effects of either temperature or pressure, or both. One has to consider, though, that eels experience extreme changes in pressure and (to differing degrees) temperature twice daily, which may have additional or adverse effects on the energetic requirements (e.g. Sébert et al., 1997; Palstra, 2006), rendering a sound estimation of COT difficult.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

J.-D.P.: Data processing, statistical analyses and drafting of the manuscript; B.P.: Planning of the project and drafting manuscript; K.W., L.M., M.F. and C.L. executed or significantly contributed towards the execution of the experiment and provided scientific input during drafting of the manuscript; R.H.: Planned and supervised the project.

#### Author contributions metadata

Conceptualization: J.-D.P., B.P., K.W., L.M., R.H.; Methodology: J.-D.P., K.W., L.M., C.L.; Validation: J.-D.P.; Formal analysis: J.-D.P.; Investigation: L.M., M.F., C.L.; Resources: R.H.; Data curation: J.-D.P., C.L.; Writing - original draft: J.-D.P., B.P., M.F.; Writing - review & editing: B.P., K.W., L.M., M.F., R.H.; Visualization: J.-D.P.; Supervision: B.P., K.W., L.M., R.H.; Project administration: B.P., K.W., R.H.; Funding acquisition: B.P., R.H.

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#### Data availability

Processed data (i.e. after running linear regressions for each measurement and removing measurements that did not fit the quality criteria) relevant to the statistical analyses are available from GitHub as RData, including R scripts used for processing data, statistical analyses and creation of output ([https://github.com/jdpo/pub\\_2022\\_travel\\_cost\\_sargasso](https://github.com/jdpo/pub_2022_travel_cost_sargasso)). Raw data are measurements over 6 weeks from 9 trials and are impractical to share because of the number and size of files, but will be made available by the authors upon request.

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