

# The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments

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## Abstract

Animals living in the intertidal zone experience regular, predictable fluctuations in physical parameters including temperature, oxygen and salinity and rely on behavioural, physiological and biochemical mechanisms to cope with environmental variation. In the present study, behavioural strategies induced by aquatic hypoxia (e.g. emergence) were performed at similar oxygen tensions across laboratory, mesocosm and field environments; the number of individuals performing these behaviours at any one time was similar in mesocosms and the field. The use of aquatic surface respiration (ASR) was more plastic than emergence behaviour, occurring at a lower oxygen tension in juveniles than adults and being influenced by the addition of alarm substance. Oxygen uptake was lower in air than in water in adults but, in contrast, oxygen uptake was not influenced by the respiratory medium in juveniles. In the laboratory, 72 h of forced emergence did not affect whole body concentrations of lactate but when ASR and emergence were prevented within mesocosm environments there was a significant elevation of lactate. The present study highlights the benefits of transcending traditional laboratory/field boundaries allowing the responses of laboratory-held animals to environmental fluctuation to be integrated with how these animals perform in their natural environment.

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## 1. Introduction

An animal's ability to tolerate environmental fluctuation requires the integration and coordination of behavioural, physiological and biochemical processes to limit potential detrimental consequences of environmental fluctuations. The intertidal zone is an archetype of a variable environment with its inhabitants experiencing regular, predictable environmental fluctuations in physical parameters such as oxygen, temperature and salinity (Davenport and Woolmington, 1981; Martin and Bridges, 1999). In particular, tidepools may oscillate between

anoxia and super-saturation with oxygen. Hypoxia becomes an issue for tidepool residents when pools become isolated from the tide at night and respiration by organisms within the tidepool lowers oxygen partial pressure with a resulting hypoxic state; an effect counteracted during the day by photosynthesis of algae (Truchot and Duhamel-Jouve, 1980).

Within the intertidal faunal assemblage, intertidal fish have fascinated researchers by their ability to live at the land/water interface. A well-studied group of temperate intertidal fish is the family Cottidae, which show different behavioural and physiological responses to hypoxia along an ecological gradient from intertidal to deep-water (Martin, 1996). Cottid fish inhabiting the upper intertidal, for example the tidepool sculpin, *Oligocottus maculosus* (Girard), experience the most extreme variations in abiotic parameters as the tidepools they inhabit can become completely isolated from the tide for long periods of

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time. Within the laboratory environment, tidepool sculpins will actively emerge from the water during hypoxic conditions (Yoshiyama et al., 1995), and have the ability to respire in air, albeit at a lower efficiency than in water (Yoshiyama and Cech, 1994). Tidepool sculpins are able to release CO<sub>2</sub> into the air, avoiding any respiratory acidosis and are able to survive air exposure in the laboratory for a period of several hours (Martin, 1993). In contrast to air-breathing freshwater fish that have typically evolved air-breathing organs, marine air-breathing species generally lack accessory air-breathing organs and utilise the skin, gills and buccal cavities as respiratory epithelia (Graham, 1997).

In *O. maculosus*, the behavioural and physiological adaptations of emergence and aerial respiration form part of a larger metabolic and ecological trade-off. Behavioural avoidance of hypoxia using aquatic surface respiration (ASR) or emergence behaviour may be beneficial in one dimension, i.e. surviving hypoxia, but it is connected to a considerable cost in another dimension, that of predation. Performance of ASR and emergence results in increased aerial predation risk (Yoshiyama et al., 1995; Shingles et al., 2005; Sloman et al., 2006), and although the duration of hypoxia exposure occurs mainly at night, the timing of emergence may coincide with the foraging of aerial predators at dawn. The ability to withstand aquatic hypoxia exposure may, therefore, be beneficial in delaying emergence behaviour and reducing predation risk but exposure to hypoxia in itself can result in physiological costs (Boutilier and St-Pierre, 2000). Similarly, aerial respiration can alleviate some of the physiological consequences of hypoxia exposure but requires costly physiological and behavioural attributes (Low et al., 1993; Graham, 1997).

Although physiological and behavioural adaptations of intertidal animals have been extensively researched (reviewed by Horn et al., 1999), in general studies have focussed on isolated variables, usually within a laboratory environment. As a consequence, we still have a limited understanding of the integrated approaches animals adopt to survive in fluctuating environments (Yoshiyama et al., 1995) and how our research methodologies might influence the picture we develop of the way that animals survive environmental fluctuations. It is extremely difficult to replicate natural environmental changes in the laboratory and consequently results from laboratory and field experiments may not be directly comparable. However, utilising a combination of field and laboratory approaches we can generate a more accurate explanation of how organisms respond to fluctuating environments.

Here we take an integrative approach to study the survival mechanisms of both juvenile and adult intertidal *O. maculosus* using laboratory, mesocosm and field environments. Examining a suite of behavioural (ASR, emergence), physiological (metabolic rates) and biochemical (by-products of anaerobic metabolism) attributes we tested the hypotheses that (i) the threshold oxygen tensions for behavioural, physiological and biochemical responses to hypoxia are similar when tested in laboratory, mesocosm and natural environments and (ii) these responses are influenced by both age (juvenile versus adult) and predation risk.

## 2. Materials and methods

Tidepool sculpins (*O. maculosus*) were collected from Wizard Islets (48°51.5'N; 125°9.4'W) and Ross Islets (48°52.4'N; 125°9.7'W), near Bamfield, British Columbia, during the day using hand held nets. The sculpins used in this study were from mid-intertidal pools. For laboratory and mesocosm experiments (see below) fish were transported back to the laboratory in buckets containing well-aerated seawater and held in outdoor flow-through tanks at the Bamfield Marine Sciences Centre. These fish were held at ambient ocean conditions (salinity 31‰; temperature: 13.5 ± 0.5 °C) under a natural photoperiod for no more than 24 h before being used in experiments to ensure that natural circadian rhythms were maintained. With the exception of fish sampled for physiology, all fish were returned to the field at the end of the study. The research adhered to the Association for the Study of Animal Behaviour guidelines for the use of animals in research, the Canadian Council for Animal Care and the regulations of the University of British Columbia and the Bamfield Marine Sciences Centre.

Behavioural responses to hypoxia were observed in laboratory, mesocosm and field environments. The effect of hypoxia on metabolism was measured (as oxygen consumption rate) under laboratory conditions in both water and air. Biochemical responses to hypoxia (whole body lactate and glycogen concentrations) were monitored in both laboratory and mesocosm environments. The methods and results are presented here in the order of laboratory, mesocosm and then field experiments.

### 2.1. Laboratory experiments

#### 2.1.1. Behavioural responses to hypoxia

(i) The oxygen tension at which juvenile (0.2 ± 0.01 g, *n* = 6) and adult (1.9 ± 0.31 g, *n* = 6) sculpins performed ASR and emerged from the water was tested. Tidepool sculpins are known to perform ASR in response to hypoxia and may eventually emerge from the water if dissolved oxygen tensions fall too low. Here we used methods similar to Yoshiyama et al. (1995) and Watters and Cech (2003) to monitor this behaviour. Fish were placed individually into 10 L aquaria containing a ramp angled at 30° allowing individuals to emerge from the water. The ramp was covered in small pebbles and the temperature in the tanks was maintained at 13.5 ± 0.5 °C by partial submergence in a water bath. Salinity was constant throughout and the tanks were screened on three sides with black plastic to minimise disturbance. Following a 1 h period (based on preliminary studies) to allow the fish to recover from handling stress, during which time the water was aerated, the oxygen tension in the water was gradually decreased (Fig. 1A) by bubbling nitrogen into the water. The oxygen concentration was monitored using an Oxyguard Handy MKIII electrode and converted to oxygen tension using the appropriate oxygen solubility coefficients. Preliminary studies measuring the oxygen tension in different areas of the tank demonstrated that the mild bubbling of nitrogen was sufficient to ensure thorough mixing of the water.

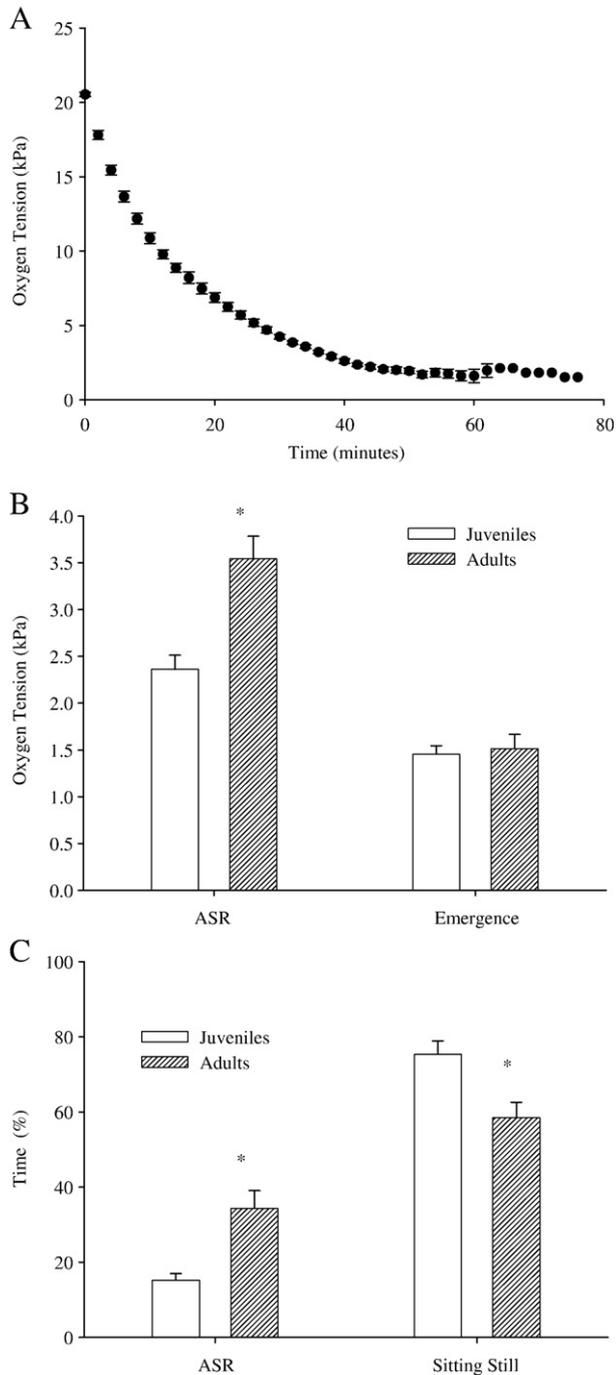


Fig. 1. (A) Decline in oxygen tensions in aquaria ( $n=12$ ) used to calculate the oxygen tension at which juvenile and adult sculpins performed ASR and emerged from the water in the laboratory. (B) Threshold oxygen tensions at which juveniles and adults performed ASR or emerged from the water ( $n=6$ ). (C) Percentage of time spent sitting still or performing ASR by adults and juveniles ( $n=6$ ). Data are presented as means  $\pm$  SEM. Asterisks denote significant differences and percentage data were arc-sin transformed before statistical analysis.

At 2 min intervals, the oxygen concentration in the tank was noted and the behaviour of the fish at the time was recorded; i.e. whether the fish was resting on the bottom or performing aquatic surface respiration (ASR). Once the sculpin had

initiated ASR it was very unusual for the sculpin to leave the water surface. The threshold partial pressures at which the fish initiated ASR and emerged from the water (where at least its whole head and gill opercula were air-exposed) were noted and the experiment terminated at emergence. Control trials were also run where fish ( $n=6$  adults and 6 juveniles) were left individually in the tank for the same amount of time as in the experimental trials but the air supply remained throughout. Behavioural observations and oxygen tensions were recorded every 2 min as above.

(ii) To test for variation of the behavioural responses in relation to trade-offs such as predation, the experiment was repeated with adults ( $1.45 \pm 0.06$  g) where 5 mL of alarm substance was added to the water to simulate a predation event. Alarm substance was generated using methods similar to those of Scott et al. (2003). Three adult sculpins were sacrificed and shallow cuts made in their skin. The dead fish were then repeatedly rinsed with seawater and then this water containing alarm substance was used as a stimulus. Oxygen tensions were reduced as above and at 4.7 kPa (a greater oxygen tension than that seen to induce ASR or emergence in the previous experiment), alarm substance was added to the deepest end of the tank via tubing attached to a syringe, so as not to disturb the fish. In control tanks, 5 mL of seawater was added instead of alarm substance ( $n=6$  for control and alarm substance). Behavioural observations and oxygen tension were recorded every 2 min as above.

### 2.1.2. $P_{crit}$ and oxygen consumption

Critical oxygen tensions ( $P_{crit}$ ), which are the environmental oxygen tensions at which oxygen uptake of the animal switches from being independent of, to being dependent on environmental oxygen (Hughes, 1981), were calculated using juvenile ( $0.25 \pm 0.02$  g) and adult ( $1.67 \pm 0.11$  g) tidepool sculpins ( $n=8$  for each). Fish were placed in flow-through custom-made respirometers and allowed to recover from handling stress overnight during which time well-aerated seawater was continually flushed through the respirometer. Respirometer sizes were scaled down for juveniles to give comparable mass/volume ratios. Fish were not fed during this time. All respirometers were partially immersed in a water bath held at  $12.3 \pm 0.1$  °C to control ambient temperature throughout the experiment. After the recovery period, an oxygen probe (FOXY-R, Ocean Optics Ltd, Florida, USA) was secured in the respirometer and the respirometer tightly sealed. The oxygen probe was connected to a data acquisition program recording the steady decline of oxygen partial pressure as the fish consumed oxygen. The fish were watched for the whole experimental period and remained quiescent. The experiment was terminated when the fish either lost equilibrium or when no further oxygen decline was recorded. Mass specific oxygen consumption was calculated over sequential 5 min periods and changes in oxygen uptake were plotted against the mean water oxygen tension and the  $P_{crit}$  was calculated using the algorithm described by Yeager and Ultsch (1989). Rates of oxygen uptake during the initial part (at water oxygen tensions greater than 11 kPa) of the experiment where oxygen uptake was

independent of environmental oxygen were used to calculate the oxygen consumption of the animal in water.

During typical  $P_{crit}$  closed respirometry trials for sculpins we have measured a  $\sim 0.8$  unit decrease in pH and  $\sim 2$  Torr increase in  $P_{CO_2}$ . However, the calculated  $P_{crit}$  values from closed respirometry trials do not differ significantly from  $P_{crit}$  values determined under partial flow-through conditions where no metabolic waste accumulates (unpublished data). Control trials with no fish present in the respirometer were carried out to account for any background drop in oxygen.

Oxygen consumption in air was also measured for juveniles and adults ( $n = 8$ ) in a similar manner. Fish were allowed to recover from handling stress overnight in the respirometers in normoxic, flow-through seawater. An oxygen probe was then secured in the respirometer, the respirometer drained of water and then tightly sealed. Fish remained quiescent during this procedure. The decline in oxygen was then measured over time as before.

### 2.1.3. Biochemical responses

The biochemical consequences of emergence were investigated by placing adult ( $1.7 \pm 0.2$  g,  $n = 56$ ) individuals on a moist substrate to prevent desiccation for up to 72 h. Fish from either normoxic water or those emerged for 6, 12, 24, 36, 48 and 72 h were euthanized by rapid decapitation and a section of the white muscle was removed and immediately frozen in liquid nitrogen ( $n = 8$  for each time point). Samples were then analysed for glycogen and lactate as described by Richards et al. (2002). During the 72 h emergence, all sculpins were quiescent but remained alert and no mortality was observed.

### 2.2. Mesocosm experiments

Four mesocosms were constructed outside at the Bamfield Marine Sciences Centre. Mesocosms were made in containers (vol. = 85 L) supplied with flowing seawater and were designed to mimic the natural tidepool habitat of sculpins. They consisted of several algae species including *Fucus distichus* (Silva), crabs (*Hemigrapsus nudus* (Dana), *H. oregonensis* (Dana)), penpoint gunnels (*Apodichthys flavidus* (Girard)), saddleback gunnels (*Pholis ornate* (Girard)) and turban snails (*Tegula funebris* (Adams)). The mesocosms were set-up to simulate oxygen fluctuations in natural tidepools by adjusting levels of algal biomass in preliminary studies until fluctuations in oxygen tensions within the mesocosms were equivalent to those measured in natural tidepools. To prevent interference from rainfall, a transparent plastic canopy was set-up over all four mesocosms but at a sufficient height to not affect temperature or sunlight. Two types of tidal simulation were carried out; the first simulated the tidepool becoming isolated from the tide at night and the second during the day. To simulate tidal isolation at night, the water flow to the mesocosm was switched off at 18:00 until 06:00 and to simulate tidal isolation during the day, water flow was turned off at 06:00 until 18:00. In all experiments, oxygen tensions, temperature and salinity were measured in each mesocosm every 3 h, including time zero when the water flow was switched off. Oxygen tension and temperature were

measured using an Oxyguard Handy MKIII electrode and oxygen concentration converted to oxygen tension using the appropriate oxygen solubility coefficients. Salinity was measured using a Reichert–Jung refractometer.

#### 2.2.1. Behavioural responses to hypoxia

Threshold oxygen tensions for sculpin behaviours were tested in the mesocosms. Ten adult ( $2.36 \pm 0.1$  g) and ten juvenile ( $0.25 \pm 0.01$  g) sculpins were lightly anaesthetised (benzocaine;  $0.05$  mg mL<sup>-1</sup>), weighed and measured and added to each mesocosm ( $n = 8$ ; four mesocosms replicated once). Again, the fish were allowed 10 h to recover from handling stress while the mesocosms were supplied with flowing, aerated seawater. The water flow to the mesocosms was then switched off either at 18:00 or 06:00 ( $t = 0$ ; simulating tidal isolation at either night or day) and behavioural observations carried out every 3 h starting at time zero. Red flashlights were used for night time observations.

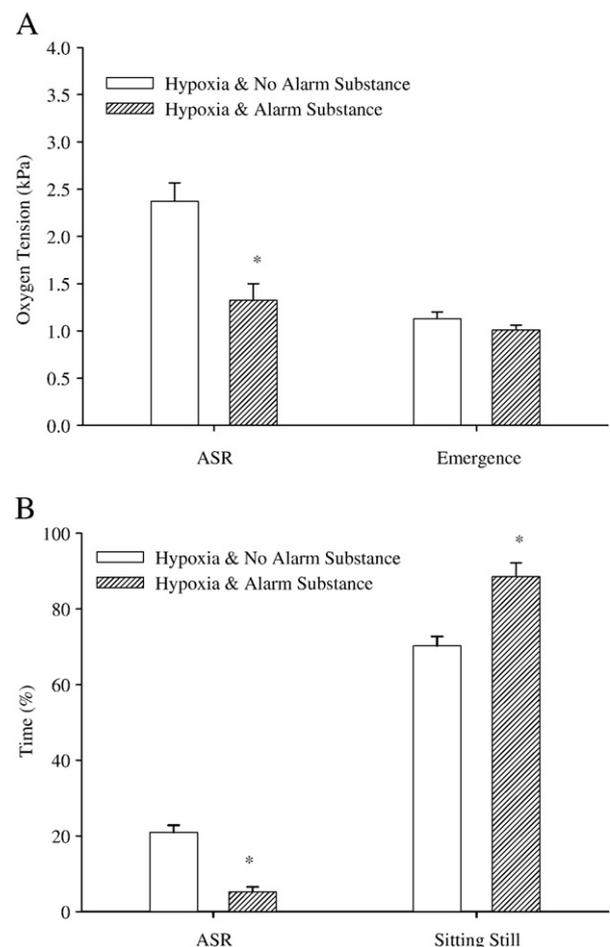


Fig. 2. (A) Threshold oxygen tensions at which adult sculpins performed ASR or emerged from the water in control and alarm substance-exposed treatments in the laboratory ( $n=6$ ). (B) Percentage of time spent sitting still or performing ASR by adult sculpins in control or alarm substance-exposed treatments ( $n=6$ ). Data are presented as means  $\pm$  SEM. Asterisks denote significant differences and percentage data were arc-sin transformed before statistical analysis.

Table 1  
Rates of oxygen uptake (in water and air) and  $P_{crit}$  values for juvenile and adult tidepool sculpins ( $n=8$ ) (at  $12.3 \pm 0.07$  °C)

	Juveniles	Adults
$P_{crit}$ (kPa)	$2.19 \pm 0.33$	$3.46 \pm 0.6$
$MO_2$ in water ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ )	$3.67 \pm 0.31$	$2.44 \pm 0.16^*$
$MO_2$ in air ( $\mu\text{mol g}^{-1} \text{h}^{-1}$ )	$3.36 \pm 0.62$	$1.87 \pm 0.07^{\dagger*}$

Data are presented as means  $\pm$  SEM.  $^{\dagger}$ Indicates a significant difference between water and air and \*indicates a statistical difference between adults and juveniles ( $p < 0.05$ ).

Two observers were involved in each behavioural observation and they sat by the mesocosm for 10 min prior to the start of observations so that fish became used to their presence (based on preliminary observations). The number of adults and juveniles at the water surface performing ASR and the number emerged from the water was then recorded every 5 min for a 20 min period (5 recordings).

### 2.2.2. Biochemical responses

To investigate the biochemical response of tidepool sculpins to hypoxia and also the consequences if access to the water surface was prevented, an experiment was carried out in mesocosm environments ( $n = 8$ ) using two types of mesh basket. The first type of mesh basket allowed access to the water surface (open) and the other was designed to hold fish below the water surface (closed). The mesh baskets allowing access to the water surface also allowed the fish to emerge from the water. All of the baskets had a plastic beaker with some pebbles at the base so that when the fish were sampled the basket could be slowly raised to the surface and a small amount of anaesthetic added to each beaker without removing it completely from the mesocosm. This method reduced disturbance to the fish during sampling. Ten baskets, five of each type, were placed into each mesocosm, each basket containing one adult ( $1.69 \pm 0.09$  g) and one juvenile ( $0.22 \pm 0.01$  g) tidepool sculpin. Fish were allowed 10 h to recover from handling stress while the mesocosms were supplied with flowing aerated seawater. The flow was then switched off at either 18:00 or 06:00 ( $t = 0$ ) and fish were sampled every 3 h for biochemical analysis, including a sample from time zero just as the water flow was stopped.

At each sampling period, one open basket and one closed basket were gently lifted from each mesocosm until the top of the beaker reached the water surface and a terminal dose of anaesthetic (benzocaine;  $0.5 \text{ mg mL}^{-1}$ ) was added to the beaker before the beaker was removed from the mesocosm. Fish were then removed, measurements of mass and length taken, and

Table 2  
White muscle glycogen, glucose and lactate in adult tidepool sculpins immersed in normoxic water or emerged for 6, 12, 24, 36, 48 and 72 h ( $n=8$  for each time point)

	Immersed	Time emerged (h)					
		6	12	24	36	48	72
Glycogen ( $\mu\text{mol glucosyl units g}^{-1} \text{ w.w.}$ )	$7.5 \pm 3.0$	$8.3 \pm 1.5$	$6.7 \pm 1.5$	$8.4 \pm 1.3$	$5.7 \pm 1.4$	$4.2 \pm 1.8$	$8.0 \pm 2.3$
Glucose ( $\mu\text{mol g}^{-1} \text{ w.w.}$ )	$0.1 \pm 0.05$	$0.4 \pm 0.13$	$0.3 \pm 0.08$	$0.5 \pm 0.21$	$0.9 \pm 0.39$	$0.3 \pm 0.2$	$0.8 \pm 0.21$
Lactate ( $\mu\text{mol g}^{-1} \text{ w.w.}$ )	$1.6 \pm 0.8$	$2.1 \pm 0.6$	$1.4 \pm 0.4$	$1.2 \pm 0.3$	$1.7 \pm 0.6$	$3.0 \pm 1.1$	$2.6 \pm 0.9$

Data are presented as means  $\pm$  SEM and there are no significant differences between emerged or immersed sculpins.

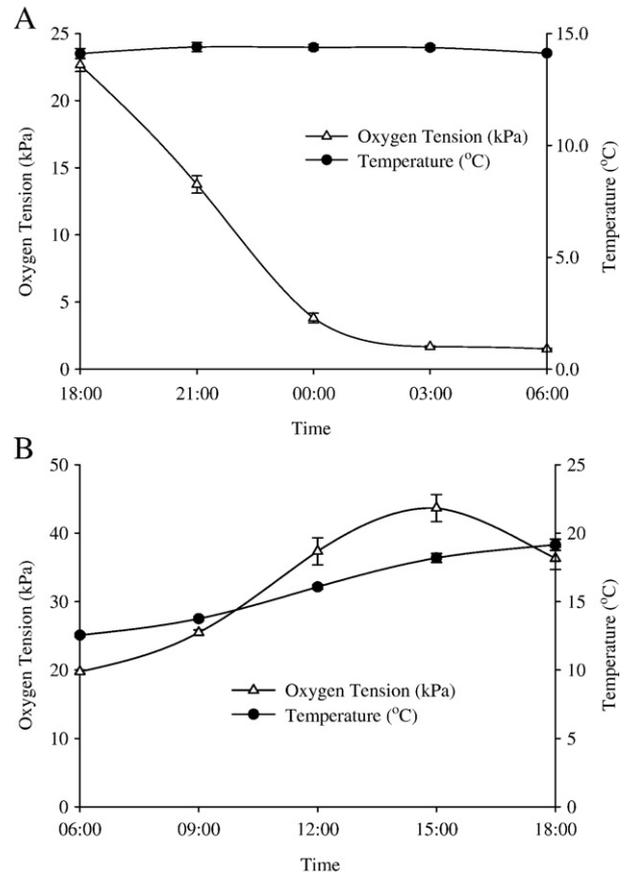


Fig. 3. (A) Oxygen and temperature profiles of mesocosms isolated from water flow for 12 h over night and (B) during the day. Measurements of physical parameters were taken every 3 h ( $n = 16$  for each time point). Data are presented as means  $\pm$  SEM.

immediately frozen in liquid nitrogen for later analysis of whole body lactate and glycogen as described by Richards et al. (2002). Whole body measurements were used in this experiment due to the small size of juvenile fish.

### 2.3. Field experiment

The behaviour of sculpins inhabiting tidepools on Wizard Islets was observed at varying oxygen tensions during the nocturnal period of the tidal cycle. Using red flashlights when necessary, observers recorded every 5 min for a 20 min period (5 recordings) the number of sculpins at the water surface performing ASR and the number emerged. Observers sat by the

edge of pools for 10 min before the start of observations to ensure that the fish had become used to their presence (as shown in preliminary mesocosm experiments). At the beginning and end of the 20 min observation period the water temperature, salinity and oxygen tension were measured. When behavioural observations were complete, all the sculpins were netted out of their individual pools to determine the total number of fish present and, therefore, the proportion of fish seen during observations could be calculated.

2.4. Statistical analyses

Comparisons between the physiology and behaviour of juvenile and adult sculpins within the laboratory environment were made using un-paired *t*-tests. Data were checked for normality using the Kolmogorov–Smirnov test, homogeneity of variance (Levene’s equality of variances) and where necessary transformations (arc-sin) or non-parametric tests were used (Mann

Whitney, Kruskal Wallis). Changes in physical parameters of mesocosms over time were compared using a Repeated Measures ANOVA as were behavioural changes of fish in the mesocosms. Mesocosm position was included as a factor in relevant statistical analyses. Field data were compared after transformation using a 2-way ANOVA with oxygen tension and age as factors.

3. Results

3.1. Laboratory experiments

3.1.1. Behavioural responses to hypoxia

- (i) Adult sculpins initiated ASR at a significantly higher oxygen tension than juveniles ( $F_{1,10} = 16.847, p = 0.002$ , Fig. 1B) but there was no significant difference in the threshold oxygen tension at which both adults and

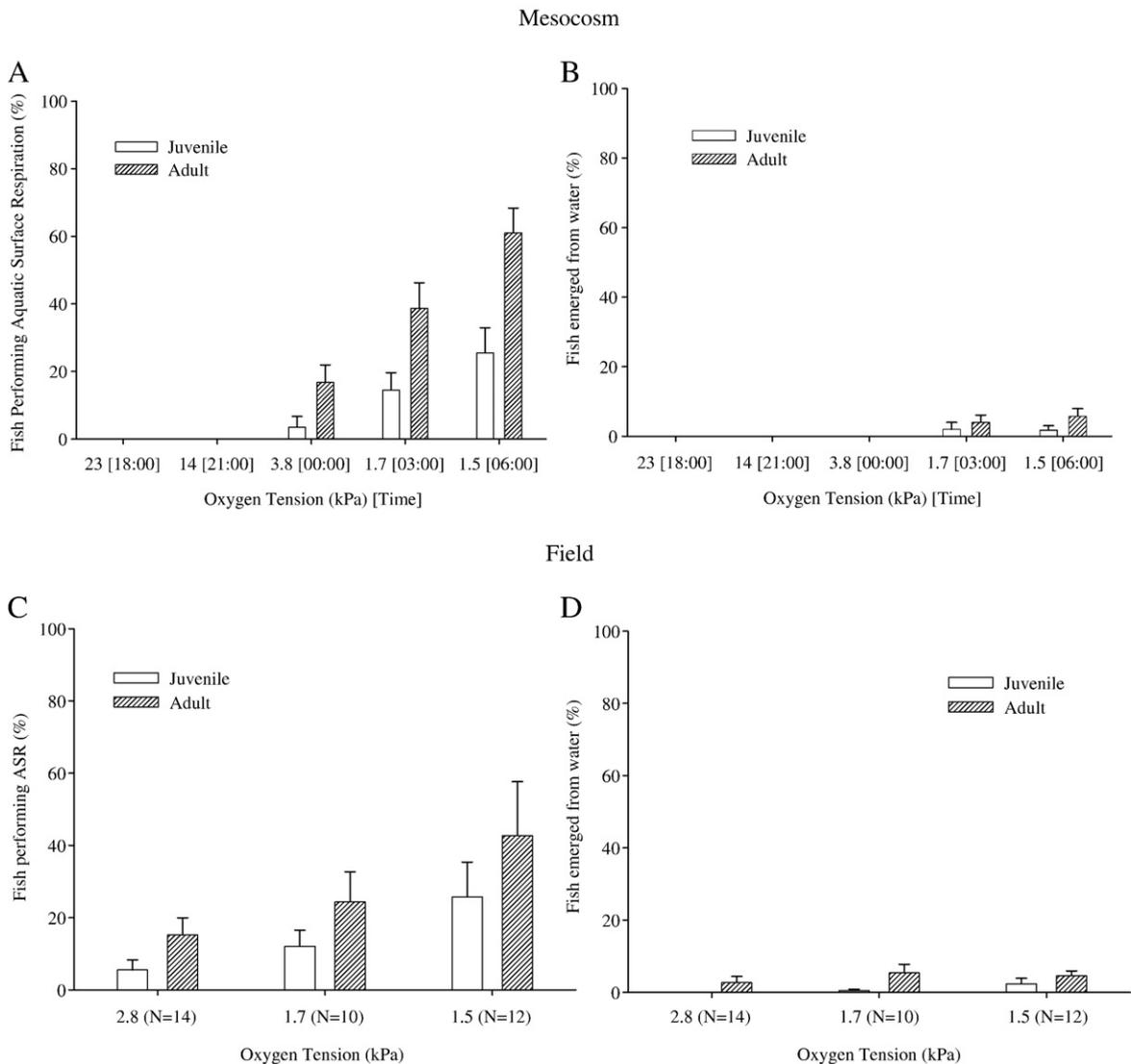


Fig. 4. Percentage of juvenile and adult sculpins (A) performing ASR and (B) emerged from the water in mesocosms isolated from water flow for 12 h over night ( $n=8$ ) and (C) performing ASR and (D) emerged from the water in tidepools observed at different oxygen tensions during the nocturnal period of the tidal cycle at Wizard Islets ( $N$  designated in parentheses). Data are presented as means±SEM and were arc-sin transformed before statistical analysis.

juveniles emerged from the water ( $F_{1,10} = 0.094$ ,  $p = 0.765$ ). Consequently, adults spent a greater percentage of the time performing ASR ( $F_{1,10} = 16.022$ ,  $p = 0.003$ , Fig. 1C) and less time sitting still on the bottom of the tank ( $F_{1,10} = 9.749$ ,  $p = 0.011$ , Fig. 1C). Control fish, not exposed to hypoxia did not perform ASR or emerge from the water and there was no statistical difference in the behaviour of juvenile and adult control sculpins ( $p > 0.1$ ).

(ii) Addition of alarm substance lowered the oxygen tensions at which adult sculpins performed ASR ( $F_{1,10} = 15.92$ ,  $p < 0.01$ , Fig. 2A) but there was no significant difference in the threshold oxygen tension at which control (no alarm substance) and alarm substance-exposed fish emerged from the water ( $F_{1,10} = 1.8$ ,  $p = 0.21$ , Fig. 2A). The threshold for ASR in control adult sculpins here tended to be slightly, but not significantly, lower in this experiment, suggesting that the addition of seawater as a control did cause a small amount of disturbance. Adult sculpins in the control group exposed to declining oxygen tensions in the absence of alarm substance spent a comparable amount of time sitting still and performing ASR as the previous experiment. However, sculpins exposed to alarm substance spent a significantly greater time sitting still ( $F_{1,10} = 15.68$ ,  $p < 0.01$ , Fig. 2B) and less time performing ASR than controls ( $F_{1,10} = 48.79$ ,  $p < 0.001$ , Fig. 2B).

### 3.1.2. $P_{crit}$ and oxygen consumption

There was no significant difference in the  $P_{crit}$  of adult and juvenile tidepool sculpins ( $t = 1.679$ ,  $p = 0.115$ ; Table 1). There was a significant effect of age on mass specific oxygen consumption ( $F_{1,30} = 13.85$ ,  $p < 0.001$ ) in both water and air, but while juveniles displayed similar oxygen consumption rates in both water and air, adults showed a significantly lower oxygen consumption rate in air compared to water (Table 1).

### 3.1.3. Biochemical responses

Fish forcibly emerged from water in the lab showed no significant changes in white muscle glycogen, glucose or lactate over a 72 h period (Table 2; glycogen:  $p = 0.668$ ; glucose:  $p = 0.125$ ; lactate:  $p = 0.562$ ).

## 3.2. Mesocosm experiments

Simulated tidal isolation at night resulted in a significant decrease in oxygen tension in the mesocosms from 23 kPa to 1.5 kPa ( $F_{1,28} = 1993$ ,  $p < 0.001$ , Fig. 3A) but with no significant change in temperature ( $F_{1,28} = 0.001$ ,  $p = 0.979$ ; max: 14.4 °C; min: 14.1 °C). Oxygen concentration was also significantly affected by tidal isolation during the day ranging from 20 kPa to 44 kPa ( $F_{1,28} = 85.96$ ,  $p < 0.001$ ) with evidence of hyperoxia (Fig. 3B). Temperature also rose significantly during the day ( $F_{1,28} = 13.81$ ,  $p < 0.001$ ; max: 19.2 °C; min: 12.5 °C) but during both day and night salinity remained constant at 32‰.

### 3.2.1. Behavioural responses to hypoxia

The percentage of fish performing ASR increased throughout the night ( $F = 73.3$ ,  $p < 0.001$ ; Fig. 4A) with more adults

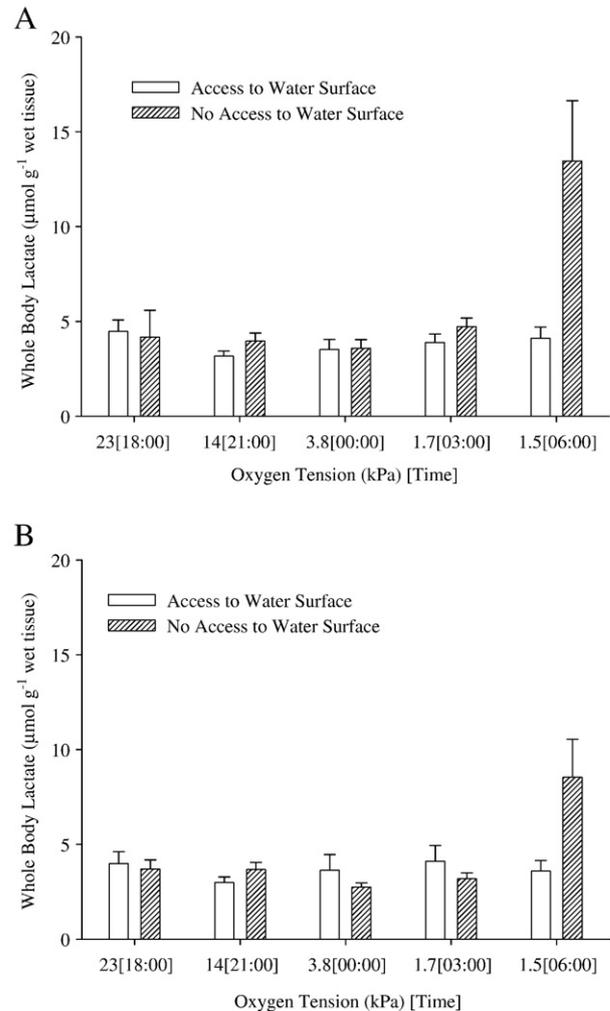


Fig. 5. Whole body lactate concentrations in (A) juvenile and (B) adult sculpins in mesocosms isolated from water flow for 12 h over night. Sculpins were sampled every 3 h ( $n = 8$ ) from baskets which either prevented (closed) or allowed (open) access to the water surface. Data are presented as means  $\pm$  SEM ( $n = 8$ ).

performing ASR (~60% by 06:00) than juveniles (~25% by 06:00;  $F = 14.47$ ,  $p < 0.01$ ). ASR was initiated at oxygen tensions around 3.8 kPa. There was a significant effect of oxygen tension on the emergence behaviour of sculpins at night with a small percentage of fish seen emerging from 03:00 [1.7 kPa] onwards ( $F = 13.36$ ,  $p < 0.01$ ; Fig. 4B). There was no significant effect of age on emergence behaviour ( $F = 2.53$ ,  $p = 0.15$ ). No fish were seen performing ASR or emerging during the day. There was no effect of mesocosm position on sculpin behaviour at night or during the day ( $p > 0.05$ ).

### 3.2.2. Biochemical responses

Denying sculpins access to the water surface at night was associated with a significant elevation in whole body lactate concentrations ( $F_{1,148} = 11.477$ ,  $p < 0.005$ ), not seen in individuals that had access to the water surface. This response was dependent on oxygen tension occurring only at 06:00 [1.5 kPa] ( $F_{1,148} = 12.732$ ,  $p < 0.001$ ) and was magnified in juveniles compared to adults ( $F_{1,148} = 4.412$ ,  $p < 0.05$ ; Fig. 5A and B). No changes in whole body lactate concentrations were seen in

mesocosms run during the day ( $F_{1,151}=2.397$ ;  $p=0.058$ ) although juveniles generally displayed higher lactate concentrations than adults ( $F_{1,151}=4.864$ ,  $p<0.05$ ). No significant differences were seen in whole body glycogen ( $p>0.05$ ; data not shown). There was no effect of mesocosm position on lactate concentrations at night or during the day ( $p>0.2$ ).

### 3.3. Field experiment

Behavioural observations were made at three different oxygen tensions ( $2.8\pm 0.46$  kPa;  $1.7\pm 0.02$  kPa;  $1.5\pm 0.04$  kPa). Salinity remained constant at 30‰ and temperature was  $15.2\pm 0.09$  °C. At lower oxygen tensions, significantly more fish were performing ASR (Fig. 4C;  $F_{2,35}=3.749$ ,  $p=0.035$ ) and more adults were performing ASR than juveniles ( $F_{1,35}=12.971$ ,  $p=0.048$ ). There were significantly more adults than juveniles emerged from the water (Fig. 4D;  $F_{1,35}=11.225$ ,  $p=0.002$ ) and a trend towards increased emergence with decreasing oxygen was seen (Fig. 5D;  $F_{2,35}=3.233$ ,  $p=0.054$ ).

## 4. Discussion

Changes in behavioural patterns are the main response of sculpins to falling oxygen tensions, the response being two-fold; performance of aquatic surface respiration as oxygen tensions fall below  $P_{crit}$ , followed by emergence if oxygen levels fall too low, or if hypoxic conditions persist for too long. During ASR, fish actively ventilate their gills at the air/water interface where aquatic oxygen tensions are raised by diffusion from the air, while emergence relies on passive diffusion of oxygen from the air across moist epithelia of the gills and skin. These behaviours have previously been documented in the laboratory environment (Yoshiyama et al., 1995; Watters and Cech, 2003) and in the present study we found that threshold oxygen tensions at which ASR and emergence were performed were consistent from the laboratory to the mesocosm and the field. While oxygen tensions at which emergence was performed did not vary with age or addition of alarm substance, threshold oxygen tensions for ASR appeared more plastic. Watters and Cech (2003) suggested that in sculpins the oxygen tension at which aquatic surface respiration is performed serves as a physiological constraint around which alternative behaviours have evolved. Here juveniles delayed performance of ASR compared to adults, potentially due to apparent differences in  $P_{crit}$  of adults and juveniles. In many species of fish, smaller individuals may be less sensitive to hypoxic stress and indeed are able to utilise hypoxic habitats as refuge from larger predators (Robb and Abrahams, 2003). However, the difference in  $P_{crit}$  of adults and juveniles in the present study is not conclusive and it is likely that the differences in ASR threshold not only reflect physiological capacity but also a difference in susceptibility to predation.

Breathing at the air/water interface can increase vulnerability to aerial predators (Kramer et al., 1983; Randle and Chapman, 2004). Although it might be predicted that ASR is triggered by environmental oxygen tensions at which respiratory mechanisms fail to compensate for environmental hypoxia (Takasusuki et al., 1998), other studies have also supported the idea that there

is an element of flexibility in the performance of ASR. Unlike adult oscar, *Astronotus ocellatus* (Cuvier), juvenile oscars postpone ASR to oxygen tensions lower than their  $P_{crit}$  and this is believed to be related to predation threat (Sloman et al., 2006). In the present study the flexibility of ASR performance was supported by the fact that detection of alarm substance under laboratory conditions delayed ASR but not emergence from the water. As *O. maculosus* release alarm substance from epithelial cells when damaged during a predation event (Hugie et al., 1991), it seems likely that the major flexibility in performance of ASR is related to predation risk.

The apparent ‘plasticity’ of ASR response, coupled with the lack of biochemical effects of confinement of fish below the water surface at oxygen tensions well below the calculated  $P_{crit}$  values, suggests an additional response mechanism is being utilised by sculpins. Only when oxygen reached the tensions associated with emergence behaviour did confinement below the water surface result in metabolic disturbances. It is likely that sculpins use ASR to effectively limit exposure to aquatic hypoxia when the  $P_{crit}$  tension is reached. However, if ASR is prevented, for example through perceived predation risk, metabolic suppression may delay the onset of anaerobic metabolism until the point when behavioural emergence would normally occur. Prevention of air exposure beyond this point results in unavoidable metabolic disturbances. In adult oscar, a suppression in metabolic rate is seen at around 20% air saturation but evidence of anaerobic metabolism does not occur until 6% air saturation (Muusze et al., 1998). In air juvenile sculpins, unlike adults, were able to maintain a metabolic rate equivalent to that in water, perhaps due to a larger surface area to volume ratio providing a larger surface area for gas exchange. However, the ability of both adult and juvenile sculpins to emerge from the water with only minor effects on oxygen consumption and no measurable biochemical effects (e.g. no activation of the accumulation of lactate) for up to 72 h, points to an extraordinary breadth of behavioural choices for sculpins to employ to avoid aquatic hypoxia exposure. The use of ASR and emergence does not appear to be limited by physiological capacity; indeed ecological factors, such as perceived predation risk, may be the primary deterrent in performance of these adaptive behaviours.

The oxygen thresholds at which adult and juvenile sculpins performed ASR and emergence was similar across laboratory, mesocosm and field environments. The number of individuals seen to perform these behaviours was also similar between mesocosm and field environments, but the percentage of individuals utilising these behaviours at any one time was surprisingly low. Additionally, no evidence of social grouping in association with ASR was seen. In complex environments such as tidepools, oxygen availability is not homogenous (Morris and Taylor, 1983) and it is possible that there are pockets of well-aerated water at different depths which the fish are able to utilise. In addition, sculpins may alternate between periods of ASR and emergence and periods of submergence, while avoiding physiological costs, thus reducing the number of individuals recorded performing these behaviours at any one time (Congleton, 1980). In mudminnows, *Umbra limi* (Kirtland), it has been suggested that they suspend respiration and close their gill covers for short periods of time in order to venture into lethally hypoxic regions

for food (Rahel and Nutzman, 1994). There is, therefore, the possibility that sculpins can suspend respiration during periods of submersion in between periods of ASR.

Although the variability of many biotic and abiotic factors, including some not measured in the present study, increases across laboratory, mesocosm and field environments, the similarities in threshold oxygen tensions at which characteristic behaviours were demonstrated in laboratory, mesocosm and field environments suggests that oxygen tension is the over-arching factor in these responses. In theory, the worst-case scenario for oxygen availability to tidepool residents involves tidal isolation occurring from dusk to dawn with no photosynthetic input of oxygen. Therefore, with tidal isolation occurring from sun-down to sun-rise, as portrayed in the present study, oxygen tensions would be predicted to reach their lowest point just before sun-rise (Morris and Taylor, 1983). Unfortunately for tidepool residents that utilise emergence as a behavioural strategy, the timing of this critical behaviour coincides with the dawn foraging of avian predators. It is likely that this survival threat plays the greatest role in modifying response to hypoxia and consequently fish will avoid emergence from the water whenever possible.

The present study is novel in utilising a range of experimental conditions (laboratory versus mesocosm versus field) to investigate how tidepool sculpins respond to hypoxia. For while laboratory studies are important in determining what an animal *can* do in response to hypoxia, in isolation they may not represent what an animal actually *does*; a combined experimental approach is essential to understand the performance of these animals in their natural environment. Studying the physiological limitations which animals face in their natural environments has recently provided a greater appreciation of integrated behavioural patterns (reviewed by Gilmour et al., 2005) and the present study identifies the necessity of multidisciplinary approaches that transcend the traditional laboratory/field boundaries.

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