

Fuel, fasting, fear: routine metabolic rate and food deprivation exert synergistic effects on risk-taking in individual juvenile European sea bass

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Summary

1. Individuals of the same species often exhibit consistent differences in metabolic rate, but the effects of such differences on ecologically important behaviours remain largely unknown. In particular, it is unclear whether there is a cause-and-effect relationship between metabolic rate and the tendency to take risks while foraging. Individuals with higher metabolic rates may need to take greater risks while foraging to obtain the additional food required to satisfy their energy requirements. Such a relationship could be exacerbated by food deprivation if a higher metabolic demand also causes greater mass loss and hunger.

2. We investigated relationships among metabolic rate, risk-taking and tolerance of food deprivation in juvenile European sea bass. Individual fish were tested for risk-taking behaviours following a simulated predator attack, both before and after a 7-day period of food deprivation. The results were then related to their routine metabolic rate (RMR), which was measured throughout the period of food deprivation.

3. The amount of risk displayed by individual fish before food deprivation showed no relationship with RMR. After food deprivation, however, the amount of risk among individuals was positively correlated with RMR. In general, most fish showed an increase in risk-taking after food deprivation, and the magnitude of the increase in risk-taking was correlated with the rate of individual mass loss during food deprivation, which was itself strongly correlated with RMR.

4. The observation that RMR was related to risk-taking behaviour after food deprivation, but not before, suggests that although RMR can influence risk-taking, the strength of the relationship is flexible and context dependent. The effects of RMR on risk-taking may be subtle or non-existent in regularly feeding animals, but may lead to variability in risk-taking among individuals when food is scarce or supply is unpredictable. This synergistic relationship between RMR and food deprivation could lead to an increased likelihood of being predated for individuals with a relatively high intrinsic energy demand during times when food is scarce.

Key-words: ecophysiology, foraging, teleost fish, physiological energetics

Introduction

Traditionally, studies of physiology have focused on the mean response of groups to particular treatments and ignored the significance of inter-individual variation within populations (Bennett 1987). Such variation may be important, however, because phenotypic diversity can be indicative of genetic diversity and the extent to which a population can

adapt to environmental change (Hayes & Jenkins 1997; Bolnick *et al.* 2003; Sears *et al.* 2009). Wide variation in certain traits may also reflect underlying behavioural or physiological trade-offs, with the costs or benefits of any phenotype being variable and dependent upon environmental factors (Mangel & Stamps 2001; Stamps 2007). Finally, studies of physiological diversity may reveal the proximate physiological causes of individual variation in behaviour or life-history characteristics (Williams 2008).

Several recent studies have documented wide, repeatable, inter-individual variation in metabolic rate across numerous animal taxa (e.g. McCarthy 2000; Broggi *et al.* 2007; Nespolo & Franco 2007; Sears *et al.* 2009), possibly stemming from

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intrinsic differences in morphology (e.g. relative sizes of metabolically active organs; Selman *et al.* 2001) or physiology among individuals (e.g. rates of protein turnover: Carter, Hoolihan & Owen 1998; leakiness of mitochondrial membranes: Brand 1990). The ecological significance of inter-individual variation in metabolic rate is not well understood, but appears to be linked to numerous physiological and behavioural traits of potential ecological importance (Biro & Stamps 2010). In ectotherms, for instance, individuals with higher metabolic rates may have an increased scope for growth (Priede 1985; Álvarez & Nicieza 2005), or be more able to out-compete conspecifics for territories or other resources (Metcalf, Taylor & Thorpe 1995; Cutts, Metcalfe & Taylor 1998).

The metabolic rate of an animal could also be linked to willingness to take risks while foraging (Finstad *et al.* 2007; Careau *et al.* 2008; Huntingford *et al.* 2010). Foraging in many animals involves an increase in activity that can make them more obvious to predators (e.g. Skelly 1994; Martel & Dill 1995; Biro *et al.* 2006; Sundt-Hansen *et al.* 2009) or reduce their vigilance towards predator attacks (Godin & Smith 1988; Watson, Aebischer & Cresswell 2007). It is possible that increased energetic requirements in individuals with a higher metabolic demand may require them to forage more often or take more risks to achieve a higher rate of food intake (Abrahams & Sutterlin 1999; Finstad *et al.* 2007; Huntingford *et al.* 2010). Conversely, differences in behavioural tendencies among individuals may actually contribute to variation in measured metabolic rate. Previous work has shown that individual animals differ consistently in their behaviour, with some being more bold or exploratory and others being more shy or docile (Wilson *et al.* 1994; Brown, Burgess & Braithwaite 2007; Réale *et al.* 2007). Bolder, more exploratory individuals are generally more active and so they may have increased energy expenditure across contexts, whether in nature, in experimental arenas or in respirometers (Careau *et al.* 2008). If intrinsic differences in personality contribute to variation in metabolic demand, then a link between metabolism and behaviour should be maintained when the same animal is exposed to different situations or environmental conditions. To date, there have been only a few attempts to examine the links between metabolic rate and risk-taking behaviour while foraging (Finstad *et al.* 2007; Huntingford *et al.* 2010), and so the direction of any causal links between them remains unknown.

Metabolic rate could be also linked to the willingness to take risks while foraging if both traits are related to inter-individual variation in the ability to tolerate food deprivation. Although rarely measured, it seems intuitive that individuals with higher energy expenditure will experience more rapid mass loss during food deprivation (Álvarez & Nicieza 2005; Bochdanský *et al.* 2005). The mass loss and hunger brought on by food deprivation could cause an increase in foraging activity and risk-taking (Gotceitas & Godin 1991; Pettersson & Bronmark 1993; Damsgird & Dill 1998; Krause *et al.* 1998), thus exacerbating any influence of routine metabolic rate (RMR) on the tendency to take risks. In nature, it

is common for animals to go for extended periods without feeding, especially fishes (Dutil & Lambert 2000; Gingerich, Philipp & Suski 2010). Individuals within a species can differ widely in their rate of mass loss during food deprivation (Dupont-Prinet *et al.* 2010), but the potential links among risk-taking, metabolic demand, and tolerance of food deprivation in individuals has not been examined.

This study investigated these relationships in individual juvenile European sea bass *Dicentrarchus labrax* Linnaeus. European sea bass display wide individual variation in RMR (Marras *et al.* 2010), tolerance of food deprivation (Dupont-Prinet *et al.* 2010) and risk-taking behaviour (Millot, Bégout & Chatain 2009). They spawn pelagic eggs at sea but, after hatch, larvae drift to inshore areas, where they colonize coastal environments such as lagoons and estuaries (Pickett & Pawson 1994). It is highly probable that juvenile European sea bass experience large fluctuations in food availability in these stochastic environments, and food deprivation may be a common feature in their life cycle (Dupont-Prinet *et al.* 2010). They also suffer intense predation in these early life stages, by birds, larger fish, and cephalopods (Quignard 1984).

Specifically, this study examined the hypothesis that RMR has an influence on risk-taking behaviour, particularly after a period of food deprivation, because of varying effects on energy status and hunger among individuals. Variability among individuals in the degree to which food deprivation affects their risk-taking while foraging, in response to inter-individual differences in metabolic rate, would indicate a synergistic interaction between metabolic demand and food deprivation, and would provide insight into the cause-and-effect relationship between metabolic demand and behaviour.

Materials and methods

ANIMALS

Juvenile European sea bass *Dicentrarchus labrax* ($n = 39$, mean wet mass = $42.54 \text{ g} \pm 1.94 \text{ SEM}$) were obtained from a local fish farm (Salses le Chateau, France; $42^\circ 49' \text{ N}$; $2^\circ 57' \text{ E}$) in February 2008 and stocked under a natural photoperiod (12-h light: 12-h dark) in a large tank (100 cm L \times 100 cm W \times 60 H) supplied with re-circulating, filtered natural sea water at a constant temperature ($20 \pm 0.5^\circ \text{ C}$) and salinity ($35.1 \pm 0.2\text{‰}$), for at least 4 weeks prior to use in experiments. The experimental fish were first-generation aquaculture fish from eggs laid and reared in captivity but obtained from wild broodstock captured in the western Mediterranean. Fish in the holding tank were fed daily with a restricted ration (feed pellets; Aphytec, Mèze, France) to limit growth in holding tanks. Despite this, fish used in experiments showed a slight increase in body mass over the course of the study (linear regression, initial body mass (g) = $33.57 + 0.15 \text{ (day)}$, $r^2 = 0.26$, $P = 0.001$). Three weeks prior to the start of experiments, all individuals were tagged for identification using VI Alpha tags (Northwest Marine Technology, Inc., Shaw Island, Washington, USA). All fish in the holding tank were eventually used in the experiment, with no bias towards bolder fish being more easily netted. Individuals were fasted for 24 h before use in experiments.

EXPERIMENTAL PROTOCOL

The overall protocol comprised: (i) testing individual fish for risk-taking behaviour, (ii) measuring their RMR over 7 days while they were deprived of food and (iii) testing risk-taking behaviour a second time, after food deprivation. The entire experiment was conducted over the course of 12 weeks, with one group of four fish being processed for measurement of risk-taking and RMR each week. All experiments were conducted at 20 ± 0.5 °C.

The 7-day period of food deprivation served two purposes: (i) it allowed for a comparison of risk-taking behaviour before and after food deprivation and (ii) it provided a measure of the rate of mass loss, which could be compared with the magnitude of the change in the behaviour of individual fish. The intention of this study was to examine the effects of short-term food deprivation, as opposed to starvation where there is wasting of muscle and structural tissues, shifts in the fuels used for metabolism and, potentially, changes in routine oxygen consumption (Wang, Hung & Randall 2006). There are no existing measures of oxygen consumption with long-term starvation in European sea bass, but a shift in the substrates used for metabolism (from dedicated energy reserves to the breakdown of structural tissues) does not occur until around 50 days of starvation in this species (Echevarría, Martínez-Bebía & Zamora 1997).

Risk-taking behaviour

Tests were conducted in an experimental raceway measuring 48 cm L \times 10 cm W \times 25 cm H. At one end of the raceway, 'cover' was provided under a horizontal PVC sheet (15 cm L \times 10 cm W). The uncovered remainder was subdivided into three sections by markings on the floor, creating three 'open' subsections, each measuring 11 cm L \times 10 cm W. The ends of the raceway were perforated PVC, and a pump generated a gentle flow of water (< 1 cm s⁻¹) from an external compartment through the walls, from the open towards the covered end. The entire setup was shielded by a black plastic blind, through which the fish could be observed via small apertures. The floor of the raceway was covered with reflective tape to improve contrast between the fish and the background for video analysis.

Individual fish were placed into separate raceways and left overnight to acclimate. Trials would commence the following morning. The experimenter would observe the fish through a small aperture in the blind, and when the fish was facing towards the open section of the raceway, it was startled by quickly releasing a short length of PVC pipe (attached to a string and released from a consistent height) vertically into the raceway 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 (birds such as cormorants *Phalacrocorax* sp., and egrets and herons *Ardea* sp., are major predators of juvenile sea bass in coastal lagoons) and almost always caused the fish to retreat under cover. Immediately following this simulated attack, five pellets of feed (2 mm diameter) were provided, via a slanting PVC tube, into the open extremity, such that they fell in the same area where the simulated attack had occurred. Fish activity was then videotaped for the next 10 min, from above (25 frames s⁻¹; Sony Mini DV camera, Tokyo, Japan). This procedure was repeated three times both before and after the 7-day period of food deprivation, with 1 hour between each trial (a total of 6 trials per fish).

Videotapes of the trials were analysed for: (i) the time taken to reappear from cover after the simulated attack (defined as the nose of the fish being visible beyond the cover), (ii) the time spent in the open area of the raceway and (iii) the number of transitions between all

sections of the raceway. Fish that did not emerge from cover after the startle stimulus in a given trial were given a maximum value of 600 s for their appearance time. Time until reappearance after being startled (hereafter referred to as 'appearance time') and total time in the open (referred to as 'open time') are indicative of boldness, while the number of transitions between raceway sections is an index of activity (referred to as 'activity'). Boldness and activity are considered two axes of animal 'temperament' and could make an individual more prone to encountering, being attacked, or captured by, a predator (Réale *et al.* 2007). Leftover food was carefully siphoned after each trial from the raceway from behind the blind. The amount of food consumed during trials was low, and it is unlikely that the fish became satiated over the course of the three trials. There was no effect of trial order on the measures of any behaviour (separate analyses were performed on data collected before or after food deprivation; linear mixed models with each measure of behaviour as the dependent variable, trial number as a categorical variable, and subject fish as a random factor, $P > 0.05$), so the mean for each behaviour across all three trials was used in subsequent analyses. Because of technical problems with video recordings for three individuals, behavioural trials were only analysed for 36 of the 39 fish that were tested for metabolic rate.

Respirometry

Following the measurement of risk-taking behaviour before food deprivation, fish were left to fast for 24 h, to allow clearance of any food consumed during the risk-taking trials, and were then transferred to individual respirometers. At the prevailing temperature, 24 h is sufficient to complete digestion in the European sea bass, with no residual effects of feeding on RMR (Dupont-Prinet *et al.* 2010). Nonetheless, the first 24 h of respirometry confinement were not included in the subsequent measurement of routine oxygen consumption.

Routine metabolic rate was measured by respirometry, as rates of oxygen uptake (Steffensen 1989; Dupont-Prinet *et al.* 2010). 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. Individual animals were carefully dried with a lint-free paper towel and then weighed using a microbalance. They were then placed in one of four polyethylene respirometers (25 cm L \times 10 cm W \times 6 cm H) immersed in an outer tank and instantaneous oxygen uptake (MO₂, in mg O₂ 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 two minutes of each closed phase). Fish could not see each other or interact while in the separate respirometers during measurement of oxygen consumption. A fifth 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 MO₂ then calculated as described by Dupont-Prinet *et al.* (2010).

The respirometers were submerged in an aerated water bath isolated in a constant temperature chamber, regulated at 20 ± 0.5 °C

and with an appropriate seasonal photoperiod (12-h light: 12-h dark). The water surface was shielded with opaque polystyrene to prevent visually disturbing the fish, and all disturbances were kept to a minimum during measurements. Fish remained in the respirometers for 7 days (168 h) of food deprivation, after which they were carefully removed from the respirometers and weighed again using a microbalance. Fish MO_2 was measured throughout this period, and the absolute routine metabolic rate (RMR; $\text{mg O}_2 \text{ h}^{-1}$) of each individual was calculated as the mean level of oxygen consumption during the last 6 days. The first 24 h of respirometer confinement were not included in analyses to avoid any potential bias due to handling stress (Steffensen 1989).

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 activity and open 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.

While appearance time, open time and activity all represent components of overall risk, they may also be confounded with one another to some extent (Réale *et al.* 2007). Therefore, we also used a principal component analysis (PCA) to combine appearance time, open time and activity into one overall measure of risk-taking behaviour. Using a correlation matrix to account for differences in the measurement scales and units among the variables, this PCA incorporated data collected before and after food deprivation. The values for this first component were used as a general index of riskiness that encompassed all behaviours quantified in the study, and is hereafter referred to as 'risk score'.

The behavioural carryover (Sih *et al.* 2004) of individuals before and after food deprivation was assessed using Pearson product-moment correlations. The intraclass correlation coefficient (τ , Sokal & Rohlf 1995) was used to examine repeatability of RMR among days of respirometer confinement and food deprivation.

Rates of routine oxygen consumption were corrected for differences in body mass by dividing the oxygen consumption of each fish ($\text{mg O}_2 \text{ h}^{-1}$) by the fish's initial 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). Absolute rates of mass loss showed a strong relationship with initial body mass (linear least squares regression, $r^2 = 0.46$, $P < 0.0001$), and so the instantaneous rate of mass loss (expressed as $\% \text{ d}^{-1}$) for each fish was calculated using the equation:

$$(\text{Ln}(m_t) - \text{Ln}(m_i))/t \times 100 \quad \text{eqn 1}$$

where m_t is the body mass at time t and m_i is the initial body mass (Hopkins 1992).

The effect of RMR and food deprivation on risk-taking was first analysed using linear mixed models with subject animal as a random factor, mass-adjusted RMR and initial body mass as covariates and measurement period (i.e. before or after the period of food deprivation) as a categorical variable. These models produced significant interactions between mass-adjusted RMR and measurement period ($P < 0.05$), and so separate models were subsequently performed for measurements collected before and after food deprivation, with mass-adjusted RMR and initial body mass as covariates. Mean differences in risk-taking behaviours before and after food deprivation were then directly analysed using paired t -tests.

Correlations between instantaneous rate of mass loss and the magnitude of change in each measure of risk-taking after food deprivation were analysed among individuals by linear least squares regression.

Results

RISK-TAKING BEHAVIOUR

There was a large degree of inter-individual variation for all measures of risk-taking behaviour (Table 1; Fig. 1). All measures of behaviour (appearance time, open time and activity) were strongly correlated with each other (Table 2).

Table 1. Summary of data collected for juvenile European sea bass in the present study

Variable	<i>n</i>	Mean	SEM	CV	Minimum	Maximum
Absolute routine metabolic rate (RMR) ($\text{mg O}_2 \text{ h}^{-1}$)	39	7.20	0.30	0.25	4.40	11.38
Mass-corrected RMR ($\text{mg O}_2 \text{ kg}^{-0.751} \text{ h}^{-1}$)	39	77.54	1.65	0.13	58.34	101.68
Initial mass (g)	39	42.54	1.94	0.27	23.41	76.79
Final mass (g)	39	40.91	1.87	0.27	22.31	73.96
Instantaneous mass loss ($\%/ \text{day}$)	39	0.56	0.02	0.25	0.25	0.79
Appearance time (s)						
Before food deprivation	36	352.08	30.10	0.51	57.33	600.00
After food deprivation	36	273.41	33.20	0.73	6.00	600.00
Open time (s)						
Before food deprivation	36	89.59	20.83	1.40	0.00	443.52
After food deprivation	36	118.30	21.63	1.09	0.00	475.82
Activity (counts)						
Before food deprivation	36	24.29	5.76	1.42	0.00	119.01
After food deprivation	36	42.23	8.11	1.15	0.00	154.99
Risk score						
Before food deprivation	36	-0.197	0.148	-	-1.156	1.869
After food deprivation	36	0.197	0.180	-	-1.156	2.521

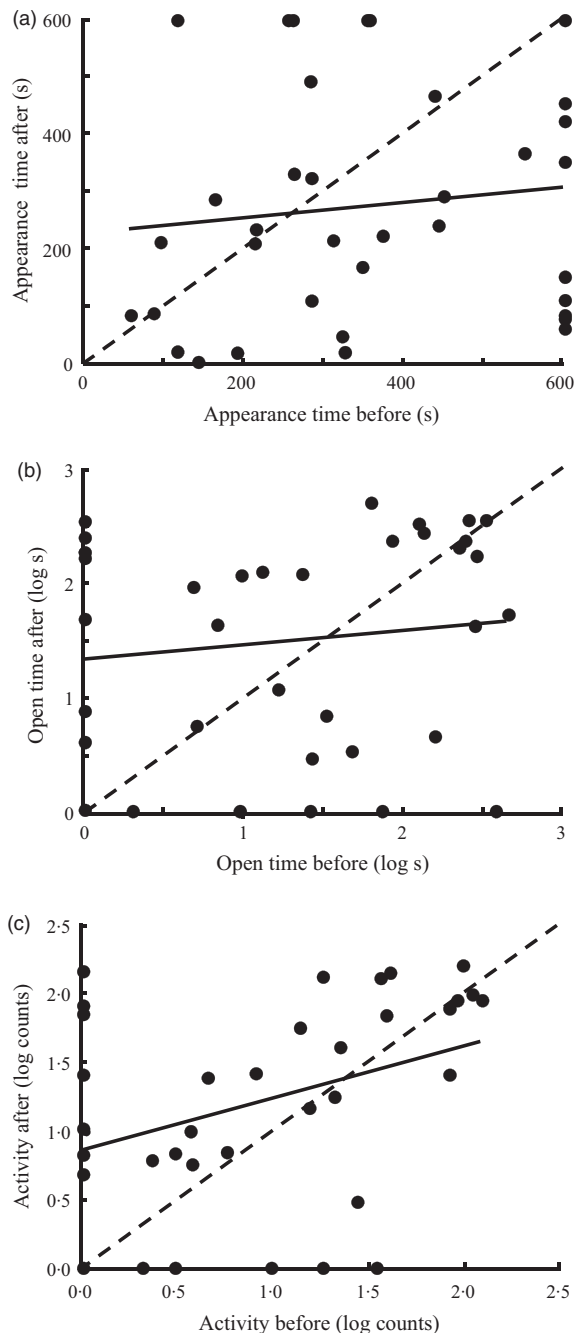


Fig. 1. Relationships between behavioural measures of risk-taking for individual juvenile European sea bass before and after a 7-day period of food deprivation ($n = 36$). Pearson product-moment correlations are as follows: (a) appearance time; $r = 0.12$, $P = 0.481$; (b) open time; $r = 0.20$, $P = 0.244$; and (c) activity; $r = 0.52$, $P = 0.0013$. For reference, the linear least squares regression line for each relationship is also shown (solid lines), as well as the line representing a theoretical 1 : 1 relationship (dashed line).

The activity of individuals showed a significant positive correlation before and after food deprivation (Pearson product-moment correlation, $r = 0.52$, $P = 0.0013$), but measures of other behaviours before and after food deprivation were not correlated (Fig. 1). Measures of behaviour did not depend on when during the course of the study a given fish was mea-

Table 2. Correlation matrix for the principle component analysis of risk-taking behaviour in juvenile European sea bass

	Variable	Appearance time	Open time	Activity
Pearson correlation r	Appearance time	1.00	-0.69	-0.61
	Open time	-0.69	1.00	0.72
	Activity	-0.61	0.72	1.00
P -value	Appearance time	–	< 0.0001	< 0.0001
	Open time	< 0.0001	–	< 0.0001
	Activity	< 0.0001	< 0.0001	–

sured (linear mixed models, with individual fish as a random factor, measurement day and body mass as covariates, $P > 0.05$).

Principle component analysis of behaviours produced a first component (risk score) accounting for 78.1% of the variance in the data set (Eigenvalue = 2.344) while loading positively for activity (0.877) and open time (0.912), and negatively for appearance time (-0.862).

ROUTINE METABOLIC RATE

The oxygen consumption of fish was elevated for approximately 24 h after transfer to the respirometers, but declined to a near constant level after this point, and remained relatively stable for the remainder of the measurement period with no detectable diurnal or nocturnal cycling of MO_2 (paired t-test of mean daytime and night time RMRs for each fish, d.f. = 38, $t = 0.413$, $P = 0.682$; overall mean daytime RMR = 80.67 ± 10.72 mg O_2 $\text{kg}^{-0.751}$ h^{-1} ; mean night time RMR = 80.20 ± 12.65 mg O_2 $\text{kg}^{-0.751}$ h^{-1}), or change in MO_2 as feed deprivation progressed (linear mixed model, subject fish as a random factor, measurement day as a categorical variable, $P = 0.624$).

Absolute RMR was correlated with initial body size according to the equation:

$$\log \text{RMR} = 0.751(\log \text{initial mass in g}) - 0.367, \quad \text{eqn 2}$$

$$r^2 = 0.68, P < 0.0001$$

After adjusting for difference in body mass, RMR still showed wide variation among individuals with a 1.8-fold difference between the minimum and maximum values. After the first 24 h following transfer into the respirometers, RMR values for individual fish showed significant repeatability among days of respirometry confinement/food deprivation ($\tau = 0.60$, $P < 0.0001$).

RELATIONSHIPS AMONG METABOLIC RATE, FOOD DEPRIVATION AND RISK-TAKING

Fish tended to display more risk after food deprivation when comparing mean values for behaviours (Table 1),

though only activity showed a significant increase (paired t -test; $t = -2.14$; d.f. = 35; $P = 0.039$). Among individuals, however, while some fish displayed a clear increase in risk-taking after food deprivation, others showed almost no change or displayed a decrease (Fig. 1). There were more fish that never reappeared from cover after the simulated attack prior to food deprivation ($n = 9$) as compared with after ($n = 6$). Appearing and non-appearing fish did not differ in terms of their body mass or RMR either before or after food deprivation (unpaired t -tests; $P > 0.05$).

The relationships between RMR and measures of risk-taking behaviour differed before and after food deprivation (linear mixed models, significant RMR \times measurement period interactions, $P < 0.05$). There was no effect of RMR on any risk-taking behaviour before food deprivation but, after deprivation, RMR had a significant effect on appearance time, activity and risk score (Table 3; Fig. 2). Risk-taking was not related to body mass before food deprivation but, after deprivation, smaller fish tended to have shorter appearance times (Table 3).

Fish that had experienced higher instantaneous rates of mass loss during food deprivation tended to show the largest negative change in appearance time after food deprivation as compared with before, and the largest positive changes in open time and activity (Fig. 3). Instantaneous rate of mass loss was, in itself, positively correlated with mass-corrected RMR (Fig. 4).

Table 3. 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, conducted before and after a 7-day period of food deprivation

	Term	d.f.	F	P
<i>Before food deprivation</i>				
Appearance time	RMR	1	0.050	0.824
	Mass	1	1.853	0.183
	Error	33		
Open time	RMR	1	0.281	0.599
	Mass	1	0.347	0.560
	Error	33		
Activity	RMR	1	0.080	0.779
	Mass	1	1.547	0.222
	Error	33		
Risk score	RMR	1	0.066	0.799
	Mass	1	1.235	0.274
	Error	33		
<i>After food deprivation</i>				
Appearance time	RMR	1	5.317	0.031
	Mass	1	6.961	0.013
	Error	33		
Open time	RMR	1	3.217	0.082
	Mass	1	1.583	0.217
	Error	33		
Activity	RMR	1	5.635	0.024
	Mass	1	2.441	0.128
	Error	33		
Risk score	RMR	1	4.263	0.032
	Mass	1	0.332	0.563
	Error	33		

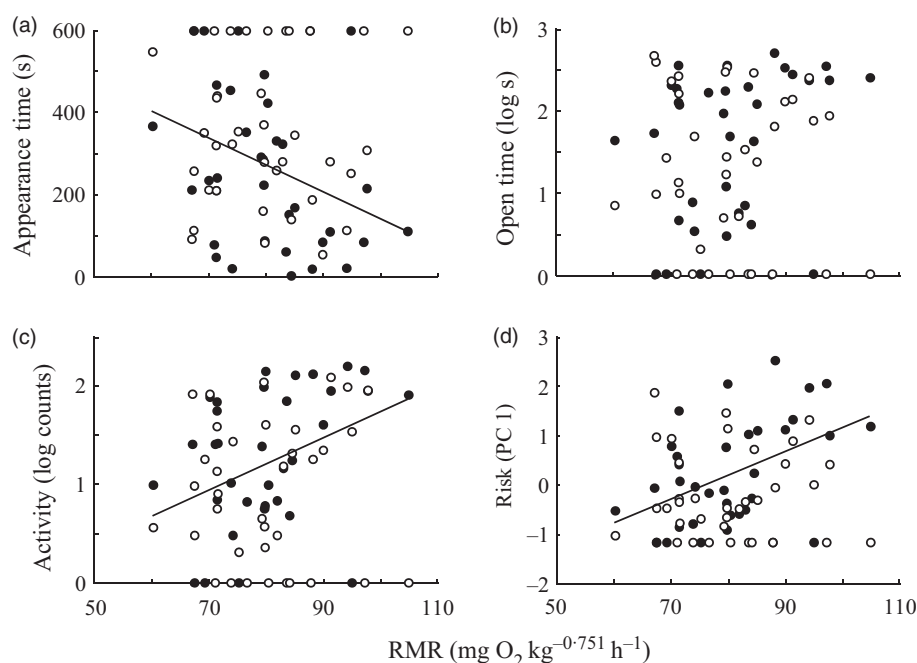


Fig. 2. The relationship between mass-corrected routine metabolic rate (RMR) and: (a) appearance time, (b) open time, (c) activity and (d) risk score. Open circles represent behavioural data collected before a 7-day period of food deprivation, while the closed circles represent data collected after food deprivation. Each data point is a mean for three trials conducted for one individual during each measurement period. There were no significant relationships between RMR and any behaviour before food deprivation. Significant linear least squares regressions for data collected after food deprivation are as follows: (a) appearance time = $783.15 - 6.57(\text{RMR})$, $r^2 = 0.11$, $P = 0.05$; (b) no significant relationship; (c) log activity = $-0.89 + 0.03(\text{RMR})$, $r^2 = 0.13$, $P = 0.029$; (d) risk score = $-3.67 + 0.048(\text{RMR})$, $r^2 = 0.21$, $P = 0.005$.

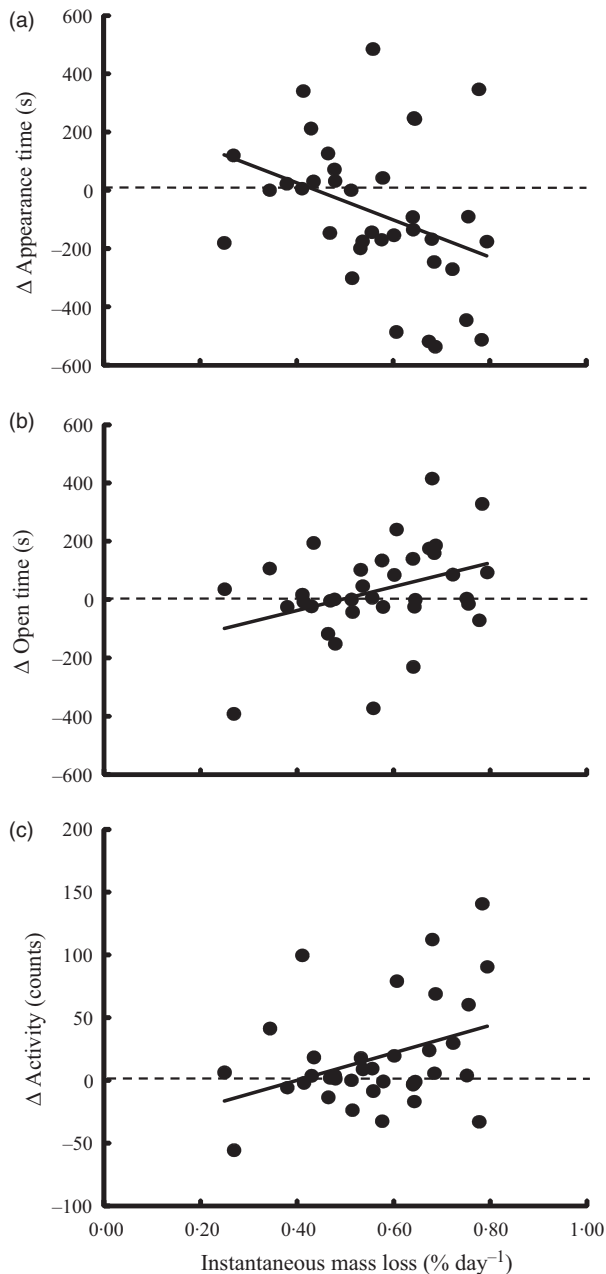


Fig. 3. Relationship between relative rate of mass loss during a 7-day period of starvation and changes in mean risk-taking behaviour before and after the period of food deprivation in individual European sea bass, measured in terms of: (a) appearance time, (b) open time and (c) activity. Linear least squares regression equations are as follows: in (a) $y = 278.84 - 634.54(\text{mass loss})$, $r^2 = 0.13$, $P = 0.031$; (b) $y = -201.34 + 408.30(\text{mass loss})$, $r^2 = 0.13$, $P = 0.031$; (c) $y = -43.77 + 109.52(\text{mass loss})$, $r^2 = 0.13$, $P = 0.029$.

Discussion

There was no evidence that RMR was correlated with risk-taking behaviour in individual fish before food deprivation but, after deprivation, fish with higher RMRs tended to display increased risk-taking. These results suggest that while metabolic demand can play a role in determining behaviour, the extent of these effects may be context dependent and vary

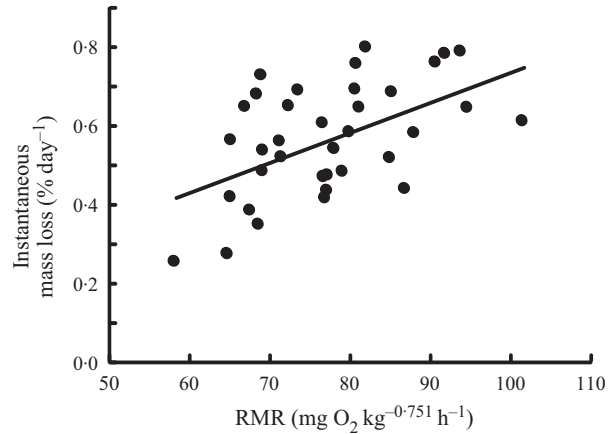


Fig. 4. The relationship between instantaneous mass loss ($\% \text{ d}^{-1}$) during a 7-day period of food deprivation and routine metabolic rate (RMR; linear least squares regression, mass loss = $-0.0266 + 0.00761(\text{RMR})$; $r^2 = 0.28$ $P < 0.001$) in individual juvenile European sea bass.

temporally within individuals. For example, RMR may have little or no effect on risk-taking under 'optimal' conditions, but individuals with increased metabolic demand may become more likely to take risks after being deprived of food.

Fish with a relatively high RMR tended to lose the most mass during food deprivation which, in turn, seemed to drive an increase in risk-taking behaviour. As such, food deprivation appears to amplify any baseline effect of RMR on risk-taking behaviour, which may be subtle (Finstad *et al.* 2007; Huntingford *et al.* 2010) or non-existent (this study) in regularly feeding fish. This will lead to a divergence in the willingness to take risks to obtain food among individuals with relatively high and low metabolic rates. In agreement with previous studies, the mean response of fish was to show an increase in risk-taking after food deprivation (Gotceitas & Godin 1991; Pettersson & Bronmark 1993; Damsgird & Dill 1998; Krause *et al.* 1998). Among individuals, however, the magnitude of the increase in risk was related to the amount of mass loss experienced by each individual. Within this general trend, some fish showed little or no change in risk-taking, and some actually decreased risk-taking after food deprivation. The possibility that some fish decreased activity as a result of being weakened from starvation is unlikely because it was those that lost the least mass that tended to have little or no change in the amount of risk displayed. Further, the duration of food deprivation in this study was far shorter than that which causes muscle wasting and changes in metabolism in European sea bass and most other fish species (Echevarría, Martínez-Bebía & Zamora 1997; Wang, Hung & Randall 2006). Instead, these results show that food deprivation does not necessarily lead to increased risk-taking in every instance, but that the magnitude of any increase in risk tends to be related to the rate of individual mass loss, which itself is driven by RMR.

This study provides an example of how risk-taking behaviour can be influenced by intrinsic metabolic demand. Individuals of the same species often display consistent

differences in behaviour, with some being more bold or aggressive and others being more shy or docile (Wilson *et al.* 1994; Brown, Burgess & Braithwaite 2007; Réale *et al.* 2007). Such differences in animal personality have been speculated to be linked with metabolic demand, although the causal direction of such a relationship is not certain. It has been suggested, for example, that some animals are inherently more active at all times, and therefore have higher energy demands because of increased physical activity (Careau *et al.* 2008). Under this scenario, intrinsic differences in behaviour would contribute to differences in metabolic demand and, in the present study, risk (which incorporated differences in activity) would have been positively associated with RMR both before and after food deprivation. This was not observed, and the results instead suggest that inherent differences in energetic requirements among individuals may cause variation in the amount of risk they are willing to accept to obtain food.

It is important to acknowledge the variability surrounding the relationships between metabolic rate and behaviour observed in this study. Some of this variability may stem from stress-associated increases in oxygen consumption in some individuals during respirometry confinement (Careau *et al.* 2008). For example, there were some fish that did not appear outside the shelter after the simulated attack either before or after food deprivation, and some of these individuals were among those with relatively high RMRs. It is possible that these fish were generally more nervous, including during respirometer confinement, thus causing an elevation in their measured RMR (Careau *et al.* 2008). The results of this study also suggest that while intrinsic characteristics such as metabolic demand or hunger play a role in determining individual behaviour, additional factors must also influence moment-to-moment decision-making. Differences in sensory ability (Giske, Huse & Fisker 1998) or perception of risk among individuals (Stankowich & Blumstein 2005), independent of RMR, could also contribute to the variability surrounding the relationship between metabolic demand and risk-taking behaviour.

Although there was no relationship between body size and behaviour before food deprivation, after deprivation smaller fish tended to appear outside the shelter sooner after the simulated attack. In the wild, smaller fish have more potential predators (Fuiman & Magurran 1994), but their higher mass-specific metabolic demand (oxygen consumption per unit body mass per unit time) can make them more prone to starvation (Post & Evans 1989; Byström *et al.* 2006). In the present study, the observed relationship between RMR and risk-taking after food deprivation is stronger when RMR is expressed on a mass-specific basis ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) and not corrected for differences in body mass using an allometric scaling relationship (Fig. S1), suggesting that mass-specific RMR is an important factor affecting foraging decisions. This could make smaller individuals even more prone to predation after overwinter periods or in other situations when there is decreased food availability (Garvey, Ostrand & Wahl 2004). Previous studies have also observed that smaller fish

tend to show greater amounts of risk (Krause *et al.* 1998; Brown & Braithwaite 2004; Brown, Jones & Braithwaite 2005); though, these authors did not relate measurements of behaviour directly to metabolic rate. The fact that smaller individuals are more likely to take risks when food-deprived, despite being more vulnerable to predators, provides further evidence that intrinsic metabolic demand has an important influence on the level of risk displayed by an individual.

The combined effects of RMR and food deprivation on risk-taking while foraging could lead to an increased likelihood of being predated for individuals with a relatively high intrinsic energy demand during times when food is scarce. Despite this apparent disadvantage, high RMR phenotypes may still persist in a population because of mortality-growth trade-offs (Stamps 2007). When food is scarce, having a low RMR may be beneficial for tolerating reduced food intake, but possessing a high RMR could be associated with increased reproductive success or growth rates when resources are abundant (Álvarez & Nicieza 2005; Bochdansky *et al.* 2005; Dupont-Prinet *et al.* 2010). Even when food is plentiful, shy phenotypes could still be competitive if they are able to adjust aspects of their behaviour to compensate for a tendency to spend more time hiding and less time foraging (Lind & Cresswell 2005). In the present study, for example, the rate of activity per minute of open time was negatively correlated with open time and positively correlated with appearance time (Fig. S2). This indicates that some individuals spend more time hiding, but when away from shelter, they are in fact more active and exploratory. This compensatory response could allow a relatively shy individual to maintain a relatively high rate of food intake, and be another means by which a range of phenotypes may persist within a population.

In conclusion, this study shows that variation in RMR is positively correlated with risk-taking behaviour in juvenile European sea bass. It is notable, however, that the effect of RMR on risk-taking was only evident after a period of food deprivation and that, among individuals, the period of food deprivation had differing effects on changes in behaviour. Additional research is needed to examine the relationship between RMR and risk-taking in other taxa – and in other situations – to see if this context-dependent effect is widespread. In situations where RMR does affect risk-taking behaviour, even under benign or optimal conditions (e.g. Huntingford *et al.* 2010), a period of food deprivation or exposure to another stressor could amplify these pre-existing tendencies. Future studies could examine how other stressors, such as exposure to acute temperature fluctuations or hypoxia, may mediate the influence of RMR on risk-taking behaviour.

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Supporting information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. The relationship between mass-specific routine metabolic rate (RMR) and behavioural indicators of risk-taking in individual juvenile European sea bass.

Fig. S2. Relationships between: (A) appearance time; and, (B) open time, with the rate of activity per unit open time.

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