

Signs of life: Oxygen sensors confirm viability, measure oxygen consumption and provide rapid, effective contamination monitoring for field-based tissue culture

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Abstract

- Understanding the ecology and evolution of wildlife and domesticated species requires knowledge of their physiological responses to environmental change and the constraints under which they operate. However, whole animal experiments are often limited in sample size and can be logistically and ethically challenging. Culture techniques represent a powerful approach, but are used infrequently in field research due to practical constraints. We used minimal tissue culture equipment in a remote field site for in vitro explant experiments using blubber from wild grey seals *Halichoerus grypus*. Assessing explant viability and detecting microbial contamination in remote field sites, where facilities are often small, unspecialised and more vulnerable to bacterial or fungal infection, present major challenges.
- We investigated whether oxygen-sensitive planar optodes (OSPO) in closed system respirometry could be used to assess oxygen consumption by blubber explants from suckling and fasting wild seal pups as a proxy for viability. We also explored whether OSPOs could provide rapid information on whole animal relevant physiological metrics by determining whether explant oxygen consumption correlated with the nutritional state of the animal, blubber depth and other tissue metabolic properties, including glucose uptake, lactate production and lipolysis.
- Vials containing blubber explants consumed significant amounts of oxygen compared to controls, showing tissues were metabolically active. Oxygen consumption differed between nutritional states and blubber tissue depth. These differences were reflected in other tissue metabolic properties. Dissolved oxygen levels remained consistent over 24 hr in 94% of control vials containing only culture media. In 6% of control vials extremely rapid oxygen consumption preceded, by 2–3 days, colour changes in the phenol-red containing media that indicate lactic acidosis from microbial metabolic activity.
- Oxygen use in control vials was, therefore, an effective monitoring system that provided vital early warning of media contamination, allowing stocks to be

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discarded, which prevented erroneous results and avoided waste of valuable field time and irreplaceable samples. OSPO are thus a useful tool for simultaneously assessing tissue oxygen consumption, investigating functional physiological differences and monitoring microbiological contamination in culture experiments, particularly in field laboratories studying live tissues from wildlife.

KEY WORDS

bacterial contamination, blubber depth, fasting, feeding, field laboratory, metabolic function, oxygen sensor, respirometry, tissue culture, viability

1 | INTRODUCTION

Wildlife species face multiple challenges: Their survival is impacted by unprecedented rates of environmental change, reduced resource availability and increasing conflict with humans (Acevedo-Whitehouse & Duffus, 2009). Similarly, domesticated species face physiological challenges from human-altered natural environments and rearing, housing and transport conditions (Chaloupkova et al., 2007; Collins et al., 2018), affecting welfare, food quality and productivity (Herbut et al., 2019; Moore & Ghahramani, 2013). Knowledge of physiological constraints and understanding animals' responses to natural and anthropogenic stressors are fundamental to predicting and mitigating stock, population and species consequences of environmental change. However, obtaining sufficient subjects, performing multiple exposures, time courses and dose responses and using known harmful treatments prohibit whole animal level investigations of key questions relevant to welfare, conservation and ecology.

Tissue and cell culture have become pivotal techniques in animal physiology (Bryant et al., 2000; Freshney, 2015), allowing complex experimental design to be undertaken using paired cells or tissue, thus increasing the scope of biological questions that can be addressed and the statistical power to answer them (Bennett et al., 2017; Bryant et al., 2000; Robinson et al., 2018). Culture techniques can be used to measure and manipulate fundamental biological processes such as growth, development, metabolic dynamics, endocrine effects and disease progression (Willmer, 2015), and are vital for exploring the impacts of potentially dangerous chemicals (Bryant et al., 2000) or drug effectiveness (Imamura et al., 2015).

Cell and tissue culture also have important applications in wildlife biology and conservation (Boroda, 2017), including investigation of disease (Smith et al., 1998); cryopreserving germ cells (de Queiroz Neta et al., 2018; Mastromonaco et al., 2014; Tovar et al., 2008; Veraguas et al., 2017); and understanding physiological responses in both wild (Bennett et al., 2017, 2021; Robinson et al., 2018) and domestic (Etherton & Ecock, 1986; May et al., 1994) animals. However, such work in remote settings is challenging because locating and retrieving samples from individuals can be invasive, stressful and logistically challenging. Transport time to dedicated tissue culture facilities may be prohibitively lengthy (Tovar et al., 2008) and cell

isolation may be too involved or complex to be practicable with limited facilities or personnel. Where cell lines can be established, experiments on cells derived from such animals can be moved back into dedicated cell culture laboratories, and used indefinitely (Wang et al., 2011). However, some cells, such as adipose, are very fragile or yield too few cells during preparation to perform robust experiments. In addition, progenitor cells may not divide and differentiate using standard conditions (Louis et al., 2015). In these cases, where a simple laboratory can be set up, straightforward tissue explant culture experiments may be more suitable (Bennett et al., 2017, 2021; Robinson et al., 2018).

Ensuring tissue viability and maintaining sterile conditions are essential for tissue culture (Lincoln & Gabridge, 1998). Standard techniques to determine viability or cytotoxicity in cell culture include trypan blue exclusion, neutral red uptake, fluorescent staining, triphenyl tetrazolium chloride reduction or luminogenic ATP assays (Riss, Moravec, et al., 2016; Riss, Niles, et al., 2016). However, these methods require the assays to be done in real time and/or tissue pieces are often too thick to allow cells to be counted individually. The additional time and equipment to undertake these assays may not be available in remote laboratories. Methods requiring explant digestion also prevent downstream applications, and the tissue disruption may itself destroy the cells, thus underestimating cell number counts. Alternatively, lactate dehydrogenase (LDH) or other intracellular enzymes in the media can be assayed (Niles et al., 2007; Riss, Niles, et al., 2016). However, elevation of such enzymes in media is inevitable because some tissue damage occurs during explant preparation making it challenging to assess the true number of viable cells. Although differences in signal could be used to assess relative time-related or treatment-induced changes, background variability would be high. In addition, LDH loses its activity after freezing (Starnes, 2008), even at -80°C, producing spurious data if the assay cannot be performed on fresh media, which is often the case in remote field labs where plate readers, especially with fluorescence or luminescence capability, are not typically available. Real time, on-site testing is vital to confirm that culture protocols are working as expected to ensure tissue obtained in the wild remains viable.

Lack of sterile conditions is often a limiting factor for tissue culture outside of dedicated facilities (Tovar et al., 2008). The separation of suitable culture laboratories from the, often remote, locations

where tissues are obtained represents a serious obstacle to conducting culture research in non-laboratory species (Tovar et al., 2008). Contamination risk is generally higher when samples are collected in natural environments, where there is less protection from bacterial and fungal spores (Marsili et al., 2000; Mastromonaco et al., 2014; Tovar et al., 2008), but fewer facilities to exclude or monitor microbial growth. Adopting new methods that can rapidly identify contamination will allow researchers to use tissue culture approaches outside of traditional model species or at distance from dedicated laboratories.

Oxygen-sensitive planar optodes (OSPO) are an established, non-invasive method for measuring dissolved oxygen. They have been used in food packaging to signal spoilage (Banerjee et al., 2016) and more recently to explore energetics of marine invertebrates in closed system respirometry (e.g. Calosi et al., 2013; Karythis et al., 2020). They have been used in cell culture (Ge et al., 2006) to measure viability over up to 168 hr (Deshpande et al., 2004; Naciri et al., 2008). OSPO have photoluminescent coatings that are quenched by oxygen, resulting in linear changes in luminescence intensity after excitation (Papkovsky & Dmitriev, 2013). OSPO luminescence does not consume oxygen and can be monitored externally through transparent vessel walls (Banerjee et al., 2016). OSPO are therefore a flexible tool that can be used in a variety of contexts to assess viability, physiological responses and contamination simultaneously and therefore possess enormous potential for deployment in field laboratories used for tissue culture.

We investigated whether OSPO could be used in a minimal tissue culture laboratory in a remote field station to assess the viability of blubber tissue explants from wild, suckling and fasting grey seal *Halichoerus grypus* pups; whether nutritional state, tissue depth and other indicators of metabolic function, such as glucose uptake, correlated with oxygen use in these experiments; whether the sensors could indicate if microbial contamination had occurred in the media and if using OSPO provides earlier indication of contamination than the changes in media colour commonly used to identify this problem.

2 | MATERIALS AND METHODS

2.1 | Study site and ethics approvals

Fieldwork to sample wild grey seal pups was conducted on the Isle of May, Scotland (56°11'N, 02°33'W) under permit from Scottish Natural Heritage (SNH) in 2017. Sample collection was performed by personal licence holders under the Sea Mammal Research Unit UK Home Office Project Licence (no. 70/7806) in compliance with Animal (Scientific Procedures) Act (ASPA) 1986 and received ethical approval from Abertay University (EMS2159).

2.2 | Study animals

Grey seal females, individually identifiable by a previously deployed brand or flipper tag, were observed from when they came ashore

to pup. Birth and weaning dates of their pups were recorded. Pups were included in this study if they were at an appropriate predicted mass for their age (at day 15 post-partum or day 12 after weaning) and if they did not have any obvious indications of ill health. Pup mass change trajectories during suckling (Anderson & Fedak, 1987; Fedak & Anderson, 1982) and the natural 1–6 week postweaning fast (Bennett et al., 2010; Hall et al., 2001) have been well studied in grey seals, and signs of infection or injury are easily detectable (Baker et al., 1980; Baker & Baker, 1988) enabling us to identify healthy individuals for the study. Blubber biopsies for explant culture were collected from nine pups at day 15 post-partum (feeding samples) and from 13 pups at day 12 after weaning (fasting samples). Three pups were sampled at both time points. All postweaning animals had weaned at a normal time and weight from their mothers.

2.3 | OSPOs in closed-system respirometry

Sterilisation was performed using 70% ethanol or ultraviolet (UV) light in a transportable PCR hood (Triple Red). UV sterilised 2-ml screw cap glass vials with flat bases and caps (Thermo Scientific, C4010-95W) were used as closed respirometry chambers. A small amount of medium viscosity silicone grease (Bayer, ACROS Organics™) was applied to the bottom inside each vial using sterile tweezers, and the open vials were UV sterilised again. One OSPO per vial (Presens, SP-PSt3-NAU) was washed in 70% ethanol and carefully adhered to the grease using sterile tweezers with the readable (red) side facing the glass (as per the manufacturer's instructions) until the spot sensor was flush with the vial surface, with no air bubbles or grease obscuring the sensor. Finally, 1.5 ml of complete cell culture media (medium 199, Hanks' balanced salts with 1% antibiotic antimycotic solution, 1% fatty acid supplement and 5% charcoal-stripped foetal bovine serum) was added to each vial, and they were sealed with a UV-sterilised screw cap (Figure 1). M199 media are HEPES buffered, which avoids the need for continual gassing to maintain pH. The pH was therefore expected to remain stable for the duration of the experiments and major deviations could be observed by colour changes in the phenol red indicator.

Once ready for use (Figure 1), vials were transferred to a sterile, humidified incubator maintained at 37°C and 5% CO₂ (Thermo Scientific, Midi 40 CO₂ Incubator, model: 3404). All vials used here were made up at least 12 hr prior to the addition of tissue to allow oxygen to equilibrate between the media and the remaining head space in the vials as they warmed.

2.4 | Blubber sampling and explant protocol

Blubber sampling and generating explants were performed as described previously (Robinson et al., 2018). Briefly, pups were given a mass-specific intravenous dose of Zoletil^{100™} and subcutaneous injections of Lignol™ in the dorsal flank. Two full depth 10-mm biopsies were taken and immediately cut into 'inner' (closest to muscle)

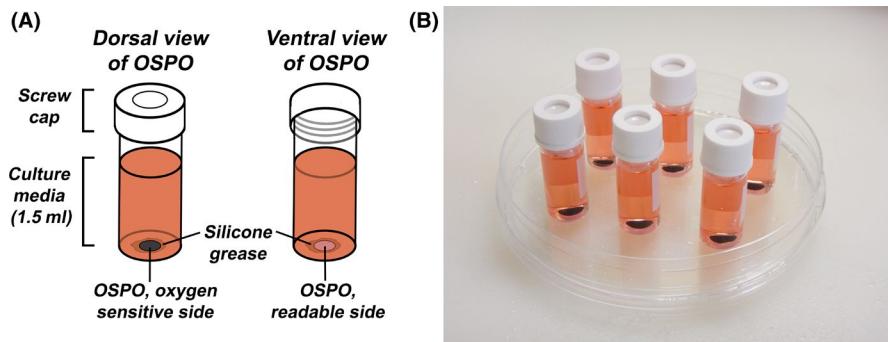


FIGURE 1 OSPO deployment in screw cap glass vials for closed system respirometry experiments using blubber explants. (A) Diagram and (B) photograph of six completed vials

and 'outer' (closest to skin) sections using sterile surgical scissors, and placed into separate 15-ml centrifuge tubes containing warm (37°C), sterile Krebs Ringer solution for transport to the field laboratory. Incisions were closed with a single suture. Pups were given an intravenous dose of Carprieve® to reduce pain and inflammation and monitored to ensure biopsy sites healed without complications.

On return to the field laboratory, tissue was washed with 1 ml warm sterile Krebs Ringer solution, minced into 5–10 mg pieces and weighed into 100 mg portions, termed explants (Robinson et al., 2018). OSPO-containing vials were removed from the incubator, opened briefly in the PCR hood to add a 100 mg explant, closed and returned to the incubator as quickly as possible to create 'filled' vials ($n = 70$). Explants floated near the surface of the media and the individual segments tended to clump, which is not possible to avoid given the properties of the tissue. Explants were not added to 33 OSPO-containing vials ($n = 33$), and these were monitored in the same way as filled vials to provide controls.

Filled vials contained inner ($n = 38$) or outer ($n = 32$) tissue, and were collected when the pup was either suckling, termed 'feeding' ($n = 32$) or fasting ($n = 38$) to give the following tissue type and nutritional state combinations: inner/feeding ($n = 14$), outer/feeding ($n = 18$), inner/fasting ($n = 24$) and outer/fasting ($n = 14$). The amount of tissue varied between animals and biopsies, and each biopsy generated tissue for an additional study (see Bennett et al., 2021). Thus, a sampling event generated one to five explants per individual for the current study.

2.5 | Oxygen measurement

Media oxygen concentration was measured using a polymer optical fibre (POF) connected to a Fibox 4 trace oxygen meter (Presens) following the manufacturer's instructions. Temperature correction was performed using input from a separate temperature sensor. To account for changes in oxygen concentrations from rapidly falling media temperature when removed from the incubator, all oxygen measurements were corrected to 35°C prior to statistical analysis using calibration data generated for each OSPO as follows. Vials containing 1.5 ml complete media and the OSPOs were made without explants, alongside vials containing 1.5 ml complete media without OSPO. Vials were warmed to 37°C in the incubator. Oxygen

concentration in OSPO vials were read immediately on removal from the incubator, concurrent with the temperature of its matched non-OSPO vial using a temperature sensor (Pt100, Presens) connected to the Fibox oxygen meter. Readings were taken every few seconds as the paired vials cooled to 24°C . A linear regression was fitted to the data for each OSPO using R 3.4.1 (R Development Core Team, 2012), to calculate a correction factor for each OSPO. Subsequent oxygen measurements in experiments were taken in tandem with a temperature reading from a non-OSPO vial to allow the temperature correction to be applied for each reading.

Oxygen concentrations and temperature were read 1, 5, 9 and 24 hr after explant addition for both control and filled vials. Vials were read in batches of six to minimise the time vials were outside of the incubator and the number of times the incubator door was opened, to avoid compromising incubator sterility and temperature stability. On eight occasions, the same control vial was used to generate data for multiple explant vials within a 48-hr period. Variation in oxygen concentrations at 0 and 24 hr reads between control vials read multiple times lay within the same range as those read once. Changes in oxygen concentrations (mg/L) between measurement intervals and over the entire 24-hr period were calculated to give oxygen use per hour ($\text{mg L}^{-1} \text{ hr}^{-1} 100 \text{ mg}^{-1}$).

2.6 | Metabolite measurement

After 24 hr, media were aspirated and aliquoted, and explants were flash frozen in liquid nitrogen. Media and explants were stored at -80°C . Media glucose and lactate concentrations were measured using Randox (County Antrim, UK) kits (glucose: GL364 and lactate: LC2389) and standards. Glucose was measured in a 96-well plate format using 2.5 μl media or standard and 250 μl reagent and measured in a BioTek (Swindon, UK) ELx800 plate reader. Media lactate was measured in an RX Monza (Randox) Clinical Chemistry analyser (Model: 328-14-0914) and media glycerol was measured using Sigma-Aldrich kit MAK117 according to manufacturers' instructions (Bennett et al., 2021; Robinson et al., 2018). Internal quality control measurements lay within $<\pm 15\%$. Intra- and inter-assay variability for sample analysis was $<15\%$ and $<10\%$ respectively. Rates of glucose removal and accumulation of lactate and glycerol were calculated per $100 \text{ mg tissue}^{-1} \text{ hr}^{-1}$.

2.7 | Statistical analysis

Analyses were performed using R 3.4.1 (R Development Core Team, 2012). A Mann–Whitney *U* test was used to compare oxygen use between filled and control vials because the data did not have a normal distribution, groups did not have equal variance and this could not be rectified using transformation.

To investigate differences in oxygen use rates across the 24 hr of the explant incubations, linear models were used. Initially, individual identities of pups were included as random effects because a number of explants came from the same individuals. However, individual did not explain a significant portion of the variance in our data and did not improve model fit, and was therefore removed. An Anderson Darling test showed that oxygen consumption rate did not have a normal distribution and was therefore logged prior to analysis. Time period between oxygen measurements (1–4, 5–9 and 10–24 hr), the type of vial (filled vials, control vials and potentially infected vials) and an interaction term between time period and vial type were included as explanatory variables.

To explore depth and nutritional state differences in oxygen use in filled vials, generalised additive mixed models (GAMMs, Wood, 2006a) were used. Tissue depth (inner or outer blubber), nutritional state (feeding or fasting) and an interaction term between nutritional state and tissue depth were included as explanatory variables. The model was fitted using the multiple generalised cross validation library mgcv (Wood, 2012). The identities of individual pups were fitted as random effect smooths (Wood, 2006b) to account for the same individual contributing multiple explants and any variation between replicates. Smoothing parameters were set by maximum likelihood to reduce overfitting (Wood, 2011). Models were fitted with a Gaussian error distribution. Model selection was performed by backwards stepwise elimination through examination of R^2 values, Akaike's information criterion (AIC) values, QQ and residual plots.

Finally, Pearson correlation coefficients were calculated to investigate associations between uptake and production of the three measured metabolic substrates (glucose, lactate and glycerol) and oxygen use.

3 | RESULTS

3.1 | Oxygen use in filled versus control vials

Filled vials used significantly more oxygen than control vials over 24 hr (Figure 2) ($\text{Mann–Whitney } U = 1,922, p < 0.001, df = 1$). Some vials (filled $n = 8$, control $n = 2$) were excluded from analysis (see Section 3.2).

3.2 | Oxygen concentration over 24 hr

Filled vials had lower oxygen concentration than control vials after explant addition across all time points measured (Figure 3). This

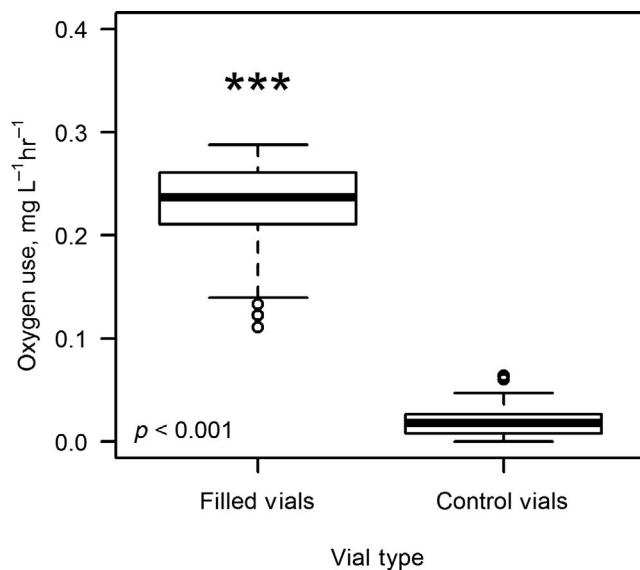


FIGURE 2 Oxygen use in filled vials containing a 100-mg blubber explant ($n = 62$) and control vials ($n = 31$). Median (central bar), upper and lower quartiles (box), 1.5x interquartile range (whiskers) and outliers (points). Significant differences between groups are denoted by asterisks, *** for $p < 0.001$

confirms that the blubber explants were viable and capable of oxidative metabolism throughout the entire experiment.

Some vials, both filled ($n = 8$) and control ($n = 2$), showed a dramatically different oxygen use profile to the other filled ($n = 62$) and control ($n = 31$) vials and were removed from the main analysis since they were presumed to be infected by microbes. Oxygen depletion occurred within the first 9 hours of the experiments in potentially infected vials, even in the absence of explants (Table S1). The culture media in the other control vials showed no rapid or large reduction in oxygen levels. Declines in other filled vials were gradual over the entire 24-hr period post explant addition (Figure 3; Table S1).

Oxygen consumption rates over the time periods between measurements (1–4, 5–9 and 10–24 hr since explant addition) differed by type of vial (filled vials, control vials and potentially infected vials; linear model: $R^2 = 0.5, p < 0.001$; Figure 4). Within the first 1–4 hr after the vials were sealed, control vials had the lowest oxygen consumption rates ($p < 0.001$). Potentially infected vials had higher oxygen consumption rates than both filled and control vials ($p < 0.001$). In control vials, oxygen consumption rate was very low and consistent, and did not change significantly throughout the 24 hr. There was substantial variability in oxygen consumption rate between explants. However, explant oxygen consumption rate was significantly higher than controls throughout the 24-hr period and showed a small but significant increase at 5–9 hr compared to 1–4 and 10–24 hr ($p < 0.001$). The rate at 10–24 hr was not different from that at the start ($p = 0.06$). Potentially infected vials showed highest levels of oxygen consumption 1–4 hr after the vials were sealed, with average oxygen consumption 65% higher than in other filled vials. There was then a reduction in oxygen consumption rate, such that values 5–9 hr after vial closure were similar in potentially infected

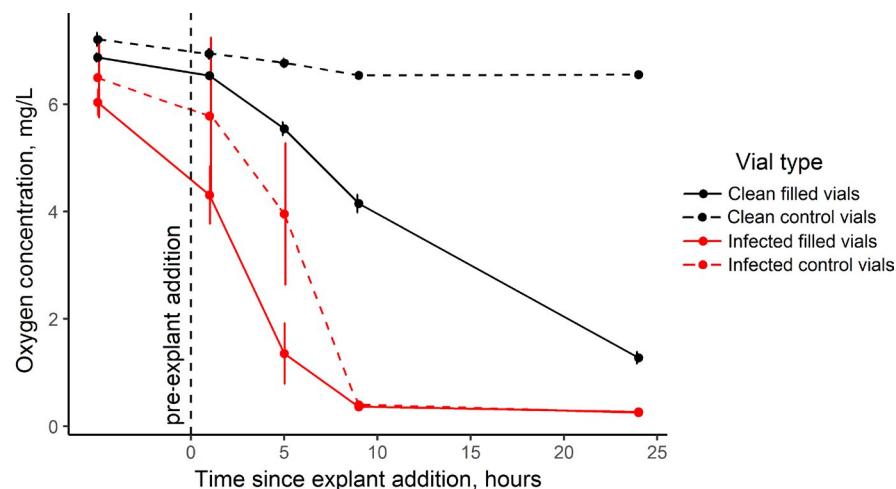


FIGURE 3 Differences in oxygen use in control ($n = 2$) and filled ($n = 8$) vials with contaminated cell culture media (red) compared to control ($n = 31$) and filled ($n = 62$) vials without contamination (black). Dots represent the mean media oxygen concentration (mg/L) in different vial types and bars represent standard errors (note that some SE bars are too small to be visible on the plot from 9 hr onwards). Pre-explant addition readings were taken at 17 hr after vial creation and 10 hr before explant addition (mean values)

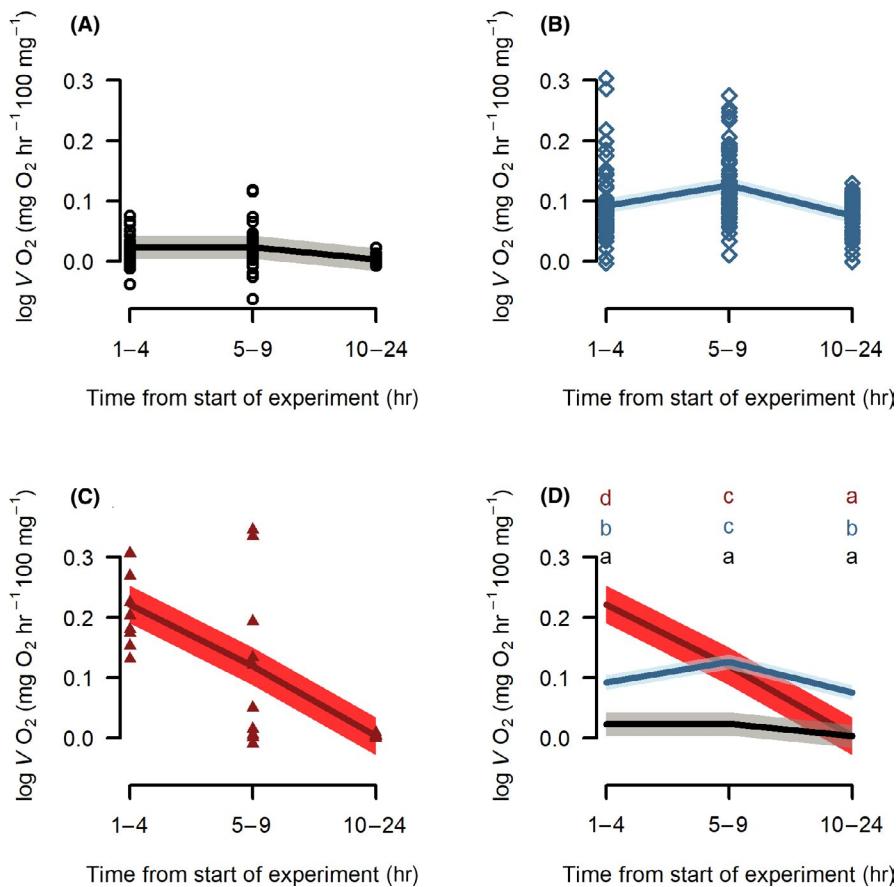


FIGURE 4 Log rates of oxygen consumption in (A) control vials ($n = 31$, black open circles); (B) vials containing explants without contamination ($n = 62$, blue open diamonds) and (C) contaminated vials ($n = 10$, red filled triangles). Lines and shading represent predicted mean response and 95% confidence intervals from the model. (D) Model fits and confidence intervals overlaid for comparison. Vial types and time points with different letters (above figure) are significantly different from each other ($p < 0.05$; LME)

vials and other explant-containing vials. By the final measurement, oxygen consumption in potentially infected vials was indistinguishable from controls, consistent with the observed oxygen depletion (Figures 3 and 4).

At the time of use, there was no observable colour difference in the media. However, in the 2–3 days after these outliers were recorded, the batches of culture media used in potentially infected vials changed from red to yellow, indicating a pH drop typical of microbial contamination. The rapid fall in oxygen levels in the vials early in the experiments thus provided a vital early warning of contamination. In the worst-case scenario, approximately 300 irreplaceable explant

experiments would have been rendered unusable if we had relied on phenol red media colour change alone as an indicator of suspected contamination.

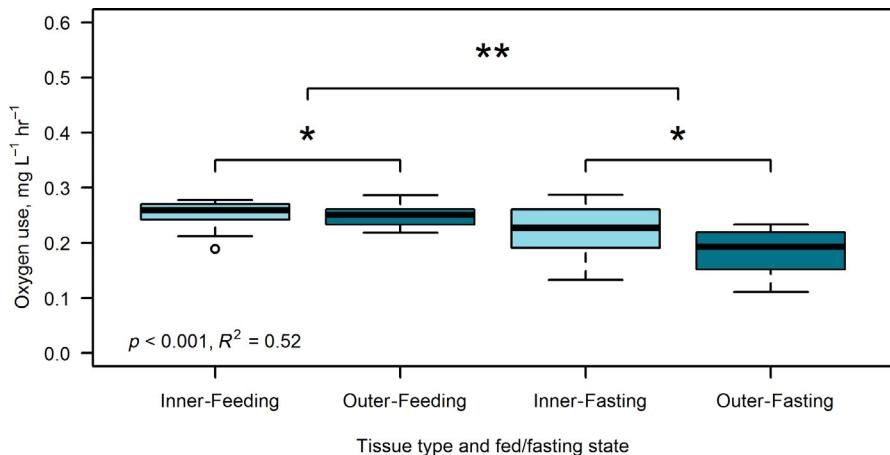
3.3 | Oxygen use and tissue characteristics

Nutritional state (feeding/fasting), blubber depth (inner/outer), an interaction term between these two variables and the individual identity smooth were retained in the final model investigating 24 hr average oxygen consumption in uncontaminated, filled vials. Blubber

TABLE 1 GAMM output for variables affecting oxygen use in blubber explants, their estimates, standard errors and *p* values. Explanatory variables with significant *p* values are highlighted in bold

Dependent variable	Explanatory variable	Estimate	SE	<i>p</i> value
Oxygen use ($\text{mg L}^{-1} \text{ hr}^{-1}$ 100 mg tissue $^{-1}$)	Nutritional state (feeding/fasting)	0.038	0.013	0.006
	Blubber region (inner/outer)	-0.031	0.013	0.02
	Interaction term between nutritional state and tissue type	0.031	0.017	0.08
	Smooth term for individual identity	N/A	N/A	0.003

FIGURE 5 Oxygen consumption in vials containing a 100-mg blubber explant of either inner or outer blubber tissue from feeding or fasting individuals ($n = 62$; inner/feeding vials ($n = 14$), outer/feeding vials ($n = 16$), inner/fasting vials ($n = 21$) and outer/fasting vials ($n = 11$)) with median (bar), upper and lower quartiles (box), 1.5× interquartile range (whiskers) and outliers (points) shown. Significant differences between groups are denoted by asterisks, ** for $p < 0.01$ and * for $p < 0.05$



from feeding individuals had ~25% higher oxygen consumption than tissue from fasting individuals ($p = 0.006$), and inner blubber had ~5% higher oxygen use than outer blubber ($p = 0.02$), (GAMM: $R^2 = 0.52$, $p < 0.001$, Table 1; Figure 5). The interaction term was not significant, showing that the magnitude of the difference between inner and outer was not different between fed and fasted states.

3.4 | Oxygen use and metabolic substrates

There was a significant positive correlation between oxygen use and glucose uptake ($r = 0.46$, $p < 0.001$), net lactate generation ($r = 0.48$, $p < 0.001$) and glycerol generation ($r = 0.39$, $p = 0.002$; Figure 6).

4 | DISCUSSION

4.1 | Explant viability

Blubber explants used oxygen at approximately $0.2 \text{ mg L}^{-1} \text{ hr}^{-1}$ or more across the entire 24-hr period, whereas oxygen use in control vials did not exceed rates of $0.1 \text{ mg L}^{-1} \text{ hr}^{-1}$. Highest rates of oxygen use by explants occurred after 5–9 hr, similar to time-related increases in oxygen consumption by rat adipose explants (Mirski, 1942). Our data thus demonstrate OSPOs can measure oxygen consumption by live, viable adipose tissue, despite its typically low metabolic activity (Wang et al., 2010). Although we were unable to measure viability using commonly used methods, for reasons outlined in the introduction, this active oxidative metabolism by

explants demonstrates tissue viability can be ascertained in real time in the field laboratory. The Fibox 4 device used here is ruggedised, has a long battery life and highly portable, making it possible to use in remote field laboratories, where other viability assays may not be possible.

The oxygen consumption rates here were similar to values from human abdominal and visceral WAT explants (Table S2; Kraunsøe et al., 2010), and, like human adipose, were lower than those measured in rodents and dog (Hallgren et al., 1990; Mirski, 1942), which may result from species differences or allometry. It is possible that our measurements underestimate the true oxygen consumption rate due to the distance between the OPSO and the explant and the lack of shaking, which may have created boundary layers in the media. Although shaking is not typically performed in short-term adipose explant experiments (Du et al., 2011; Grivel & Margolis, 2009; Miller et al., 1991), further optimisation could involve modification to vessel dimensions, positioning of the optode and shaking to minimise oxygen depletion close to the explant.

4.2 | Oxygen use, metabolites and tissue properties

Oxygen consumption differed between explants from feeding and fasting pups and between ‘inner’ and ‘outer’ blubber, which is consistent with biochemical information from glucose uptake, lactate production/use and glycerol production (Robinson et al., 2018) and with previous energetic studies at whole animal and tissue levels in seals and rats (Mirski, 1942; Nordøy et al., 1990; Reilly, 1991; Robinson et al., 2018). Our data provide further empirical support

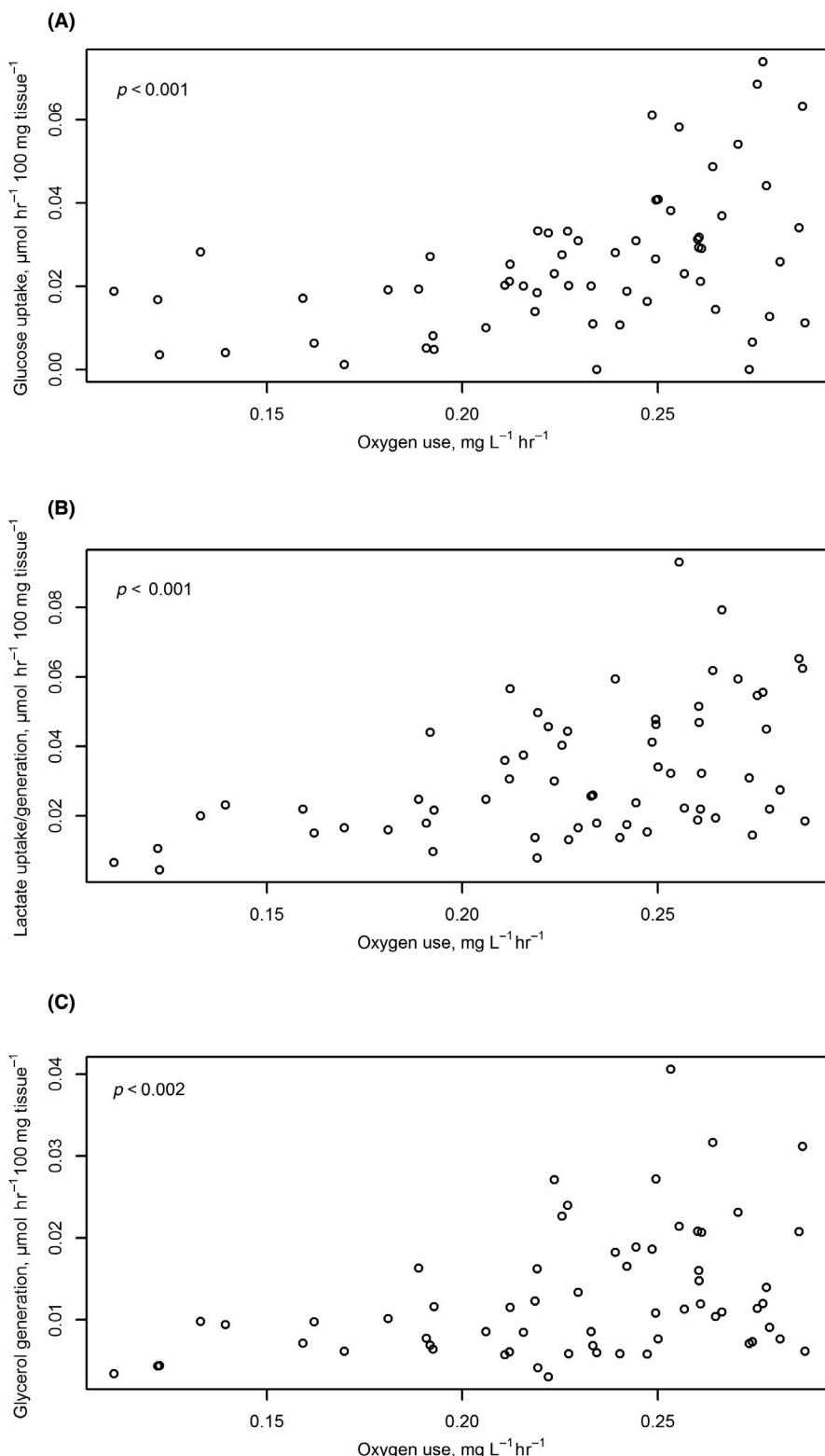


FIGURE 6 Relationships between oxygen use in blubber explants and glucose uptake (A), lactate uptake/generation (B) and glycerol generation (C) showing Pearson's correlation p value

for higher metabolic rate in blubber tissue closer to the core in marine mammals. Although we were unable here to identify the specific energy sources used by the explants and determine the fate of the carbon, all three measurements of metabolic substrate dynamics, and the nutritional and tissue type variation are consistent with the oxygen consumption measured by the OSPOs. This is a strong indication that tissue-level oxygen consumption

measurements may be useful as proxies for tissue metabolic activity in tissues and may be indicative of whole animal metabolic state and responses. Tissue oxygen consumption could thus be used to assess important physiological differences between animals in different states.

High glucose uptake and lactate generation could indicate that explants were hypoxic and glycolytic (Mulukutla et al., 2010).

However, the positive relationship between lactate production and oxygen consumption shown here suggests explants with high lactate production were not hypoxic, and instead both metrics simply indicate higher metabolic activity. Additionally, hypoxia inducible factor 1 (HIF1 α) protein content was not different between paired snap frozen and 24 hr incubated explants, suggesting they did not experience hypoxia (Supplementary Information file 3, Figure S1). Oxygen limitation occurs in mouse adipose explants when media oxygen concentration reaches 60–80 μM (Wan et al., 2012). In our study, measurements were well above these values for the first 9 hr of the study, suggesting oxygen was not limiting aerobic respiration for at least that duration. After 24 hr, oxygen content was, on average, $79.7 \pm 7.6 \mu\text{M}$ and $<80 \mu\text{M}$ in 34 of the 62 uninfected filled vials. Oxygen limitation may thus have been responsible for the slowing of oxygen consumption in the later portion of the measurement period for some of the explants. Viability assessment and physiological measurements using this closed system respirometry method should thus be performed within 10 hr of explant creation, or, if longer incubation times are needed, media must be refreshed. The centre of the explant could have become necrotic, even though we have no evidence for hypoxia. This could be assessed to provide additional information on tissue health by subsequent measurement of necrosis markers in the tissue or media.

4.3 | Rapid oxygen depletion can be used for early identification of contaminated media

Field researchers need to detect contamination as early as possible to avoid wasting valuable samples, time and resources, which may be irreplaceable (Ryan, 1994). The development of turbidity or colour changes in phenol red indicator when media pH drops are typically used as contamination indicators (Langdon, 2004). However, phenol red indicators, particularly in HEPES-buffered media, such as that used here, are slow to respond to lactate production by microbial growth and may not produce an observable colour change at all if media are refrigerated or during fungal infection (Chu et al., 2000). Exponential growth of microbe populations can rapidly deplete local oxygen levels, which can be identified promptly and in real time with the appropriate sensors (Papkovsky & Dmitriev, 2013). The rapid oxygen depletion detected by the OSPOs in our explant experiments indicated contamination 2–3 days before the infection was established enough to cause acidosis-driven colour change in the cell culture media. Although we were unable to confirm that our samples were contaminated by microbial infection because we did not have a microscope or microbiological culture facilities, there are no other likely explanations for the rapid oxygen consumption observed. Our data show that vials in which oxygen concentrations have reached zero within 9 hr of blubber explant addition are likely to be contaminated, while uncontaminated vials never reach zero within 24 hr. Therefore, alongside assessing metabolic function and confirming explant viability, these oxygen sensors could also be used

in quality control to detect contamination in tissue culture in challenging locations.

5 | CONCLUSIONS

Understanding factors that alter or impair cellular processes in non-model species will only become more important as anthropogenic environmental impacts increase. The need for methods to assess physiological responses to ecological changes without the need for whole animal experiments in wildlife is paramount. Such methods may also facilitate work on domesticated species' responses to physiological challenge in remote locations. OSPOs can provide a valuable, real-time method for assessing these responses while enabling quality control of tissue experiments at field sites without dedicated tissue culture laboratories. Their use could expand the tools available for such research, furthering our knowledge of physiological mechanisms that underpin adaptation, ecology and evolution.

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CONFLICT OF INTEREST

The authors declare they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

K.A.B. and A.J.H. conceived the ideas for the study; K.A.B., K.J.R., H.C.A., S.E.W.M. and A.J.H. designed the methodology; K.J.R., H.C.A., L.O. and S.E.W.M. collected the data; K.J.R. and K.A.B. analysed the data; K.J.R. and K.A.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/2041-210X.13710>.

DATA AVAILABILITY STATEMENT

The data used in this study are available in Dryad Digital Repository 10.5061/dryad.wh70rxwnz (Robinson et al., 2021). Data will also be deposited into the British Oceanographic Data Centre (BODC), a Natural Environment Research Council (NERC) data centre.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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