CORRECTION

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RESEARCH ARTICLE

To boldly gulp: standard metabolic rate and boldness have context-dependent influences on risk-taking to breathe air in a catfish

David J. McKenzie[1,2,*], Thiago C. Belão[3], Shaun S. Killen[4] and F. Tadeu Rantin[1]

ABSTRACT

The African sharptooth catfish *Clarias gariepinus* has bimodal respiration, it has a suprabranchial air-breathing organ alongside substantial gills. We used automated bimodal respirometry to reveal that undisturbed juvenile catfish (N=29) breathed air continuously in normoxia, with a marked diurnal cycle. Air breathing and routine metabolic rate (RMR) increased in darkness when, in the wild, this nocturnal predator forages. Aquatic hypoxia (20% air saturation) greatly increased overall reliance on air breathing. We investigated whether two measures of risk taking to breathe air, namely absolute rates of aerial O_2 uptake ($\dot{M}_{O_2,air}$) and the percentage of RMR obtained from air (%$\dot{M}_{O_2,air}$), were influenced by individual standard metabolic rate (SMR) and boldness. In particular, whether any influence varied with resource availability (normoxia versus hypoxia) or relative fear of predation (day versus night). Individual SMR, derived from respirometry, had an overall positive influence on $\dot{M}_{O_2,air}$ across all contexts but a positive influence on %$\dot{M}_{O_2,air}$ only in hypoxia. Thus, a pervasive effect of SMR on air breathing became most acute in hypoxia, when individuals with higher O_2 demand took proportionally more risks. Boldness was estimated as time required to resume air breathing after a fearful stimulus in daylight normoxia (T_{res}). Although T_{res} had no overall influence on $\dot{M}_{O_2,air}$ or %$\dot{M}_{O_2,air}$, there was a negative relationship between T_{res} and %$\dot{M}_{O_2,air}$ in daylight, in normoxia and hypoxia. There were two T_{res} response groups, 'bold' phenotypes with T_{res} below 75 min (N=13) which, in daylight, breathed proportionally more air than 'shy' phenotypes with T_{res} above 115 min (N=16). Therefore, individual boldness influenced air breathing when fear of predation was high. Thus, individual energy demand and personality did not have parallel influences on the emergent tendency to take risks to obtain a resource; their influences varied in strength with context.

KEY WORDS: Bimodal respiration, Energy metabolism, Hypoxia, Personality, Respiratory partitioning, Risk-taking

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INTRODUCTION

In many animal species, individuals show wide individual variation in traits of both energy metabolism and personality (Burton et al., 2011; Careau et al., 2008; Sih et al., 2012). It has been argued that this variation persists because it shapes life-history trade-offs between production (growth, maturation, reproduction) and mortality (Biro and Stamps, 2008; Burton et al., 2011; Killen et al., 2011; Quinn et al., 2012; Sih et al., 2012; Stamps, 2007). Foraging has received attention because, although it brings advantages for energy acquisition, it increases risk of predation (Anholt and Werner, 1998; Biro et al., 2004; Mangel and Stamps, 2001). Variation in the intensity of such risky behaviour could reflect physiology, with individuals with higher metabolic rate being able, or driven, to forage more (Killen et al., 2011, 2013). It could also reflect personality, with bolder individuals willing to accept greater risks to secure energetic benefits (Conrad et al., 2011; Sih et al., 2012; Wolf and Weissing, 2012). As variation in both sets of traits would contribute to a similar life-history trade-off, it has been predicted that correlational selection should co-adapt physiology and temperament: animals with higher basal oxygen demands should be bolder (Biro and Stamps, 2010; Careau and Garland, 2012; Jenjan et al., 2013; Réale et al., 2010; Stamps, 2007). There is evidence, however, that reality is more complex and that the relative strength of these drivers of risk-taking can vary with context, such as resource availability or perceived risks of predation (Frost et al., 2013; Killen et al., 2011, 2012, 2013; Thomson et al., 2012).

Air-breathing fishes are interesting models to investigate how individual energy metabolism and personality influence risk-taking to obtain a key resource, oxygen. Oxygen has low solubility in water and aquatic habitats are prone to episodes of hypoxia (Diaz and Breitburg, 2009; Rahn, 1966; Randall, 1982). Many fish species have evolved a capacity to sustain aerobic metabolism by gulping oxygen-rich atmospheric air, to store in air-breathing organs (ABOs) from which oxygen diffuses into the blood (Graham, 1997; Johansen, 1968, 1970; Randall et al., 1981). The species are all 'bimodal' breathers that possess both gills and an ABO, and they differ in their relative reliance upon obtaining oxygen from water versus air (Graham, 1997). Although a capacity to gulp air might seem advantageous, the adaptations are relatively rare among fishes and, it has been argued, one major reason is that rising to the water surface brings significant risk of predation (Chapman and Mckenzie, 2009; Kramer et al., 1983; Smith and Kramer, 1986). It follows that, in bimodal fishes, risk-taking to obtain atmospheric air may be driven by an individual's oxygen demand but, at the same time, the tendency to surface may also reflect boldness. Context has profound effects on air breathing in bimodal fishes. A reduced availability of resources – aquatic hypoxia – is a profound stimulant of air breathing (Chapman and McKenzie, 2009; Graham, 1997; Johansen, 1968; Lefevre et al., 2014a,b) but surfacing is

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List of symbols and abbreviations

ABO	air-breathing organ
CV	coefficient of variation
LMEM	linear mixed-effect model
M_b	body mass
$\dot{M}_{O_2,air}$	absolute rate of O_2 uptake from air
$\%\dot{M}_{O_2,air}$	percentage of SMR obtained from air
$\dot{M}_{O_2,water}$	absolute rate of O_2 uptake from water
RMR	routine metabolic rate
SMR	standard metabolic rate
T_{res}	time to resume air breathing after a simulated predator attack

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inhibited if fishes perceive a risk of predation (Chapman and McKenzie, 2009; Shingles et al., 2005; Smith and Kramer, 1986).

The African sharptooth catfish *Clarias gariepinus* uses a suprabranchial chamber as an ABO. It also possesses substantial gills and is a 'facultative' air breather, meaning that, if denied access to the surface in normoxic water, it can maintain routine metabolic rate (RMR) by gill ventilation alone. Aquatic hypoxia stimulates profound increases in air breathing (Belão et al., 2011). It is a nocturnally active hunter; juveniles seek cover in daylight, apparently to avoid detection by predators (Britz and Pienaar, 1992; Bruton, 1979; Hecht and Pienaar, 1993). Although the catfish can meet routine oxygen requirements by gill ventilation in well-aerated water, it will spontaneously gulp air at irregular intervals (Belão et al., 2011). It has been suggested that such aperiodic air gulping might be a reflex driven by peripheral oxygen chemoreceptors (Milsom, 2012; Shelton et al., 1984) and therefore could reflect individual oxygen demand. Given, however, that the catfish is a facultative air breather (Belão et al., 2011), this risky surfacing in normoxia might also be driven by individual boldness, perhaps to increase the flux of oxygen for aerobic activities such as foraging, digestion and growth.

In a population of individually tagged juvenile catfish (*N*=29), automated bimodal respirometry (Lefevre et al., 2016) revealed continuous air breathing in normoxia, with a marked diurnal cycle. Air breathing and RMR increased during the night when, in the wild, the species forages in darkness. Aquatic hypoxia (20% air saturation) markedly increased proportional reliance on air breathing, contributing more to RMR throughout the diurnal cycle. We then investigated the general hypothesis that individual variation in taking risks to gulp air would show dependence on both standard metabolic rate (SMR) and intrinsic boldness. We investigated two slightly contrasting specific hypotheses: (1) that SMR and boldness are correlated and will exert parallel influences on risk taking to breathe air, or (2) that they act independently and their influences differ in strength with prevailing context. We investigated the influence of resource availability by comparing normoxia with aquatic hypoxia, and the influence of perceived risk of predation by comparing periods when fish were more visible to predators (day versus night).

MATERIALS AND METHODS
Animals
Feral wild-caught juvenile *Clarias gariepinus* (Burchill 1822), with a mass of approximately 250 g, were obtained from a farm in São Paulo state and transported to the Department of Physiological Sciences, Federal University of São Carlos (São Carlos, SP, Brazil). There, they were maintained together in a single 1 m³ tank supplied with well water at 25±1°C under a natural photoperiod and fed commercial feed daily at 2% body mass day⁻¹, for 6 weeks. Animals were then tagged (with a passive integrated transponder or PIT) into the dorsal epaxial muscle under mild anaesthesia

(0.1 g l⁻¹ benzocaine), for individual identification, after which they recovered in routine holding conditions for 1 week before experiments. All experiments were performed at 25°C. Experiments were approved by the Ethics in Animal Experimentation Committee (CEUA) of the Federal University of São Carlos, according to Federal Law 11.794 (authorisation no. 067/2011).

Bimodal respirometry
Intermittent stopped-flow respirometry (Steffensen, 1989) modified for bimodal breathers (Lefevre et al., 2016) was used to measure RMR and respiratory partitioning over 24 h in both normoxia and aquatic hypoxia (4 kPa P_{O_2}, ~20% air saturation). The tagged catfish were exposed to aquatic conditions on separate occasions with half of the animals exposed to normoxia first, half to hypoxia, with at least 96 h recovery between exposure, during which they were returned to standard holding conditions. Catfish were fasted for 24 h prior to measurements in either condition, then individuals were transferred gently to respirometers without air exposure, to minimise effects of handling. There were four respirometer chambers immersed in well-aerated water in an outer bath (1 m² surface area, 21 cm water depth) with the entire setup screened behind tarpaulin so that routine air-breathing behaviour was not inhibited by fear of human presence (McKenzie et al., 1991, 2007; Shingles et al., 2005). Fish were placed in the respirometer in the evening between 18:00 h and 20:00 h and fish were then allowed 16 h to recover from handling. Collection of respirometry data started the following morning and continued with the animals undisturbed for 24 h.

The bimodal respirometers were partially immersed in the outer bath, with an underwater water phase of volume 5.72 l and an air phase that projected above the water surface with a volume of ~200 ml. For each respirometer, the exact volume of the air phase was derived from the effects on O_2 partial pressure of a 20 ml bolus of 100% N_2 delivered into the sealed space. The intermittent stopped-flow technique alternated two periods within a 15 min cycle. In the first, water and air chambers were closed to the exterior for 10 min, so that O_2 uptake from both phases could be recorded (see below). The alternate period was when the two phases, aquatic and aerial, were flushed simultaneously for 5 min to replenish O_2 levels. Aquatic hypoxia was created and controlled as described previously, with corrections made for rates of passive diffusion of O_2 from air to water (McKenzie et al., 2007, 2012). Details of the system are provided in Lefevre et al. (2016).

Oxygen levels in the water and air were measured with an optode system (OXY-10 Micro, Pre-Sens Precision Sensing GmbH, www.presens.com), using eight channels such that four individuals were measured for their metabolic rate at any one time. Optodes were positioned to sample water and air for each respirometer, with all data stored on a PC using the manufacturer's software. Absolute rates of O_2 uptake from air ($\dot{M}_{O_2,air}$) and water ($\dot{M}_{O_2,water}$) were calculated (in mmol O_2 kg⁻¹ h⁻¹) for each 15 min respirometry cycle, based upon the decline in O_2 content in each phase during the closed period, as described previously (McKenzie et al., 2007, 2012). The $\dot{M}_{O_2,water}$ and $\dot{M}_{O_2,air}$ were summed to calculate RMR. The proportion of RMR derived from air breathing was calculated as the percentage of $\dot{M}_{O_2,air}$. The $\dot{M}_{O_2,air}$, $\dot{M}_{O_2,water}$ and the resultant RMR and $\%\dot{M}_{O_2,air}$ measures, were averaged over four sequential respirometry cycles to provide hourly means. These were averaged for the experimental population to reveal general diurnal patterns over 24 h.

Individual variation in air-breathing behaviour
The hourly values for the absolute rates of oxygen uptake from air, and the proportion of metabolism derived from aerial respiration, were averaged for each individual from 07:00 h to 18:00 h for daylight and from 19:00 h to 06:00 h for hours of darkness. This was repeated for normoxia and hypoxia.

Standard metabolic rate
The SMR was estimated by the quantile method (Dupont-Prinet et al., 2010; Chabot et al., 2016). This assumes that a certain proportion of all measured rates of RMR are below true SMR because of temporal variability and possible measurement errors. The quantile splits the dataset into the

q smallest and the $1-q$ largest values, where q is a proportion chosen by the experimenter. Here, q was fixed at 0.12 such that 12% of values fell below true SMR (Chabot et al., 2016). All measures of RMR were considered for each individual, both in normoxia and hypoxia, hence four measures per hour over 48 h, or 192 measures.

Boldness

Boldness was evaluated as the time required to resume air breathing (T_{res}) after a fearful stimulus, delivered when air breathing was especially risky but not physiologically essential: daylight normoxia. Following the respirometry in normoxia, the screen covering the respirometers was lifted for 1 min and each fish was disturbed by knocking sharply on the lid of their box 10 times, causing them to retreat to the bottom of the water phase. The T_{res} was taken as the time, in minutes, for the individual to resume oxygen uptake from air (Chapman et al., 2011). This represents the propensity to resume a risk-taking behaviour in a potentially dangerous environment (Huntingford et al., 2010; Killen et al., 2011, 2012). This was always performed between 14:00 h and 15:00 h to avoid confounding effects of diurnal rhythms in activity or air breathing.

T_{res} was evaluated for evidence of a bimodal distribution, indicative of 'bold' versus 'shy' phenotypes (Frost et al., 2013; Huntingford et al., 2010; Thomson et al., 2012) because such typing into personalities (or 'coping styles') can be used to inform interpretation of individual variation in phenotypic traits in fishes (MacKenzie et al., 2009; Rey et al., 2013, 2015). The T_{res} data were inspected visually for evidence of two groups, one with relatively short T_{res} indicating bold individuals, and a second with relatively longer T_{res} indicating shy individuals (Frost et al., 2013; Huntingford et al., 2010; Thomson et al., 2012).

Data analysis and statistics

Statistics were performed with SPSS Statistics v17.0 (www.ibm.com/software/analytics/spss). The level of significance for all tests was $\alpha=0.05$. To understand the variables that influenced air-breathing behaviour, linear mixed-effect models (LMEMs) were constructed with either absolute ($\dot{M}_{O_2,air}$) or proportional ($\%\dot{M}_{O_2,air}$) rates of air breathing as dependent variables, and fish mass, O_2 availability (normoxia versus hypoxia), time of day (day versus night), SMR and T_{res} as fixed effects. Because each fish was tested under multiple conditions, fish identity was included as a random effect in the models, with time of day and oxygen availability treated as repeated factors. Initial models included all two-way interactions between SMR/T_{res} and each context of O_2 availability and time of day. Non-significant interactions were sequentially dropped and the models re-run. Model assumptions of homogeneity, linearity and normality of residuals were verified by inspection of residuals–fits plots. T_{res} was log transformed prior to use in the model. When apposite, dependence of the air breathing upon SMR or T_{res} was demonstrated visually using ordinary least-squares regression under each time of day and level of oxygenation.

Following classification of individuals by their T_{res}, namely resuming air breathing either relatively fast (bold) or relatively slowly (shy), a separate LMEM was constructed to focus on daylight $\dot{M}_{O_2,air}$ and $\%\dot{M}_{O_2,air}$ as dependent variables. Fish mass, O_2 availability (normoxia versus hypoxia) and T_{res} type (bold versus shy) were fixed effects. Once again, fish identity was included as a random effect in the models, with oxygen availability treated as a repeated factor.

RESULTS

Data were collected for 29 individuals in normoxia, with a mean (±s.d.) mass of 292±60 g. Among these, data were collected for 24 fish in aquatic hypoxia, with a mean mass of 300±58 g.

General patterns of respiratory partitioning

In normoxia, the respirometry data showed a clear diurnal pattern in routine metabolism. Mean hourly RMR was relatively low during the day and much higher during the night (Fig. 1A). There were

measurable rates of mean O_2 uptake from air ($\dot{M}_{O_2,air}$) throughout the diurnal cycle, but these increased markedly in darkness, contributing to a marked increase in RMR. The mean rates of uptake from water ($\dot{M}_{O_2,water}$) were considerably less variable over the diurnal cycle. The mean SMR of the population was always below mean $\dot{M}_{O_2,water}$ in normoxia (Fig. 1A). This presumably indicates that the catfish could meet their O_2 demands for SMR from gill ventilation alone (Belão et al., 2011).

In aquatic hypoxia, there was still a clear diurnal rhythm in routine metabolism (Fig. 1B). The mean RMR was, however, visibly lower than in normoxia; this was linked to very low mean hourly rates of aquatic respiration, and $\dot{M}_{O_2,w}$ varied very little over the diurnal cycle (Fig. 1B). Furthermore, mean $\dot{M}_{O_2,w}$ was always below mean SMR in aquatic hypoxia, which indicates that the catfish would need to resort to air breathing to meet their basal O_2 demands.

Calculation of diurnal patterns of the percentage of routine metabolism that was due to O_2 uptake from air ($\%\dot{M}_{O_2,air}$) in normoxia and hypoxia (Fig. 2) confirmed that this showed a large increase at night, especially in normoxia, and also that there was an overall much greater proportional reliance on air breathing in hypoxia.
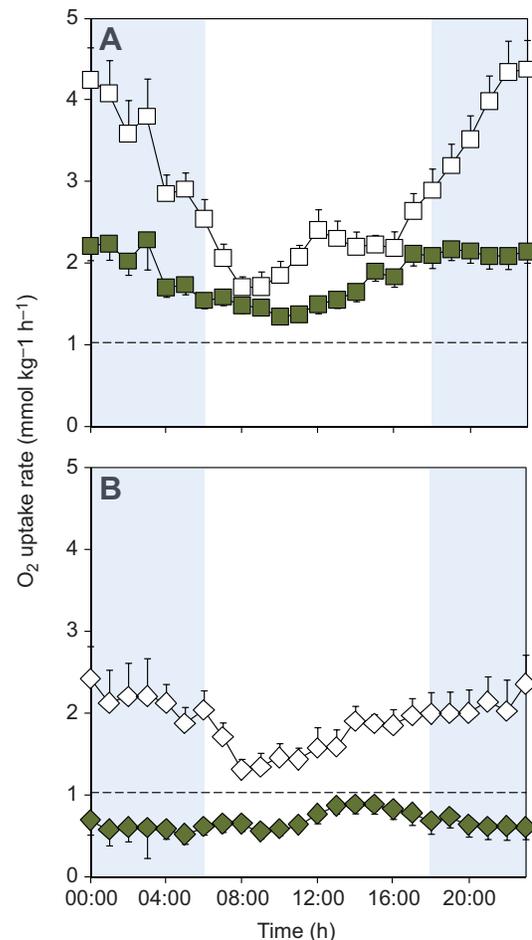
Fig. 1. Routine metabolic rate and aquatic oxygen uptake in juvenile *Clarias gariepinus*. Routine metabolic rate (white symbols) and aquatic oxygen uptake (green symbols) measured over 24 h in (A) normoxia (squares, N=29) or (B) aquatic hypoxia at 20% air saturation (diamonds, N=25). The difference between aquatic oxygen uptake and RMR is due to oxygen uptake from air. Shaded blue areas represent night-time hours. The dotted line is mean standard metabolic rate. Values are means±s.e.
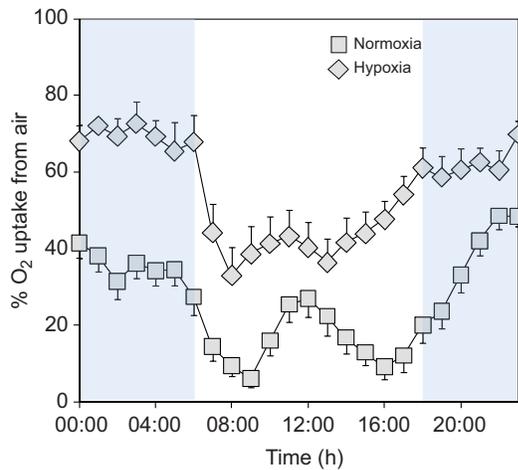
Fig. 2. The percentage of routine metabolic rate derived from air (%$\dot{M}_{O_2,air}$) over 24 h in juvenile *Clarias gariepinus*, in either normoxia (*N*=29) or aquatic hypoxia at 20% air saturation (*N*=25). The shaded blue areas represent night-time hours. Values are means±s.e.

Table 1 carries descriptive statistics for the two measures of risk-taking to breath air, namely $\dot{M}_{O_2,air}$ and %$\dot{M}_{O_2,air}$, which were calculated separately for daylight hours (07:00 h to 18:00 h) and night-time darkness (19:00 h to 06:00 h), in normoxia and then hypoxia. It also shows mean SMR. These traits all varied at least two-fold within the sample population.

Boldness as T_{res}

Table 1 carries descriptive statistics of the measure of boldness, T_{res}, which varied widely within the experimental population. There was evidence of a bimodal distribution (Fig. S1), whereby individual catfish either resumed air breathing in less than 75 min (*N*=13) or in more than 115 min (*N*=16). Two groups, with mean (±s.e.) T_{res} of 37±5 versus 181±14 min, were therefore designated as bold versus shy phenotypes, respectively (Frost et al., 2013; Huntingford et al., 2010; Rey et al., 2013; Thomson et al., 2012).

Associations between SMR and T_{res}

There was no correlation between body mass (M_b) and SMR or T_{res}, over the small range in mass of the experimental population. Individual SMR and T_{res} were, however, negatively correlated

Table 1. Descriptive statistics of rates of oxygen uptake from air and percentage total metabolic rate derived from air by juvenile *Clarias gariepinus*

|	Statistic			
	N	mean±s.d.	CV	Range
Normoxia				
Day $\dot{M}_{O_2,air}$	29	0.52±0.41	0.791	0.00–1.43
Night $\dot{M}_{O_2,air}$	29	1.59±0.85	0.537	0.59–3.66
Day %$\dot{M}_{O_2,air}$	29	16.3±10.9	0.664	0.0–34.6
Night %$\dot{M}_{O_2,air}$	29	37.6±12.1	0.320	15.4–64.1
Hypoxia				
Day $\dot{M}_{O_2,air}$	25	0.98±0.52	0.528	0.36–2.84
Night $\dot{M}_{O_2,air}$	25	1.49±0.39	0.257	0.71–2.31
Day %$\dot{M}_{O_2,air}$	25	46.5±17.6	0.378	14.5–83.3
Night %$\dot{M}_{O_2,air}$	25	66.1±12.4	0.187	42.5–91.6
SMR	29	1.02±0.32	0.308	0.48–1.85
T_{res}	29	117±83	0.715	7–325

$\dot{M}_{O_2,air}$ and SMR are in mmol O_2 kg^{-1} h^{-1}; T_{res} in minutes. CV, coefficient of variation.

(Spearman Rank *R*=−0.391, *P*=0.036, *N*=29), such that catfish with higher basal O_2 demands resumed risky air breathing relatively more quickly after the fearful stimulus (Fig. 3). Interestingly, the bold and shy phenotypes did not distribute at opposing ends of this relationship. In fact, SMR did not differ between them, being 1.11±0.10 versus 0.97±0.08 mmol O_2 kg^{-1} h^{-1}, for bold and shy fish, respectively, with a very similar range of values (Fig. 3). It is noteworthy, however, that all of the fish with bold phenotypes had a T_{res} that fell below the line describing the least-squares linear correlation, while almost all of the fish with shy phenotypes were above it (Fig. 3). The mean residuals for bold fish were −73±8 min, significantly less than the residuals for shy fish, which were 60±13 min (*t*-test *P*<0.00001). That is, the bold fish were resuming air breathing an average of 73 min faster than would be predicted from their SMR, whereas the shy fish resumed air breathing an average of 60 min slower.

Influence of individual SMR and T_{res} on variation in risk-taking to breathe air

The LMEMs did not reveal that SMR and T_{res} co-varied in their influence upon $\dot{M}_{O_2,air}$ or %$\dot{M}_{O_2,air}$ (Tables 2 and 3). In terms of absolute rates of oxygen uptake from air, there was a significant dependence of $\dot{M}_{O_2,air}$ on time of day, water O_2 availability and SMR, but not upon T_{res} (Table 2). There were no interactions among the factors. The estimates of effects confirmed that the catfish breathed significantly less air during the daytime, with a significantly lower $\dot{M}_{O_2,air}$ than at night. In hypoxia, there was a general increase in $\dot{M}_{O_2,air}$ compared with normoxia. Across all these contexts, there was a positive dependence of $\dot{M}_{O_2,air}$ on SMR: individuals with higher basal O_2 demands had higher rates of aerial respiration (Table 2).

In terms of the percentage of total metabolic rate that was met by air breathing, the model revealed no significant main effects of time of day, oxygen availability, SMR or T_{res} on %$\dot{M}_{O_2,air}$ (Table 3). There were, however, significant interactions amongst these explanatory variables. Thus, there was a significant interaction between effects of O_2 availability and SMR, whereby an effect of
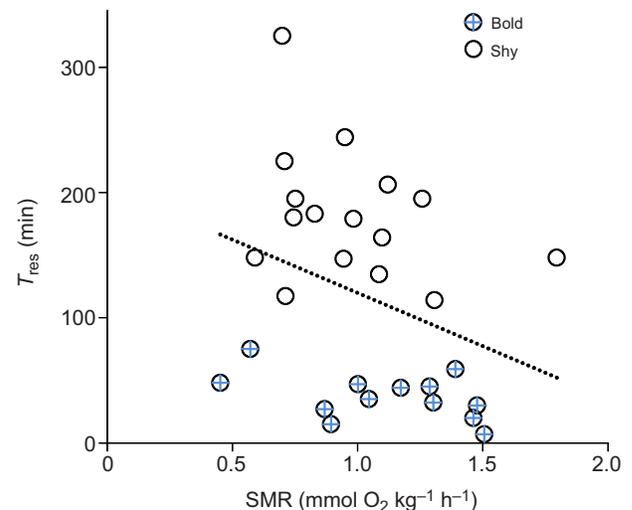
Fig. 3. Relationship between individual standard metabolic rate and time to resume air breathing after a simulated predator attack in juvenile *Clarias gariepinus*. Scatterplot of the relationship between SMR and time to resume air breathing after a simulated predator attack (T_{res}). There was a significant negative Spearman rank correlation (*R*=−0.391, *P*=0.036, *N*=29). The line is a least-squares linear fit. Fish with bold T_{res} phenotypes resumed air breathing in less than 75 min (see text for further details).

Table 2. Estimates of fixed effects from a linear mixed-effect model to evaluate the dependence of individual variation in rates of oxygen uptake from the air

A. Fixed effects

Source	Numerator d.f.	Denominator d.f.	F	P
Intercept	1	21.7	2.17	0.155
M_b	1	20.0	0.22	0.643
Water O_2 level	1	58.8	12.52	**<0.001**
Time of day	1	46.8	56.06	**<0.001**
SMR	1	22.2	13.58	**0.001**
log T_{res}	1	67.0	0.54	0.466

B. Estimates of fixed effects

Parameter	Estimate±s.e.	d.f.	t	P
Intercept	0.19±0.43	44.0	0.45	0.656
M_b	0.87±0.85	22.7	1.02	0.321
Water oxygen=hypoxia*	0.35±0.10	73.6	3.38	**0.001**
Time=day*	−0.66±0.09	48.9	−7.35	**<0.001**
SMR	0.61±0.17	22.2	3.68	**0.001**
log T_{res}	0.08±0.11	67.0	0.734	0.466

Rates of oxygen uptake from air were measured with bimodal respirometry for every hour over 24 h, either in normoxia (N=29 individuals) or in aquatic hypoxia (20% of air saturation, N=25 individuals). For each oxygen level, averages were calculated for each individual for either daylight hours (07:00 h–18:00 h) or darkness (19:00 h–06:00 h). SMR was assessed from the respirometry, T_{res} as the time in minutes to resume air breathing after a simulated predator attack in daylight normoxia. See text for further details. *Alternative parameter is set to zero because it is redundant. Parameters with significant effects are bold. M_b, fish body mass.

Table 3. Estimates of fixed effects from a linear mixed-effect model to evaluate the dependence of individual variation in percentage total metabolic rate derived from air

A. Fixed effects

Source	Num. d.f.	Den. d.f.	F	P
Intercept	1	23.7	5.88	**0.023**
M_b	1	22.5	1.06	0.314
Water O_2 level	1	66.4	3.30	0.074
Time of day	1	48.6	0.39	0.537
SMR	1	20.9	3.45	0.077
log T_{res}	1	24.0	0.63	0.436
Water O_2×SMR	1	66.5	5.18	**0.026**
Time of day×log T_{res}	1	48.4	7.23	**0.010**

B. Estimates of fixed effects

Parameter	Estimate±s.e.	d.f.	t	P
Intercept	20.9±14.0	32.7	1.50	0.144
M_b	23.5±22.8	22.5	0.62	0.537
Water O_2=hypoxia*	13.4±7.4	66.3	1.81	0.074
Time=day*	6.3±10.1	48.6	0.62	0.537
SMR	1.0±5.2	32.2	0.19	0.853
log T_{res}	4.0±4.4	35.9	0.92	0.366
Hypoxia×SMR*	15.7±6.9	66.5	2.28	**0.026**
Day×log T_{res} *	−13.8±5.1	48.4	−2.69	**0.010**

Rates of oxygen uptake from air and water were measured with bimodal respirometry for every hour over 24 h, either in normoxia (N=29 individuals) or in aquatic hypoxia (20% of air saturation, N=24 individuals), to derive $\%\dot{M}_{O_2,air}$. For each oxygen level, averages were calculated for each individual for either daylight hours (07:00 h–18:00 h) or darkness (19:00 h–06:00 h). SMR was assessed from the respirometry, T_{res} as the time in minutes to resume air breathing after a simulated predator attack in daylight normoxia. The LMEM tested for interactions between water oxygen level and SMR, and between time of day and T_{res}. See text for further details. *Alternative parameter is set to zero because it is redundant. Significant effects are in bold. M_b, fish body mass.

individual basal O_2 demand on $\%\dot{M}_{O_2,air}$ was only statistically significant in aquatic hypoxia (Table 3). Regressions of $\%\dot{M}_{O_2,air}$ against SMR for the four context combinations (Fig. 4A,B) demonstrate a significant positive dependence of $\%\dot{M}_{O_2,air}$ on SMR in hypoxia only (Fig. 4B), for both times of day. That is, in hypoxia, individuals with higher basal O_2 demands tended to obtain proportionally more of their O_2 from the air.

The LMEM also revealed a significant interaction between T_{res} and time of day, whereby a significant dependence of $\%\dot{M}_{O_2,air}$ on individual T_{res} was found, but only during the daytime (Table 3). Regressions of $\%\dot{M}_{O_2,air}$ against T_{res} for the four different context combinations (Fig. 5A,B) revealed significant negative dependence of $\%\dot{M}_{O_2,air}$ on T_{res} during the day but not the night, in both normoxia and hypoxia. That is, during the daytime, individuals that had shorter T_{res} tended to obtain proportionally more of their O_2 from the air.

Influence of T_{res} phenotype on variation in risk taking to breathe air

The model did not reveal a significant dependence of daylight $\dot{M}_{O_2,air}$ on T_{res} phenotype (data not shown), but did reveal a dependence for daylight $\%\dot{M}_{O_2,air}$ (Table 4). The proportion of O_2 obtained from air was significantly greater in bold compared with shy phenotypes during the day, with no interaction with level of oxygen availability (Table 4). That is, further to the complex influences of individual SMR and T_{res} on $\%\dot{M}_{O_2,air}$, bold fish breathed proportionally more air than shy fish during daylight hours, in both normoxia and hypoxia.

DISCUSSION

The results support the general hypothesis that the tendency to take risks to gulp air depends upon both basal O_2 demand and intrinsic boldness in a fish with bimodal respiration. Context itself, namely

O_2 availability (normoxia versus hypoxia) or level of perceived risk (day versus night) had profound effects on air-breathing behaviour. The results provided apparently paradoxical support for both specific hypotheses because, although SMR and T_{res} were correlated, there was clear evidence of independent influences that varied in strength according to the context.

General patterns of respiratory metabolism and boldness

The marked circadian rhythm in RMR and air-breathing behaviour by *C. gariepinus* is consistent with reports of diurnal variation in activity and a pronounced nocturnal habit in this species (Britz and Pienaar, 1992; Bruton, 1979; Willoughby and Tweddle, 1978). In the wild, juvenile catfish emerge from cover at night, to forage (Bruton, 1979; Willoughby and Tweddle, 1978). Therefore, the overall increase in RMR and air breathing at night presumably reflects an increase in activity by the animals (Lefevre et al., 2014a, b), which is at least partly due to a reduced fear of detection by predators (Britz and Pienaar, 1992).

The fact that SMR was below $\dot{M}_{O_2,air}$ in aquatic normoxia confirms that, in well-aerated water, the species is able to meet and exceed basal O_2 demands by gill ventilation alone and is, indeed, a facultative air breather (Belão et al., 2011). In aquatic hypoxia, the overall increase in air breathing was expected (Belão et al., 2011) and the data demonstrate that the catfish needed to breathe air because aquatic respiration could no longer support their basal metabolic demands, mean $\dot{M}_{O_2,water}$ was consistently below mean SMR. The overall decline in RMR that was also visible in aquatic hypoxia, over the entire daily cycle when compared with normoxia,
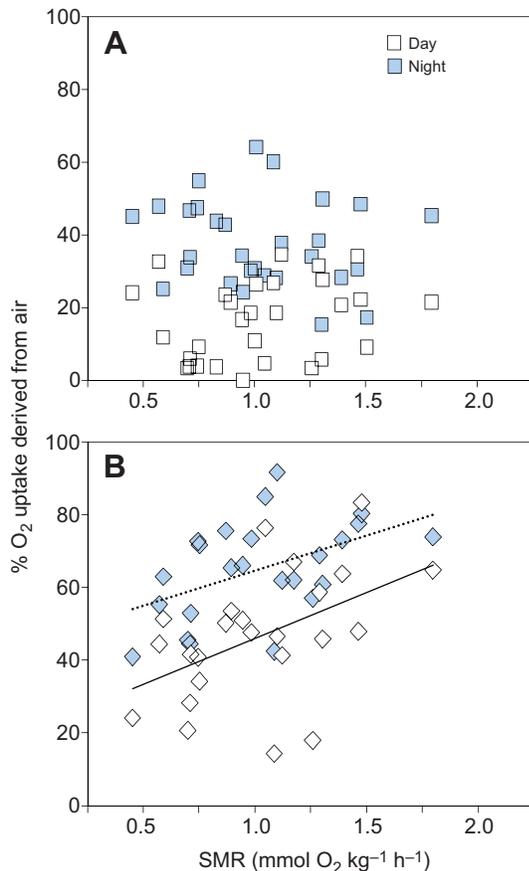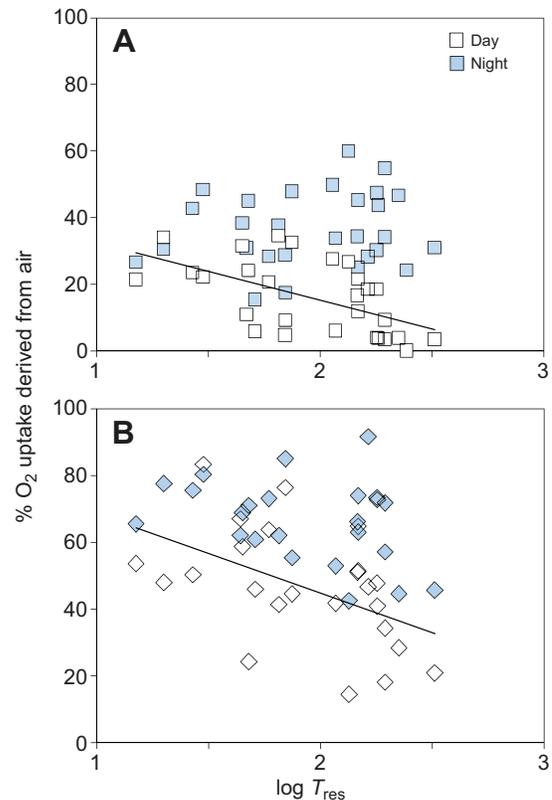
Fig. 4. The dependence of individual percentage of oxygen uptake obtained from air on standard metabolic rate of juvenile *Clarias gariepinus*. Scatterplots of the relationship between individual SMR and % $\dot{M}_{O_2,air}$ in (A) normoxia (N=29) and (B) aquatic hypoxia at 30% air saturation (N=24). For normoxic daytime, %$\dot{M}_{O_2,air}$=7.64+8.45×SMR, R^2=0.063, P=0.187; for normoxic night-time, %$\dot{M}_{O_2,air}$=43.90–6.11×SMR, R^2=0.030, P=0.395; for hypoxic daytime, %$\dot{M}_{O_2,air}$=20.86+25.22×SMR, R^2=0.318, P=0.017 (B, solid line); for hypoxic night-time, %$\dot{M}_{O_2,air}$=45.42+19.27×SMR, R^2=0.231, P=0.017 (B, dotted line).

Fig. 5. The dependence of individual percentage of oxygen uptake obtained from air on time to resume air breathing after a simulated predator attack of juvenile *Clarias gariepinus*. Scatterplots of the relationship between log T_{res} and %$\dot{M}_{O_2,air}$ in (A) normoxia (N=29) or (B) aquatic hypoxia at 25% air saturation (N=24) in the day or night. For normoxic daytime, %$\dot{M}_{O_2,air}$=49.83–17.28×log T_{res}, R^2=0.285, P=0.002 (A, solid line); for normoxic night-time, %$\dot{M}_{O_2,air}$=27.08+4.91×log T_{res}, R^2=0.013, P=0.431; for hypoxic daytime, %$\dot{M}_{O_2,air}$=92.23–23.71×log T_{res}, R^2=0.206, P=0.015 (B, solid line); for hypoxic night-time, %$\dot{M}_{O_2,air}$ =83.46–9.03×log T_{res}, R^2=0.028, P=0.211.

whereby SMR and T_{res} were correlated but also exerted independent and context-dependent effects on risk taking.

T_{res} is correlated with SMR but also possesses an independent element of boldness

The significant negative correlation between SMR and T_{res} is consistent with proposals that individuals with higher basal oxygen demands should also be bolder (Biro and Stamps, 2010; Careau et al., 2008; Réale et al., 2010). It would be predicted, therefore, that catfish with the shortest T_{res}, with bold phenotypes, would have a significantly higher mean SMR than the shy fish. Instead, they had a similar overall range of values for basal metabolism. Within the overall correlation between SMR and T_{res} there was, however, clear evidence that the bold fish were indeed intrinsically more risk-prone than shy fish, because the former resumed air breathing relatively sooner than would be predicted from their SMR.

One way of interpreting these results is that there are two mechanisms at work. The general correlation of SMR and T_{res} may, in fact, be a consequence of a chemoreflexive respiratory drive to gulp air in the catfish. As discussed in detail below, the overall dependence of $\dot{M}_{O_2,air}$ on SMR reveals such a chemoreflexive drive, whereas the context-dependent influence of SMR on %$\dot{M}_{O_2,air}$ in hypoxia confirms it. The second mechanism appears to involve an independent and intrinsic difference in boldness, causing individuals to resume air breathing either relatively faster or

has been observed previously in facultative air-breathing fishes and may reflect a decline in spontaneous activity levels (Lefevre et al., 2012, 2014a,b; McKenzie et al., 2012).

Our measure of boldness – the time to resume air breathing after a fearful stimulus – is similar to previous studies that have, for example, evaluated boldness as the time required to emerge from cover into a potentially threatening environment (Huntingford et al., 2010; Killen et al., 2011, 2012; MacKenzie et al., 2009). T_{res} should be a valid measure of intrinsic boldness because our respirometry data confirm that the catfish is a facultative air breather in aquatic normoxia. By definition, therefore, the animals should not, in theory, have had any physiological drive to take risks to obtain oxygen from air.

The evidence of a bimodal distribution in T_{res}, with bold and shy phenotypes, is consistent with previous studies on fishes, which have used various tests, such as emergence from cover or response to novel objects, to classify phenotypes as bold or shy (Frost et al., 2013; Huntingford et al., 2010; Rey et al., 2013; Thomson et al., 2012). Consideration of this apparent difference in personality improved our ability to interpret other elements of individual variance in our data (MacKenzie et al., 2009; Rey et al., 2013, 2015). In particular and as discussed below, the apparent paradox

Table 4. Estimates of fixed effects from a linear mixed-effect model to evaluate the dependence of individual variation in percentage total metabolic rate derived from air during daylight hours

A. Fixed effects

Source	Numerator d.f.	Denominator d.f.	F	P
Intercept	1	27.3	13.91	**<0.001**
M_b	1	24.1	0.04	0.950
Water O_2 level	1	24.1	64.14	**<0.001**
T_{res} phenotype	2	25.1	4.29	**0.025**

B. Estimates of fixed effects

Parameter	Estimate±s.e.	d.f.	t	P
Intercept	11.3±8.4	24.1	1.35	0.191
M_b	1.8±28.1	24.1	0.06	0.950
Water O_2=hypoxia*	30.2±3.7	24.1	8.01	**<0.001**
T_{res} phenotype=bold*	9.8±3.5	24.6	2.78	**0.010**

Rates of oxygen uptake from air and water were measured with bimodal respirometry for every hour over 24 h, either in normoxia (N=29 individuals) or in aquatic hypoxia (20% of air saturation, N=24 individuals), to derive %$\dot{M}_{O_2,air}$. For each oxygen level, averages were calculated for each individual for daylight hours (07:00 h–18:00 h). T_{res} is the time in minutes to resume air breathing after a simulated predator attack in daylight normoxia, where fish with bold phenotypes resumed in less than 75 min (N=13), shy fish in greater than 115 min (N=16). The LMEM tested for interactions between water oxygen level and boldness phenotype. See text for further details.
*Alternative parameter is set to zero because it is redundant. Significant effects are bold. M_b, fish body mass.

slower than their SMR and respiratory drive would predict. This difference in personality was then linked to clear differences in risk-taking during the daytime, as discussed in detail further below.

Individual standard metabolic rate exerts a pervasive influence on air breathing

The fact that the absolute rates of O_2 uptake from air were dependent upon individual SMR, across all contexts, was unexpected. A facultative air-breathing fish should not, in theory, have a physiological drive to take risks to gulp air in normoxia. One possible explanation for this apparent inconsistency would be that the surfacing responses are, at least in part, inescapable neurophysiological reflexes (Lefevre et al., 2014a). In freshwater fishes, air breathing is believed to have evolved as a response to aquatic hypoxia (Graham, 1997; Johansen, 1968, 1970; Randall et al., 1981). Hypoxic surfacing responses are reflexes, stimulated by oxygen-sensitive chemoreceptors in the gills, which monitor water and blood O_2 levels (Chapman and McKenzie, 2009, 1991; Milsom, 2012). Although these reflex circuits may have evolved because they allow bimodal fishes to increase air breathing and regulate their metabolic rate in aquatic hypoxia, it is conceivable that they also cause them to perform surfacing responses at irregular intervals in normoxia (Lefevre et al., 2014a). It has been proposed that the aperiodic gulping of air by animals with bimodal respiration is indeed a chemoreflex, whereby blood oxygen levels decline progressively over time after an air breath, this is monitored by oxygen-sensitive chemoreceptors in the vasculature that eventually stimulate the animal to surface and take another gulp (Milsom, 2012; Shelton et al., 1984). The overall positive relationship between SMR and $\dot{M}_{O_2,air}$ could be explained by such a physiological mechanism: if the decline in blood oxygen occurs more rapidly in individuals with higher basal metabolic demands, this will stimulate more reflex air-breathing responses.

This physiological basis to air-breathing responses was almost certainly the mechanism underlying the dependence of %$\dot{M}_{O_2,air}$ on

SMR in aquatic hypoxia. It seems logical to presume that, the higher an individual's SMR, the more their metabolic equilibrium was challenged in aquatic hypoxia (Killen et al., 2012, 2013). Chemoreflexive responses would presumably be stimulated relatively more profoundly and frequently in individuals that were consuming oxygen from their blood at the highest rates.

This evidence of a pervasive chemoreflexive drive to breathe air, even in normoxia, could then explain why SMR and T_{res} were correlated. Individuals with higher SMR would have a stronger chemoreflexive drive to breathe air and, therefore, would exhibit a reflex response more rapidly after the fearful stimulus, irrespective of any influence of their personality.

Individual boldness exerts a context-dependent influence on air breathing

The current study provides the first evidence that personality can influence risky air-breathing behaviour by an aquatic vertebrate. It is particularly interesting that this effect was context dependent and independent of any respiratory drive. Our measure of intrinsic boldness, T_{res}, was clearly linked to the proportion of O_2 obtained from air at a time of day when, in the wild, surfacing would be especially risky, because of the danger of visual detection by predators (Bruton, 1979; Willoughby and Tweddle, 1978). The effect of intrinsic boldness on risk taking was confirmed by the fact that bold T_{res} phenotypes breathed proportionally more air than fish with shy phenotypes during the daytime.

It is conceivable that choosing to surface and tap O_2-rich air may allow individuals to complete metabolic activities such as digesting meals more rapidly, and so sustain higher rates of foraging, energy flux and growth in their environment. This would be analogous to previous reports of bolder animals achieving faster growth via increased foraging rates (Biro et al., 2004). It is also conceivable that these air-breathing responses provided other benefits to bolder animals, such as improved maintenance of buoyancy (Hedrick and Jones, 1993) or even improved auditory sensitivity (Shao et al., 2014).

Conclusions and perspectives

The current data contribute to the ongoing debate regarding the relationships between physiological and behavioural traits, although they indicate that these are not simple. Although our finding that SMR and T_{res} were correlated is consistent with suggestions that selection should co-adapt physiology and temperament (Biro and Stamps, 2010; Careau and Garland, 2012; Réale et al., 2010; Stamps, 2007), in the current study, the correlation probably reflects our method of measuring boldness. Although we were able to show separate independent influences of metabolic demand and intrinsic boldness on risky air-breathing behaviour, future experiments in air-breathing fishes should utilise measures of boldness that are not confounded by chemoreflexive respiratory drive. This first attempt to disentangle the influences of physiology and personality on air breathing in an aquatic vertebrate highlights the complexities of designing experiments to evaluate causal relationships between energy metabolism and personality (Killen et al., 2013).

Chemoreflexes can be invoked to explain why individuals with high SMR had higher absolute rates of aerial respiration in all situations. This became most prominent during aquatic hypoxia, when the need to obtain atmospheric O_2 constrained risk-taking behaviour, forcing individuals with higher basal metabolic demands to surface relatively more frequently. The question remains as to why, if individual energy metabolism has such a pervasive

influence on risky air-gulping, animals with high SMR did not all have bold T_{res} phenotypes. Instead, independent effects of intrinsic boldness were observed during the daytime, including in normoxia when surfacing was seemingly non-essential, but potentially more risky. The relative influences of SMR and boldness were much more complex than might be predicted from correlational selection on a similar life history trade-off.

While the relative costs and benefits of foraging activity in relation to predation risk have been well studied (Biro and Stamps, 2008; Biro et al., 2004; Sih et al., 2012; Stamps, 2007), the trade-offs involved in access to other vital resources, such as oxygen for fishes, are little understood and require further investigation. The complex associations among energy demand, personality and the emergent tendency to take risks, indicate that cause–effect links between physiology and behaviour are dynamic and dependent upon context.

Supplementary information
Supplementary information available online at
http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.122903/-/DC1

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