Immune-challenged fish up-regulate their metabolic scope to support locomotion

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Abstract

Resource-based trade-offs occur when investment in one fitness-related trait diverts energy away from other traits. The extent to which such trade-offs are shaped by limits on the rate of conversion of energy ingested in food (e.g. carbohydrates) into chemical energy (ATP) by oxidative metabolism rather than by the amount of food ingested in the first place is, however, unclear. Here we tested whether the ATP required for mounting an immune response will lead to a trade-off with ATP available for physical activity in mosquitofish (*Gambusia holbrooki*). To this end, we challenged fish either with lipopolysaccharide (LPS) from *E. coli* or with Sheep Red Blood Cells (SRBC), and measured oxygen consumption at rest and during swimming at maximum speed. Relative to saline-injected controls, only LPS-injected fish showed a significantly greater resting metabolic rate two days post-challenge and significantly higher maximal metabolic rates two and seven days post-challenge. This resulted in a significantly greater metabolic scope two days post-challenge, with LPS-fish transiently overcompensating by increasing maximal ATP production more than would be required for swimming in the absence of an immune challenge. LPS-challenged fish therefore increased their production of ATP to compensate physiologically for the energetic requirements of immune functioning. This response would avoid ATP shortages and allow fish to engage in an aerobically-challenging activity (swimming) even when simultaneously mounting an immune response. Nevertheless, relative to controls, both LPS- and SRBC-fish displayed reduced body mass gain one week post-injection, and LPS-fish actually lost mass. The concomitant increase in metabolic scope and reduced body mass gain of LPS-challenged fish indicates that immune-associated trade-offs are not likely to be shaped by limited oxidative metabolic capacities, but may instead result from limitations in the acquisition, assimilation or efficient use of resources.

42 **Key-words:** LPS, maximal metabolic rate, metabolic scope, mosquitofish, muscle, resting
43 metabolic rate, SRBC, trade-off

Introduction

Pathogens can represent intense selection pressures for their hosts [1, 2]. Despite this, the evolution of host resistance through the activation of a protective immune response may be constrained by the energetic costs of immune functioning and trade-offs with other fitness-related traits [3-6]. A growing number of studies have provided evidence of costly immune responses to inert antigens, with immune-challenged individuals showing increased nutritional intake [7, 8] and body mass loss [9, 10] compared to controls. Because hosts usually have access to limited resources, the energy allocated to fuel immune responses may be diverted away from other fitness-related functions, such as growth, reproduction and maintenance [3]. Accordingly, immune challenges have been shown to be associated with reduced locomotor performance [11, 12] and lowered investments in reproduction and growth [13, 14]. However, while energetic constrains are often assumed to mediate life history trade-offs [3], the physiological processes by which this might occur still remain to be elucidated.

Previous studies quantifying the energetic cost of immunity have focused on measuring changes in basal metabolic rates following immune challenge [9, 15, 16]. Basal (or resting when measured under less restrictive circumstances) metabolic rates reflect the rate of ATP hydrolysis required to maintain cellular processes during physical inactivity [17]. For instance, vaccinated rainbow trout (*Oncorhynchus mykiss*) displayed higher routine metabolic rates, which in fish can be indicative of basal metabolism, than controls [18], with rates increasing by ~20% 223° days post-vaccination [19]. Similarly, four species of insects (*Tenebrio molitor*, *Acheta domesticus*, *Cotinis nitida* and *Periplaneta americana*) showed an increase in metabolic rate by up to 28% following the induction of an encapsulation response [20]. Cutrera et al [21] reported that tuco-tucos (*Ctenomys talarum*) experimentally-challenged with Sheep Red Blood Cells (SRBC) experienced a 20-35% increase in metabolic

rate, which was equivalent to a 15% increase in daily energetic expenditure. Martin et al. [22] and Eraud et al. [23] found that the increase in basal metabolic rate displayed by collared doves (*Streptopelia decaocto*) and house sparrows (*Passer domesticus*) following injections with inert antigens was of a magnitude similar to that required to produce half an egg per day and to maintain an optimal body temperature at an ambient temperature 1-2°C below thermo-neutrality, respectively. While immune responses have thus been shown to increase the resting metabolic rate, it remains to be determined whether such immune-triggered increase in ATP use at rest means that less ATP is available for engaging in other aerobically-challenging activities.

The level of energy that an individual can allocate to fitness-related activities such as reproduction, foraging and engaging in behavioural interactions, can be estimated by the aerobic metabolic scope [24-26]. The aerobic metabolic scope is defined as the difference between the maximal metabolic rate, which reflects maximal mitochondrial flux [24], and the resting metabolic rate, and therefore represents the metabolic capacity to engage in aerobically-challenging activities [27]. Metabolic scopes may decline either when there is an increase in the resting metabolic rate or when there is a decrease in the maximal metabolic rate. Hence, if metabolic maintenance costs increase, the resultant reduction in metabolic scope can create an allocation trade-off [28]. Decreases in the amount of energy available to other activities may, however, be counterbalanced physiologically though an increase in the capacities of mitochondrial metabolic pathways [26, 29, 30]. While such compensatory responses may abolish ATP shortages, trade-offs may still persist if investment in multiple aerobically-challenging activities depletes storages of ingested chemical energy with detrimental consequences for body condition.

Here we examined the energetic basis of immune-associated resource allocation trade-offs in wild-caught, female mosquitofish (*Gambusia holbrooki*). Individuals were challenged either with lipopolysaccharides (LPS) isolated from *Escherichia coli* or with Sheep Red Blood Cells (SRBC) to investigate how differences in the type of immune response elicited may constrain energetic investments in other functions [21, 31-33]. Although fish are thought to be less sensitive to endotoxins such as LPS than other vertebrates [34-36], LPS-challenges in fish *in vivo* and *in vitro* nevertheless induced a strong inflammatory response [37, 38], and increased the production of cytokines and acute phase proteins, as well as stimulated T and B lymphocytes, macrophages and complement systems [25]. For example, the expression of cytokines in a monocyte-macrophage lineage of rainbow trout was detectable 6h after exposure to *E. coli* LPS and increased over 24h [29, 32]. LPS also induces antibody production, which in the brown trout (*Salmo trutta*) was detectable on day 14 post-injection [39]. However, antigen-binding and antibody-secreting cells were detected in the spleen and kidney of these fish as early as 2 and 4 days after injection, with peaks reached between day 14 and 18 post-injection [40]. SRBC, on the other hand, induced a non-pathogenic T and B-cell dependent antibody response [31]. Brown trout immunized with SRBC displayed detectable levels of antigen-binding and antibody-secreting cells on day 6 post-injection, with levels peaking on day 12 [40]. Levels of antibody-producing cells were detectable as early as day 5 and peaked on day 10 in the Mozambique tilapia (*Oreochromis mossambicus*) [41].

First, we predicted that if immune responses were energetically costly, then immune-challenged individuals would exhibit higher resting metabolic rates than controls. Second, we tested whether limits on ATP production and the amount of ATP available can give rise to immune-associated trade-offs. We predicted that, if this were the case, immune-challenged individuals should experience a reduction in metabolic scope relative to controls. Conversely,

119 if immune-associated trade-offs are shaped by a limited availability of ingested energetic
120 resources, then immune activation should instead be associated with a reduction in body
121 mass.
122
123 **Material and methods**
124 STUDY ANIMALS
125 Wild invasive mosquitofish were captured in July 2011 from natural ponds on private land
126 near Perpignan (42.698°N, 2.895°E) in southern France, with permission from local
127 landowners. Animals were immediately brought back to the laboratory at the Station
128 d'Ecologie Expérimentale du CNRS in Moulis (Ariège, France), where they were housed in
129 large containers (100 fish at approximately 1 fish/L), with a mixture of water from their
130 capture sites and aged tap water. The females used in this study were house individually (in
131 300×250×200 mm tanks) so that we could follow individuals. Females were habituated to
132 their individual tanks for two weeks before the start of the experiment. At the time of capture,
133 water temperature at the sites of capture varied between 28-30^{o}C; fish were therefore kept at
134 30^{o}C in the laboratory throughout the habituation and experimentation phases. Because
135 female mosquitofish store sperm and will be mated immediately upon reaching maturity [19],
136 we categorized females based on the shape and distension of their abdomen to ensure that all
137 individuals used were at a similar early stage of pregnancy [for detailed methods see 42] [26].
138 Fish were fed to satiety with commercial fish flakes once per day. Food was withheld 24 h
139 prior to immune-treatment and prior to all measurements of metabolic rates. This ensured that
140 fish were in a post-absorptive state and therefore fit the requirement for basal metabolic rate
141 measurement (i.e., calm, motionless and post-absorptive).
142
143

144 IMMUNE CHALLENGE

145 After approximately two weeks in captivity, females were randomly assigned to experimental
146 or control groups (experimental LPS-injected: N=10; experimental SRBC-injected: N=9;
147 controls: N=10). Individuals were challenged with an intra-peritoneal injection of LPS
148 (Sigma; St. Louis, USA; L4005; 10µg/g fish at 1g/L [43]) or SRBC (Sigma R3378; 20µg/g
149 fish at 1g/L [41, 44]) for experimental treatments, and 0.01mL of saline solution (PBS) for
150 controls. Prior to injection, all fish were lightly anesthetized in a clove oil solution, and after
151 injection animals were placed immediately in aged tap-water where they recovered within 1-2
152 minutes. We recorded total body length using callipers (Mitutoyo, Kawasaki, Kanagawa,
153 Japan; precision: ±0.05 mm) at the start of the experiment, and we measured body mass at the
154 time of injection (hereafter: initial mass) and again after 7 days (~168 hours) using an
155 electronic balance (Ohaus, Brooklyn, NY, USA; precision: ±0.01 g).

156

157 OXYGEN CONSUMPTION

158 In fish, the immune response to LPS is known to occur within hours of injection and last for
159 at least 2 weeks [29, 32, 39]. On the other hand, antibody responses to SRBC can become
160 detectable 5-6 post-immune challenge and peak 10-12 day post-injection [40, 41].
161 Consequently, we measured resting and maximal metabolic rates at 24h, 48h and 168h (7
162 days) post-challenge in LPS-exposed individuals and control fish, and at 7 days only in
163 SRBC-exposed ones. Oxygen consumption is a commonly-used, indirect measure of
164 metabolic rates [45]. Oxygen consumption was measured according to published methods
165 [42] with a fibre-optic oxygen system (Fibox 3, Presens, Regensburg, Germany) monitoring
166 sensor spots (Presens, Germany) attached to the insides of respirometers according to the
167 manufacturers' instructions. For resting oxygen consumption measurements, we allowed fish
168 to rest in a cylindrical glass respirometer (245 ml volume) placed into a darkened tank for 45-

60 minutes. We then sealed the respirometer, making sure not to disturb the fish, and let the fish rest for a further 10-15 minutes before recording the decrease in oxygen over a 7-10 minute period or until a steady rate of oxygen decrease was established; we followed the decrease in oxygen levels within the respirometer in real-time.

Maximal rates of oxygen consumption were determined in a cylindrical glass respirometer (415 ml) placed on a magnetic stirrer [42]. A magnetic stirbar within the respirometer created water flow that could be adjusted with the control on the magnetic stirrer. Turbulence and eddies within the respirometer were minimised by a central column suspended from the lid. Fish were placed into the respirometer and the speed was ramped up slowly until fish swam steadily, but occasionally had to struggle to maintain their position in the water column, i.e. fish occasionally went backwards in the water column and had to engage in burst swimming to regain their position, indicating near maximal swimming speeds. Oxygen consumption was measured during 6-8 minutes of swimming at that speed. Rates of oxygen consumption (in $\mu mol\ g^{-1}\ min^{-1}$) were determined as the slope of the decrease in oxygen content divided by the fish body mass and multiplied by the volume of the container [45]. We calculated exercise-induced metabolic scope as the difference between resting and swimming oxygen consumption.

STATISTICAL ANALYSES

All statistical analyses were performed using SAS software version 9.3 (SAS Inc., Cary, NC). When required, data were log_{10}-transformed before analyses to fulfil assumptions of normality and homoscedascity. Because metabolic rates are generally influenced by body mass (Hill et al 2012), we initially tested such an association using a general linear model with a normal error structure and by specifying initial mass as an explanatory term. We then

tested whether resting and maximal metabolic rates, and metabolic scopes of LPS-injected fish differed from those of controls using multivariate general linear mixed models with treatment (LPS or PBS), time and their interaction, and with initial mass as fixed effects, and with individual as the random effect; for each time point, differences between LPS-challenged and control fish were contrasted within the same model using the "estimate" statement. Non-significant interactions were removed from final models. To examine how SRBC-injected fish differed in their resting and maximal metabolic rates and in their metabolic scope relative to controls and to LPS-fish, we conducted general linear models with treatment (SRBC, LPS or PBS) and initial mass as fixed effects; between-treatment differences were contrasted within the models using the "estimate" statement. Treatment effects on changes in body mass over the course of the experiment were conducted using a general linear model with the difference in body mass between 0 and 7 days post-injection as the dependent variable and with treatment and initial mass as fixed effects; between-treatment differences were contrasted using the "estimate" statement.

Results

EFFECTS OF IMMUNE TREATMENT ON RESTING AND MAXIMAL METABOLIC RATES

Resting and maximal rates of oxygen consumption and the metabolic scope were all significantly associated with body mass, regardless of the treatment (GLM; resting metabolic rate: $F_{1,67}=9.77$, $p=0.0026$, maximal metabolic rate: $F_{1,67}=45.01$, $p<0.0001$, metabolic scope: $F_{1,67}=26.6$, $p<0.0001$). First, we investigated the metabolic cost of an immune response to LPS. LPS-injected fish displayed higher resting and maximal rates of oxygen consumption than saline-injected controls over the course of the experiment (GLMM; resting rates of oxygen consumption: treatment: $F_{1,38}=8.8$, $p=0.005$, time: $F_{2,38}=1.2$, $p=0.319$; initial mass:

219 $F_{1,38}$=9.32, p=0.004; maximal rates of oxygen consumption: treatment: $F_{1,38}$=6.14, p=0.018,
220 time: $F_{2,38}$=1.1, p=0.334, initial mass: $F_{1,38}$=13.7, p<0.001; Table 1; Fig. 1a, b). Separate
221 analyses of each time point revealed significant between-treatment differences in resting
222 metabolic rates 24 h post-challenge and in maximal metabolic rate 48 h and 7 days post-
223 challenge (resting metabolic rate: 24 h: t_{36}=2.41, p=0.021; 48 h: t_{36}=1.22, p=0.230; 7 days:
224 t_{36}=1.97, p=0.057; maximal metabolic rate: 24h: t_{36}=0.02, p=0.986; 48h: t_{36}=2.88, p=0.007; 7
225 days: t_{36}=1.90, p=0.066).
226
227 **Table 1:** Metabolic measures for immune-challenged (with LPS or SBRC) and control
228 (saline-injected) mosquitofish. We provide raw means (in μmol/min/g) and standard
229 deviations for resting and maximal metabolic rates and for metabolic scopes at 24h, 48h and
230 7 days post-challenge with LPS, and at 7 days post-challenge with SRBC.

Time post-treatment	**Metabolic measure**	**Treatment**		
		Control	LPS	SRBC
24h	Resting	0.163 ± 0.053	0.293 ± 0.174	
	Maximum	0.831 ± 0.147	0.932 ± 0.255	
	Scope	0.668 ± 0.147	0.640 ± 0.224	
48h	Resting	0.202 ± 0.060	0.282 ± 0.083	
	Maximum	0.692 ± 0.124	1.038 ± 0.198	
	Scope	0.490 ± 0.110	0.756 ± 0.207	
7 days	Resting	0.205 ± 0.078	0.322 ± 0.150	0.209 ± 0.082
	Maximum	0.809 ± 0.133	1.196 ± 0.675	1.696 ± 0.786
	Scope	0.605 ± 0.127	0.874 ± 0.579	1.488 ± 0.750

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232
233 **Fig. 1:** Resting (a) and maximal (b) rates of oxygen consumption and metabolic scope (c) of
234 LPS-injected and PBS (control) fish 24h, 48h and 168h (7 days) after treatment. Values show
235 predicted means (in μmol/min/g) with standard errors (* indicates p<0.05).
236

Second, we examined the metabolic cost of an immune response to SRBC and compared the metabolic rates of SRBC- and LPS-fish. Resting rates of oxygen consumption 7 days post-injection differed significantly between treatments (GLM, treatment: $F_{2,25}$=5.6, p<0.010, initial mass: $F_{1,25}$=4.9, p=0.036; Table 1; Fig. 2a, b). However, between-treatment tests revealed that differences were significant between LPS and SRBC-fish only (t=3.17, p=0.004), with SRBC-fish displaying resting rates of oxygen consumption that were 50% lower than LPS-injected ones, but not between SRBC-fish and saline-injected controls (t=1.4, p=0.171). Treatment did not affect maximal rates of oxygen consumption 7 days post-injection (GLM, treatment: $F_{2,25}$=0.5, p=0.600, initial mass: $F_{1,25}$=8.3, p=0.008), with no significant difference detected between SRBC-fish and either LPS-fish or controls (p>0.1).

Fig. 2: Resting (a) and maximal (b) rates of oxygen consumption and metabolic scope (c) of SRBC-, LPS- and PBS-injected (control) fish 7 days after treatment. Values show predicted means (in μmol/min/g) with standard errors (** indicates p<0.01).

EFFECTS OF IMMUNE TREATMENT ON METABOLIC SCOPES AND BODY MASS

LPS-injected fish displayed a significantly greater metabolic scope over the course of the experiment than sham-injected controls (GLMM, treatment: $F_{1,36}$=1.44, p=0.238, time: $F_{2,36}$=0.8, p=0.460, treatment × time: $F_{2,36}$=3.3, p=0.050, initial mass: $F1_{,36}$=4.7, p=0.037; Table 1; Fig. 1c). Separate analyses of each time point revealed a significant difference between LPS-challenged and control fish 48h after treatment (24h: t_{36}=-1.01, p=0.321; 48h: t_{36}=2.32, p=0.026; 7 days: t_{36}=1.04, p=0.306). Although SBRC-injected fish displayed higher metabolic scopes than controls 7 days post-injection, the effect of treatment on metabolic scopes at that time point was not significant (GLM, treatment: $F_{2,25}$=0.8, p=0.443, initial mass: $F_{1,25}$=5.7, p=0.025; Table 1; Fig. 2c), with no significant differences detected in any of

the pairwise comparisons (all p>0.1). There was a significant effect of treatment on mass change over the course of the experiment (GLM, treatment: $F_{2,25}$=7.3, p=0.003, initial mass: $F_{1,25}$=5.0, p=0.034; Fig. 3), with mass change differing significantly between LPS- and control fish (t=-3.7, p=0.001) and between SRBC- and control fish (t=2.8, p=0.010). LPS-fish lost on average over 4% of their body mass over the 7 days of the experiment (initial mass=0.73±0.21g; final mass=0.70±0.18g), but SRBC and control-fish increased their body mass by 5% and 8%, respectively (SRBC: initial mass=0.42±0.14g, final mass=0.44±0.17g; control: initial mass=0.90±0.24g, final mass=0.97±0.21g).

Fig. 3: Mass change between the day of the injection (day 0) and 7 days after injection. Values show predicted means (in g) with standard errors (** indicates p≤0.01 and *** indicates p≤0.001).

Discussion

Our results show that experimental challenges with an inert antigen can give rise to changes in resting and maximal metabolic rates, with measurable consequences for metabolic scopes and hence the overall amount of ATP available to aerobically-challenging activities other than those at rest. Resting and maximal metabolic rates and the metabolic scope were significantly increased relative to controls in LPS-challenged fish only. Furthermore, immune-challenged fish gained significantly less body mass over the course of the experiment than controls, with LPS-fish actually losing body mass. Our results indicate that oxidative metabolic capacities can be increased in immune-challenged individuals, such that these individuals actually produce more ATP than would be needed for engaging in aerobically-challenging activities in the absence of an immune challenge.

The higher resting metabolic rate displayed by LPS-challenged individuals relative to controls corroborates the existence of an energetic cost associated with the immune response to LPS. LPS are immunogenic molecules found in the cell wall of gram-negative bacteria that can rapidly trigger a strong inflammatory response without causing infection [46]. In endotherms, injections with *E. coli* LPS are commonly used to assess the acute phase response, which occurs within hours of challenge and includes changes in body temperature (e.g., fever) and the expression of sickness behaviours [47-50]. Responses to LPS have been indirectly shown to be costly. For example, LPS-challenged individuals displayed decreased food intake, activity and growth, and exhibited reduced reproductive output [14, 15, 49, 51-53]. Furthermore, direct energetic costs of LPS injections have been demonstrated as a 10 and 20% increase in the resting metabolic rates of zebra finch and rats, respectively [15, 54]. In fish, LPS has been shown to induce a depletion of liver glycogen levels in yearling coho salmon (*O. kisutch*) and rainbow trout [55], and it is likely to be a potent agent of anorexia in gold fish (*Carassius auratus auratus*) [56]. Our results verify the metabolic cost of a response to LPS in fish, which can be maintained over the course of a week, but is higher 24h than 7 days post-challenge.

On the other hand, the lack of significant difference in resting metabolic rates between SRBC-treated and control fish suggests either that the cost of immunity to SRBC is negligible or that it becomes detectable later than 7 days post-challenge. SRBC is a T cell-dependent antigen commonly used to assay humoral immune responses, and anti-SRBC antibodies reach a peak 10 and 12 days after injection in brown trout and Mozambique tilapia, respectively [40, 41]. SRBC-injections were previously found to produce a 8.5% increase in the basal metabolic rate of collared doves 7 days after challenge [23], yet they did not affect body mass and molting in house sparrows (*Passer domesticus*) [57]. Similarly,

SRBC-injected greenfinches (*Carduelis chloris*) were shown to exhibit reduced activity, but no mass loss, 4 and 8 days post-challenge [58], suggesting that SRBC induces only a mild sickness response. While the metabolic costs and consequences of the immune response mounted by mosquitofish against SRBC still remain to be determined, the fact that SRBC-fish gained significantly less mass over the course of the experiment than controls suggests that a challenge with SRBC induces measurable energetic costs the first week post-injection.

Immune-challenged mosquitofish did not display a decreased metabolic scope relative to saline-injected controls, indicating that immune functioning did not give rise to a trade-off at the level of ATP production and use. In fact, not only were metabolic scopes not decreased in LPS-injected fish, but they were actually greater than those of controls, indicating that individuals actually boosted their levels of energy available to physical activity. This increase in metabolic scope was significant only at 48h post-injection in LPS-fish, indicating that this process is not immediate and may only be transitory. One explanation for such overcompensation is that immune-challenged fish require more ATP to sustain swimming than saline-injected controls. LPS has indeed been shown to decrease the efficiency of carbohydrate catabolism by skeletal muscles, that is the ratio between ATP use and power output, giving rise to greater oxygen consumption during activity, and to stimulate muscle wasting leading to muscle dysfunction [59-61]. In fish, white skeletal muscle is mostly anaerobic and used primarily in burst swimming, while red skeletal muscle is aerobic and involved in sustained swimming speed [62]. Gilthead seabream (*Sparus aurata*) challenged with LPS displayed strong transcriptomic responses in their white and red skeletal muscles 24h and 72h post-injection [11]. Protein synthesis and carbohydrate catabolism were strongly increased in white muscle 24h post-LPS administration, which suggested in part that LPS may initially stimulate energy production through glycolysis; these patterns were, however,

reversed at 72h possibly indicating muscle atrophy. Genes involved in aerobic metabolism and protein synthesis, on the other hand, were up-regulated 72h post-challenge in red muscle [11]. Similar increases in carbohydrate metabolism were observed in the fast muscle fibres of rainbow trout following challenge with LPS [63]. Whether the increased aerobic metabolism that we detected in mosquitofish 48h post-immune challenge with LPS, allowed fish to swim at an equivalent speed or faster than control fish was not explored in this study. The capacity to increased aerobic metabolism may be positively selected for if it facilitates parallel increases in two or more concurrent, aerobically-demanding activities, such as immunity and locomotion, that maximize survival and hence residual reproductive values [64, 65]. Future work is required to better understand the consequence of immune-associated increases in metabolic scope on locomotor performance (e.g., swim speed and duration) as well as its evolutionary significance for individual fitness.

While immune functioning did not give rise to a trade-off in terms of metabolic scope, the lowered relative body mass of immune-challenged fish at the end of the experiment suggests that limited acquired food resources, or that impaired food assimilation or mitochondrial use of those resources [66], may instead drive trade-offs between immunity and other aerobic traits. For e.g., inflammatory responses have indeed been found to cause mitochondrial dysfunction [67] and a lower efficiency of mitochondria as a result of proton leak, for example, would mean that greater amounts of substrate and ultimately food or energy reserves) have to be oxidised to achieve a given ATP output [66]. Fish that were challenged with LPS- or SRBC- both exhibited reduced body mass gain relative to controls over the 1 week duration of the experiment, with LPS-fish actually loosing mass and SBC-fish gaining >4 times less mass than controls. Divergence in mass change between SRBC- and LPS-fish is likely to stem, in part, from LPS-induced adaptive anorexia, which may have prevented a

362 compensation of the energetic costs of immunity through greater food intake [56]. Anorexia
363 is, indeed, a host defence mechanism against bacterial infections [68] and a typical
364 component of the sickness response to LPS [15, 51], but not SRBC [57, 58].
365
366 Our data show that plasticity in oxidative metabolism is at least a short term response to an
367 immune challenge that can increase the fitness of individuals by maintaining locomotor
368 performance, reproductive activity, and similar aerobically-demanding functions. The
369 benefits of such upregulated ATP production, however, are expected to diminish with muscle
370 loss and as body condition decreases below critical levels.
371
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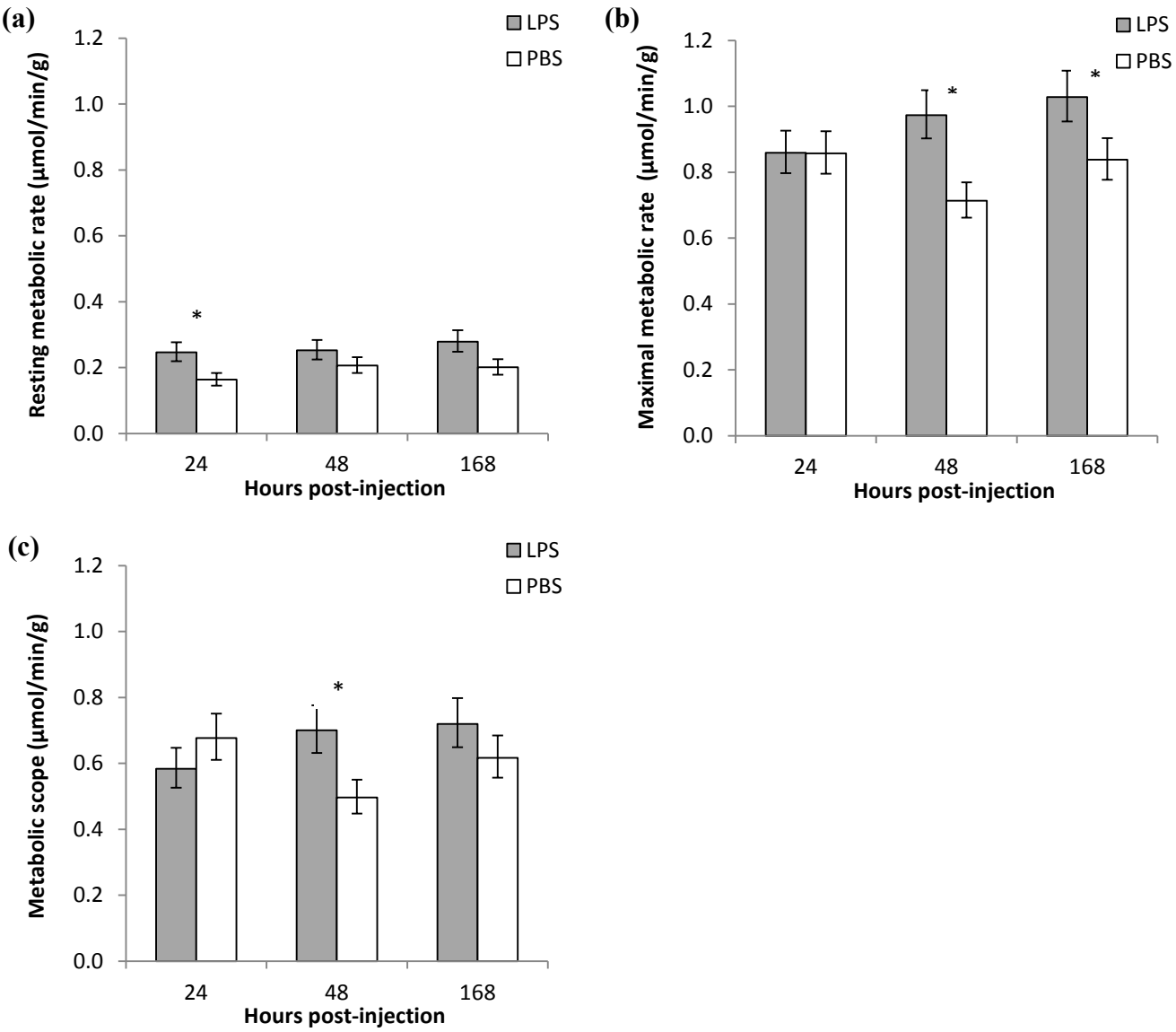
572

573

Fig 1


(a)
LPS
PBS
Resting metabolic rate (µmol/min/g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
24
48
168
Hours post-injection
(b)
LPS
PBS
Maximal metabolic rate (µmol/min/g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
*
*
24
48
168
Hours post-injection
(c)
LPS
PBS
Metabolic scope (µmol/min/g)
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0.6
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0.2
0.0
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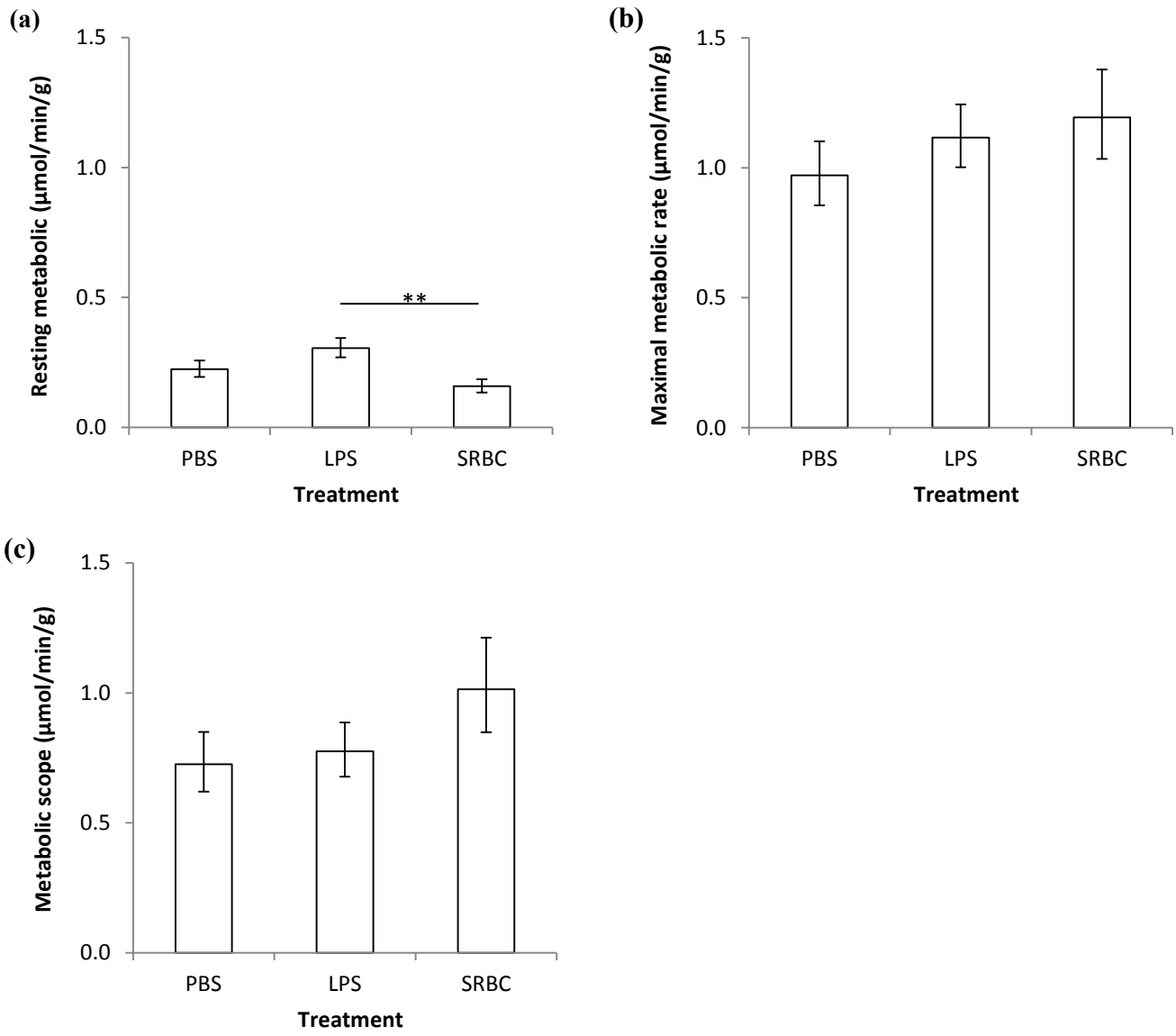
24
48
168
Hours post-injection

Fig 2


(a)
Resting metabolic (µmol/min/g)
1.5
1.0
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**
PBS
LPS
SRBC
Treatment
(b)
Maximal metabolic rate (µmol/min/g)
1.5
1.0
0.5
0.0
PBS
LPS
SRBC
Treatment
(c)
Metabolic scope (µmol/min/g)
1.5
1.0
0.5
0.0
PBS
LPS
SRBC
Treatment

Fig 3