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ORIGINAL RESEARCH

A theoretical evaluation of the respiration rate partition in the *Gasterosteus aculeatus-Schistocephalus solidus* host-parasite system

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Abstract The oxygen consumption rate in a wild Mediterranean non-migratory population of a teleostean fish, the three-spined stickleback (*Gasterosteus aculeatus)*, was measured to investigate if the infection by the cestode worm *Schistocephalus solidus* plerocercoids increases the oxygen consumption (Vo_2) of the host fish. Previously, an increment in Vo_2 has been reported but never found to be statistically significant. A new approach was carried out in the present study, using a small, but statistically representative, sample of uninfected and infected fish. We used the data on the parasite oxygen consumption available in the current literature to estimate the possible contribution of plerocercoids to the oxygen consumption of our experimental animals. The maximal plerocercoid putative contribution to the measured Vo_2 of the infected host (i.e., host plus parasite) was assessed to be around 2.8%. Interestingly, a key role in the respiration rate of infected sticklebacks turned out to be played by the infection burden. In other words, the host specific Vo_2 (sVo_2) increment was significantly correlated with the number of plerocercoids harboured by the host. The observation implies that a reliable comparison of the physiological parameters between infected and uninfected stickleback populations can be only carried out if the number of parasites among the single individuals is homogeneous. Unfortunately, the random occurrence of the parasites makes this effort unpractical.

Keywords Allometric equation . Host-parasite interaction . Co-evolution . Energy allocation . Oxygen consumption

Introduction

The life of each species is a continuous trade-off among the energies that can be allocated to several activities, like foraging, reproduction, migration, intra- and interspecific interactions, etc. Parasitism is one peculiar way of interspecific interaction between the parasite and its host, in which the two organisms can be seen as a single functional entity. The teleost fish *Gasterosteus aculeatus* (L. 1758), commonly named the three-spined stickleback, and the cestode worm *Schistocephalus solidus* (Müller 1776), form a well-known experimental model to investigate host-parasite interactions (Barber and Scharsack 2010).

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S. solidus undergoes a three stages/three hosts life cycle. The first stage transpires in the haemocoel of the primary host, i.e., a freshwater copepod, which ingests the free-swimming coracidia of the cestode worm. The second occurs in the intermediate host, i.e. *G. aculeatus*, via alimenting on infectious copepods (Bråten 1966). During this stage, the parasite migrates to the abdomen of the fish and grows into an adult size plerocercoid. Sexual maturity is achieved only in the third stage, when an endothermal organism, a piscivorous bird, swallows the infected stickleback, resulting in the settlement of *S. solidus* in the intestine of the final host (Clarke 1954). The fertilized eggs are eventually excreted into the water thus completing the life cycle (Barber and Scharsack 2010).

A parasite infection can impact all aspects of a host's life, including immunity, physiology, and behaviour (Clarke 1954; Khan 2012; Lafferty and Shaw 2013). When a parasite infection modulates a host's behaviour it can result in unpredictable ecological outcomes. For instance, parasitism has been shown to influence fish habitat selection and regulate host activity levels (Lafferty and Shaw 2013). However, the relationship between parasite and host, being a mosaic of factors, is not easy to quantify. The altered responses observed in parasitized fishes are usually linked to the increased opportunity of the parasite to complete its life cycle.

An essential aspect to understand a host-parasite interaction encompasses the quantification of the energy expenditure of each organism. In this context, the boundaries between the two parts of the system are rather undefined. The parasite cannot live as a free form, only relying on the host physiology. The host cannot avoid paying its toll to the parasite presence, mainly in terms of allocated energy, as one of the key factors driving the evolution and adaptation of a species to a changing environment. Thus, it is necessary to draw a functional partition within a host-parasite system. This is a crucial step in the light of the ecological consequences of the energy budget erosion due to an excessive parasite burden. In the *G. aculeatus*-*S. solidus* host-parasite system several lines of evidence support that parasitism by *S. solidus* plerocercoids may influence the anti-predator behaviour of the stickleback (Lester 1971; Giles 1983; Barber et al. 2000), even modulating the circadian surfacing habit of the fish (Quinn et al. 2012). Therefore, the altered behaviour observed in infected sticklebacks was hypothesized to be linked to a parasite-induced increment in their oxygen consumption rate. This was further supported by observational data in which the infected sticklebacks were seen swimming closer to the oxygen-rich surface of the pond, to satisfy their increased requirement for oxygen (Lester 1971; Giles 1983). Despite some evidence suggesting that the oxygen consumption rate of infected fish tended to be higher than that of uninfected ones, the differences were never proven to be statistically significant (Walkey and Meakins 1970; Lester 1971; Meakins and Walkey 1975). Thus, the hypothesis that the plerocercoids affect the energy expenditure of the host (Lester 1971; and as reviewed in Barber et al. 2008), is not supported by a robust assessment. Whether the infected fish show a higher resting respiration rate because of a mere additional effect of the parasite respiration to that of the host, or whether the parasites can drive an increment of the host metabolism remains an open question.

As far as we know, Walkey and Meakins (1970) were the first to suggest a possible role of the parasite number as a parameter to keep into account when evaluating the additional effect over the host respiration rate. However, such a parameter was disregarded from subsequent studies carried on the infected/uninfected comparison of stickleback populations.

In the present study, we started from the following working hypothesis: if the host specific metabolism was not affected by the parasite, its specific oxygen consumption (sVo_2) should be independent from both the parasite number and the parasite index (PI). It is worth to recall that the PI was defined first by Arme and Owen (1967) as the weight of parasites over the total fish plus the parasites weight. Owing to the negative allometry of specific metabolism, we expected that if PI is constant there will be a decrease in the sVo2 of the host when the number of parasites increases.

To test our hypotheses, we took advantage of the published allometric curve of *S. solidus* plerocercoid metabolism (Davies and Walkey 1966) to estimate the dependence of total *S. solidus* Vo2 (tVo_2) from both number and PI.

The results obtained did not support our starting hypothesis and demonstrated that the increasing number of parasites trigger the increment of the host specific metabolic rate. A factor that should be considered in future studies devoted to shed light on the different metabolic rate between infected and

uninfected hosts.

Materials and methods

Specimen collection and maintenance

Considering the need to minimize the sampling of live animals in a protected area, a limited number of adult three-spined sticklebacks (11) were collected by a fish trap in the Nature Reserve of Posta Fibreno (FR, Italy) between October 2013 and January 2014. Fish were maintained in the facilities of the Dept. of Biology of the University of Naples Federico II and were acclimated for a minimum of 14 days prior to experiments in 50-l aquaria with dechlorinated, filtered, and aerated freshwater (20 °C, pH 7.0, 10h:14h L:D photoperiod). All the collected specimens did not show any breeding colouration, and thus, assumed to be reproductively quiescent. During the acclimation period, the animals were daily fed ad libitum with Chironomus' larvae (Eschematteo S.R.L., Italy), then left to fast for 48 h prior to the experiments. None of the animals displayed morphological abnormalities typical of heavy infection (Barber and Svensson 2003).

Respiration rate

The routine oxygen consumption rate was measured using an oxygen electrode probe in a closed system (YSI 5357 Micro Probe, USA; accuracy<0.1% dissolved oxygen concentration). Individual fish were randomly chosen from the tank they were fasting in. After measuring the whole wet body weight (BW), they were introduced into an insulated respiration chamber (volume of 320 ml; constant temperature of 20 °C) and left undisturbed to acclimate under a constant air-saturated water flow, from 0.5h up to 1h. Closing time varied with the weight of the animal, according to the individual total consumption rate. However, the total fall in oxygen concentration was never allowed to exceed ≈20%. The linear regression of oxygen concentration decreasing over time gives the amount of oxygen consumed by the animal per unit of time. Specific routine oxygen consumption (sVo_2) was calculated as $mlO_2\ h^{-1}\ Kg^{-1}$. At the end of the oxygen consumption experiment, each fish was dissected and visually inspected to check for the presence of the *S. solidus*. Fish were then separated into infected and uninfected experimental groups. All procedures were approved by the Animal Care Review Board of the University Federico II of Naples. Respiration rate data from Walkey and Meakins (1970) were retrieved from the original Fig.3 by using a web plot digitizer available at https://apps.automeris.io /wpd/.

Contribution of the parasite respiration rate

The published allometric curve of *S. solidus* plerocercoid metabolism (Davies and Walkey 1966), allowed us to test if the *S. solidus* metabolism per se could account for the experimentally observed differences between infected and uninfected fish. The authors reported the following equation at 20 °C (the experimental temperature of the present study): $Log_n(sVo_2) = -0.631 \times Log_n(BW) + 0.3219$

Where the variables sVo_2 and BW were the specific oxygen consumption and the dry weight of the parasite, respectively.

In the above equation the BW was expressed as dry weight, that was converted to wet weight using the dry weight/wet weight ratio of 0.268 (Tierney and Crompton 1992).

Starting from the above allometric equation, the variation of the *S. solidus'* tVo_2, as a function of both parasite individual weight and number of parasites harboured by the infected fish, was analysed. The putative parasite weight range of 0.05-0.5g (at 0.01g steps), and a parasite number ranging from one to eleven, were investigated. The extremes of the weight range corresponded, respectively, to the minimum infective size (Barber and Svensson 2003) and the maximum plerocercoid average size registered in wild population of stickleback (Confer et al. 2012), whereas the maximum parasite number was that observed in our sampling (Table 1).

A hypothetical PI range was also defined. Considering our samples (Table 1), the minimum possible

Table 1 Body mass, total and specific oxygen consumption rate of *G. aculeatus*, and number of plerocercoids for each infected specimen.

	Wet Body Weight (g)	tVO_2 ($mlO_2\ h^{-1}$)	sVO_2 ($mlO_2\ h^{-1}\ kg^{-1}$)	# *S. solidus*
Uninfected	0.91	0.68	744.48	-
	1.27	0.58	455.08	-
	1.34	0.54	403.69	-
	1.46	0.57	389.13	-
	2.65	0.64	242.84	-
	3.15	0.85	268.97	-
Mean±SD	1.79±0.89	0.64±0.11	417.36±179.94	
Infected	2.34	1.11	473.85	3
	2.60	1.15	440.62	5
	2.94	0.96	327.47	2
	3.58	2.70	752.96	11
	5.37	1.59	295.49	7
Mean±SD	3.36±1.21	1.49±0.71	458.07±180.97	
(Mann-Whitney) P, U, z	NS, 5, -1.73	<0.01, 0, -2.65	NS, 11, -0.64	

PI was 3% (i.e., corresponding to the minimum plerocercoids weight of 0.05g in a fish infected by two worms). Regarding the maximum PI, Barber and Svensson (2003) reported that, at the end of a 16-week experiment, plerocercoids BW was up to 0.159g, with a corresponding parasite index of 25.8%, and that an apparent maximum plerocercoid size was reached at 12 weeks post-infection, growing little after this time. Moreover, Arme and Owen (1967) reported an average PI value of 31.1%, over a 5-year sampling in a wild population.

Based on these reference data, the putative percent contribution of *S. solidus* to the tVo_2 of the infected sampled animals in a hypothetical parasite index (PI) range of 3-30%, was calculated. The actual number of parasites found in each fish was considered, using the formula:

$$\text{Host}\, sVo_2 = \frac{\left(\text{host-parasite}\, tVo_2\right) - \left(\text{S. solidus}\, tVo_2\right)}{\left(\text{host-parasite BW} - \text{total parasite BW}\right)}$$

Where the total parasite BW (g) varied according to the PI. This approach allowed us to compare the specific metabolic rate of parasitized host with that of uninfected fish, as well as to evaluate the putative dependence of the host specific Vo_2 in the infected fish from the parasite index and the number of parasites infecting the fish. Details about the procedure described above, and the data obtained, are available in the supplementary materials (S1).

Statistical analyses

The relationship between body weight and Vo_2 has been massively studied (see Agutter and Wheatley 2004 for a review), and is known to follow the equation $Y = aX^b$, where $Y = Vo_2$, and X = body weight. Comparison between equations was tested by one-way ANCOVA (BW as covariate variable). Regarding the mass specific oxygen consumption, sVo_2 (expressed as $mlO_2\ Kg^{-1}\ h^{-1}$) and the whole-body oxygen consumption, tVo_2 (expressed as $mlO_2\ h^{-1}$), as well as the specimens body weight, the statistical significance of the differences was assessed by the non-parametric Mann-Whitney test. Non-linear regression was used to analyse the relations of *S. solidus* and host Vo_2 with PI. The F-test was used to evaluate the best fit-model: i) in which one curve fits all data sets (p-value N 0.05), or ii) in which each species is fitted by a different curve (p-value b 0.05). All the statistical analyses were performed by GraphPad Prism version 9.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

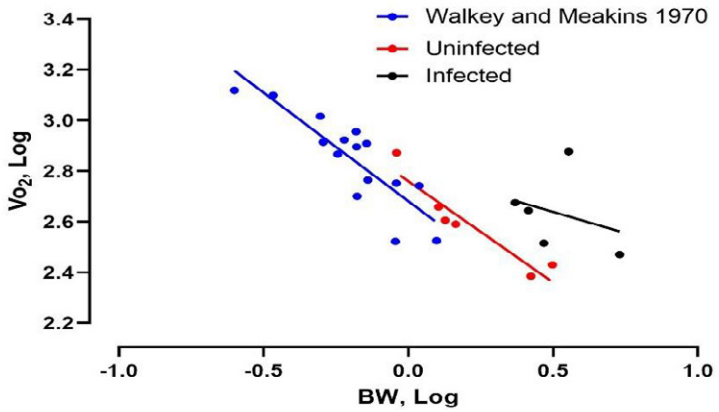

Fig. 1 Correlation between specific oxygen consumption (Vo_2, $mlO_2\ h^{-1}\ kg^{-1}$) and wet body weight (BW, grams) of uninfected (red dots) and infected (black dots) three-spined sticklebacks. For comparison, the data for uninfected fish from Walkey and Meakins (1970) are also reported (blue dots).

Results

Oxygen consumption

The BW of all sampled three-spined stickleback (*Gasterosteus aculeatus)* ranged from 0.9g to 5.4g (Table 1). The average value and the standard deviation (SD) were 2.51g ± 1.24 (Table 1). After dissection, in five out of eleven fish, the presence of plerocercoids was detected. The number of worms in each infected fish ranged from two to eleven (Table 1). The BW of each infected and uninfected sticklebacks, ranging from 2.34g to 5.37g (average 3.37g ± 1.08 SD), and from 0.91g to 3.15g (average 1.80g ± 0.89 SD), respectively, were also reported (Table 1). The difference between the BW averages of two groups was not statistically significant. No significant correlation was found between the number of worms found in the abdominal cavity and that of infected animals BW.

The average and standard deviation of sVo_2 values of infected and uninfected groups were 458.08±180.97 and 417.37±179.74 $mlO_2\ h^{-1}\ kg^{-1}$, respectively (Table 1). Those of the tVo_2 were 1.49±0.71 and 0.64±0.11 $mlO_2\ h^{-1}$, respectively (Table 1). According to the Mann-Whitney test, the difference was statistically significant for tVo_2 ($P < 0.01$; $U=0$; $z=-2.65$), but not for sVo_2.

The allometric correlation between BW and the corresponding sVo_2 was analysed (Fig. 1). In the group of uninfected fish, the equation of the regression line was $\text{Log}\ (sVo_2) = -0.80\ \text{Log}\ (BW) + 2.76$, $r^2=0.89$ and $P < 0.01$. In the group of infected fish, the equation was $\text{Log}\ (sVo_2) = -0.33\ \text{Log}\ (BW) + 2.81$, $r^2=0.09$ and *p*-value=N.S.. Since a significant negative correlation between sVo_2 and BW was found in uninfected fish, but not in infected ones, a statistical difference between the two regressions could not be assessed.

The dataset used in the present analyses was 2.5-3 times smaller than those previously published on the same topic (Walkey and Meakins 1970; Lester 1971; Meakins and Walkey 1975). However. comparative statistical analyses showed that our allometric equation was not significantly different from that of Walkey and Meakins (1970) (F-test, $P > 0.05$, Fig. 1). Furthermore, the slope of the regression line for uninfected fish reported in Lester (1971), neither was significantly different from ours, s=-0.85 and s=-0.80, respectively.

A comparison of the sVo_2 between infected and uninfected fish of the same BW, was performed. An infected stickleback of a BW of 3.37g (average value of the group) would display a sVo_2 consumption of 458.08 $mlO_2\ Kg^{-1}\ h^{-1}$ (average value of the group). The sVo_2 of an uninfected fish with the same BW, would be 221.01 $mlO_2\ Kg^{-1}\ h^{-1}$. The difference between the two values was ≈237.00 $mlO_2\ h^{-1}\ Kg^{-1}$, corresponding to a 52% difference between infected and uninfected fish of the same body mass. Note that this difference is about six times higher than that obtained by simply comparing the average values of the two groups, i.e., ≈40 $mlO_2\ h^{-1}\ Kg^{-1}$. However, since the body mass of the infected fish is partially influenced by the body mass of the parasite, a given amount of the sVo_2 difference was likely due to the parasite.

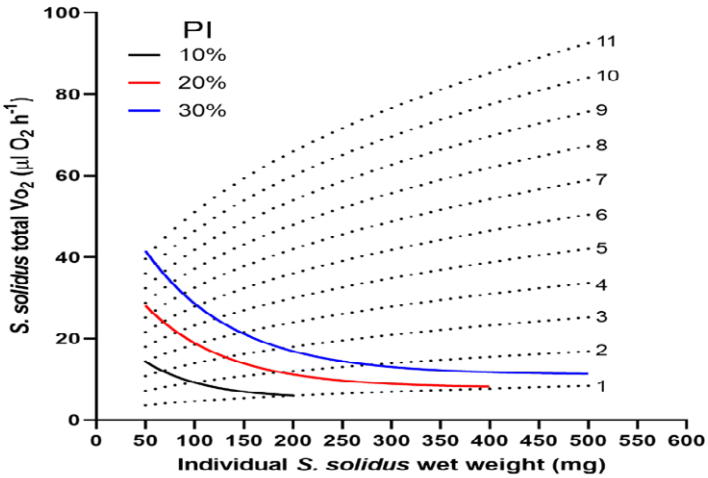

Fig. 2 Relationship between *S. solidus* total Vo_2 and plerocercoids individual body mass (range 50-500 mg, see text). Dot curves account for increasing number of parasites per individual (from 1 to 11). Values were derived from the allometric curve reported by Davies and Walkey (1966). Solid curves represent the variation of *S. solidus* tVo_2 induced by an increasing number of parasites at constant parasite Index (PI).

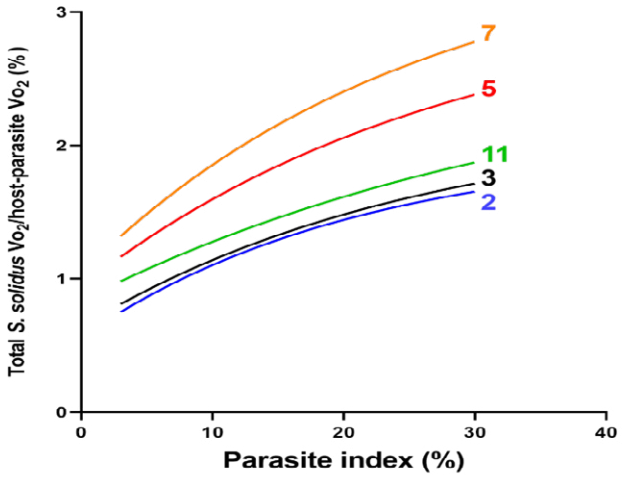

Fig. 3 The curves describe, in the putative PI range of 3-30% (see text), the tVo_2 of *S. solidus*, expressed as percent of the host-parasite total respiration rate (Vo_2), in five infected sticklebacks (Table 1). Numbers on each curve correspond to the number of parasites harboured by each infected fish.

Parasite contribution to the respiration rate

By using the allometric equation of Davies and Walkey (1966), the variation of parasite tVo_2, at 20 °C, as function of parasite BW was derived. To simulate the additive effect resulting from a multiple worm infection, the procedure was applied to an increasing number of parasites, from one to eleven, assuming parasites of the same size. Plotting the parasite tVo_2 as function of parasite BW according to the number of worms, the additive effect of increasing number of worms was highlighted (Fig. 2, dotted lines). Indeed, the slope of the curve of a single parasite was by far less steep than those of multiple parasite infection. To highlight the dependence of the *S. solidus* tVo_2 on the number of parasites, three arbitrary PI (10%, 20% and 30%) were chosen (Fig. 2, solid lines). At each PI threshold, the BW of the infected fish was constant, and was assumed to be 2 grams. Whit a plerocercoids BW of 50 mg (minimum infective size), the difference in the plerocercoids tVo_2 between 10% and 30% of PI is 28.76 $\mu lO_2\ h^{-1}$. Moving along the X-axis toward increasing BW values, this difference becomes smaller and smaller. At plerocercoids BW of 200 mg, the difference in tVo_2 between 10% and 30% is 11.99 $\mu lO_2\ h^{-1}$.

The putative tVo_2 contribution of the parasite to the experimentally measured whole (host+parasite) tVo_2 of the infected fish was assessed. Knowing the parasite tVo_2, and the number of parasites in each infected

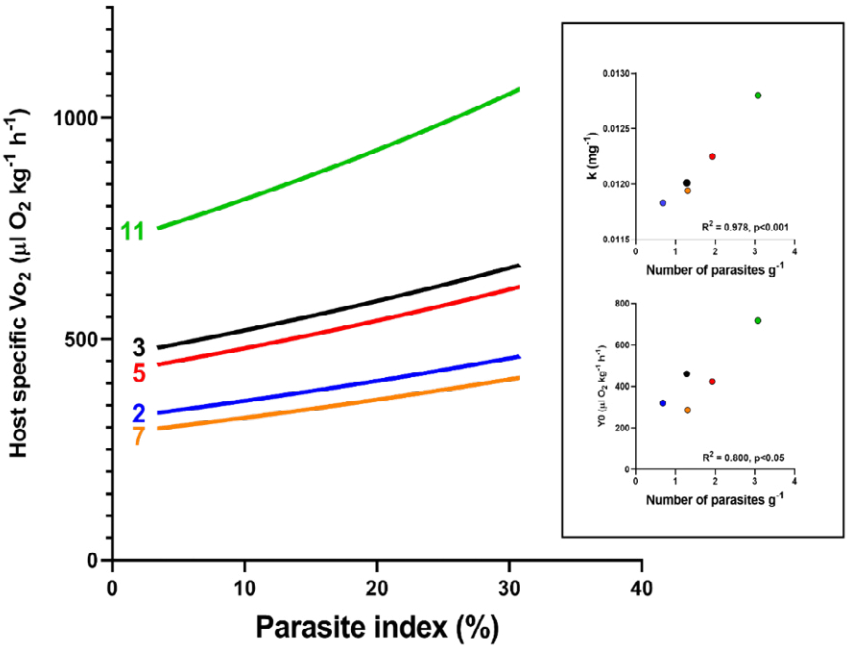

Fig. 4 The plot describes, in the putative PI range of 3-30% (see text), the sVo_2 variation of the host, net of the Vo_2 and BW parasite contribution, in the five infected sticklebacks (Table 1). Numbers on each curve correspond to the number of parasites harboured by each infected fish. Data were fitted to the exponential equation . Inlet plots: upper plot, the equation coefficient K (the rate constant), lower plot, the Y_o (the intercept on the Y axis), both as function of the number of parasites per grams of fish.

individual, the percentage of parasite tVo_2 contribution over a parasite body wet weight up to a PI of 30% was calculated. The maximum theoretical contribution of the parasite to the total oxygen consumption of the individual infected animals was about 2.8%. The value corresponds to the curve of the fish infected by seven plerocercoids at its maximum (Fig. 3, orange line).

The contribution of the parasite to the tVo_2 was used to calculate back the net host specific sVo_2 for each of the infected individuals, over the same range of PI (Fig. 4). When the correlation between sVo_2 of the host (net of the parasite burden) and the PI is plotted, the curve fitting was described by an exponential model ($Y = Y_0 \cdot e^{k \cdot X}$). The parameters that described the model are: Y_0, i.e., the intercept on the Y axis, expressed as $\mu lO_2\ kg^{-1}\ h^{-1}$, and k, i.e., the constant rate, expressed as mg^{-1}. According to the extra sum-of-squares F-test, both Y_0 and k of the five curves were significantly different ($P < 0.05$).

To test the independence of the host sVo_2 from the number of plerocercoids, both Y_0 and k were plotted against the number of plerocercoids. Since Y_0 and k are independent from the weight, the number of parasites was calculated over the net weight of the host (i.e., number of worms/host weight). The Spearman's rank correlation results show that both Y_0 ($R^2 = 0.800$, $P < 0.05$) and k ($R^2 = 0.978$, $P < 0.01$) were significantly correlated with the number of parasites per gram of fish (Fig. 4, inlet plots).

Discussion

One of the key points of the host-parasite system is to demonstrate and quantify any possible alteration of the global metabolic requirements of the host induced by the presence of the parasite. The host-parasite system *Gasterosteus aculeatus-Schistocephalus solidus* has been largely studied, mainly in migratory populations of stickleback (Barber and Scharsack 2010). The respiration rate was reported to be slightly higher in the infected population compared to the uninfected one, but the comparisons of the two groups were never statistically significant (Walkey and Meakins 1970; Lester 1971; Meakins and Walkey 1975).

An accurate evaluation of the host-parasite respiration rate should take into account an inference of fish and worm(s) tissues, separately. To date, the attempts to measure the *S. solidus* respiration rate are rare, mainly due to the difficulties to acclimatize the parasite to a suitable environmental condition to perform the measurement (Mccaig and Hopkins 1965). As far as we are aware, only Walkey and Meakins (1970)

attempted to assess the contribution of *S. solidus* on the fish respiration rate, performing a study based on a theoretical estimation of the worm growth rate from an *in vitro* growth curve described by Sinha and Hopkins (1967). Unfortunately, they did not attempt to include the *S. solidus* oxygen consumption rate estimation in their models. Afterward, Meakins and Walkey (1975) estimated the difference between parasitized and unparasitized sticklebacks, showing that the difference in routine oxygen consumption rate could vary between 11.1 and 114.6 $\mu lO_2/h$. As stated by the authors, "the host-parasite system cannot be effectively partitioned into host and parasite components" (Meakins and Walkey 1975). In fact, the range of difference was so wide that it was hard to draw any clear-cut conclusions concerning the question of "how much" the parasite presence influenced the sVo_2 of the host.

Several factors affected the previous studies carried out on the respiration rate of infected/uninfected stickleback populations. Those factors are linked to the intrinsic eco-physiological characteristics of the host-parasite system, namely: a) the variable body mass of the host (Barber and Svensson 2003), according to the infective stage; b) the variable number of parasites found in each specimen of stickleback (Arme and Owen 1967); c) the different parasite susceptibility observed in different stickleback populations (Scharsack et al. 2016), and d) the stickleback ecotype, since migratory populations are characterized by a standard respiration rate higher than that of resident ones (Tudorache et al. 2007). To rule out the effect of migration on the basal respiration rate of the fish, a Mediterranean lake resident ecotype population (Munzing 1963; Mäkinen et al. 2006; Lucek and Seehausen 2015) was studied here. Nevertheless, we faced again the difficulty to draw out a robust assessment on the different oxygen consumption rate between infected and uninfected fish. In fact, the correlation coefficient of the allometric equation of infected fish was not statistically significant. Thus, a comparison with the allometric equation of uninfected fish was not possible. It is worth stressing that the above impasse was not due to the small sample size of the present investigation. A comparison of our allometric equation with that reported by Walkey and Meakins (1970), obtained with a 2.5 bigger sample size than the present one, showed that the two equations were not statistically different, highlighting that sample size was not a critical point (Fig. 1).

Our attention was captivated by the consideration of Walkey and Meakins (1970) that "*comparisons of energy relations in single and multiple infections with S. solidus, is essential for further refinement of the energy budget*". Hence, in the present study a new approach in the evaluation of the stickleback-*S. solidus* respiration rate was carried out. More precisely, our strategy was to assess a putative percent Vo_2 contribution of the parasites, in a given range of parasite-index (Arme and Owen 1967), based on the sole available, but reliable, correlation between sVo_2 and BW for *S. solidus* (Davies and Walkey 1966).

Starting from the allometric equation calculated by Davis and Walkey (1966), we extended their observation by calculating the tVo_2 of an increasing number of plerocercoids (Fig. 2). The result graphically depicted the additive effect of a multiple infection of *S. solidus* over the tVo_2. Indeed, as shown in Fig. 2, even if the PI was kept constant, the tVo_2 decreases quickly as the individual parasites' BW increases (i.e., the total number of worms decreases). This is an obvious corollary of the allometric equation of metabolic rate. However, the number of parasites in each individual specimen was usually neglected in previous studies. Even the study of Meakins and Walkey (1975), one of the most comprehensive, did not report the number of parasites, disregarding the possibility that the huge Vo_2 variation observed could also depend on the different number of mature plerocercoids infecting the sampled sticklebacks' populations.

Considering that the measured tVo_2 of the host-parasite system is the sum of the two tVo_2, the one of the fish plus that of the parasite, the first hypothesis was that the difference between parasitized and unparasitized sticklebacks was due to the mere additive effect of the parasite tVo_2 to the host-parasite tVo_2. The calculation of the contribution of the maximum putative parasite tVo_2 to the host-parasite system is unexpectedly low (2.8%), confirming that the sole tVo_2 of the parasites cannot account for the difference between infected and uninfected stickleback (52% on average in this study).

In the attempt to demonstrate that the difference between infected and non-infected sticklebacks was only due to the additional effect of the parasite Vo_2, we observed on the contrary that the number of parasites, rather than the PI, mainly accounts for the increment of the host specific metabolic rate. In fact, as shown in Fig. 4, the host sVo_2 strongly correlates to the number of parasites per gram of animal, independently from the PI. For instance, a 3-fold increase in the number of plerocercoids will bring to a more than 2-fold increase in the host sVo_2. This increment is not comparable to the calculated contribution of parasites to the

tVo_2, being indeed very low (≈3%). The increment of sVo_2 resulting from our theoretical exploration can only be explained by some sort of active stimulation of the parasite to the sVo_2 of its host.

In past studies, the parasite BW and the PI were used as reliable parameters to evaluate the parasite Vo_2 contribution. Present data suggested that the number of mature parasites is a further value to be considered. To explore the dependence of the host sVo_2 from the number of parasites, the equation between the PI and the host sVo_2 has been partitioned in its two parameters, k and Y_o. The constant rate k represents the effect of increasing PI on the sVo_2 of the host, or in other words how the host sVo_2 changes at increasing PI. Y_o describes the host sVo_2 at constant PI (the only variables being the number of the parasites). Here we showed that both the variables positively corelated with the number of parasites in each specimen. Given the small set of parasitized fishes analysed, this is a surprising strong correlation.

The above observation supports the hypothesis of Meakins and Walkey (1975) that the increased host respiration rate could be the response to the CO_2 produced by the parasites. Higher CO_2 levels, in fact, increment the acidosis in the host, ultimately lowering the haemoglobin affinity toward oxygen. At a constant PI, a multiple-warm infection would produce more CO_2 than a single-warm infection.

Recently, in a study carried out on *Macrocheles subbadius*, a *Drosophila nigrospiracula* parasite, the authors reached the conclusion that "[the parasite] caused significant metabolic changes in the host, that scaled with infection intensity (…) this increase was not strictly linear, there was a threshold above which infection exerted a more substantial impact." (Brophy and Luong 2021). The converging results of two phylogenetically distant host-parasite systems open new perspectives on the effect of parasitism on the metabolic rate of the host. In this context, the comparison of the metabolic rate of infected/uninfected populations should take into consideration the effect of the parasite burden on the homogeneity of the population. Indeed, in all studies till now carried out on the *G. aculeatus*-*S. solidus* model, as well in the present one, the allometric correlation was statistically significant for uninfected animal populations, but not for the infected ones. The result, most probably, is due to a different homogeneity of the two population types. For a reliable comparison of the physiological parameters between infected and uninfected populations, homogeneity is a fundamental prerequisite to be considered.

Conclusions

Although we did not assess the exact contribution of parasitism to the individual oxygen consumption rate of the sticklebacks, our theoretical approach using pilot data lends weight to the hypothesis that the specific metabolic rate of three-spine sticklebacks is increased by the presence of the *S. solidus* plerocercoid per se, likely bringing to a significant metabolic cost of parasitism, with possible eco-physiological consequences for the species. We encourage the use of this approach for future assessment on the parasite contribution to the stickleback-schistocephalus system to overcome the problem of parasite BW contribution.

Authors' contributions AT Performed experiments/data collection, data analysis and interpretation, provided revisions to scientific content of the manuscript, provided stylistic/grammatical revisions to the manuscript; CB performed data analysis and interpretation, provided revisions to scientific content of manuscript, provided stylistic/grammatical revisions to the manuscript; CA Conceived the ideas or experimental design of the study, data analysis and interpretation, provided revisions to scientific content of the manuscript, provided stylistic/grammatical revisions to the manuscript: GDO Conceived the ideas or experimental design of the study, data analysis and interpretation, drafted the paper and provided revisions to scientific content of the manuscript, provided stylistic/grammatical revisions to the manuscript.

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