# Challenging popular belief, mosquito larvae breathe underwater

Running tittle: *Mosquito larvae breathe underwater*

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## Summary statement

We present the first quantitative analysis of mosquito larvae respiration in air and water, unravelling the unknown capacity of larvae of the most cosmopolitan disease vector of breathing underwater.

## Abstract

It is taken for granted that immature mosquito only breathe atmospheric air through their siphons. However, there is no quantitative study that demonstrated it. We analysed the survival of the last instar larvae of *Aedes aegypti* fully submerged at different temperatures, and measured oxygen consumption from air and dissolved in water, of larvae and pupae of this species under different conditions. Results revealed that under water, larvae survived much longer than expected, reaching 50% mortality only after 58, 10, and 5 days at 15°, 25° and 35°C, respectively. Interestingly, whereas we registered moults to pupae in larvae with access to air, individuals kept submerged never moulted. When remaining at the water surface, larvae obtained 12.72% of $O_2$ from the water, while pupae only 5.32%. When completely submerged, larvae consumed less oxygen than in contact with the surface, but enough for surviving, while pupae did not. At both media, temperature affected larvae respiration rate, with relatively close $Q_{10}$ values. In the related species, *Ae. albopictus*, a similar pattern of $O_2$ consumption were observed. Larvae got 12.14% of their oxygen from the water. Interestingly, no significant differences in total $O_2$ consumption were found between water $O_2$ consumption, when *Ae. albopictus* larvae were submerged, or when they also have access to air (dual $O_2$ consumption). Our findings not only challenge the classical idea that mosquito larvae only breathe atmospheric $O_2$, but also force us to reconsider the potential effectiveness of control methods based on asphyxiating larvae by detaching from water surface.

***Keywords:*** *Aedes*, metabolism, survival, respiration, temperature, $Q_{10}$

## Introduction

As an originally terrestrial group and from an evolutionary standpoint, when insects colonized the freshwater, have to deal with many vital functional problems. Respiration was probably the greatest one. As an environment, water is a less favourable medium for respiration because its oxygen concentration is twenty times lower, and its rate of oxygen diffusion is lower by a factor of 105 (Dejours, 1988). In this sense, insects that had adapted to water environments developed plenty of different strategies to acquire oxygen. Among them, there are examples of cutaneous respiration, developed spiracular, tracheal, and blood gills, the use of air stores carrying air bubbles, or the use of hydrofuge structures to remain attached to the water surface and breathe atmospheric air (Wigglesworth, 1972). It is taken for granted that the last is the case of mosquito larvae and pupae, who would use their siphons as "snorkels" when they rest attached to water surface. This statement is widely affirmed in textbooks, internet scientific and dissemination sites, and also in protocols for controlling mosquito populations at juvenile stages. Yet, the vital role of aerial respiration in immature mosquitoes has been challenged by sporadic observations since long time ago (e.g., Da Costa Lima, 1914; MacFie, 1917; Ramsey and Carpenter, 1932; Wang, 1938; Richards, 1941).

Even though it seems reasonable that atmospheric air would constitute the main source of oxygen, it cannot be excluded that mosquito larvae and pupae could gather some oxygen from the water. On the one hand, the survival underwater of larvae belonging to different mosquito species, along with some measurements of oxygen consumption from the water (Sen, 1914; Fraenkel and Herford, 1938) are pieces of evidence that water-dissolved oxygen could be sufficient for maintaining vital functions during periods without contact with the surface. However, the relative contribution atmospheric and water-dissolved oxygen to the metabolism of mosquito larvae and pupae has not been yet investigated.

In our study, we attempt to shed light on respiratory gas exchange in immature mosquito stages. We analysed the survival and oxygen intake of mosquito larvae and pupae having or not having access to air and measured the effect of the temperature on these variables. For the first time, we provide quantitative data on oxygen consumption from air and water, measuring them both, separately and simultaneously. Finally, we discuss the evidence obtained during our experiments in the framework of mosquito respiratory physiology, as well as the possible consequence of our findings for control methods based on the suffocation of juvenile mosquitoes

## Materials and methods

### *Mosquitoes*

Eggs of *Aedes aegypti* of the Bora strain (insecticide susceptible) and *Ae. albopictus* of Vectopole strain reared at Vectopole (Montpellier, France) were provided by the European network InFravec2 (https://infravec2.eu/). Eggs were put in dechlorinated tap water for hatching, adding traces of ascorbic acid and tropical fish food, and kept at 26°C (± 1°C) in a climatic chamber, under a light/dark cycle 12h:12h/ (lights on at 08:00 am). Food was regularly provided until they reached the 4$^{th}$ instar (6-10 days in our conditions), and used for experiments. Individuals were handled by aspirating them with plastic Pasteur pipettes with their tip cut. Each larva was tested only once and discarded afterward.

### *Survival experiments*

The survival time of fourth-instar larvae of *Ae. aegypti* was evaluated at three temperatures (15°, 25°, and 35ºC). A climatic chamber was set at the experimental temperature, and these values were kept constant at nearly 1°C. A 12h:12h light cycle was imposed with lights on at 08:00 am. Relative humidity was kept at 70% to reduce the evaporation of the water in the recipients. In case some evaporation occurred, deionised water was daily added to return to the initial volume.

For each temperature, two larvae were placed in recipients with 300 ml of dechlorinated water under two conditions, submerged or with access to air. In both cases, a unique larva was placed into a glass cylinder (0.6 cm in diameter and 2 cm in length), both ends closed by a tissue mesh kept with the aid of an O-ring; to allow water circulation, but keeping the larva caged at the same time. In the first condition (submerged), the cylinder was completely sunk at the bottom of the recipient, taking care that no air remained captive inside. The control condition consisted of a larva placed in the same cylinder maintaining half of it in contact with the air, and the other half underwater (Fig. S1). No food was provided, but the water was neither changed, nor the development of microorganisms impeded. Recipients were placed inside a climatic chamber at 15°, 25°or 35°C (± 0.5°C), under a light/dark cycle at 12h:12h/ (lights on at 08:00 am). The number of dead and moulted insects was recorded daily.

### *Oxygen consumption*

The individual oxygen consumption of immature *Ae. aegypti* was measured using optodes. We employed two 4-channel Firesting $O_2$ meter (Pyro Science, Aachen, Germany) using 4 ml vials with an integrated optical oxygen transducer (OXVIAL 4). Briefly, flashes of light of specific wavelengths generated in the interface are guided through a light fibre to excite a transducer inside the vial from the outside. The fluorescens of the substance, which is proportional to the oxygen concentration in the medium (air or water) is gathered by the same optic guide and analysed at the interface. The temperature of the vials was controlled using a Peltier element and controller (QuickCool 34W; Peltron Gmbh, Germany). A temperature sensor from the oxygen meter measured the temperature inside an empty vial, and its signal allowed the system to adjust the values of the measured $O_2$ concentrations.

Fourth-instar larvae of *Ae. aegypti* and *Ae. albopictus*, and pupae of *Ae. aegypti* were evaluated. Most measurements were carried out on *Ae. aegypti* under three vial conditions: submerged, closed vial, and open vial, at 15°, 25° and 35°C. The condition submerged consisted in a closed vial filled with water with an individual inside, and water $O_2$ concentration was registered for four hours. In this treatment, the total absence of air bubbles was carefully checked. For the closed vial condition, an individual of the mosquito immature stage was placed in a closed vial half filled with water, and the water and air $O_2$ concentration was registered for four hours. Finally, the open vial condition consisted of an open half-filled water vial with an individual mosquito inside, and the water $O_2$ concentration was registered for four hours. Each treatment was replicated between 12 and 30 times. The rate of oxygen consumption was calculated for each replicate, calculating the slope of a linear regression of the $O_2$ concentration versus the time of the experiments. For *Ae. albopictus* same assays were performed except for open vial condition and close vials at 15 and 35°C.

*Statistics*

To analyse differences in survival across temperatures and conditions (submerged and control) a Kaplan-Meier analysis was performed.

For the analyses of oxygen consumption, the effects of vial conditions (submerged, closed vial, and open vial), temperature (15°, 25° or 35°C), the medium where $O_2$ was taken (air or water) and stage (larva or pupa) in $O_2$ consumption one or two-ways ANOVAs were performed. *A posteriori* comparisons of significant ANOVAs were performed by means of Tukey test. The significance threshold was chosen at 0.05 for all analyses.

## Results

### *Survival experiments*

The survival of *Ae. aegypti* larvae were significantly affected by the temperature and immersion conditions ($p<0.05$). In the control treatment (i.e., with access to air) at 25°C, no death was registered, and the survival curve was significantly higher than the curve of the submerged treatment at the same temperature. At 35ºC, the survival curve of the submerged larvae presented the lowest values with the higher negative slope, differing significantly from its control at the same temperature. Surprisingly, at 15 ºC the submersion treatment did not differ from the control (Fig. 1A). The 50% mortality of submerged larvae differed with the temperature, being 58, 10, and 5 days at 15°, 25°, and 35ºC, respectively (Fig. 1B). Remarkably, some individuals remained alive for as long as 30 days at 25ºC and 68 days at 15ºC. Finally, whereas we registered moults to pupae in control larvae, individuals kept submerged never moulted (Table 1).

### *Oxygen consumption*

#### *Larvae and pupae at 25ºC*

In all three conditions: submerged, closed vial and open vial, *Ae aegypti* larvae and pupae evinced to consume measurable amounts of oxygen from the water (Fig. 2). A significant difference in $O_2$ consumption from the water was observed between the interaction of immature stages and the vial conditions (two-way ANOVA F: 107.31, DF: 5, $p<0.0001$). Larvae under the submerged condition presented the highest rate of water-dissolved $O_2$ consumption, followed by pupae under the same vial conditions. In addition, larvae under closed vial and open vial conditions presented higher rates of $O_2$ consumption than pupae under the same vial conditions, but their $O_2$ consumption rates were always lower than larvae and pupa under submerged conditions (Fig. 2). No significant difference was found between closed and open vial treatments for both immature stages, allowing us to employ closed vials for next measurements.

Pupae presented higher oxygen consumption from the air than larvae under closed vial conditions (two-way ANOVA, F: 119.75, DF: 3, $p<0.0001$, Fig. 3). The air $O_2$ consumption of

both immature stages tested was significantly higher than their water $O_2$ consumption ($p<0.05$, Fig. 3). The dual rate of $O_2$ consumption, adding air and water $O_2$ consumption of closed vial treatment, were 0.111 and 0.128 moles/h for larvae and pupae respectively. Larvae obtained most of their oxygen from the air, but about 13% from water, while pupae obtained almost 95% from the air and the rest from water (Fig. 4B, C). Finally, the dual (air + water) $O_2$ consumption in closed vial condition was significantly higher than $O_2$ consumption from the water of the submerged condition (two-way ANOVA, F: 68.85, DF: 3, $p<0.0001$, Fig. 4).

*Effect of temperature on oxygen consumption ($Q_{10}$ of larvae)*

The consumption of water-dissolved oxygen of *Ae. aegypti* larvae when they were submerged, also significantly varied with temperature (one-way ANOVA, F: 33.46, DF: 2, $p<0.0001$). The highest $O_2$ consumption was registered at 35ºC, followed by 25ºC, and the lowest one was registered at 15ºC. The $Q_{10}$ calculated between 15° and 25ºC was 1.47 and 1.66 between 25° and 35ºC, so the mean $Q_{10}$ across the experiment was 1.56. Also, $O_2$ consumption from air of *Ae. aegypti* larvae significantly varied with temperature (one-way ANOVA, F: 18.09, DF: 2, $p<0.0001$). The highest $O_2$ consumption was registered at 35ºC, followed by 25ºC, and the lowest was registered at 15ºC. The $Q_{10}$ calculated between 15° and 25ºC was 2.21 and between 25° and 35ºC was 1.45, so the mean $Q_{10}$ across the experimental temperatures was 1.83.

*Aedes albopictus*

Similar patterns of $O_2$ consumption were observed in *Ae. albopictus*. A significant difference in $O_2$ consumption from the water was observed between vial conditions (one-way ANOVA, F: 278.36, DF: 1, $p<0.0001$, Fig. 5). *Ae. albopictus* larvae from the submerged condition treatment presented significantly higher $O_2$ consumption than larvae from the closed vial (Fig. 5). In the closed vial condition, the mean dual (air + water) rate of $O_2$ consumption was 0.0185 $\mu$moles of $O_2$/h for larvae. They consumed a significantly higher amount of $O_2$ (around 88%) from the air than from water (one-way ANOVA, F: 27.97, DF: 1, $p< 0.0001$, Fig. 6). Interestingly, no significant differences in the total $O_2$ consumption of *Ae. albopictus* larvae were found between water-dissolved oxygen consumption when they were submerged or when they also had access to air (dual $O_2$ consumption) in the closed vial condition (one-way ANOVA, F: 0.99, DF: 1, $p= 0.325$, Fig. 7).

On the other hand, the temperature affected the rate of $O_2$ consumption from water (one-way ANOVA, F: 77.52, DF: 2, $p<0.0001$). The rate of $O_2$ consumption did not differ significantly between 15° and 25°C, but a significant increase was registered between either 15° or 25° and 35°C. The $Q_{10}$ calculated between 15° and 25ºC was 1.47, and between 25° and 35ºC was 3.01, so the average $Q_{10}$ between 15° and 35ºC was 2.24.

## Discussion

We report the first quantitative data on oxygen consumption, both from the air and from the water, by larvae and pupae of two major disease vectors, *Aedes aegypti* and *Ae. albopictus*. In both cases, far from a great surprise, most of the oxygen consumed comes from atmospheric air, but not all of it. The portion gathered from the water is low, but physiologically significant, which means it is just enough for survival, and much lower in pupae, which obtain practically the totality of the oxygen they consume from the air.

Even though underwater respiration by mosquito larvae has been repeatedly reported, this phenomenon remained anecdotal, deserving no attention by most people. In fact, not only there is no evidence to what extent oxygen gathered from water might be physiologically significant to mosquito larvae, but it has been completely ignored as a potential drawback in control procedures involving larval suffocation. Indeed, being detached from the surface and losing contact with the air, does not mean the immediate death of the larvae by asphyxiation. In complete submersion, they were capable of surviving for days, weeks, or even months, depending on the temperature of the water.

As can be expected for a poikilotherm and ectotherm organism, water temperature has a significant impact on larval metabolism, which is reflected in the intensity of both aerial and aquatic respiration. This dependence is quantitatively expressed by the calculation of $Q_{10}$ values for oxygen consumption, and also by the differential survival time of completely submerged larvae across a wide range of temperatures. As expected, the lower the temperature, the longer the survival time observed. This fact can be explained by the modulation of larvae metabolism, reflected on the $Q_{10}$ different from 1, together with a higher $O_2$ concentration in the water, since the solubility of oxygen increases as temperature decreases. So, in tropical

areas or areas where human activities result in relatively warm temperatures, we could expect a lower capacity to tolerate immersion for prolonged periods.

Interestingly, the moult cycle was markedly affected by the deprivation of access to atmospheric air. No larva was capable of accomplishing a normal moult in the prolonged submersion experiments. The oxygen gathered exclusively from the water resulted to be sufficient for surviving and swimming (larvae did not remain immobile nor in akinesis into the tubes), but not enough for moulting. Only larvae kept at the lower temperature showed signs of incomplete ecdysis after many weeks under water, which indicates that the first steps of the moult process took place. Probably, ecdysis is excessively expensive in terms of energetic demands to complete it under these conditions.

Aquatic respiration was reported several times in mosquito larvae. One of the first scientists to turn his interest towards water respiration in mosquito larvae was the Brazilian entomologist Ângelo Moreira Da Costa Lima more than a century ago. He performed a series of experiments placing larvae of different Culicidae species under complete submersion and reporting day by day the status of each individual (Da Costa Lima, 1914). The author observed that some larvae survived for several days and that one larva whose "leaflets" (i.e., anal papillae) had been removed, returned at the surface more often than another intact used as control. These results led the author to assert: *"The results of my experiments convinced me that mosquito larvae, while generally breathing mainly free air by the two tracheae of the respiratory syphon, also respire the oxygen of the air dissolved in water, the gaseous exchanges being made by the branchial leaflets and the general integument of the body."*. This assertion was criticised by colleagues, who distrusted the results due to poor control of the experimental conditions (Sen, 1914). Da Costa Lima (1916) replicated some of the original experiments taking additional care and reporting similar observations to his previous ones. On the other hand, Da Costa Lima noted the resemblance to gills of anal papillae, in line with other colleagues considering these structures as respiratory organs in aquatic Diptera (Koch. 1938). The demonstration of the osmoregulatory function of the papillae (Wigglesworth, 1932, 1933, review by Bradley, 1987), together with the general assumption that the potential contribution of oxygen dissolved in the water should be insignificant, rapidly made aquatic respiration to be disregarded as physiologically relevant for mosquito larvae (Thorpe, 1933).

Other early investigations, such as those conducted by MacFie (1917), Ramsey and Carpenter (1932), Wang (1938), and Richards (1941) focussed on the oxygen requirements of

mosquito larvae by submerging them in oxygenated or deoxygenated water, observing differential survival. According to our results (Fig. 5), and those obtained by Fraenkel and Herford (1938), fully submerged *Ae. aegypti* larvae would consume only half as much oxygen as larvae swimming in the normal manner. However, this is not the case in *Ae. albopictus*, whose larva can gather similar amounts of oxygen when fully submerged or attached to the surface (Fig. 8).

Using a different experimental approach, Krogh (1941) noticed that the gaseous pressure in the trachea of *Culex* larvae reduced during submersion; according to the author, this could be caused by the withdrawal of oxygen for respiration and the loss of $CO_2$ through the external cuticle. This author estimated the volume of the tracheae close to 1.5 µlitre, suggesting that the content of oxygen would be enough for surviving 5-10 min underwater Krogh (1941).

Hagstrum (1970) measured the aerial and aquatic respiration of larvae of different species, in the presence of petroleum oils in the water. His study suggested that for *Ae. aegypti* aquatic respiration could represent *ca.* 5-20% of aerial respiration, rather than 50% as formerly proposed by Fraenkel and Herford (1938). This agrees with our results where the aquatic respiration of *Ae. aegypti* larvae constituted about 13%. Hagstrum (1970) also reported delayed mortality in *Ae. aegypti* larvae, although their tracheae were blocked with petroleum oil, noting that these larvae were unable to pupate.

Later on, different authors started to focus their attention on the fact that the amount of oxygen dissolved in the water might impact on the survival of larvae and pupae, and, as a consequence, eventually affect their control based on larval suffocation. Reiter (1978) kept submerged larvae of three mosquito species in water with fixed dissolved oxygen contents and at different temperatures. The author concluded that those larvicides which kill by anoxia are likely to be effective only when the water is less than 30% saturated with oxygen, the exact value depending on the species. Westwood et al. (1983) and Silberbush et al. (2015) extended this idea to larvae living in natural environments with access to the air, providing additional quantitative data related to survival and oxygen saturation in the water.

Interestingly, different authors commenced in recent years to insinuate that breathing in mosquito larvae may have been misunderstood, raising questions about canonical assumptions and the real effectiveness of larval suffocation as a method for controlling natural

311 populations of mosquitoes. For instance, in an attempt to understand the underlying mechanism
312 of breathing cut-off (e.g., wettability of the siphon), Lee et al. (2018) exposed larvae of *Aedes*
313 *togoi* to water treated either with oil-film layers or with surfactants (i.e., substances impeding
314 larvae to remain attached to the water surface). The survival times recorded were variable
315 according to the treatment, but reached times of about one day. The authors concluded that
316 cutaneous respiration dependent on the oxygen concentration in the water could affect the
317 efficacy of larval asphyxiation methods. In this sense, Lee et al. (2018) underlined the need to
318 prevent oxygen dissolution by blocking the exchange between the water and the atmospheric
319 air at the surface. It is clear that this procedure would negatively affect not only mosquitoes,
320 but also the rest of the organisms living in the same environment. More recently, Nyberg and
321 Muto (2020) investigated the mechanism of action of what authors called *"mosquito acoustic*
322 *larviciding"*. They reported that their acoustic treatment provoked the tracheal system a series
323 of what they reported as *"previously unobserved phenomena"* According to Nyberg and Muto
324 (2020), these phenomena would be difficult to explain based on the present knowledge of
325 mosquito respiration, eventually concluding that the respiratory function in mosquitoes is far
326 from being completely understood.

327 Our study sheds light on the respiratory physiology of the mosquito aquatic instars by
328 analysing the survival and oxygen intake of mosquito larvae and pupae having or not having
329 access to the air, and measuring the effect of the temperature on these variables. For the first
330 time, we provide quantitative data on oxygen consumption from air and water, which have
331 been measured separately and simultaneously. Our work also provides evidence demonstrating
332 how our limited knowledge of crucial aspects of mosquito respiratory physiology may
333 compromise control methods based on the suffocation of juvenile mosquitoes.

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## Competing interest

Authors declare no competing nor financial interests.

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398 **Table 1.** Proportion of pupae ± s.e.m. (number of individuals) of *Ae. aegypti* larvae at control
399 and submerged treatments at three temperatures: 15°, 25°, and 35°C.

| Temperature (°C) | Control | Submerged |
|---|---|---|
| 15° | 0.09 ± 0.09 (11) | 0 (7) |
| 25° | 0.52 ± 0.11 (21) | 0 (36) |
| 35° | 0.18 ± 0.10 (16) | 0 (28) |

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405 **Figure Captions**

406 **Figure 1.** (**A**) Survival of *Ae. aegypti* larvae: control (solid line) and submerged treatments
407 (dotted line) at three temperatures: 15°, 25°, and 35°C (blue, green, and red respectively). **(B)**
408 Survival time 50 (estimated and 95 % confidence intervals) of *Ae. aegypti* larvae at control and
409 submerged treatments at three temperatures: 15°, 25°, and 35 °C (blue, green, and red
410 respectively). Different letters indicate significant differences between conditions (submerged
411 and control) and between temperatures in the submerged condition ($p<0.05$). *It was not
412 possible to estimate the survival time 50 due to the lack of mortality in the control treatment at
413 25 ºC, so the value shown is the maximum survival time registered.

414 **Figure 2**. Consumption of water-dissolved $O_2$ (mean and 95 % confidence intervals) by *Ae.*
415 *aegypti* larvae and pupae under three conditions: submerged, closed vial and open vial.
416 Different letters indicate significant differences between treatments (Tukey multiple
417 comparison).

418 **Figure 3.** (**A**) $O_2$ consumption (mean and 95 % confidence intervals) obtained from air and
419 water of *Ae. aegypti* larvae and pupae in the closed vial condition. Different letters indicate
420 significant differences between treatments (Tukey multiple comparison). Percentage of oxygen
421 consumption of *Ae. aegypti* larvae (**B**) and pupae (**C**), obtained from air and water.

422 **Figure 4**. Total $O_2$ consumption (mean and 95 % confidence intervals) of Ae. aegypti larvae
423 and pupae with (dual) and without (submerged) access to air. Different letters indicate
424 significant differences between treatments (Tukey multiple comparison).

425 **Figure 5.** Consumption of O2 from water (mean and 95 % confidence intervals) by Ae.
426 albopictus larvae under two conditions: submerged and closed vial. Different letters indicate
427 significant differences between treatments.

428 **Figure 6.** (**A**) $O_2$ consumption (mean and 95 % confidence intervals) obtained from air and
429 water of *Ae. albopictus* larvae in the closed vial condition. Percentage of oxygen consumption
430 of *Ae. albopictus* larvae (**B**) obtained from air and water. Different letters indicate significant
431 differences between treatments.

**Figure 7.** Total $O_2$ consumption (mean and 95 % confidence intervals) of *Ae. albopictus* larvae with (dual) and without (submerged) access to air. Different letters indicate significant differences between treatments.

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438 Supplementary material

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440 Fig S1. Setup for testing larval survival at different temperatures.

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442

Figure 1

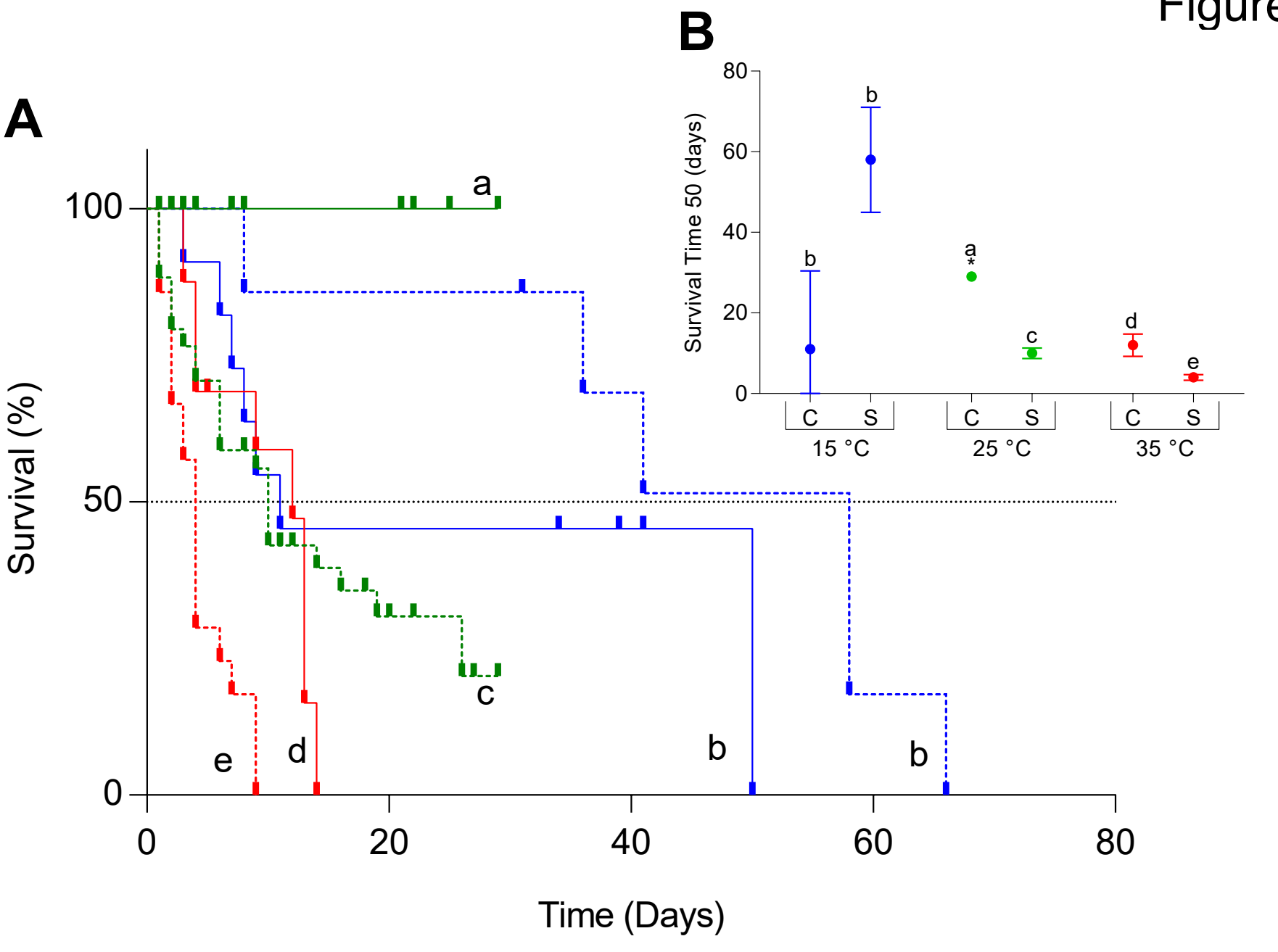

A
B
Survival (%)
100
50
0
0
20
40
60
80
Time (Days)
a
b
c
d
e
b
b
Survival Time 50 (days)
80
60
40
20
0
b
b
a
*
c
d
e
C
S
C
S
C
S
15 °C
25 °C
35 °C

Figure 2

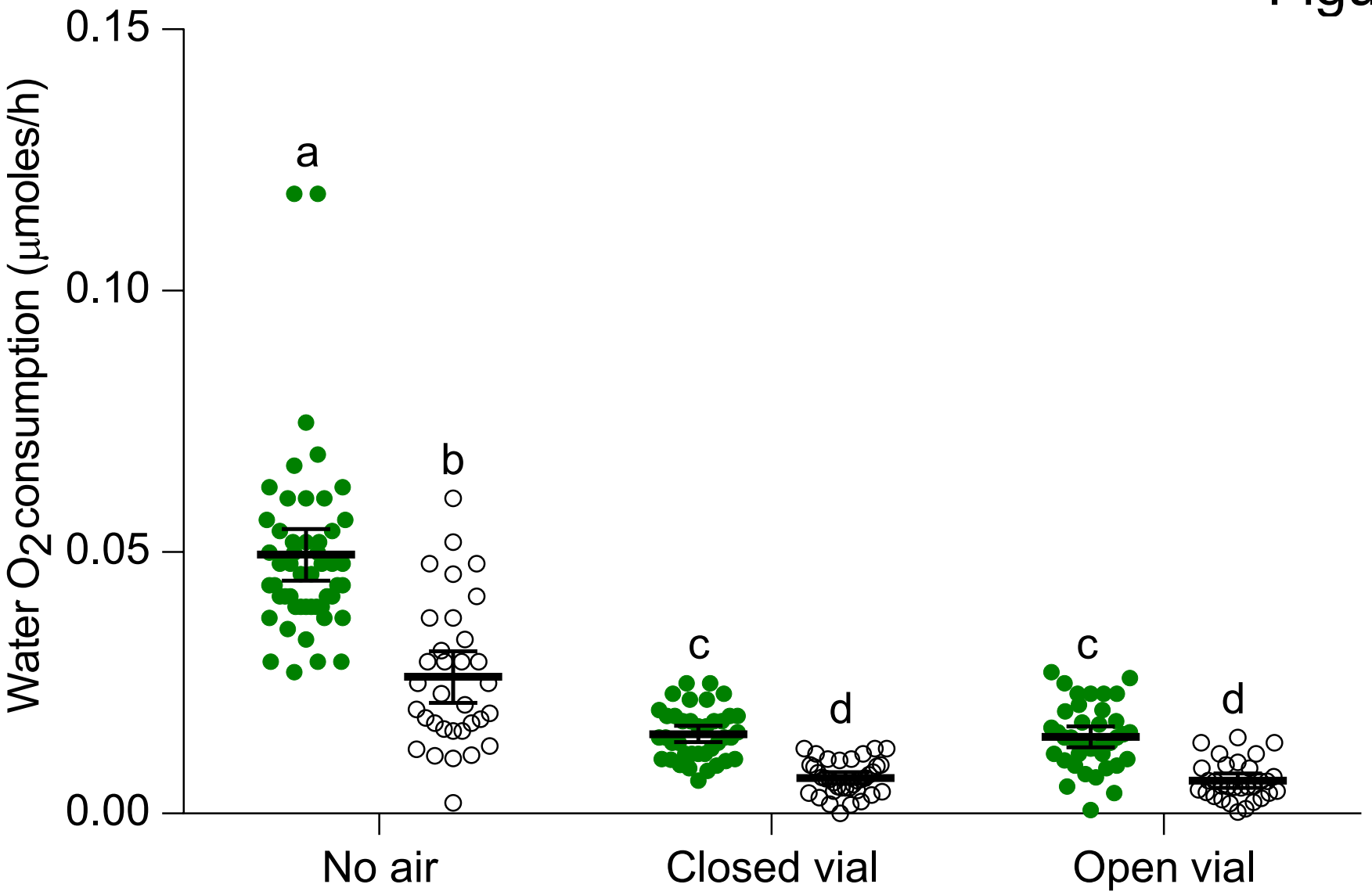

0.15
0.10
0.05
0.00
Water $O_2$ consumption (µmoles/h)
a
b
c
d
c
d
No air
Closed vial
Open vial

Figure 3

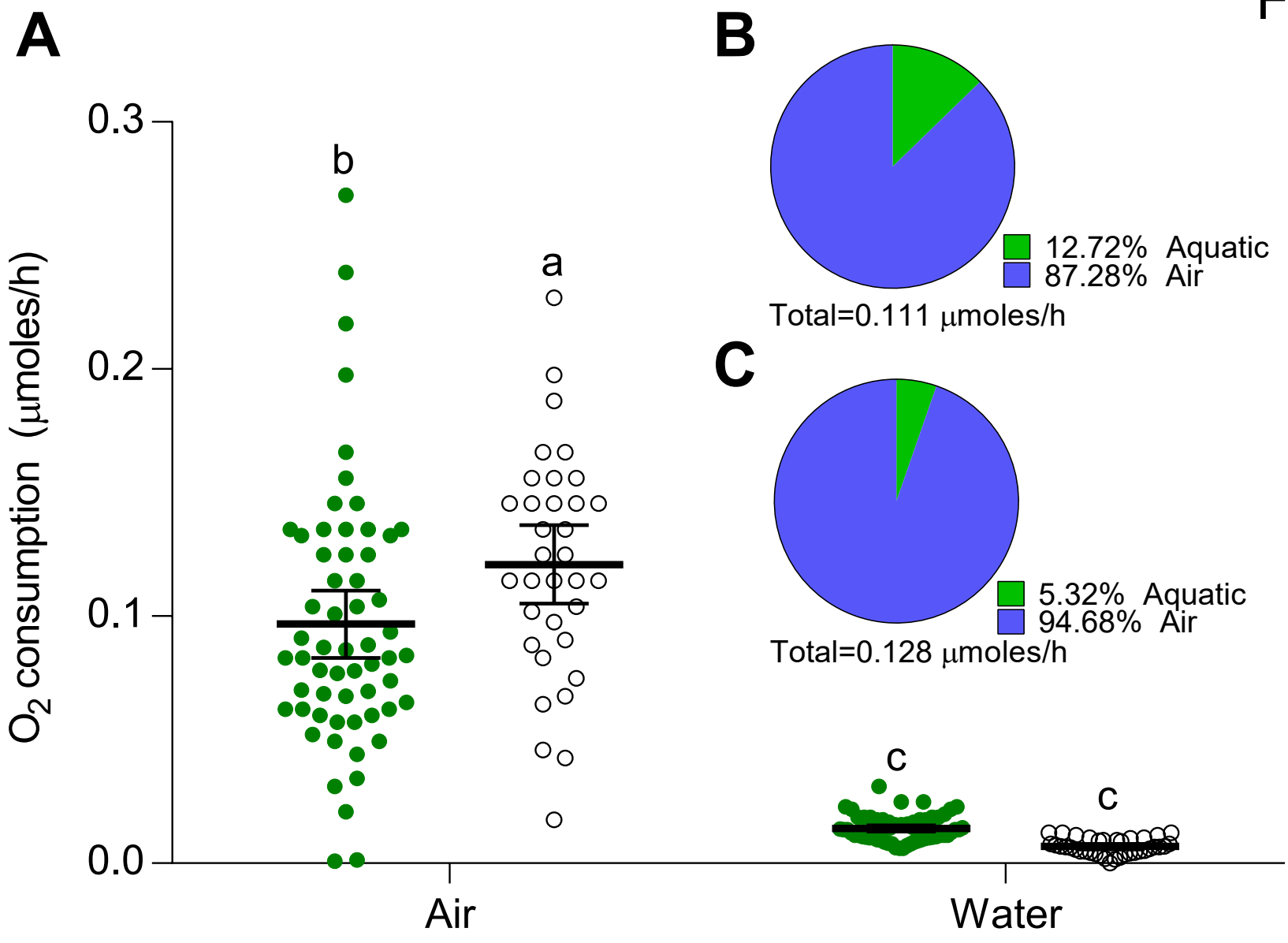

A
B
C
0.3
0.2
0.1
0.0
$O_2$ consumption (μmoles/h)
b
a
c
c
Air
Water
12.72% Aquatic
87.28% Air
Total=0.111 μmoles/h
5.32% Aquatic
94.68% Air
Total=0.128 μmoles/h

Figure 4

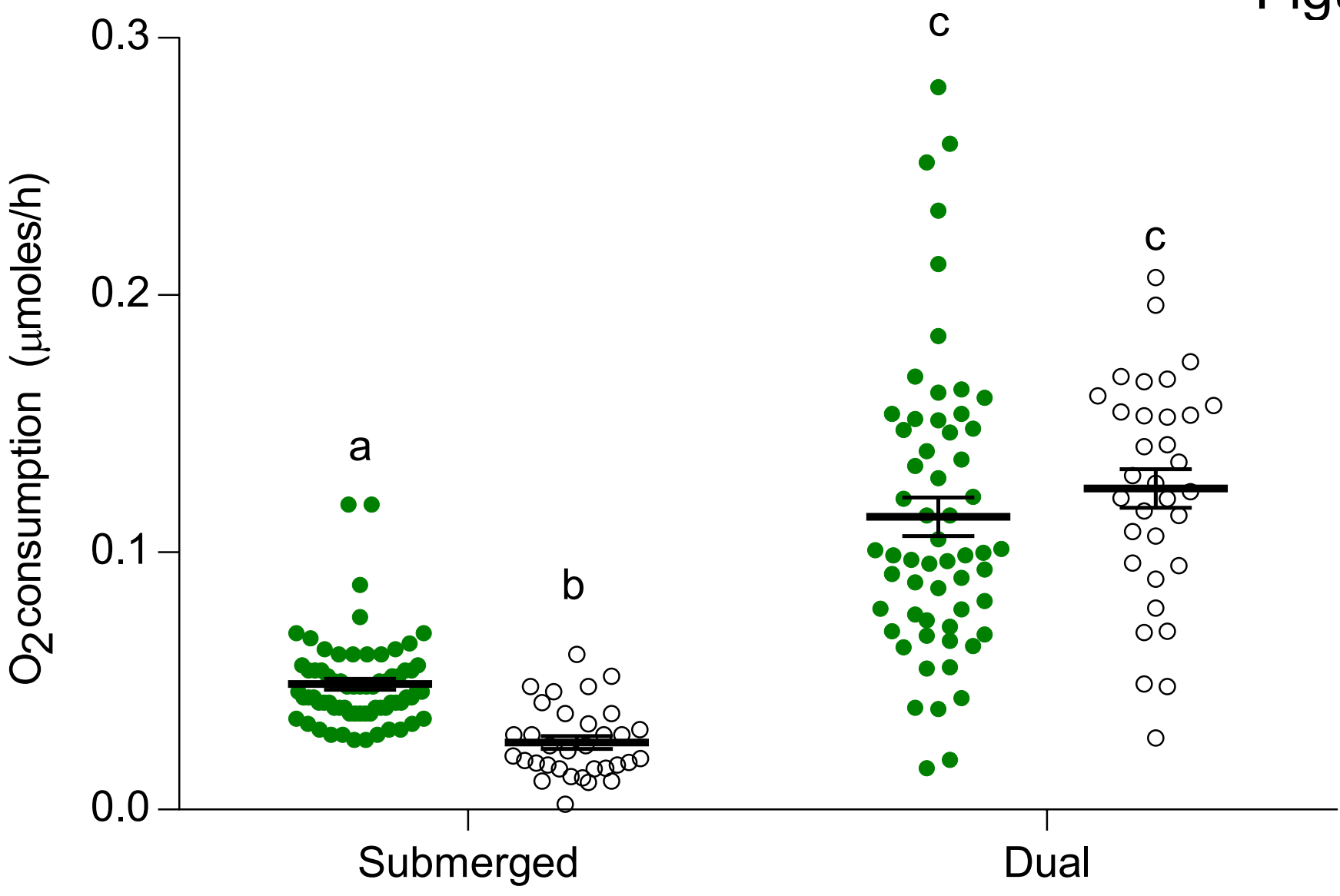


Figure 5

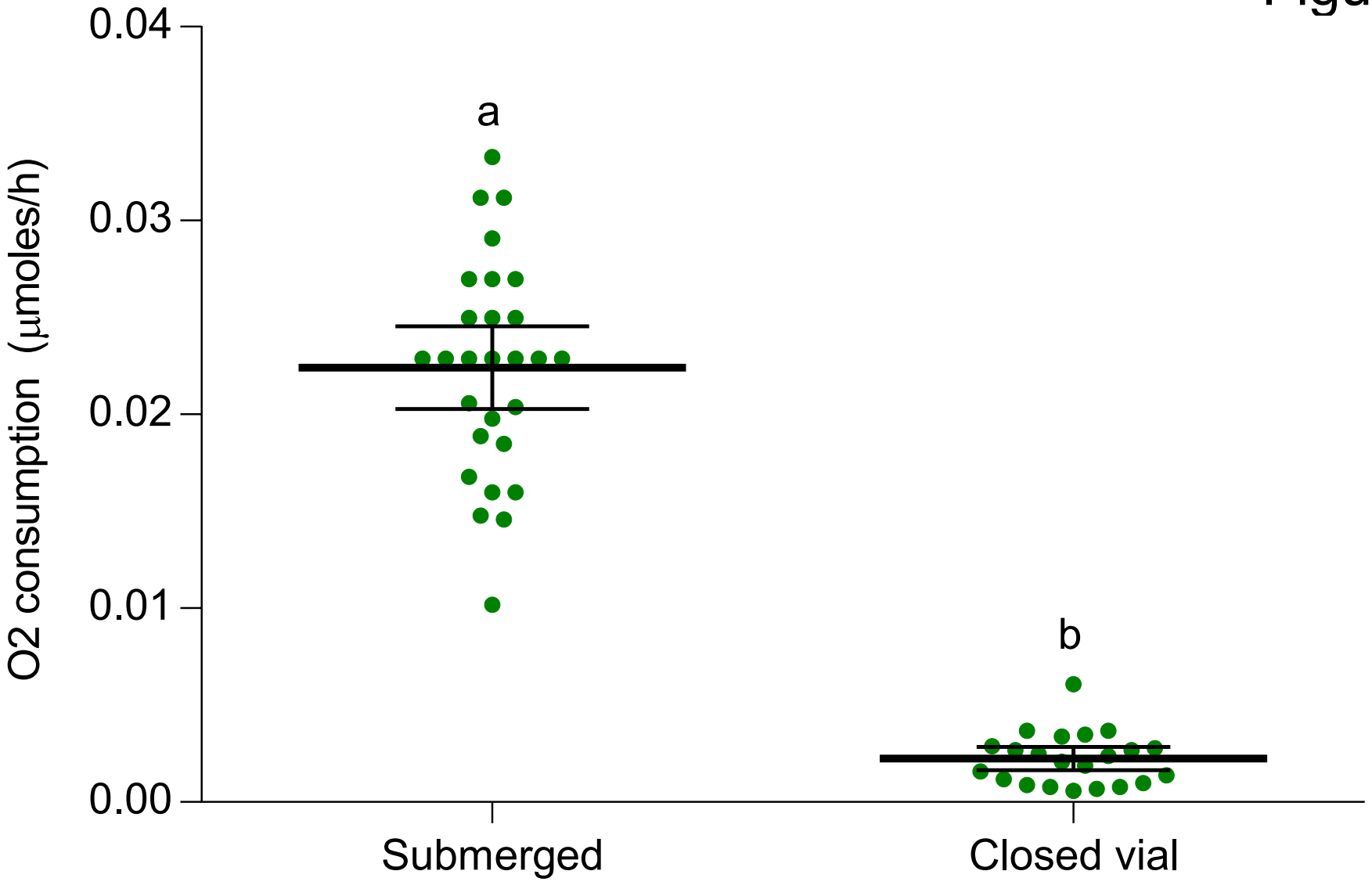

0.04
0.03
0.02
0.01
0.00
O2 consumption (µmoles/h)
a
b
Submerged
Closed vial

Figure 6

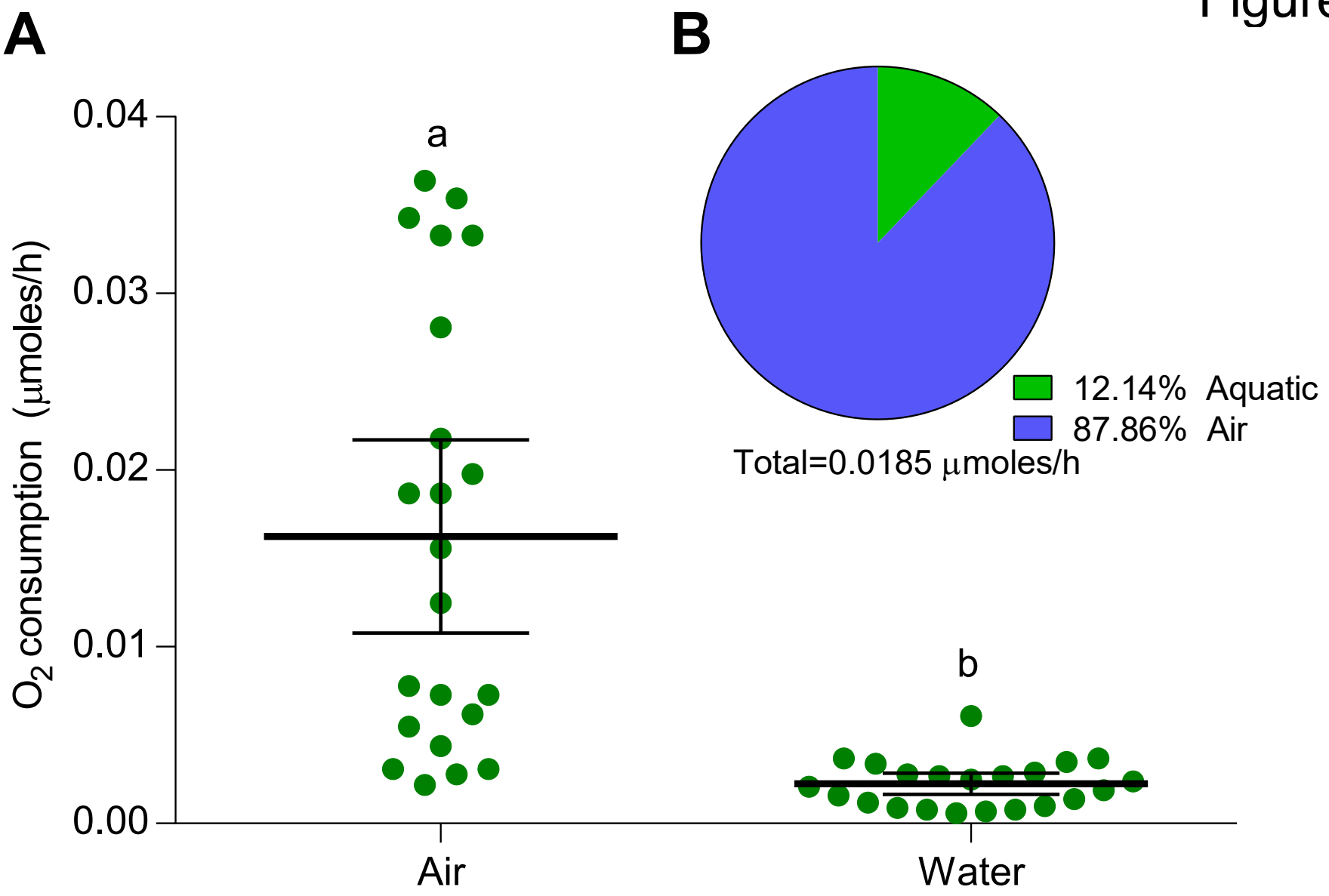
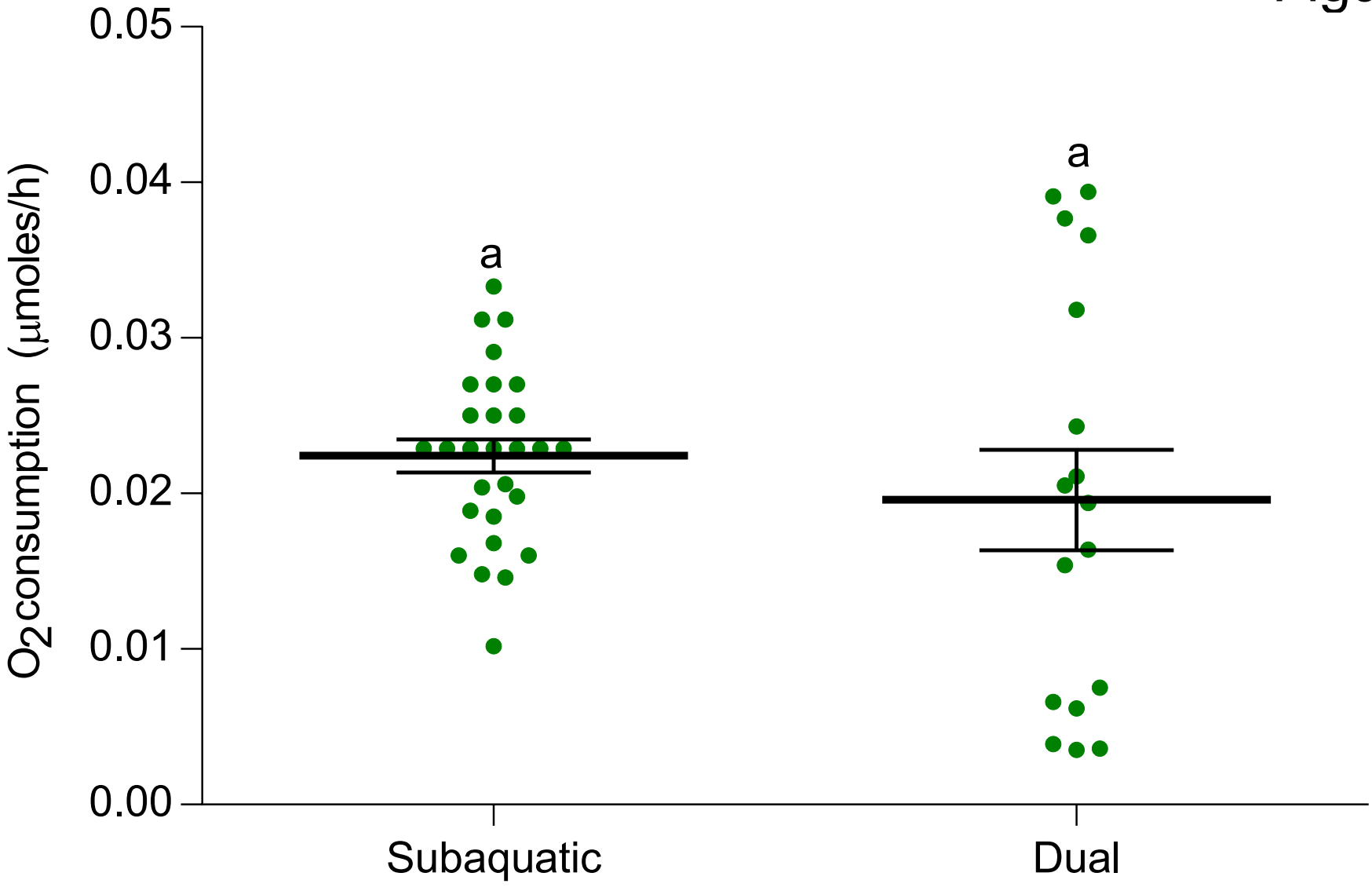

A
B
0.04
0.03
0.02
0.01
0.00
$O_2$ consumption (μmoles/h)
a
b
Air
Water
12.14% Aquatic
87.86% Air
Total=0.0185 μmoles/h

Figure 7


0.05
0.04
0.03
0.02
0.01
0.00
$O_2$ consumption (µmoles/h)
a
a
Subaquatic
Dual