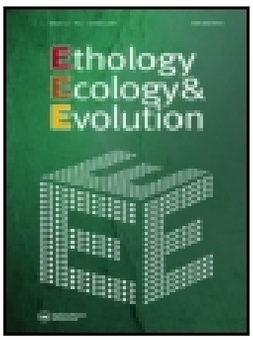

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Postcopulatory cost of immune system activation in *Poecilia reticulata*

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The function and maintenance of the immune system impose costs that can take the form of energy consumption and/or physiological side effects. These, in turn, can affect the expression of several traits, including traits closely related to fitness. The interaction of the immune system with other life-history traits (e.g. growth and survival) and with precopulatory sexually selected traits (e.g. male ornaments) has been widely studied, whereas little is known about its interplay with postcopulatory sexually selected traits, such as sperm characteristics. We studied the effects of an immune challenge on the expression of precopulatory and postcopulatory traits in the guppy, *Poecilia reticulata*. We injected two groups of males with a lipopolysaccharide (LPS) solution or a saline solution (control) and compared the effects of the immune system activation on traits' expression. The results showed a specific negative effect of the LPS injection on sperm traits, whereas precopulatory ornaments were lowered merely by manipulation (injection). We discuss the possible mechanisms underlying our findings, and the evolutionary implications that follow.

KEY WORDS: lipopolysaccharides, *Poecilia reticulata*, immunocompetence, postcopulatory sexual selection, sperm velocity, ejaculate.

INTRODUCTION

Activation of immunological machinery in response to pathogens is known to impose significant costs on individuals (Lochmiller & Deerenberg 2000). These costs could be direct (mediated by the physiological products and by-products of immunological machinery), indirect (due to the energy consumption) or both (von Schantz et al. 1999; Hanssen 2006). Independently of the nature of these costs, their presence may generate trade-offs between immune response and other life-history traits (e.g. Moret & Schmid-Hempel 2000; Rigby & Jokela 2000; Soler et al. 2003; Jacot et al. 2004; Kilpimaa et al. 2004), in particular with those traits related to reproduction (Lawniczak et al. 2007). As a consequence of these trade-offs, a reduced expression of sexually selected traits,

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which are energetically costly to maintain and often phenotypically flexible (Griffith & Sheldon 2001; Piersma & Drent 2003; Oufiero & Garland 2007), is expected when a higher investment in the up-regulation of the immune system occurs. This is a keystone concept in parasite-linked sexual selection theories that link the expression of specific male traits to the resistance to parasites (Hamilton & Zuk 1982). Following this hypothesis, for which there is significant empirical evidence (Faivre et al. 2003; McKean & Nunney 2008; Lopez et al. 2009; Kelly 2011; McNamara et al. 2013), only resistant males are expected to have sufficiently low parasite burdens to produce high-quality sexual signals (Hamilton & Zuk 1982). However, a positive correlation between reproductive traits and immune response may be also observed (Jacobs & Zuk 2011), for example when ornaments act as signals of immunological capabilities to prospective mates (Blount et al. 2003; Ahtiainen et al. 2004; Peters et al. 2004; Locatello et al. 2012; Zanollo et al. 2012). In general, a positive covariance between traits is expected when variability in resource acquisition is greater than the variability in resource allocation to traits (Reznick et al. 2000). Despite the increasing attention devoted to investigating the effect of immune system activation on precopulatory traits (as "armaments" or "ornaments"; see e.g. Folstad & Karter 1992; Lozano 1994; Roberts et al. 2004; Schmid-Hempel 2011; Kelly 2014), very few studies have focused on traits that concur with reproductive fitness after copulation, such as ejaculate characteristics. However, these traits also may be affected by immune system activation. Indeed, as producing good-quality ejaculate is costly (Dewsbury 1982; Parker & Pizzari 2010) a trade-off in resource allocation between ejaculate traits and other traits, not least immunity, may arise (Folstad & Skarstein 1997; Devigili et al. 2015a; Cattelan et al. 2016). Moreover, an up-regulation of the immune system is also expected to directly reduce sperm quality as gametes represent non-self material (e.g. Liljedal et al. 1999) and are normally protected via immunosupression (Menkveld 2004; but see Meinhardt & Hedger 2011). The interplay between immunocompetence and ejaculate quality has been less widely addressed, and, except in a handful of cases (Liljedal et al. 1999; Santiago-Moreno et al. 2010; Losdat et al. 2011; Figenschou et al. 2013; Kekalainen et al. 2014), most of the evidence comes from invertebrates (see e.g. Kerr et al. 2010; Dowling et al. 2012; Radhakrishnan & Fedorka 2012; Simmons 2012; Nystrand & Dowling 2014; Sturup et al. 2014). Here, we adress this dearth by exploring the effects of an immune challenge on precopulatory (coloured ornaments) and postcopulatory (sperm number and velocity) traits in the guppy, *Poecilia reticulata* (Peters 1859).

P. reticulata is a small teleost fish in which polyandrous females choose partners primarly on the basis of coloured ornaments (orange, iridescent and black spots) and sexual displays (Houde 1997). As a consequence of female promiscuity and male mating coercion, sperm competition (the competition of sperm of two or more males in fertilising eggs; Parker & Pizzari 2010) and cryptic female choice (the capacity of females to favour sperm of some males over others; Eberhard 1996) play a crucial role in determining male fitness, making ejaculate traits particularly important (Pilastro et al. 2004; Boschetto et al. 2011; Evans & Pilastro 2011; Devigili et al. 2015b). In this species, ornaments and ejaculate are strictly connected as more colourful males produce better ejaculate (Locatello et al. 2006; Pitcher et al. 2007) being thus favoured in competition both before and after mating. In the population used in this study (originally from lower Tacarigua river, Trinidad) sperm number is costly and condition dependent, and responds to food deprivation more strongly than body colouration does (size of colour spots, Gasparini et al. 2013). Studies on other populations have revealed that sperm velocity and viability are also affected by food deprivation (Devigili

et al. 2013; Rahman et al. 2013; Evans et al. 2015). In this species, the selective pressures imposed by parasites have been studied both in controlled conditions (Cable & van Oosterhout 2007; Richards et al. 2010) and in the wild (van Oosterhout et al. 2007). Results demonstrated how the resistance to parasites is variable and heritable, with parasites affecting group life, migration and demography (Cable 2011). The interplay between parasites and the expression of orange colouration (Houde & Torio 1992) and between parasites and behaviour (Kolluru et al. 2009) has been also well documented in this species. With few exceptions (Martin & Johnsen 2007), studies confirm predictions that more colourful individuals better resist parasites and that, on the other hand, parasites can negatively affect the expression of precopulatory sexually selected traits (Kolluru et al. 2009). To our knowledge, however, the effect of infections on postcopulatory traits in this species has not been investigated so far. Moreover, previous studies have considered the response of individuals to live parasites, making it impossible to disentangle the cost of immune system activation from the cost of a direct pathogenic infection, potentially making the effective manipulation further vary from one individual to another (Bonneaud et al. 2003). Bacterial lipopolysaccharides (LPS) are potent immune elicitors that mimic a bacterial infection by triggering various immune parameters, such as T and B lymphocytes, macrophages and complement systems (Swain et al. 2008). The use of LPS, well documented in teleost fish (reviewed by Swain et al. 2008), allows the raising of a general immune response while avoiding the introduction of a replicating, metabolically active pathogen into the host (Bonneaud et al. 2003). The costs driven by an immune elicitor are set in place at the initial time of exposure in an effort to immediately clear the infection, whereas a live pathogen implicates costs that would be brought about by the sustained infection. A live pathogen can, for example, invoke disease phenotypes via replication, manipulation of host resources and the cellular environment, and interference in host signalling pathways, as well as direct tissue damage (see Nystrand & Dowling 2014).

We compared the expression of precopulatory (colouration) and postcopulatory (sperm number and velocity) sexually selected traits of a group of guppy males injected with LPS solution ("LPS group") with those of a control group injected with saline solution ("SAS group").

MATERIALS AND METHODS

Fish maintenance

Experimental fish were descendants of wild-caught fish from the lower part of Tacarigua River, Trinidad. Fish were reared under standard conditions in 125-L stock aquaria where the sex ratio was approximately 1:1 (see Gasparini et al. 2010 for the details of fish maintenance). We used 40 males between 6 and 8 months old, randomly collected from stock aquaria and randomly assigned to one of two experimental groups (n = 20 in each group). All fish were fed ad libitum before and during the experiment with commercial dry food (DuplaRin) and fresh *Artemia salina* nauplii.

Experimental design

At the beginning of the experiment (day 0; Fig. 1), we proceeded with the morphological measures of all individuals (see the next section on "Male size and ornamentation"). The same day

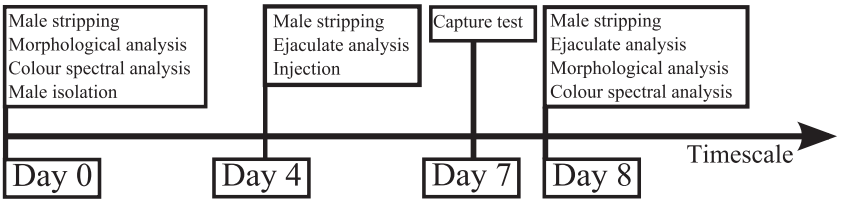

Fig. 1. — Graphical representation of the experimental setup and timing.

sperm was stripped from males but not used for analysis, as sperm age was not known and sperm reserves were possibly affected by recent copulations in the stock tank (Gasparini et al. 2014). Males were then randomly divided into two groups which underwent the same manipulation except for the treatment solution (treated males: LPS; control males: SAS). Pre-treatment measurement of sperm traits (see the section below on "Sperm analyses") was performed after 4 days of isolation (day 4; Fig. 1). During this period, males were reared in standard conditions in 2-L tanks, physically but not visually isolated from females. This procedure ensured a complete replenishment of sperm reserves with fresh gametes (Bozynski & Liley 2003; Gasparini et al. 2009). Immediately after sperm collection, males were injected with 1.25 µL of LPS solution or saline solution. LPS solution was obtained by dissolving lyophilised LPS (*Escherichia coli* lipopolysaccharides serotype O55:B5, Sigma Chemical) in 0.9% saline solution (final concentration LPS/SAS: 0.8 µg/µL). As male guppies weigh on average 0.1 g, the final amount of LPS injected in each male was 1 µg, corresponding to a dose of LPS of 10 mg/kg of fish body mass (this dosage is comparable to those of other studies; Swain et al. 2008). Injection was performed with commercial insulin syringes with a 0.26-mm needle diameter. A screw plunger was used to ensure the injection of a precise amount of solution. Different syringes were used for injecting males of the two groups. Before each injection the needle was sterilised. For the injection each male was placed on a polystyrene support covered with medical sterile tape. Injection was then performed in the dorsal part of the left side of the fish, near the caudal peduncle. After this procedure, fish were isolated in individual tanks (2 L). Three days after treatment (day 7; Fig. 1) we measured male escape ability (see the section below on "Male escape ability"), and 4 days after treatment we re-measured male morphological traits and sperm traits (day 8; Fig. 1).

Before any manipulation, males were anesthetised with tricaine methanesulfonate (MS-222) at 150 mg/L concentration (Chambel et al. 2013).

Two males (one in each group) died before the end of the experiment. The final sample size was, therefore, 38: 19 LPS-treated and 19 control individuals. Males used in the experiment did not differ in any of the parameters measured before the injection (Student *t*-tests; all $P > 0.105$; see Table 1).

This experiment was conducted according to the Italian legal requirements and was approved by the Ethics Committee of the University of Padua (permit no. 55/12 to M.B. Rasotto).

Male size and ornamentation

Anesthetised males were photographed near a ruler (Canon 450D camera, equipped with Canon EFS 60 mm MACRO lens and circular flashlight). Pictures were analysed using UTHSCSA ImageTool software (http://ddsdx.uthscsa.edu/dig/itdesc.html). Standard length (SL), body area and colour spot area were measured on the left side of each male. Spots were assigned to three different categories (Brooks & Endler 2001): carotenoids and pteridine (orange, red and yellow spots, hereafter "orange"), melanin (black and dark spots, hereafter "black") and structural spots (iridescent, green-white or blue-violet spots, hereafter "iridescent"). After photography males were gently placed on a dark plastic support, and spectral characteristics of a single orange spot were measured 3 times. The biggest spot, sized by eye, was chosen. We used an S2000 spectrometer

Table 1.

Descriptive statistic of male traits measured and used in the analysis. For each group, means ± standard deviations are given. Measurements both before and after treatment (if measured) are given.

	SAS group		LPS group	
Male traits	Before	After	Before	After
Sperm number (^10^6)	6.75 ± 4.71	5.39 ± 3.43	6.25 ± 4.63	3.06 ± 2.72
Sperm number (Log10)	6.71 ± 0.35	6.63 ± 0.33	6.59 ± 0.54	6.19 ± 0.63
VAP (μm/sec)	87.58 ± 10.56	91.57 ± 9.03	88.13 ± 11.48	81.17 ± 12.42
VSL (μm/sec)	80.00 ± 10.56	83.80 ± 9.00	81.17 ± 10.90	72.77 ± 13.45
VCL (μm/sec)	116.17 ± 8.23	118.14 ± 8.85	115.81 ± 10.65	111.84 ± 12.36
Sperm velocity (PC)	– 0.02 ± 0.95	0.42 ± 0.78	0.02 ± 1.07	– 0.42 ± 1.04
Orange %	8.15 ± 3.4	7.14 ± 2.72	9.22 ± 3.15	8.35 ± 2.71
Black %	2.76 ± 1.31	2.12 ± 1.27	3.11 ± 1.57	2.92 ± 2.17
Iridescent %	7.91 ± 2.1	8.08 ± 3.34	6.58 ± 2.81	7.20 ± 2.51
Orange chroma	0.31 ± 0.02	0.30 ± 0.02	0.32 ± 0.02	0.30 ± 0.03
Standard length (mm)	17.59 ± 1.24		17.45 ± 1.15	
Capture time (sec)[1]		13.31 ± 9.19		8.3 ± 3.6
Capture time (Log10)[1]		1.06 ± 0.324		0.88 ± 0.18

[1]One outlier of SAS group (94 sec) was excluded. VAP: average path velocity; VSL: straight line velocity; VCL: curvilinear velocity; PC: principal component.

equipped with an ADC1000-USB (Ocean Optics, Inc., USA; work range: 400–1000 nm) connected with a halogen light source (HL-2000-FHSA-LL, Ocean Optics, Inc., USA, range: 360–1700 nm). Spots were illuminated and their reflectance measured with a bifurcated 400-μm fibre-optic cable (Ocean Optics, Inc., USA). The distal end of the fibre probe was fitted with an opaque, black plastic tube that allowed us to sample 1.5 mm Ø area, to maintain the probe end at a constant distance of 4 mm from the fish surface, and to exclude ambient light. Before measuring each male, we calibrated the spectrometer using a white standard (WS-1, Ocean Optics). Data were digitalised with SpectraSuite software (OceanOptics) and subsequently analysed using AVICOL software (freely available at https://sites.google.com/site/avicolprogram, last update October 2013; Gomez 2006). Raw spectra consisted of an array of data points (≈ 2000) representing the reflectance in the 360–1000 nm wavelength interval (one measurement every 0.325 nm). We considered only the visible light range (400–700 nm). The three spectral measurements of each spot were averaged. Data were then reduced by averaging every 2 nm to obtain 151 measurements of reflectance for each sample. Orange chroma, calculated as the proportion of reflectance occurring between 550 and 625 nm over the total reflectance ($R_{550–625}/R_{400–700}$), represents the colour purity in the region of the spectrum corresponding to orange, and was used in subsequent analysis as an index of orange spot colour quality (Montgomerie 2006).

Sperm analyses

Males were stripped for baseline sperm counts and sperm velocity analysis on day 4 and day 8, with a standard protocol (Matthews et al. 1997; Gasparini et al. 2010). Males were placed on a

Petri dish under a dissection microscope with 0.5 mL of physiological solution, and a gentle pressure was applied to the side of the abdomen with a mall probe. In this species, sperm are released in packages (spermatozeugmata) called sperm bundles and containing an average of 22,000 sperm (Gasparini et al. 2013). Computer-assisted sperm analysis (Hamilton-Thorne CEROS) was performed for each male using six bundles, collected with a Drummond Micropipette, placed on a multi-well slide and activated with 150 mM potassium chloride (KCl) and 4 mg/mL bovine serum albumin (BSA) (Gasparini et al. 2013). Three standard measures of sperm velocity were obtained: average path velocity (VAP), straight line velocity (VSL) and curvilinear velocity (VCL). On average, a total of 152 (± 9 standard error, SE) sperm were used for each male to estimate sperm velocity. As VAP, VSL and VCL are highly correlated in this species (Devigili et al. 2013) and in this population (Boschetto et al. 2011), we decided to reduce the variables with a principal component analysis (PCA; Devigili et al. 2013). Sperm velocities measured before and after the treatment were considered separately in two distinct PCA, both of which led to a single principal component (PC; see Table 2) subsequently used for statistical analysis. All other bundles were collected in a known volume of saline solution for sperm counting using an "improved Neubauer haemocytometer" under 400× magnification. The average of 10 counts per male was used to estimate sperm reserves at rest (Devigili et al. 2013).

Male escape ability

Three days after injection, males were tested for predator escape ability (called "capture test"; adapted to adults from Evans & Magurran 2000), in order to test for the effectiveness of immune stimulation on male condition. Predator escape ability is a good estimate of condition used in guppy and other fishes (Grorud-Colvert & Sponaugle 2006; Gasparini et al. 2013). We measured individual condition as a proxy of the efficacy of the immune challenge, since the small body size of our study species did not allow any direct measurement of immunological parameters through blood sampling. However, it is proven that the energetic expenditures associated with immune response may have a non-trivial impact on an individual's condition (e.g. Ots et al. 2001). The "capture test" consisted in recording the time needed to capture the fish with a hand fishnet using a standardised procedure (see Gasparini et al. 2013 for a detailed description). Briefly, each fish was placed in a 20-L tank with multicolour gravel and left for 10 min of acclimatisation. After this period, the same person measured the time needed for capturing the fish, chasing it with the hand fishnet at a speed kept as constant as possible. Inbred and food-deprived guppies show a significantly reduced capture time (Gasparini et al. 2013), and this test is highly repeatable in this species ($r = 0.68 \pm 0.13$ SE, $F_{18,37} = 5.30$, $P < 0.001$; Gasparini et al. 2013).

Table 2.

Principal component analysis (PCA) of sperm velocity parameters. PCA have been performed separately for the three parameters of sperm velocities (VAP, VSL and VCL) measured before and after treatment. Only components with a score greater than 1 are retained. Loading factors and percentage of variance explained are given.

	PC pre	PC post
VAP	0.987	0.985
VSL	0.984	0.948
VCL	0.943	0.854
Variance explained	94.4%	86.6%

VAP: average path velocity; VSL: straight line velocity; VCL: curvilinear velocity; PC: principal component.

Statistical analysis

Male ornamentation and sperm traits were all compared between the two groups using univariate repeated measures analyses of variance (RMANOVA), with a two-level "within-subject" factor representing the change in the traits before and after the treatment (named "time"). Group treatment was used in the model as fixed factor "between subjects". Colour spot size, colour index, sperm number and sperm velocity were entered as dependent variables. For the analysis of sperm number, standard length was entered as covariate in the model since the number of sperm produced was positively correlated with male size (Pearson correlation, number of sperm before the treatment: Pearson = 0.367; $P < 0.023$; number of sperm after the treatment: Pearson = 0.372; $P < 0.022$). Male escape ability was measured after the treatment and compared between groups with Student *t*-test. Paired-sample *t*-tests were used to evaluate the differences within groups, before and after injection, where the two groups respond differently. Capture time and sperm number (millions) were log10 transformed before statistical analysis in order to meet normality assumptions. All analyses were performed with PASW 18.

RESULTS

After removing one outlier (in the SAS group), whose capture time was ca 7 times longer than the average (see Table 1 for descriptive statistic), results of the capture test showed that LPS males were captured more rapidly than their control counterparts were (Fig. 2A; Student *t*-test; $t_{1,35} = 2{,}489$, $P = 0.017$).

Both LPS and saline-solution injections had an effect on precopulatory traits. Orange area (but not iridescent and black spot size, all $P > 0.101$) was significantly and equally reduced after treatment in both experimental groups (RMANOVA; Time: $F_{1,36} = 5.043$, $P = 0.031$; Time*Treatment: $F_{1,36} = 0.025$, $P = 0.876$). The same pattern was observed for orange chroma (RMANOVA; Time: $F_{1,36} = 9.555$, $P = 0.004$; Time*Treatment: $F_{1,36} = 0.918$, $P = 0.344$).

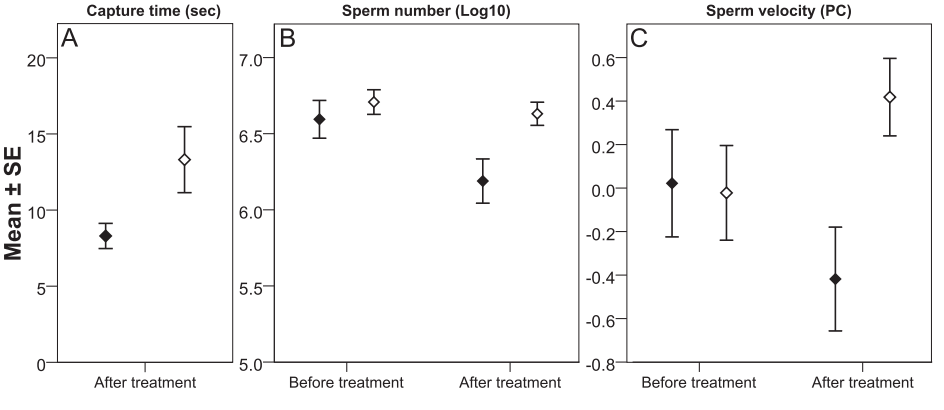

Fig. 2. — Effect of injection on A: capture time, B: sperm number and C: sperm velocity (after principal component analysis). Open diamonds represent males of SAS group and black diamonds represent males of LPS group. Sperm number and sperm velocity were measured both before and after the treatment (injection), whereas capture time was measured only after the treatment. Means ± standard errors (SE) are given.

Postcopulatory traits were strongly affected by LPS treatment (Fig. 2B–C). Sperm number decreased in both experimental groups (see Table 1) but this decrement was significantly stronger in LPS-treated males (RMANCOVA (repeated measure analysis of covariance); Time: $F_{1,35} = 1.103$, $P = 0.301$; Time*Treatment: $F_{1,35} = 4.891$, $P = 0.034$; Time*SL: $F_{1,35} = 0.692$, $P = 0.411$). Sperm velocity (expressed by Sperm PC1, see "Material and Methods" section) changed differently between groups (RMANOVA; Time: $F_{1,36} = 0.000$, $P = 1.000$; Time*Treatment: $F_{1,36} = 5.936$, $P = 0.020$): while in the LPS group sperm velocity decreased, in the SAS group the velocity increased (see Table 1 and Fig. 2). Paired-sample *t*-tests show that in the LPS group the change in sperm number was statistically significant ($t_{1,18} = 3.14$, $P = 0.006$), whereas the decrease in sperm velocity was close to the significant threshold ($t_{1,18} = 1.82$, $P = 0.084$). No such differences were observed in the SAS group ($P > 0.120$).

DISCUSSION

With this experiment we evaluated a possible cost of immune system activation, focusing on its consequences for pre- and postcopulatory sexually selected male traits. To precisely estimate this cost we elicited an inflammation through LPS. This allowed us to exclude the component related to the parasite and to focus only on the host side.

We found that our treatment strongly affected escape ability (capture time), suggesting that LPS injection reduced the general health condition of individuals (Fig. 2A). We also observed a decrease in the size and intensity of orange colourations which, however, occurred almost equally in both groups. On the other hand, the detrimental effect on sperm number was significantly stronger in the group treated with LPS. Sperm velocity was oppositely affected in the two groups (Fig. 2B–C).

The effect observed on the expression of precopulatory traits, which was equal in both the control and LPS-treated groups, did not support our expectation of a stronger decrease in immune-challenged animals. Indeed, in the guppy, yellow/orange ornamentations are carotenoid based, and, as in many animal species, they may represent an honest signal of the bearer's present condition (Vinkler & Albrecht 2010; Svensson & Wong 2011). These pigments are, in fact, implicated in anti-inflammatory response as they function as anti-oxidants (Krinsky 1989), and may be mobilised from ornaments in response to the oxidative stress caused by infection (Faivre et al. 2003; Vinkler & Albrecht 2010; Svensson & Wong 2011; Locatello et al. 2012). As colouration in guppy is a flexible trait (sensu Piersma & Drent 2003) that readily responds to experimental stress (Devigili et al. 2013; Rahman et al. 2013), including parasite infection (Houde & Torio 1992), a greater decrease in coloured ornaments (spots' colour, size and intensity) of LPS males was expected. It is possible that, in our case, the time interval between LPS injection and trait measurements (4 days) was insufficient to observe the mobilisation of carotenoids from ornaments. Indeed, treatment duration of previous studies performed to test the effect of stress and infection on carotenoids in guppies lasted longer than our experiment (from 9 days up to 1 month; Houde & Torio 1992; Devigili et al. 2013; Rahman et al. 2013). However, in other species, such as the peacock blenny, *Salaria pavo*, a fast reduction of precopulatory traits expression after LPS injection (within 5 days) was observed (Locatello et al. 2012). An alternative explanation to our findings, given the small size of the species used, may lie in the effect of the

manipulation (injection) per se, which could have masked the effect of the induced inflammation.

Postcopulatory traits, on the contrary, were differently affected in the two experimental groups: sperm number and velocity were reduced in LPS-injected fish, whereas changes in the control group were not significant. Different, non-mutually exclusive, explanations could account for these results. An energetic trade-off between the investment in postcopulatory traits and immune response may have arisen, confirming the results found in other vertebrate species (e.g. Liljedal et al. 1999; Losdat et al. 2011; Radhakrishnan & Fedorka 2012). Indeed, both immune response (Lochmiller & Deerenberg 2000; Raberg et al. 2002) and ejaculate production are energetically costly (Dewsbury 1982) and, because energy reserves and resource acquisition are limited, an overinvestment in one trait may affect the pattern of resource allocation to the other (e.g. Simmons 2012). Moreover, as sperm cells are commonly perceived as non-self material by the vertebrate immune system, the immune system activation could have a negative effect on sperm cells (Roitt et al. 1993), thus affecting ejaculate quality (e.g. Liljedal et al. 1999). Lastly, the products of inflammation that are formed as defence against pathogens, such as reactive oxygen species (ROS), are known to undermine sperm motility (Helfenstein et al. 2010; Bucak 2011), sperm morphology (Aziz et al. 2004) and sperm viability (Windsor et al. 1993), finally reducing male fertilisation ability (Guthrie & Welch 2012). Unfortunately, our experiment does not allow us to disentangle the role of these not mutually exclusive mechanisms, and the observed reduction in sperm number and velocity might even be driven by a combination of them.

However, irrespective of the involved mechanisms, considering that guppy male fitness strongly depends on fertilisation success (Evans & Pilastro 2011; Devigili et al. 2015b), the observed postcopulatory costs associated with an immune challenge may have important evolutionary consequences. For example, these costs could enhance ejaculate traits' variability between individuals (Liljedal et al. 1999). Since, in this species, sperm traits are involved in reproductive success (e.g. Boschetto et al. 2011), an increase in ejaculate variability may enhance male fitness variability, with a reinforcement of selection potential (i.e. fewer individuals will have a higher success; Lande & Arnold 1983; Arnold & Wade 1984; Howie et al. 2013; Johnston et al. 2013). An indirect selection based on a good-genes-like hypothesis might even occur, if males of better genetic quality and in better condition could more strongly resist pathogens at lower costs for the ejaculate quality (Liljedal et al. 1999; Lewis et al. 2008). This would make natural and sexual selection reinforce each other by favouring more resistant and better fertilising individuals (Hillgarth et al. 1997; Skau & Folstad 2003), further enhancing variability in male fitness and the potential of selection.

Although far from being fully explicative of the whole scenario of host-parasite interactions in the guppy, our study describes a relevant aspect of the individual response to infections: the effect of an elicited inflammatory response on a series of male sexually selected traits. To our knowledge this is one of the few studies exploring the postcopulatory costs associated with immune system activation in vertebrates.

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DISCLOSURE STATEMENT

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