A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass

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Summary

1. Inter-individual variation in metabolic rate exists in a wide range of taxa. While this variation appears to be linked to numerous aspects of animal behaviour and personality, the ecological relevance of these relationships is not understood. The behavioural response of individual fish to acute aquatic hypoxia, for example, could be related to metabolic rate via influences on oxygen demand or the willingness to take risks. Individuals with higher metabolic rates could show greater hypoxia-associated increases in activity that could allow them to locate areas with increased oxygen availability but that also make them susceptible to predation. Any relationship between metabolic rate and risk-taking behaviour among individual fish could therefore be modulated by environmental oxygen levels, perhaps becoming stronger as oxygen availability declines.

2. We measured spontaneous swimming activity as an index of risk-taking by juvenile European sea bass in normoxia, moderate hypoxia (40% air saturation) and severe hypoxia (20% air saturation) after being startled by a predator model. All fish were also separately measured for routine metabolic rate by measuring oxygen uptake.

3. In hypoxia, fish re-emerged from cover sooner after a simulated attack and were generally more active than when the same fish were startled in normoxia. In addition, individual activity and risk-taking in severe hypoxia were positively correlated with metabolic rate. Aquatic surface respiration was a major contributor towards increased activity in hypoxia and was positively related to metabolic rate during severe hypoxia. There were no relationships between risk-taking and metabolic rate in moderate hypoxia or normoxia.

4. Relative measures of risk-taking among individual fish were not consistent among oxygen levels, further suggesting that individuals differ in sensitivity to hypoxia and the degree to which this environmental stressor affects risk-taking behaviour.

5. These results suggest that fish with relatively high metabolic rates become more active during acute hypoxia, possibly leading to increased susceptibility to predation in response to differences in metabolic demand. In addition, the relationship between metabolic rate and risk-taking may only be observable during exposure to a physiological stressor or such a stressor may strengthen any relationships observable under more benign conditions.

Key-words: boldness, ecophysiology, foraging, personality, physiological energetics, predation, teleost fish

Introduction

Numerous species display considerable inter-individual variation in resting metabolic rate (McCarthy 2000; Nespolo & Franco 2007; Broggi *et al.* 2009). The persistence of this

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variation may reflect numerous behavioural and physiological trade-offs. For example, individuals with higher metabolic rates may be able to attain higher rates of growth or physical activity (Priede 1985; Álvarez & Nicieza 2005), but may also be more prone to starvation because of increased energetic requirements (Álvarez & Nicieza 2005; Bochdansky *et al.* 2005; Killen, Marras & McKenzie 2011). Metabolic rate is also related to animal personality, as individuals with higher metabolic rates tend to be more bold, dominant and aggressive (Metcalfe, Taylor & Thorpe 1995; Cutts, Metcalfe & Taylor 1998; Huntingford *et al.* 2010; Killen, Marras & McKenzie 2011). These traits can lead to increased resource acquisition but can also make individuals more susceptible to predation. Although there appear to be such links between metabolic rate and behaviour, there is uncertainty about the causal direction of these relationships (Careau *et al.* 2008; Biro & Stamps 2010).

Physiological and behavioural trade-offs can become more apparent in situations where resources are limited or during exposure to an environmental stressor (Killen, Marras & McKenzie 2011). Hypoxia, for example, is a common stressor in aquatic environments to which fishes have evolved various physiological and behavioural responses. Aquatic hypoxia is now a serious global problem, with the frequency and severity of acute hypoxic episodes in coastal zones increasing over the last 50 years (Diaz & Rosenberg 2008; Zhang *et al.* 2010). The behavioural response to acute hypoxia involves a trade-off between increasing swimming activity to increase the probability of finding more oxygenated water and decreasing swimming activity to lower oxygen requirements (Bushnell, Steffensen & Johansen 1984; Schurmann & Steffensen 1994; Domenici, Steffensen & Batty 2000; Chapman & McKenzie 2009; Lefrançois *et al.* 2009). Some fish species that frequently experience environmental hypoxia increase their activity to perform aquatic surface respiration (ASR), whereby individuals swim close to the surface and ventilate their gills with the relatively oxygenated water that is in contact with the air (Kramer 1987; Verheyen, Blust & Decleir 1994; Shingles *et al.* 2005; Chapman & McKenzie 2009). An important consequence of any hypoxia-associated increase in activity is a greater likelihood of being killed by a predator, as activity is known to put prey at a greater risk by increasing their obviousness and decreasing their vigilance (Godin & Smith 1988; Skelly 1994; Martel & Dill 1995; Watson, Aebischer & Cresswell 2007). This may be especially true of ASR, which puts a fish at great risk of being detected and attacked by aerial predators (Kramer, Manley & Bourgeois 1983; Kersten *et al.* 1991; Chapman & McKenzie 2009).

The possibility that individuals within species vary in their behavioural response to hypoxia has not been investigated, particularly in response to intraspecific variation in metabolic rate. In hypoxia, individuals with a higher metabolic rate could show greater increases in spontaneous swimming activity if they are (i) less tolerant of hypoxia, because of increased oxygen requirements, thus motivating them to leave the hypoxic area or perform ASR or (ii) generally more willing to accept the greater risk of predation that accompanies an

Fig. 1. Juvenile European sea bass *Dicentrarchus labrax*. Photograph by Shaun S. Killen.

increase in swimming activity. Conversely, individuals with lower metabolic rates could display a relatively small increase in activity during hypoxia, either because they are less sensitive to hypoxia or because they take fewer risks (Krause *et al.* 1998; Finstad *et al.* 2007; Huntingford *et al.* 2010; Killen, Marras & McKenzie 2011). Such individuals may even show a reduction in activity and adopt an energy-conserving response to hypoxia, especially if they experience a decreased motivation to forage because of a decreased aerobic scope and ability to digest food (Chabot & Claireaux 2008). Variable behavioural responses to hypoxia among individuals stemming from inter-individual variation in metabolic demand could mean that the influence of metabolic rate on risk-taking by individual fish is situation-dependent, becoming more pronounced when individuals are exposed to a stressor such as hypoxia. It could also make individuals with higher metabolic rates more susceptible to predation during acute hypoxic episodes.

This study investigated the hypothesis that the relationship between risk-taking and metabolic rate is influenced by oxygen availability in individual juvenile European sea bass *Dicentrarchus labrax* (Fig. 1). Juvenile European sea bass often inhabit coastal lagoons and estuaries that experience acute hypoxic episodes, and they are frequently preyed upon by aerial predators and larger fishes. The species also displays wide, repeatable individual variation in metabolic rate (Marras *et al.* 2010; Killen, Marras & McKenzie 2011). To investigate whether the relationship between metabolic demand and risk-taking behaviour was dependent on the severity of hypoxia, we measured the activity after a simulated predator attack in individual fish under normoxia and two levels of hypoxia. It was predicted that individuals with higher metabolic rates would show the most swimming activity and associated risk and that strength of this relationship would increase with the severity of hypoxia.

Materials and methods

ANIMALS

Juvenile European sea bass *Dicentrarchus labrax* ($n = 23$, mean wet mass $= 81.20 \pm 2.37$ g SEM) were captured from the wild (Stagno di Cabras, Sardinia, Italy) in May 2009. They were transported to the laboratory and held under a 12-h light/12-h dark photoperiod in a

large cylindrical tank (2 m diameter, 1·5 m water level) supplied with re-circulating, filtered natural sea water at a constant temperature (22 °C) for 2 weeks prior to use in experiments. After 1 week, all fish were tagged for identification using VI Alpha tags (Northwest Marine Technology, Inc., Shaw Island, WA, USA). Fish in the holding tank were fed daily with a maintenance ration consisting of dried feed pellets supplemented with dead sand smelt *Atherina boyeri* that had been frozen and thawed. Individuals were fasted for 36 h before use in experiments.

EXPERIMENTAL PROTOCOL

Respirometry

Routine metabolic rate (RMR) was measured by respirometry, as rates of oxygen uptake (Steffensen 1989; Dupont-Prinet *et al.* 2010; Killen, Marras & McKenzie 2011). In fishes, RMR is the metabolic rate of post-absorptive, undisturbed, resting animals that also includes the costs of random activity and the maintenance of posture and equilibrium (Jobling 1994). Individual animals were placed in one of three polyethylene respirometers (25 cm L × 10 cm W × 6 cm H) immersed in an outer water bath regulated at 22 ± 0·5 °C and with an appropriate seasonal photoperiod (12-h light/12-h dark). The water bath was surrounded by opaque polystyrene to prevent visual disturbance to the fish. Instantaneous oxygen uptake (MO_2, in mg O_2 h^{-1}) was measured by intermittent stopped-flow respirometry (Steffensen 1989) once every 30 min. Water flow from the external bath through the respirometers was driven by an external pump that was set to turn on and off for alternating 15-min periods. This allowed decreases in water oxygen content to be measured every 15 s for 15 min while the respirometer was in the closed state, and then, the respirometer was flushed with aerated water for 15 min. The oxygen consumption during each closed phase was calculated using linear least-squares regression, excluding the first and last 2 min of each closed phase. A fourth respirometer was left empty, to provide parallel measurements of background microbial respiration in the system. Water oxygen levels were measured with optodes (Oxy-4 mini; PreSens Precision Sensing GmbH, Regensburg, Germany) and associated software (Pre-Sens Oxy 4v2), and MO_2 then calculated as described by Dupont-Prinet, Claireaux & McKenzie (2009). Fish remained in the respirometers for 24 h. Oxygen consumption typically stabilized after approximately 20 h of respirometer confinement, and so only measurements taken during the last 4 h of this period were used to estimate metabolic rate (between 09:00 and 13:00 hours the day after the fish was first introduced in to the respirometer). The absolute RMR (; mg O_2 h^{-1}) of each individual was calculated as the mean level of oxygen consumption during this time and was then corrected for differences in body mass by dividing the absolute RMR of each fish (mg O_2 h^{-1}) by the fish's body mass (in kg) raised to the power of *b,* the slope of the log–log allometric relationship between absolute RMR and body mass (Rodnick *et al.* 2004; Killen, Marras & McKenzie 2011).

Behavioural response to hypoxia

After being measured for RMR, each fish was tested for activity and risk-taking behaviour over the course of three successive days, under conditions of normoxia (>95% air saturation), moderate hypoxia (40% air saturation) and severe hypoxia (20% air saturation; one treatment each day). Prior to the study, a treatment schedule was designed so that each treatment-order combination would be used a nearly equal number of times. Fish were then randomly assigned to fill out this trial schedule. Of the six possible treatment-order combina-

tions, five were used four times and one was used three times. Tests were conducted in an experimental arena measuring 55 cm L × 37 cm W × 30 cm H (filled to 17 cm high). At one end of the tank, cover was provided under a horizontal PVC sheet (11 cm L × 37 cm W × 16 cm H). Fish were unable to access the surface of the water to perform ASR while in this covered area. The remainder of the arena was without cover and was subdivided into three sections by markings on the floor, each measuring 14·6 cm in width (these marking permitted the quantification of activity; described in detail later). The arena was located in a temperature regulated room maintained at 22 °C. The entire setup was surrounded by a black plastic blind, through which the fish could be observed via small openings. Before testing, individual fish were placed into the arena and left to acclimate for 4 h. During this acclimation period, the water in the arena was aerated with an air stone and a small circulating pump located in the far open section of the arena. For trials in which fish were exposed to either moderate or severe hypoxia, the water oxygen concentration in the arena was gradually decreased by bubbling nitrogen into the water instead of air. This was performed towards the end of the acclimation period, with the water oxygen content decreasing at a rate of approximately 1·0% air saturation per minute (over approximately 55 min for moderate hypoxia and 75 min for severe hypoxia, timed such that both treatments reached their target oxygen concentration 30 min before the beginning of the observation period). The water oxygen concentration was measured continuously during this time using a submersible oxygen probe attached to the outflow of the circulating pump.

After this acclimation period, a trial would commence by releasing a model cormorant head (*Phalacrocorax* sp.; attached to a retort stand and released from a consistent height) vertically into the arena at the farthest end of the open section, so that it broke the water surface. It was then immediately removed. This was meant to simulate an attack from an aerial predator and always caused the fish to retreat to the covered area. Birds such as cormorants, egrets and herons *Ardea* sp. are the main predators of juvenile sea bass living in lagoon environments (Addis & Cau 1997). To provide motivation for fish to emerge after being startled across all treatments, a food item was placed into the far open end of the arena in the same area where the simulated attack had just occurred (a dead sand smelt, 1·92 ± 0·08 g wet mass, tethered to the wall of the arena by a piece of 1-mm steel wire). The air stone and circulating pump were then carefully removed from behind the surrounding blind. Fish activity was then videotaped for the next hour, from above (Sony Mini DV camera; 25 frames s^{-1}).

Videotapes were later analysed for (i) the time taken to reappear from cover after startling, defined as the nose of the fish being visible beyond the edge of the cover (hereafter referred to as appearance time); (ii) the time taken to completely emerge from cover after startling, defined as the entire fish being visible in the open area of the arena (emergence time); (iii) the total time spent in the open area of the arena (open time); (iv) the number of transitions between subsections of the arena (activity); and (v) the total amount of time spent performing ASR (ASR time). Appearance time, emergence time and open time are indicative of boldness, while the number of transitions between arena sections is an index of activity. Boldness and activity are considered two axes of animal temperament and could increase the risk of an individual encountering or being attacked by a predator (Réale *et al.* 2007). While each of these measures represents a component of overall risk-taking, they may also be confounded with one another (Réale *et al.* 2007). We therefore combined appearance time, emergence time, open time, activity and ASR time into one overall measure of risk-taking using principal component analysis (PCA; see Data and Statistical Analyses). Fish that did not emerge from cover after the startle stimulus in a given trial were given a value of 3600 s

(the total duration of the observation period) for their appearance and emergence times.

Between trials at the various oxygen concentrations, fish being tested were kept overnight in aerated holding tanks measuring 60 cm L × 40 cm W × 40 cm H. After a fish had been tested under all three oxygen levels, it was measured for total length and carefully dried with a lint-free paper towel and weighed using a microbalance.

Data and statistical analyses

Statistics were performed with SPSS Statistics v17.0 (SPSS Inc. and IBM, Chicago, Illinois, USA). The level of significance for all tests was $\alpha = 0.05$. For parametric tests, the normality, linearity and homogeneity of residuals were verified by inspection of residual-fit plots. For measures of open time, activity and ASR time, log transformation of the data (after adding 1·0 to the raw values to account any values equal to zero) was necessary to satisfy these requirements.

Principle component analysis was used to produce an overall index of risk-taking behaviour in individual fish using data collected under all three levels of oxygen availability. Using a correlation matrix (to account for differences in the measurement scales and units among the variables), this PCA incorporated (i) appearance time, (ii) emergence time, (iii) open time, (iv) activity and (v) ASR time. The scores for the first component were used as a general index of riskiness ('risk score').

Comparisons of the overall mean values for behaviours among treatments (normoxia, moderate hypoxia and severe hypoxia) were performed using linear mixed models, with subject fish as a random factor and treatment as a categorical variable, followed by Fisher's LSD tests for pairwise comparisons between treatments. The effect of RMR on each behaviour was first analysed using linear mixed models, with subject animal as a random factor, RMR and body mass as covariates and treatment as a categorical variable (five terms: the main effects of treatment, RMR, mass, the treatment × RMR interactions and treatment × mass interactions). These models produced no significant interactions between mass and treatment ($P > 0.05$), but had significant interactions between RMR and treatment ($P < 0.05$) for some behaviours. The presence of such interactions prevents an interpretation of lower-order main effects (Quinn & Keough 2002), so separate general linear models for each behaviour were performed within each treatment with RMR and body mass as covariates (main effects only). Consistency in the rank order of behavioural measures among individuals was examined with Spearman correlations and the intraclass correlation coefficient (τ, Sokal and Rohlf 1995). The effect of RMR on the probability that individuals would perform ASR during moderate and severe hypoxia was examined using binary logistic regression.

To explore individual variability in the behavioural strategy utilized to cope with moderate and severe hypoxia (i.e. an increase or decrease in activity), a four-quadrant plot was constructed with the change in activity between normoxia and moderate hypoxia on the *x*-axis and the change in activity between normoxia and hypoxia on the *y*-axis. A chi-square test was used to determine whether the distribution of the points differed from the expected random distribution across all four quadrants.

Results

ROUTINE METABOLIC RATE

Absolute RMR was correlated with body mass according to the equation:

$$\log \text{RMR} = 0 \cdot 906 \, (\log \text{initial mass in g}) - 0 \cdot 492, \, r^2$$
$$= 0 \cdot 30, P < 0 \cdot 007 \qquad \text{(eqn 1)}$$

After adjusting for difference in body mass (which ranged from 56·73 to 114·39 g), there remained a two-fold difference between the minimum (118·93 mg O_2 kg$^{-0.906}$ h^{-1}) and maximum (236·45 mg O_2 kg$^{-0.906}$ h^{-1}) values for RMR among individuals.

BEHAVIOUR

There was no effect of treatment sequence on any behaviour, at any level of oxygenation (ANOVA, treatment sequence as a categorical variable, $P > 0.05$). In particular, risk score, which incorporates all behaviours quantified in the study, displayed no dependence on whether a treatment was received 1st, 2nd or 3rd, during normoxia (ANOVA, $F = 0.257$, $P = 0.776$), moderate hypoxia ($F = 0.901$, $P = 0.422$) or severe hypoxia ($F = 1.100$, $P = 0.352$).

After the simulated attack during normoxia, fish often inspected or attacked the food item, although it was not possible to precisely quantify this behaviour as it was sometimes unclear whether the fish was biting the smelt or simply passing by it. During both mild and severe hypoxia, however, fish never inspected or attacked the dead smelt and appeared disinterested in eating. Despite this, fish exposed to severe hypoxia appeared and emerged from cover sooner after the simulated attack (Fig. 2a, b), spent more time in the open and displayed more activity as compared to during normoxia (Fig. 2c, d), and during moderate hypoxia, fish displayed more activity than during normoxia (Fig. 2d). During severe hypoxia, fish showed greater indicators of risk-taking for all behaviours as compared to moderate hypoxia (Fig. 2). The increased activity during hypoxic exposure was at least in part because of the performance of ASR, a response that was more pronounced in severe than in moderate hypoxia (Fig. 2e). ASR was never observed during normoxia.

The majority of fish conformed to this overall trend of increasing activity and risk-taking with increasingly severe hypoxia (Fig. 3, significantly different from an expected random distribution across all four quadrants, $\chi^2 = 20.49$, $P < 0.001$). The rank order among individuals was not consistent for any measure of behaviour when comparing between normoxia, moderate hypoxia and severe hypoxia (Spearman correlations, $P > 0.05$). Estimates of consistency across treatments using the intraclass correlation coefficient were also low (appearance time: $\tau = 0.164$, $P = 0.041$; emergence time: $\tau = 0.132$, $P = 0.069$; open time: $\tau = 0.129$, $P = 0.054$; activity: $\tau = 0.163$, $P = 0.025$; ASR time: $\tau = 0.079$, $P = 0.147$; risk score: $\tau = 0.207$, $P = 0.006$).

Principal component analysis of behaviours produced a first component accounting for 63·30% of the variance in the data set (eigenvalue = 3·165) while loading positively for activity (0·476), open time (0·445) and ASR time (0·323) and negatively for time until first appearance (−0·461) and time until first emergence (−0·508).
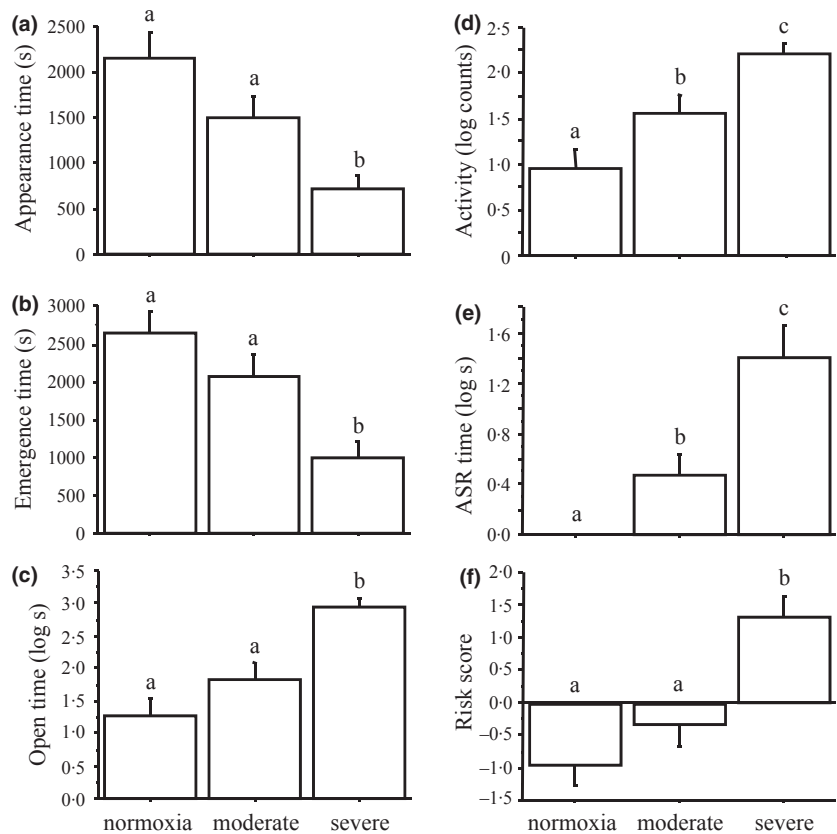
Fig. 2. Measures of risk-taking behaviour during normoxia, and moderate and severe hypoxia in juvenile European sea bass. Bars with a different letter within each behaviour indicate a significant difference (linear mixed models, $P < 0.05$, Fisher's LSD tests for pairwise comparisons).
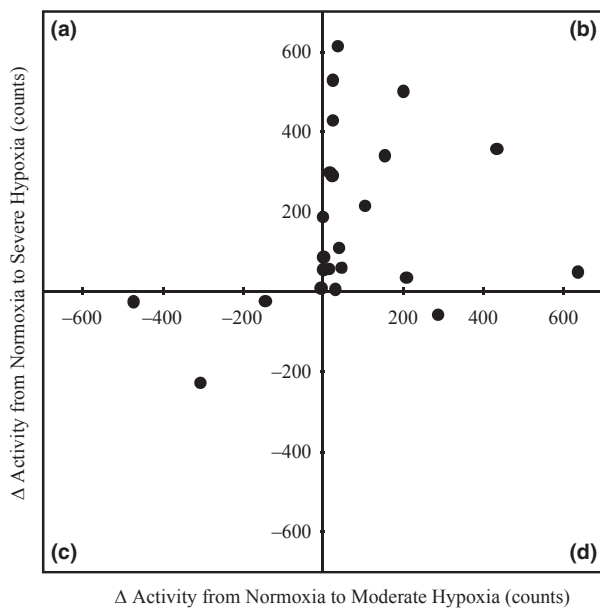

Fig. 3. Activity after a simulated attack for European sea bass in moderate and severe hypoxia, relative to normoxia. Points in quadrant (b) represent individuals that consistently increase activity relative to normoxia regardless of the level of hypoxia; points in quadrant (c) represent individuals that consistently decrease activity regardless of the level of hypoxia; points in quadrants (a) and (d) represent individuals that increase activity during either moderate or severe hypoxia, but then decrease activity when exposed to the opposite level of hypoxia.

RELATIONSHIPS BETWEEN METABOLIC RATE AND BEHAVIOUR

The relationships between RMR and measures of risk-taking behaviour differed between normoxia, moderate hypoxia and severe hypoxia (linear mixed models, significant RMR × treatment interactions, $P < 0.05$). Within treatments, RMR was not associated with any behavioural measure during normoxia or moderate hypoxia, but was associated with all behaviours under severe hypoxia (Table 1, Fig. 4). In addition, the probability of an individual performing ASR showed a significant increase with RMR during exposure to severe hypoxia (binomial logistic regression, Wald $\chi^2 = 5.744$, $P = 0.017$; Fig. 5). The probability of performing ASR during moderate hypoxia was not related to RMR.

Behaviour was not linked with body mass under normoxia, but under moderate hypoxia larger fish had higher open times, activity and risk score (general linear models, $P < 0.05$, Table 1). Under severe hypoxia, larger fish tended to emerge sooner after the simulated attack and have a higher risk score (general linear model, $P < 0.05$, Table 1). ASR showed no association with body size in any treatment.

Discussion

The vast majority of individuals increased activity in response to both mild and severe hypoxia. This response probably reflected attempts to escape the hypoxic area and the

performance of ASR. Importantly, metabolic demand was not linked with any behavioural measure during normoxia or moderate hypoxia, but in severe hypoxia, fish with higher RMR tended to be those that showed the greatest activity and associated risk. This could lead to individuals with relatively high metabolic rates becoming more vulnerable to predation during acute hypoxic episodes and suggests that the influence of metabolic rate on risk-taking behaviour can be stressor-dependent and exacerbated during exposure to hypoxia.

Previous studies have indicated a link between metabolic rate and behaviours associated with boldness and aggression (Metcalfe, Taylor & Thorpe 1995; Cutts, Metcalfe & Taylor 1998; Finstad *et al.* 2007; Huntingford *et al.* 2010; Killen, Marras & McKenzie 2011). The causal direction of this association, however, is unclear (Careau *et al.* 2008). On the one hand, increased metabolic demand may cause some individuals to be more bold and aggressive so that they can obtain more food and other resources. On the other hand, intrinsic differences in behaviour may drive variation in metabolic rate if some individuals are more active and therefore require increased routine energy expenditure. If this latter supposition were the case in the current study, there should have been a relationship between RMR and behaviour under normoxia, mild hypoxia and severe hypoxia. This did not occur, suggesting that the relationship between RMR and behaviour under severe hypoxia resulted from increased oxygen requirements in some individuals, which became more important as oxygen availability decreased. This situation is analogous to previous findings with juvenile European sea bass whereby the relationship between RMR and risk-taking while foraging was stronger when fish were food-deprived than when they were fed (Killen, Marras & McKenzie 2011). Together with the present study, these results suggest that the effects of RMR on behaviour may only manifest during exposure to a stressor or that a stressor may amplify pre-existing differences in behavioural tendencies because of intrinsic variation in RMR.

The hypothesis that increased oxygen requirements for individuals with the higher RMR make them more sensitive to hypoxia is supported by the observation that RMR was correlated with time spent performing ASR under severe hypoxia. The probability of performing ASR during severe hypoxia also increased with RMR. The performance of ASR can be beneficial during acute hypoxia, because it allows fish to obtain more oxygen and possibly avoid hypoxia-associated mortality. There is a trade-off, however, in that ASR is known to make fish prone to aerial predation (Kramer, Manley & Bourgeois 1983; Kersten *et al.* 1991). The danger inherent to ASR is emphasized by the fact that fish will temporarily delay the onset of ASR after exposure to a predatory threat or relocate to safer areas (Kramer, Manley & Bourgeois 1983; Kersten *et al.* 1991; Shingles *et al.* 2005). In environments with numerous predators, frequent and intense hypoxic episodes could lead to differential survival of individuals as a function of their RMR.

In normoxia, the activity displayed by fish was associated with foraging behaviours, including exploration of the arena and attempts to inspect or consume the provided food item. Under moderate and severe hypoxia, however, fish showed no interest in food and any activity was probably associated with attempts to find an area with increased oxygen availability or perform ASR. Fish experience a reduction in appetite during hypoxia, which is likely due to a reduced aerobic scope for digestion and nutrient assimilation (Chabot & Claireaux 2008). As a result, the presence of food becomes less important as a factor driving risk-taking behaviour during hypoxia, while the need to either increase or decrease activity to cope with reduced oxygen availability becomes a priority. This suggests that the difference in activity between hypoxic and normoxic conditions would be even greater in the absence of food as a motivating factor during normoxia.

There was no consistency in the relative activity or risk score between normoxia and either level of hypoxia, or between hypoxia levels, indicating that relative risk displayed among individuals did not carry over among situations or between contexts (foraging vs. the behavioural response to hypoxia; refer to the definitions of 'situation' and 'context' provided by Sih, Bell & Johnson 2004). These results contrast with the concept of behavioural syndromes (Sih, Bell & Johnson 2004; Réale *et al.* 2007). Instead, the findings of the current study are analogous to those of Coleman & Wilson (1998), who observed that relative boldness among pumpkin-seed sunfish *Lepomis gibbosus* was not consistent across contexts, which in that study consisted of behavioural trials conducted in the presence of various threatening and non-threatening stimuli. The lack of consistency in the rank order of risk-taking among situations in the present study suggests varying degrees of behavioural plasticity and sensitivity to hypoxia among individuals (Dingemanse *et al.* 2009), probably resulting from the absence of a relationship between RMR and activity under normoxia, contrasted with the increasingly strong influence of individual RMR on activity with higher degrees of hypoxia. Again, these results suggest that, at least in juvenile European sea bass, routine metabolic demand only becomes relevant for differences in activity and risk-taking when in the presence of a stressor such as severe hypoxia.

Despite the overall relationship between metabolic rate and activity under severe hypoxia, there was a high degree of variation surrounding this general trend. This suggests there are additional factors, independent of metabolic rate, which also play a role in determining the amount of activity and risk displayed by individual fish during hypoxia. Differences in sensory ability (Giske, Huse & Fiksen 1998) or perception of risk (Stankowich & Blumstein 2005), for example, could influence behaviour after an attack. Differences between individuals in stress experienced during respirometer confinement could also increase variation in RMR estimates, especially for shy individuals that may become nervous in respirometers (Careau *et al.* 2008; Martins *et al.* 2011).It should also be noted that while most fish consistently increased activity in response to hypoxia, five fish (22% of the individuals used in

Table 1. General linear model results for the effects of routine metabolic rate (RMR) and body mass on behavioural indicators of risk-taking in juvenile European sea bass after a simulated attack from an aerial predator during normoxia, moderate hypoxia and severe hypoxia

Term	d.f.	F	P
Normoxia			
Appearance time			
RMR	1	0·005	0·946
Mass	1	0·715	0·408
Error	20		
Emergence time			
RMR	1	0·741	0·400
Mass	1	3·810	0·065
Error	20		
Open time			
RMR	1	0·790	0·385
Mass	1	2·009	0·172
Error	20		
Activity			
RMR	1	0·237	0·632
Mass	1	2·094	0·163
Error	20		
ASR time			
RMR	1	–	–
Mass	1	–	–
Error	20		
Risk score			
RMR	1	0·064	0·803
Mass	1	2·908	0·104
Error	20		
Moderate hypoxia			
Appearance time			
RMR	1	0·407	0·531
Mass	1	1·700	0·207
Error	20		
Emergence time			
RMR	1	0·002	0·962
Mass	1	2·048	0·168
Error	20		
Open time			
RMR	1	0·087	0·771
Mass	1	7·587	0·012*
Error	20		
Activity			
RMR	1	0·054	0·819
Mass	1	9·070	0·007*
Error	20		
ASR time			
RMR	1	3·165	0·090
Mass	1	1·467	0·240
Error	20		
Risk score			
RMR	1	0·005	0·947
Mass	1	6·794	0·017*
Error	20		
Severe hypoxia			
Appearance time			
RMR	1	5·414	0·031*
Mass	1	2·317	0·144
Error	20		
Emergence time			
RMR	1	6·837	0·017*
Mass	1	6·957	0·016*
Error	20		

Table 1. (Continued)

Term	d.f.	F	P
Open time			
RMR	1	6·188	0·022*
Mass	1	3·062	0·095
Error	20		
Activity			
RMR	1	5·283	0·032*
Mass	1	2·384	0·138
Error	20		
ASR time			
RMR	1	6·644	0·018*
Mass	1	0·709	0·410
Error	20		
Risk score			
RMR	1	10·819	0·004*
Mass	1	4·756	0·041*
Error	20		

ASR, aquatic surface respiration
*Significant effect ($P < 0.05$).

this study) either decreased activity at both levels of hypoxia or varied their response depending on the severity of the hypoxia. This suggests that there may be a proportion of the population that utilizes an energy-conserving strategy for coping with acute hypoxia. There was nothing obviously different about these individuals with regard to characteristics such as RMR or body mass, although the uneven sample sizes between groups of fish showing avoidance and energy-conserving responses prevented a statistical analysis. It is also possible that these fish were responding to a reduced motivation to forage in response to a hypoxia-induced reduction in appetite, especially if these individuals possessed a reduced aerobic scope (Chabot & Claireaux 2008).

Although this study included animals encompassing a relatively small size range, there were notable relationships between body size and behaviour that needed to be considered to isolate the effects of RMR on behaviour, with larger fish having higher risk scores in both moderate and severe hypoxia. While it is possible that smaller individuals are more physiologically tolerant to hypoxia (Robb & Abrahams 2003), other authors have argued that, because of the similar allometric scaling of metabolic rate and gill surface area in fishes, body size should have little physiological effect on hypoxia tolerance (Nilsson & Östlund-Nilsson 2008). In the latter case, the results of the present study would suggest that smaller fish are more willing to suffer the physiological cost of remaining in a hypoxic zone if the associated reduction in activity leads to increased security from predation (Krause *et al.* 1998; Sloman *et al.* 2006).

If metabolic rate is correlated with growth rate during the early life stages of fishes (Álvarez & Nicieza 2005; Bochdansky *et al.* 2005), this could cause a trade-off between growth and hypoxia tolerance. Individuals with higher metabolic rates may grow faster given the right environmental conditions (Álvarez & Nicieza 2005; Bochdansky *et al.* 2005), but may also be less tolerant of hypoxia, which could make them more susceptible to predation due to hypoxia-associated
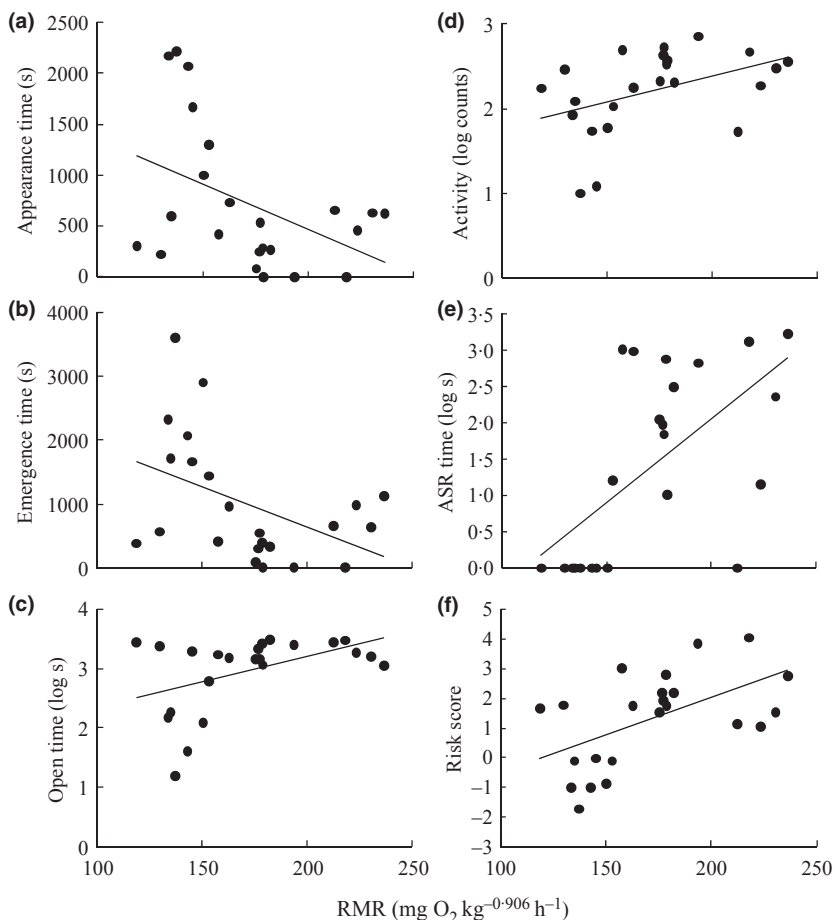
Fig. 4. The relationship between mass-corrected routine metabolic rate (RMR) and risk-taking during severe hypoxia in European sea bass. There were no significant relationships between RMR and behaviour observed in normoxia or moderate hypoxia (data not shown). Significant linear least-squares regressions in the panels are as follows: (a) appearance time = $2243 \cdot 81 - 8 \cdot 90$ (RMR), $r^2 = 0 \cdot 192$, $P = 0 \cdot 036$; (b) emergence time = $3173 \cdot 26 - 12 \cdot 63$ (RMR), $r^2 = 0 \cdot 198$, $P = 0 \cdot 034$; (c) log open time = $1 \cdot 494 + 0 \cdot 009$ (RMR), $r^2 = 0 \cdot 208$, $P = 0 \cdot 029$; (d) log activity = $1 \cdot 143 + 0 \cdot 006$ (RMR), $r^2 = 0 \cdot 188$, $P = 0 \cdot 039$; (e) log aquatic surface respiration time = $-2 \cdot 577 + 0 \cdot 023$ (RMR), $r^2 = 0 \cdot 380$, $P = 0 \cdot 002$; (f) risk score = $-3 \cdot 007 + 0 \cdot 025$ (RMR), $r^2 = 0 \cdot 300$, $P = 0 \cdot 007$. See Table 1 for the full results of the statistical models.
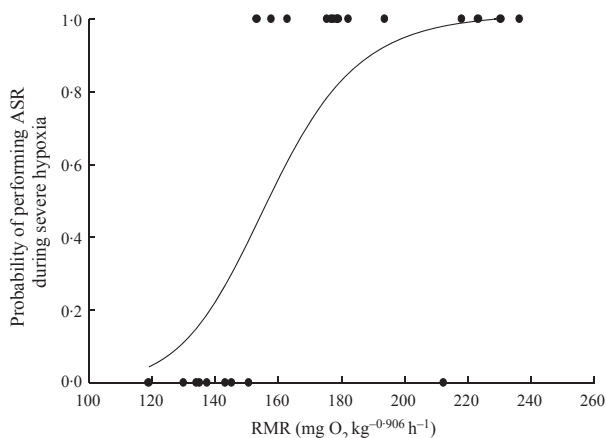

Fig. 5. The probability that an individual would perform aquatic surface respiration (ASR) after a simulated predator attack during severe hypoxia. The probability (P) of performing ASR at any given value for routine metabolic rate (RMR) was calculated according to the equation $P = 1/(1 + e^{-z})$, where $z = -11 \cdot 27 + 0 \cdot 072$ (RMR), Wald chi-square = $5 \cdot 74$, $P = 0 \cdot 017$.

increases in activity. Future research could examine the importance of adaptation for tolerance of environmental hypoxia as a factor constraining growth rates in young fishes. There could also be trade-offs associated with social status

and hypoxia tolerance in species that form dominance hierarchies. For example, individuals with higher metabolic rates have been found to be more dominant (Yamamoto, Ueda & Higashi 1998; Mccarthy 2001), but the present study suggests they could also be more vulnerable to hypoxia or experience increased predation during hypoxic episodes. Alternatively, subordinate fish can experience stress-induced increases in metabolic rate that could reduce their hypoxia tolerance, at least temporarily (Sloman *et al.* 2000; Millidine, Metcalfe & Armstrong 2009). It has previously been observed that juvenile European sea bass held in a common tank with abundant food do not form dominance hierarchies (Di-Poï *et al.* 2007), and so social status is unlikely to have influenced the results of the present study. Dominance hierarchies could form in situations where food availability is more limited, however, and so this is another potential area for further study.

The exposure of the same animals to three different levels of oxygen availability provides information on how individual behaviour changes across an environmental gradient in relation to variation in physiological traits and is therefore more relevant to the study of animal personalities and behavioural plasticity than if different fish had been used for each treatment (Dingemanse *et al.* 2009). A concern associated with this approach, however, is that individuals may habituate to simulated attacks during exposures to multiple treatments. In the current study, the order of treatments was

balanced among individuals, and notably, there were no significant effects of treatment order on any behaviour, at any level of oxygenation (see Results). Further, although habituation to prolonged or repeated simulated threats can occur in fish (e.g. Magurran & Girling 1986), attacks in the current study were rapid and fish would have been exposed a maximum of only twice previously. In nature, any fish that stopped responding after only one or two unsuccessful attacks from a predator over a 24–48 h period would be easily captured in the future. Another potential issue is that some animals could have acclimated to hypoxia during the study. Again, this is unlikely, as the major contributor to increased swimming activity in hypoxia – ASR – is a chemoreflex driven by oxygen-sensitive receptors on the gills or head area (Shingles *et al.* 2005). The intensity of this response depends on the stimulation of these receptors and is not likely to be influenced by a transient hypoxic episode 24–48 h earlier.

In conclusion, most individuals showed an increase in activity in response to acute hypoxia. The fact that almost all fish showed an increase in activity is significant, because it suggests that in the wild, acute hypoxic episodes could increase the risk of predation compared to normoxia. Perhaps more important, however, is that there was substantial variation in the magnitude of the response to hypoxia and that this variation was related to metabolic demand. In a broader context, the results of this study suggest that the effects of RMR on individual behaviour and aspects of animal personality are situation and context dependent, in that exposure to a stressor such as severe hypoxia appears to increase the influence of RMR on risk-associated behaviours.

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