

1 **Oxygen consumption rate in *Gasterosteus aculeatus*-*Schistocephalus solidus***  
2 **system from a non-migratory naturally infected population.**

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12 **Keywords:**

13 GASTEROSTEUS ACULEATUS; SCHISTOCEPHALUS SOLIDUS; HOST-PARASITE SYSTEM; CO-EVOLUTION;  
14 MIGRATION; LIFESTYLE; RESPIRATION RATE; OXYGEN CONSUMPTION.

15

16 **Abstract**

17 The three spine stickleback *Gasterosteus aculeatus* is a specific obligatory intermediate host  
18 for the cestode worm *Schistocephalus solidus*. This system is commonly used to investigate the  
19 host-parasite interaction in fishes. Despite the interesting attempts which have been made to  
20 quantify the impact of the parasite over the respiration rate of the host fish, none of the previous  
21 reports took in consideration that stickleback is diversified in different ecotypes according to its  
22 ability to made reproductive migration, from and to the sea. Here the oxygen consumption rate in  
23 specimens of three-spine stickleback collected from a non-migratory population was quantified with  
24 the aim to test if the *S. solidus* infection drives a change in the oxygen consumption level of the host  
25 fish. The results showed that the infected fishes have a higher rate of oxygen consumption  
26 compared with the uninfected one. The differences were due to a direct effect of the parasite, not  
27 merely to its contribution to the whole oxygen consumption rate. The data were compared with  
28 previous reports, showing that the non-migratory population was characterized by a different level  
29 of oxygen consumption rate. The differences were interpreted in terms of divergence in  
30 physiological adaptations which had to be appeared in different populations.

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## 32 Introduction

33 Parasitic relationships are very common among living organisms. The *Gasterosteus*  
34 *aculeatus-Schistocephalus solidus* system is a widely used experimental model for investigating the  
35 host-parasite interaction in fish (Barber and Scharsack, 2010). The life cycle of the cestode worm *S.*  
36 *solidus* is characterized by three stages. The first starts in the haemocoel of freshwater copepods,  
37 which ingest the free swimming embryos of the worm. The *G. aculeatus*, commonly known as  
38 three-spine stickleback acquires the cestode larvae alimenting on infectious copepods. *G. aculeatus*  
39 is a species-specific host for *S. solidus*. At this stage, the parasite grows up to the adult size within  
40 the abdomen cavity of the fish. Sexual maturity is achieved in the third definitive host, which must  
41 be an endotherm, normally a piscivorous bird that, by ingesting the infectious stickleback, allow the  
42 settlement of *S. solidus* in the intestine. The fertilized eggs are finally released into the water  
43 through the bird feces, and a second-generation cycle can occurs. However, the *S. solidus* life cycle  
44 can develops in fresh- or brackish waters only. Three-spine stickleback, on its side, repeatedly  
45 evolved in freshwater-resident ecotypes from ancestral marine/anadromous populations, which  
46 could colonize newly inland waters only formed after the retreat of glaciers (Bell and Foster, 1994).

47 The study of the host-parasite system encompasses the quantification of its energy budget.  
48 Many efforts, indeed, have been made to evaluate the influence of *S. solidus* on stickleback energy  
49 expenditure (reviewed in Barber et al., 2008), including interesting attempts to determine the whole  
50 respiration rate of the infected fishes in comparison with non-infected individuals (Walkey and  
51 Meakins, 1970; Lester, 1971). However, virtually no data were available throughout the literature  
52 on the whole respiration rate of infected and non-infected sticklebacks from non-migratory  
53 populations. However, it has been reported that the level of activity drives the evolution of higher  
54 levels of resting oxygen consumption and respiratory surfaces in fishes (Tarallo *et al.*, 2016). Within  
55 this frame, the evolution of different levels of respiration rate could results in differential responses  
56 to the *S. solidus* infection.

57 In the following study the oxygen consumption rate in the *G. aculeatus-S. solidus* system  
58 was investigated. The respiration rate in specimens of three-spine stickleback collected from a non-  
59 migratory population was measured. Infected and uninfected fishes showed different routine  
60 respiration rate, being that of of the former significantly higher than that of the latter.

61 Comparing our results with those available in the current literature (Walkey and Meakins,  
62 1970; Lester, 1971), the observation that the presence of *S. solidus* drives the increment in the  
63 oxygen consumption of *G. aculeatus* was further supported. In both the migratory and the resident  
64 ecotypes, independently from the different respiratory adaptations due to their different lifestyles,  
65 the same effect is observed.

66

## 67 Materials and Methods

68 Adult individuals of three-spine stickleback *Gasterosteus aculeatus* were collected by fish  
69 trap in the Nature Reserve of Posta Fibreno (FR, Italy) between October and January. Specimens  
70 were maintained in the facilities of the Dept. of Biology of the University of Naples Federico II, and  
71 were acclimated for a minimum of 14 days prior to experiments in 50-l aquaria with dechlorinated,  
72 filtered and aerated freshwater (20°C, pH 7.0, 10 h:14 h L:D photoperiod). During the acclimation  
73 period the animals were fed daily with Chironomus' larvae, then left to fast for 48 h prior to the  
74 experiments.

75 Rate of routine oxygen consumption, i.e. fish spontaneously activity, were measured using  
76 oxygen electrode probe in a closed system. Specimens were weighed, introduced into an insulated  
77 respiration chamber (volume of 320ml; constant temperature of 20°C) and left undisturbed to

78 acclimate under a constant air-saturated water flow, from 0.5h up to 1h. Closing time varies with the  
79 weight of the measured sample, never exceeding a total fall in oxygen concentration of about 20%.  
80 The linear regression of oxygen concentration decreasing over time gives the amount of oxygen  
81 consumed by the animal per unit of time. Soon after the oxygen consumption experiment, each  
82 specimen was checked for the presence of the parasite *Schistocephalus solidus*.

83 The procedures described above were approved by the Animal Care Review Board of the  
84 University Federico II of Naples.

85 Data from Walkey and Meakins (1970) regarding routine respiration rate of stickleback  
86 specimens, collected in UK areas, were recalculated. According to the same authors, dry weights  
87 were converted in wet weight. Data regarding Canadian migratory sticklebacks were recalculated  
88 from Lester (1971). Fish length provided by the author were converted to fish weight using the  
89 empirical model of Length-Weight relationship for *G. aculeatus* available in FishBase (Froese et al.,  
90 last access February 2017). The above relationship was based on more than thirty thousand  
91 observations from different authors. Knowing the worm/fish weight ratio (Lester, 1971), the worm  
92 weight were obtained and added to the fish weight. The resulting weight was referred to the whole  
93 parasite-host system wet weight.

94 Statistical analyses were performed using the software R and the VassarStats facilities  
95 available from the website (<http://www.vassarstats.net/index.html>).

96

## 97 **Results and discussion**

98 The weight of the sampled fish ranged from less than one gram up to more than five grams  
99 (Table 1). On average, the whole body weight of the infected sticklebacks is slightly higher than  
100 that of uninfected fishes (Mann-Whitney test  $p$ -value<0.1). This difference could be due to the  
101 presence of *Schistocephalus solidus* found in the abdominal cavity of the infected fishes, which  
102 increase their total body weight.

103 Regarding the respiration rate, the log-log equation between mass of uninfected fishes and  
104 their specific oxygen consumption was  $O_2 \text{ consumption} = -0.79\text{Mass} + 2.76$  ( $r^2=0.89$ ,  $p$ -value<0.01;  
105 Figure 1). The regression line of the respiration rate from infected fishes showed an increased  
106 residual standard deviation, and the corresponding equation was  $O_2 \text{ consumption} = -0.33\text{Mass} + 2.81$   
107 ( $r^2=0.09$ ,  $p$ -value N.S.; Figure 1). Infected fishes showed a higher respiration rate than that of the  
108 uninfected ones. The statistically significant difference was about  $40 \text{ mlO}_2 \cdot \text{h}^{-1} \cdot \text{Kg}^{-1}$  (One-Way  
109 ANCOVA test  $p$ -value<0.05).

110 The finding was in quite good agreement with previous measurements from Walkey and  
111 Meakins (1970). Indeed, the authors reported an intercept of 3.16 and 3.22 for the resting oxygen  
112 consumption rate in uninfected and infected *G. aculeatus*, respectively (the equation obtained from  
113 their results has been shown in Figure 1), while we observed respectively 2.76 and 2.81. Hence, the  
114 difference of intercept between the two regression lines was of the same order of magnitude.  
115 Despite that, Walkey and Meakins, contrary to our present results, found no statistically significant  
116 difference between infected and uninfected sticklebacks. The authors did not made any statement  
117 about the place where the animals sampling was done, though some years later they published a  
118 more detailed report on the same topic (Meakins and Walkey, 1975) in which sticklebacks were  
119 reported to have been sampled from Eccles, Kent (UK). This, it is not unlikely that the authors  
120 repeatedly performed experiments on the same population. Unfortunately, this information could  
121 not help to elucidate if the fishes were migrating ecotypes or not, since both the forms are present in  
122 that area.

123 Previous reports highlighted the positive link between level of activity and oxygen uptake in  
124 fishes (De Jager and Dekkers, 1974; Tarallo *et al.*, 2016). In particular, migratory sticklebacks are  
125 characterized by a standard respiration rate higher than that of non-migratory ones, also when the  
126 two ecotypes were sampled from the same river and acclimated at the same laboratory conditions  
127 (Tudorache *et al.*, 2007). The authors, extrapolating the standard oxygen consumption from the  
128 measured respiration rate during active swimming, revealed an oxygen consumption of  $198 \text{ mlO}_2 \cdot \text{h}^{-1} \cdot \text{kg}^{-1}$   
129  $^1 \cdot \text{kg}$  in the migratory ecotypes, and of  $126 \text{ mlO}_2 \cdot \text{h}^{-1} \cdot \text{kg}^{-1} \cdot \text{kg}$  in the non-migratory ones (the values are  
130 recalculated for sake of comparison with the data here presented). In this frame, the resulting  
131 difference between the respiration rate in non-migratory sticklebacks here presented against those  
132 produced by Walkey and Meakins (1970) shown a comparable amplitude. The theoretical values for  
133 a 1 gram stickleback recalculated from Walkey & Meakins (1970) accounted for  $69 \text{ mlO}_2 \cdot \text{h}^{-1} \cdot \text{kg}$ .  
134 Following the same rationale, the respiration rate for a 1 gram-animal sampled in Posta Fibreno lake  
135 accounted for  $27 \text{ mlO}_2 \cdot \text{h}^{-1} \cdot \text{kg}$ . The difference could be even greater, considering that the  
136 temperature at which the experiments were performed by Walkey & Meakins (1970) was lower  
137 ( $15^\circ\text{C}$ ) than those for the Posta Fibreno sticklebacks ( $20^\circ\text{C}$ ). Thus, it could be argued that the  
138 ecotype of fish measured by from Walkey & Meakins (1970) was the migratory one.

139 Lester (1971) produced data on migratory ecotype of sticklebacks fished in Alouette Lake  
140 (Canada). He found slightly or no differences between infected and uninfected fish (the data  
141 obtained from the measures produced for infected fish only were shown in figure 1 for sake of  
142 comparison). However, the author pointed out that the mass of the fish carrying the parasite cestode  
143 *S. solidus* should be scaled down by the burden of the parasites. Taking this in consideration would  
144 increment the estimate of the resting oxygen consumption rate of the host fish alone. Using the  
145 equations produced by (Davies and Walkey, 1966) on the respiration rate in *S. solidus*, it can be  
146 calculated than a fish should be infected by about 5000 worms 10 grams each to elevate the host-  
147 parasite system respiration rate by 1 ml/h. This calculation suggest that the presence of the parasite  
148 *S. solidus* drives an increment in the respiration rate of the host through an induced effect, i.e. is not  
149 a merely indirect consequence of the presence of the worms consuming more oxygen than the host  
150 fish.

151 Interestingly, infected migratory sticklebacks from Aulette Lake (Canada) (Lester,  
152 1971) have lower oxygen consumption than non-migratory ecotype (Figure 1). The difference was  
153 partially due to the lower temperature at which the experiments has been carried on with migratory  
154 sticklebacks. The experimental temperature reported by the author was in the range of 10 to  $21^\circ\text{C}$ .  
155 Also taking this into consideration, the differences were still high. One possible explanation could  
156 be that migratory population at Aulette Lake, which cannot migrate anymore because of dams that  
157 close the way to the sea, acclimatized by lowering their respiration rate. In seawater fish, often a  
158 better growth rate is linked with a lowered surrounding salinity. Usually, this is correlated with a  
159 lower standard metabolic rate (Boeuf and Payan, 2001).

160 As observed by Clarke (1954), the surface of the ponds are preferentially populated by  
161 parasitized sticklebacks. This observations has been used as a working hypothesis by Lester (1971)  
162 to test if the different distribution of infected and uninfected fish were a consequence of a different  
163 respiratory needs between the two categories, i.e. the hypothesis that infected fish tend to swim  
164 close to the oxygen rich surface of the pond to satisfy their need for oxygen. Moreover, it has been  
165 shown that the higher oxygen consumption was not the only effect of the presence of *S. solidus* in  
166 the stickleback abdomen. Barber and coworkers, studying the escape response of experimentally  
167 infected *G. aculeatus* to bird predation, reported an altered response of the fish due to the presence  
168 of *S. solidus* (Barber *et al.*, 2004). The infected fish showed a slower and sluggish response to fake

169 bird beak. In the wild this behavior should result in an increased risk to be predated by a bird. The  
170 net outcome from the *S. solidus* point of view would be the incremented chance to reach the  
171 intestine of a third-host bird, where it can complete its life cycle achieving the sexual maturity. No  
172 effort has been made to quantitatively clarify if a real segregation of infected fish took place in the  
173 water column. As a matter of fact, oxygen concentration is higher at the ponds surface respect to the  
174 bottom. The eager of oxygen, typical for parasitized stickleback, should have an influence on their  
175 distribution within the water column. It is known that fish are able to sense the oxygen  
176 concentration and adjust their behavior to minimize the adverse effects of low oxygen on their  
177 fitness (Costa *et al.*, 2014), selecting aquatic oxygen tensions that maintain their metabolic scope  
178 for growth and activity (Burlerson *et al.*, 2001). In this frame, a lower routine respiration rate of non-  
179 migratory ecotypes could counteract the negative effect of the rise of respiration rate.

180 In mammals, the elevation of metabolic rate driven by the parasite is well documented,  
181 though the exact mechanisms are still unclear (Morand and Harvey, 2000). In ectotherm species  
182 exposed to the parasite, an enhancing in the levels of metabolic rate has been reported (Shinagawa  
183 *et al.*, 2001; Khokhlova *et al.*, 2002). The energetic costs of maintenance and activation of the  
184 immune system probably account, at least in part, for the difference. In fishes, there is no golden  
185 rule for respiration rate after parasitic infection. In Arctic charr *Salvelinus alpinus* it has been  
186 observed the metabolic depression due to the parasite infection (Seppänen *et al.*, 2008 and  
187 references therein). Hence, the host-parasite system *G. aculeatus*-*S. Solidus*, still a very interesting  
188 model, cannot be generalized to other fish-parasite systems.

189 More effort would be devoted for the unveiling of the full picture, particularly to the study  
190 of the physiology of the *G. aculeatus*-*S. Solidus* system, both in field and in lab controlled  
191 experiments.

## 193 **Acknowledgements**

194 Authors are grateful to Dr. C. Bailey for challenging discussions that greatly improved the  
195 manuscript. AT has been supported by the SZN A. Dohrn research fellowship #04/2016.

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**Table 1. Body mass and specific oxygen consumption rate for infected and uninfected *G. aculeatus***

	<b>Wet Body Weight (g)</b>	<b>Ox Consumption (mlO<sub>2</sub>*h<sup>-1</sup>)</b>	<b>Specific Ox Consumption (mlO<sub>2</sub>*h<sup>-1</sup>*kg<sup>-1</sup>)</b>
<b>Uninfected</b>	0,91	0,6775	744,48
	1,27	0,5780	455,08
	1,335	0,5389	403,69
	1,464	0,5697	389,13
	2,65	0,6435	242,84
	3,15	0,8473	268,97
<b>Infected</b>	2,34	1,1088	473,85
	2,6	1,1456	440,62
	2,94	0,9628	327,47
	3,58	2,6956	752,96
	5,37	1,5868	295,49

251 **Figure 1. Log-log equation between whole wet body mass versus specific oxygen consumption**  
252 **in infected (full symbols) and uninfected (empty symbols) sticklebacks. UK population are**  
253 **represented by squares (data from Walkey and Meakins 1970); Posta Fibreno lake (Italy) are**  
254 **represented by circles (this study); Aulette lake population by triangles (data from Lester**  
255 **1971).**

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