CAN OCTOPUSES BREATHE AIR:

INVESTIGATION OF IN-AIR RESPIRATION IN OCTOPUS RUBESCENS

By

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Abstract

Several species of octopus have been known to spend extended periods of time outside of water, often quite actively. However, virtually no investigation has been made into what sort of physiological processes allow octopuses to survive these terrestrial excursions. This study examined the ability of the ruby octopus (*Octopus rubescens*) to consume oxygen while out of water. When octopuses were enclosed in an air filled respirometer for fifteen minutes, no significant drop in oxygen concentration was measured. No significant difference was found in oxygen debts between octopus exposed to air compared to octopus exposed to anoxic water, nor was any significant difference found in arm muscle metabolites for octopus exposed to air or anoxic water. This study found no evidence that octopus are consuming oxygen while in air, and instead indicates these octopuses rely entirely on their anaerobic metabolism during terrestrial movement.

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Introduction

There are written references to octopus moving on land since Aristotle described the octopus in his book, *Historia Anamalium*, In which he cites the octopus as "the only mollusc that ventures onto dry land" (Aristotle). This citation in 300 BCE is followed by others from Pliny the Elder and Aelian which, though possibly less scientifically accurate, tell of very large octopus moving out of water to feed on fish drying on the shore (Lee 1875). Though these stories may be fantastical, octopus moving across land by the shore continue to be reported in natural science literature. This ability to move readily out of water, combined with a tendency to explore, make octopus difficult to keep in captivity, and their tendency to escape their tanks and be found moving outside of their aquariums is well known (Wood and Anderson 2004). Naturalist Henry Lee, in his book *Aquarium Notes*, describes an octopus (likely *Octopus vulgaris*) at the Brighton Aquarium which would regularly leave its tank to feed on its neighbors and then return to its home tank before its keepers caught on to its excursions (Lee 1875).

Despite the large number of references historically, very little has been published in scientific literature about the terrestrial movement of octopuses, and there have been no studies directly evaluating the physiology of this behavior. *Octopus vulgaris* has been observed to leave the water onto exposed rocks to collect immobile gastropods before returning to the water to feed on them (Wodinsky 1971). In fact, these terrestrial excursions appear to be a normal behavior for some species (Wood and Anderson 2004). Perhaps the most extensive documentation of this behavior of octopuses comes from non-scientific sources such as amateur videos, which are frequently posted online. As the availability of cameras and footage has increased and the ease of accessing these videos has increased, more sightings of octopus moving on land have become available.

The predominant number of recorded sightings of octopus moving on land consists of two species, *Octopus vulgaris* and *Octopus rubescens* (Table 1). This may not reflect an increased likelihood for these species to leave water, but perhaps both the abundance of these species, and their proximity to humans with readily available cameras. Other sighted species include *Octopus alpheus*, *Octopus* cf. *tetricus*, and *Abdopus aculeatus* in Australia, *Enteroctopus dofleini* along the pacific coast of North America, and *Octopus briareus* in the Caribbean. Most sightings occur on rocky or coral rubble beaches, where octopus have been discovered hiding under rocks before they begin their movement. In some of these videos octopuses appear to leave the water without being pursued or disturbed from their hiding places. Fewer recordings take place on sandy beaches than on rocky areas, and of these, several may be the result of storm or wave action depositing the octopus on the beach, forcing it to make its way back to the water. The maximum time spent out of water in these videos is about 2 minutes and 30 seconds, although some videos are incomplete, not showing when the octopus began or ended its movement on land. The octopus in the video containing the longest terrestrial excursion appears to have emerged voluntarily, and then returned to the water. These observed periods out of water are shorter than some records which cite octopus surviving three or four hours after escaping their tanks at a public aquarium (Anderson and Martin 2002). In many of these videos the octopuses appear to be healthy, holding their bodies upright and moving quickly. Several of these octopuses are seen dragging prey along with them and in one instance capturing prey, which would make their movement even more energy demanding.

Movement over land is likely demanding for an aquatic organism. During these terrestrial expeditions octopuses are often very active, and are sometimes seen dragging prey items along with them. This raises the question of how octopuses perform such a high energy task if they are forced to rely on their anaerobic metabolic pathways. It has been assumed that octopus have some mechanism for gas exchange while in air (Courage 2016). It is very likely that octopuses cannot match their in-water respiration rate but could perhaps consume enough oxygen to extend their terrestrial excursions. This is a strategy employed by many air-breathing amphibious fish (Graham 1997).

Indeed, it seems to a likely possibility that octopus are consuming oxygen while in air. While some amphibious fish use their gills on land, it is unlikely gas exchange could occur through octopus gills while in air, because the mantle cavity, where the gills are located, is collapsed while on land, separating the gills from the environment (Graham 1997). Octopus have thin, highly vascularized skin and this makes the skin well suited for gas exchange (Madan and Wells 1996). Cutaneous respiration makes up a substantial portion of octopuses respiration rate while in water (41% at rest, 33% during exercise) (Madan and Wells 1996). In addition, similar adaptations have been observed in other amphibious species. Highly vascularized skin may supply over 50% of the total oxygen uptake by amphibious fishes. (Graham 1997). Other amphibious species, such as intertidal crabs like *Birgus latro* similarly have attenuated cuticles along the dorsal surface of their abdomens, which are used for gas exchange via a network of respiratory vessels (Greenaway 2003). It seems likely that octopus could be performing similar cutaneous respiration while in air, so long as the octopus' skin remained moist.

I hypothesized that *Octopus rubescens* is able to consume oxygen while out of water. Based on this hypothesis, I predicted that octopuses would rely less on their anaerobic metabolism while out of water than when in a completely anoxic environment. I also predicted that I would observe a lower oxygen debt, lower muscular phospho-L-arginine concentration and higher octopine concentration in octopuses that spent periods of time out of water than those exposed to comparable periods of time without oxygen.

Materials and methods

Octopus ability to consume oxygen while in air was assessed using three measurements: direct measurement of oxygen consumption in an air filled respirometer, comparison of oxygen debt following equal periods in air or anoxic water, and quantitation of accumulation of metabolites associated with metabolism after equal periods in air or anoxic water (Figure A1). *Octopus rubescens* was chosen as a study organism because it is easy to collect and keep in captivity, and is one of the most documented species of octopus to move on land.

Octopus collection and maintenance

Thirty-eight *Octopus rubescens* of both sexes ranging in size from 40 to 359 grams were collected by SCUBA at a depth of 45’ at Driftwood Park in Admiralty Bay on Whidbey Island, Washington (48°16’ 38.06”N -122°63’70.18”W) between June 2014 and August 2014 (Figure 1). Divers located glass bottles, and examined each for the presence of octopus (Anderson et al. 1999). Bottles containing octopus were placed into plastic bags and transported to Rosario Beach Marine Laboratory. Octopuses were held in flow through seawater tanks, isolated in 30.8cm by 24.5cm by 36.2cm (27.3 L) plastic storage containers modified to allow water to flow through. Octopus were kept with an approximately natural day:night cycle.

The octopus were given one week to acclimate before they were used in any experimental trials and were fed *Hemigrapsus nudus*. Routine metabolic rates were measured for each octopus in a water filled, 2.8L flow through, water jacketed respirometer. Oxygen concentration of inflow and outflow water was measured using a Pyroscience Firesting O2 oxygen optode. Flow rate through respirometers was calculated by collecting outflow of chamber in a graduated cylinder for a period of 30 seconds.

In-air respiration

Oxygen consumption of each octopus was directly measured while in air using a 1 gallon sealed polyethylene terephthalate (PET) plastic chamber equipped with a Pyroscience contactless oxygen sensor spot. Prior to use chambers were filled with nitrogen and oxygen pressure increase in the chambers was measured for 24 hours to ensure that they were air-fast. Each chamber receiving an octopus was paired with an identical, empty, ‘blank’ chamber equipped with an oxygen sensor spot to account for background respiration and pressure changes due to temperature change or physical interaction while sealing the respirometer. Octopus were sealed in the respirometer for a period of 15 minutes while oxygen concentration was measured. Both the respirometer and blank chamber were placed in a dark, room temperature (~13°C) water bath to prevent photosynthesis by microorganisms and to limit temperature disturbances (Figure A2).

Oxygen debt comparison

Oxygen debts of each octopus were measured using flow-through respirometers as described above following 15 minutes in air and also following 15 minutes in anoxic water. Anoxic water exposures were performed in the same chambers used for the in-air trials. Anoxic water was generated by bubbling nitrogen into seawater and using oxygen optodes to ensure an undetectable amount of oxygen. Following a period of 15 minutes in air or in anoxic water, the octopus were placed into a flow-through respirometer and allowed to recover for a period of 6 hours while oxygen consumption was measured (Figure A3 & A4). Octopuses were sorted randomly to receive anoxic or in-air treatment first to ensure that repeated exposure to hypoxic conditions did not bias results. Octopus were then exposed to the opposite treatment (in-air or anoxic water) and respirometry following a one week recuperation period.

To quantify oxygen debt from the respirometry data, routine metabolic rate, measured for each octopus, was subtracted from the oxygen debt respirometry data. A LOESS regression was determined for each oxygen debt and area under the regression was integrated for all time periods prior to the LOESS reaching 0 for the first time (Figure A5). Each plot was manually examined to ensure that this calculation worked as expected.

Tissue analysis

After the second oxygen debt trial was complete, concentration of molecules involved in anaerobic metabolism following in-air or anoxic water periods was done by taking a tissue sample immediately following an additional 15 minutes of in-air or anoxic water period. Following the period of exposure, octopus were sedated using MgCl at 27 g.L−1 for a period of 6 minutes (Gonçalves et al. 2012). Once sedated a single arm was collected from each animal using a scalpel. This sample was immediately frozen in liquid nitrogen. Once the sample had been taken, the octopus was returned to oxygenated seawater and allowed to recover. Octopuses were not out of water for longer than 3 minutes. Arm samples were stored at -80°C, and sent to Washington State University Laboratory for Cellular Metabolic Engineering (LCME) for quantification of octopine and phospho-L-arginine by UPLC-MS.

Statistical analysis

Data was tested with a Shapiro-Wilk's test to determine normality. When data was found to be non-normal, I examined the skew and kurtosis to help determine appropriate transformations to achieve normality. The levels of oxygen change in air-filled respirometers were compared to 0 using a one-sample wilcoxon-signed rank test because the data was not normally distributed. Data from oxygen debt calculations were organized by treatment (in-air or anoxic water) and by order of which test each octopus received first. These were compared by two-way repeated measure ANOVA on log transformed mass-corrected data to correct for positively skewed data. Metabolite data from UPLC-MS was analyzed using a PERMANOVA of octopine and phospho-L-arginine by treatment. All statistical analyses were performed in R (R Core Team 2015).

Results

Mean routine metabolic rate (RMR) was 2.89±2.22 μmolO2 g^{-1} hr^{-1} (mean±SD, Figure 2), similar to previous studies measuring RMR in this species (Onthank and Cowles 2011). Distribution of measured routine metabolic rates showed a positive skew of 2.207.

Mean oxygen change in respirometers during 15 minutes of in-air respirometry was an increase of 2981±13317 μmolO2 g^{-1} hr^{-1} (mean±SD, Figure 3). This is significantly different than a mean change of zero (Wilcoxon signed rank test, V=659, p=0.0086). This is a much larger magnitude than would be expected from normal octopus respiration. It also seems unlikely that this is due to off-gassing from the octopus because the starting oxygen pressure in the respirometer was approximately 100% atmospheric saturation in all runs.

Distribution of oxygen debts showed a positive skew, which was expected due to the skew in the distribution of the routine metabolic rates. I therefore performed a log transformation on our oxygen debt values to give the data a normal distribution. I found no significant difference in oxygen debts between octopus exposed to air and octopus exposed to anoxia (Figure 4), and there was no significant effect based on which treatment the octopus received first (anoxia or in-air) (Figure 5).

Octopine and phospho-L-arginine demonstrate an inverse relationship in octopus arm muscle tissue as expected (Figure 6). Arm muscle octopine concentrations following in air treatments were 1601±688 μg g^{-1} tissue and following anoxic water trials were 1180±725 μg g^{-1} tissue. Arm muscle phospho-

L-arginine concentrations following in air treatments were 4.1±4.4 µg g^{-1} tissue and following anoxic water trails were 12.9±21.1 µg g^{-1} tissue. Data was not normally distributed and transformation was not able to make all treatments simultaneously normal (Royston's Multivariate Normality Test on untransformed data, H=15.6, p=0.0008), therefore a PERMANOVA was used to compare concentrations of metabolites by treatment (PERMANOVA, df=19, f-statistic=1.9215, p=0.2057). I found no evidence of significant difference in levels of octopine or phospho-L-arginine between octopus exposed to air and octopus exposed to anoxia (Figure 7).

Discussion

Despite numerous records of octopus moving out of water, there have been no investigations into the mechanisms that allow this behavior. It has been assumed that octopus are exchanging oxygen while out of water, but it appears this is not so. I measured no significant decrease of oxygen concentration while octopuses were in air filled respirometers (Figure 2). I did find a significant increase of oxygen concentration during these tests. It is possible that the octopus were off-gassing oxygen through their skin. However, because the starting oxygen pressure in the respirometer was approximately 100% atmospheric saturation in all runs, it seems unlikely that this is happening. It is also possible that microorganisms within the chamber are performing photosynthesis or the change of oxygen concentration is an artifact of changes in temperature. These two explanations seem unlikely however because the respirometers were kept in a dark water bath to prevent these things from occurring. This data indicates those octopuses are not consuming oxygen from their environment while out of water.

Furthermore, I have looked for and failed to find evidence that octopus rely less on anaerobic metabolic pathways while in air, compared to completely anoxic water. Octopus exposed to air did not have a significantly different oxygen debts when compared to octopus exposed to anoxic water (Figures 3).In addition arm muscle metabolite analysis and the lack of significant difference between oxygen debts of octopus in-air compared to those in anoxic water suggests a similar reliance on anaerobic metabolism in both treatments (Figure 7).

Unexpectedly, some octopuses exposed to 15 minutes of complete anoxia had very high arm muscle concentrations of octopine and low levels of octopine, suggesting very low levels of reliance on anaerobic metabolism (Figure 6). It's possible that these octopus may be suppressing their routine metabolic rate, as has been observed in other cephalopods such as the Humboldt squid, *Dosidocus gigas,* when exposed to hypoxic conditions (Rosa and Seibel 2010; Seibel 2011). This adaptation might be adaptive in water, allowing the organisms to wait until more oxygenated water arrives, but seems unlikely that this same strategy would be practical for an aquatic creature while in air.

This failure to find evidence does not preclude the ability to consume oxygen out of water in other octopus species, and may not even rule out the possibility of this ability in *Octopus rubescens*. It is possible that there are subpopulations within *O. rubescens*. Most of the video recordings of *O. rubescens* occur in California, and the activity is only known in Washington due to anecdotal evidences. It's possible that the Washington population has not developed the same adaptations as those in California, and don’t emerge from the water at the same rate. In addition, I collected our sample organisms from a subtidal populations of octopus, which, based on their location, are unlikely to be in a position to leave the water. Further studies should be done, testing octopuses from other locations and from other species in order to corroborate our findings.

In addition, it’s possible that that oxygen consumption while in air is a facultative response in *O. rubescens*. Octopus may need a period of time to prepare themselves for land movement, and the rapid transition into air during our experiments may either cause too much stress, or occur too quickly for the octopus to react as they would naturally. This seems unlikely however, as octopus move onto land on their own quite suddenly as they escape from predators or chase prey.

Octopuses relying on their anaerobic metabolism during terrestrial movement has several interesting implications and raises a number of questions. The movement across land must provide some offsetting benefit. It's possible that octopus have some sort of coping adaptation similar to other aquatic organisms that move on land.

When mitochondria are exposed to hypoxic conditions, they produce reactive oxygen species (ROS) (Turrens 2003). If octopus are relying anaerobic metabolism while in air, they are exposing themselves to hypoxia, this means their tissues and mitochondria within their tissues are hypoxic, and likely producing excess ROS. Do octopuses have mechanisms to cope with these surplus ROS when moving out of water?

Other molluscs do not provide adequate models because unlike the octopuses, most marine molluscs do not move while out of water, instead securing themselves in place until the tide returns (Nybakken and Bertness 2005). An analogous group of organisms with similar behavior to octopus while on land are amphibious fishes such as the walking catfish or the mudskipper. There is little data describing the length of times spent out of water by many amphibious air breathing fishes, however the air-breathing periods of

Erpetoichthys calabaricus were a mean of 2.3 minutes, but excursions lasted up to 74 minutes (Graham 1997). It appears that a greater threat than suffocation to amphibious organisms (as it may be for octopus) is desiccation, and amphibious fishes will regularly make use of any available pools of water during their amphibious expeditions (Graham 1997). To be able to spend long periods of time out of water, amphibious fish have developed several adaptations. In addition to being able to take up significant amounts of oxygen through their highly vascularized skin, some species of mudskippers such as *Pseudapocryptes* and *Blennius* seal a small amount of water within their operculae or branchial chambers. The amphibious crab *Sudanonautes (Convexonautes) aubryi monodi* uses a unique pulsatile reversed air flow after moving onto land, moving stored water from its branchial chambers over its gills (Cumberlidge 1986). Similar adaptations have not yet been observed in *O. rubescens* or any other species of octopus, but our data suggests that storing oxygenated water within the mantle cavity is unlikely. Such an adaptation should result in a reduced reliance on anaerobic metabolism while in air.

If octopus do not have similar adaptations to other amphibious organisms, they are unusual, relying solely on their anaerobic metabolism while moving on land. This makes them more analogous to terrestrial organisms and marine mammals, which rely completely on their aerobic metabolisms while underwater or have internal oxygen stores that can be accessed independent of the oxygen availability in the environment. I were unable to find recorded examples of other marine or amphibious organisms which rely on breath holding and anaerobic metabolism while moving actively in air.

Whatever the case, the fact that octopus move on land is still very unusual. Terrestrial movement is very risky, exposing the octopus to predators, suffocation, and desiccation. If octopuses are relying solely on their anaerobic metabolism while in air they compound this, setting a distinct limit on how long they can spend out of water.

Conclusion

I found no evidence that these *Octopus rubescens* can consume oxygen while in air. This research is just the very first part of a much larger possible exploration of the terrestrial movement of octopus. Since there has been so little study on this subject, I can only theorize on the purposes and of the metabolic adaptations that allow octopuses to be so active while on land. I hope that this study helps to promote interest into a continued exploration of this unexplored topic.

Acknowledgements

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Table 1. Records of octopus species moving on land, from amature online videos, published videos, online written references, and literature references.

Species	Country	Location	Duration	Source
Online Videos				
Octopus cf tetricus	Australia	Rottnest Is	:19	1
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	2:38	2
Octopus cf tetricus	Australia	Yallingup	:22	3
Octopus rubescens	USA	Pillar Point, CA	2:23	4
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	1:03	5
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	*:20*	6
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	*:35*	7
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	*:30*	8
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA	*:33*	9
Hapalochlaena sp.	Australia	Central coast, NSW, Australia	*:36*	10
Octopus rubescens	USA	Near Pacifica, CA	*:18*	11
Octopus rubescens	USA	Monterey Bay, CA	:52	12
Octopus rubescens	USA	Cape Mendocino, CA	*1:31*	13

Octopus rubescens	USA	Santa Barbara, CA	*:40*	14
Octopus rubescens	USA	San Fransisco, CA	*:23*	15
Octopus rubescens	USA	Lincoln Park Beach, LA, CA	*:24*	16
Octopus rubescens	USA	Big Sur, CA	*:42*	17
Octopus rubescens	USA	USA	:29	18
Octopus vulgaris	Cayman Islands	Cayman Island	:05	19
Octopus vulgaris	Jamaica	Montego Bay	:45	20
Published Videos				
Abdopus aculeatus	Unknown	Australia		BBC The Hunt: Race against Time (Episode 6 of 7)
Online References				
Octopus rubescens	USA	San Simeon, CA		21
Unknown	USA	Humbolt, CA		22
Unknown	Ecuador	Galapagos Islands		23
Octopus rubescens	USA	Fitzgerald Marine Reserve, CA		24
Octopus vulgaris	Bermuda	Bermuda		25
Literature References				
Octopus alpheus	Australia	Australia, East Coast		(Norman 2000)

Unknown	Japan	Bonin Islands	(Lee 1875)
Unknown	Australia	Blackwood's Bay, Torres Strait	(Lee 1875)
Unknown	Bermuda	Bermuda	(Lee 1875)
Likely *Octopus vulgaris*	Unknown	Mediterranean	(Aristotle)
Octopus rubescens	USA	North-eastern Pacific	(Wood and Anderson, 2004)
Enteroctopus dofleini	USA	North-eastern Pacific	(Wood and Anderson, 2004)
Octopus briareus	Caribbean	Caribbean	(Wood and Anderson, 2004)

1. https://www.youtube.com/watch?v=qYxd9erB6Ng, 2. https://www.youtube.com/watch?v=FjQr3lRACPI, 3. https://www.youtube.com/watch?v=F5fZu-1bt6Y, 4. https://www.youtube.com/watch?v=_Wkl3exDmys, 5. https://www.youtube.com/watch?v=pD81VOLSmV8, 6. https://www.youtube.com/watch?v=UsNAQ-bPhEw, 7. https://www.youtube.com/watch?v=Eoy2hBpePPQ, 8. https://www.youtube.com/watch?v=shAMYcGPiNA, 9. https://www.youtube.com/watch?v=xOXm-asjIdc, 10. https://www.youtube.com/watch?v=roOZDpZcQQE, 11. https://www.youtube.com/watch?v=cuLXVcqsVLI, 12. https://www.youtube.com/watch?v=zloMXeEAXT8, 13. https://www.youtube.com/watch?v=hjoHhcjK3Lo, 14. https://www.youtube.com/watch?v=4Lp5a-r3MJU, 15. https://www.youtube.com/watch?v=X00tmxswLNk, 16. https://www.youtube.com/watch?v=6Gvh7kKwBwo, 17. https://www.youtube.com/watch?v=VHiA06HV49s, 18. https://www.youtube.com/watch?v=DdRh3xjC1_Q, 19. https://www.youtube.com/watch?v=_F-ag5nMvD0, 20. https://www.youtube.com/watch?v=6ICQxNnJ-iE, 21. http://www.wallawalla.edu/academics/departments/biology/rosario/inverts/Mollusca/Cephalopoda/Octopus_rubescens.html, 22. http://smilesandsunrays.tumblr.com/post/74670809843/go-birding-find-an-octopus-but-seriously-why, 23. http://www.ms-starship.com/journal/jun99/21.htm, 24. http://www.oregontidepooling.com/tidepooling_on_the_southe/octopus/, 25. http://blogs.scientificamerican.com/octopus-chronicles/land-walking-octopus-explained-video/

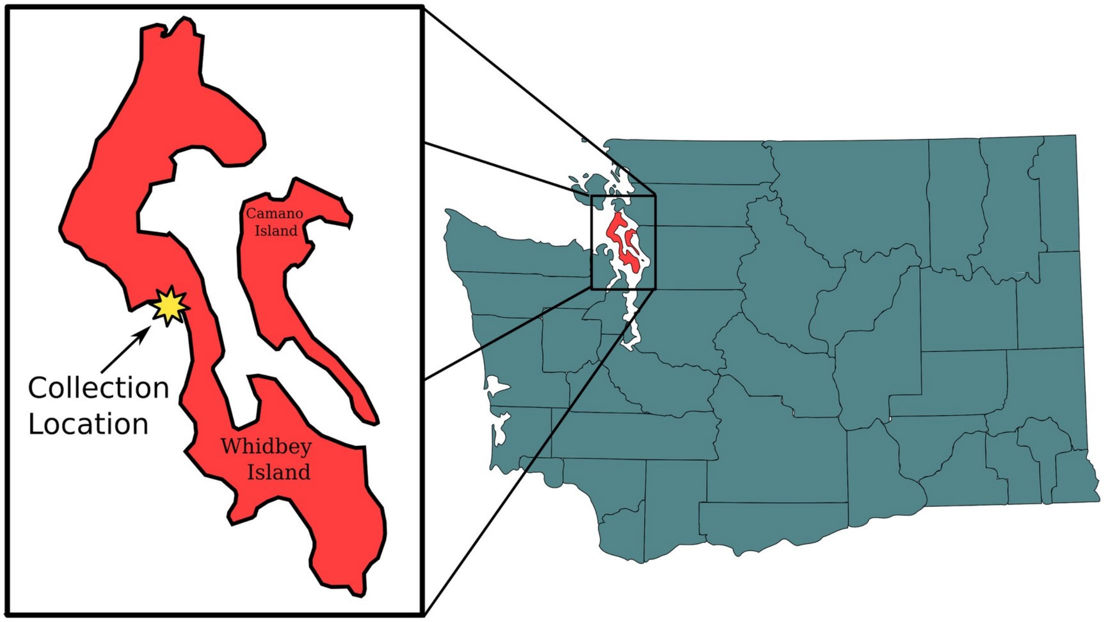

Figure 1. Map of Study Location. Yellow star indicates collection site at Driftwood Park in Admiralty Bay on Whidbey Island, Washington (48°16’ 38.06”N - 122°63’70.18”W).

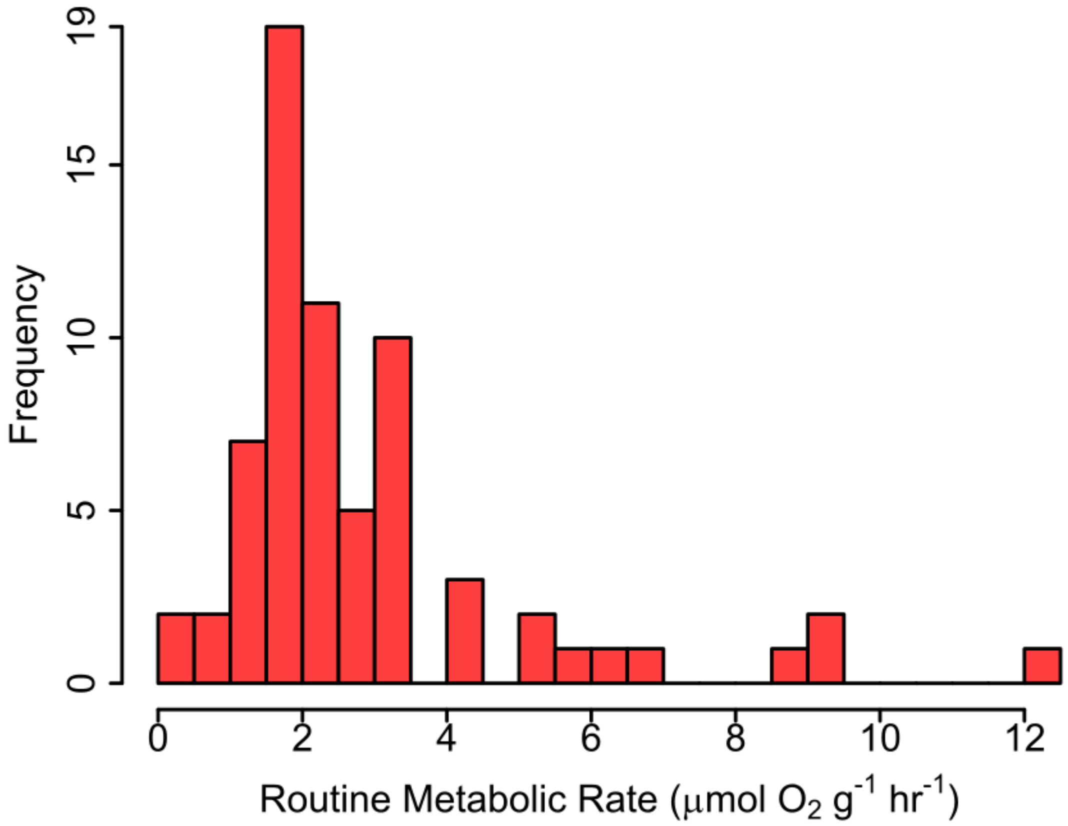

Figure 2. Routine metabolic rates of octopuses in water filled flow-through respirometers. Mean routine metabolic rate was 2.89±2.22 μmolO2 g-1 hr-1 (mean±SD). Skew of this distribution was +2.207.

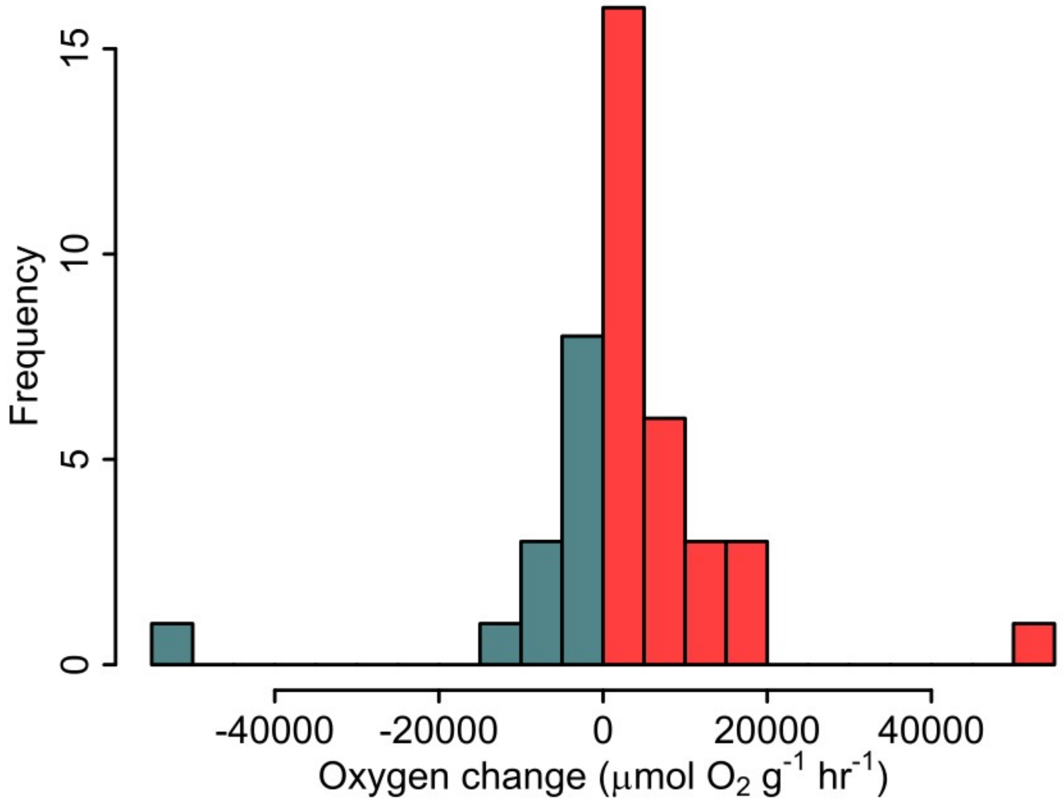

Figure 3. Change in oxygen concentration of in-air respirometers containing octopuses less the oxygen concentration change in paired blank respirometers. Median is not equal to 0 (Wilcoxon signed rank test, V=659, p=0.0086). Dark blue indicates values below zero, red indicates values above zero.

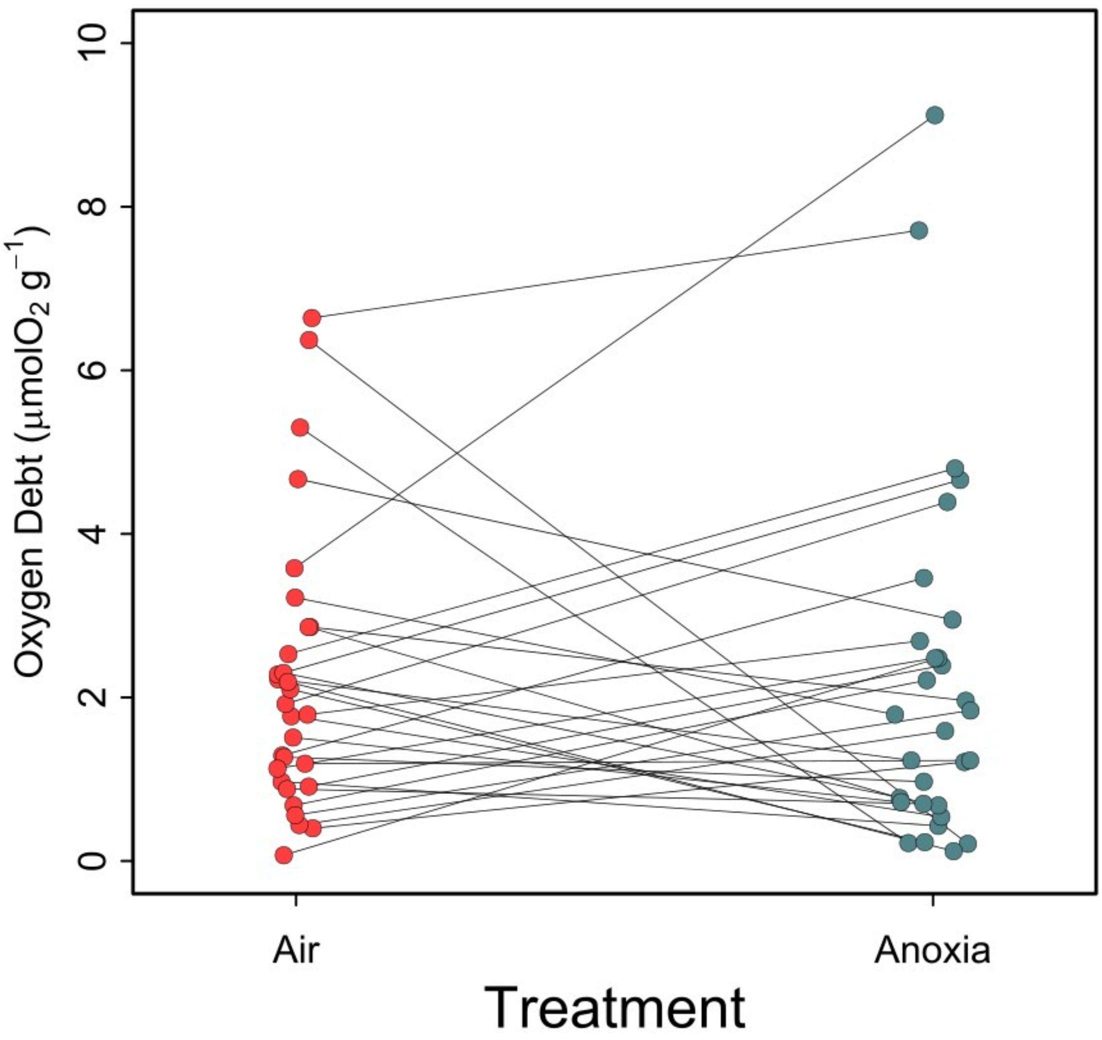

Figure 4. Oxygen debts following period of 15 minutes in air and 15 minutes in anoxic water. Lines indicate debts for the same octopus. Debts are not significantly different (Paired 2-way ANOVA on log-transformed data, df=28, Order of treatments F=0.018, p=0.893, Treatment F= 0.119, p=0.733, Order of treatment and treatment interaction F=0.456, p=0.505).

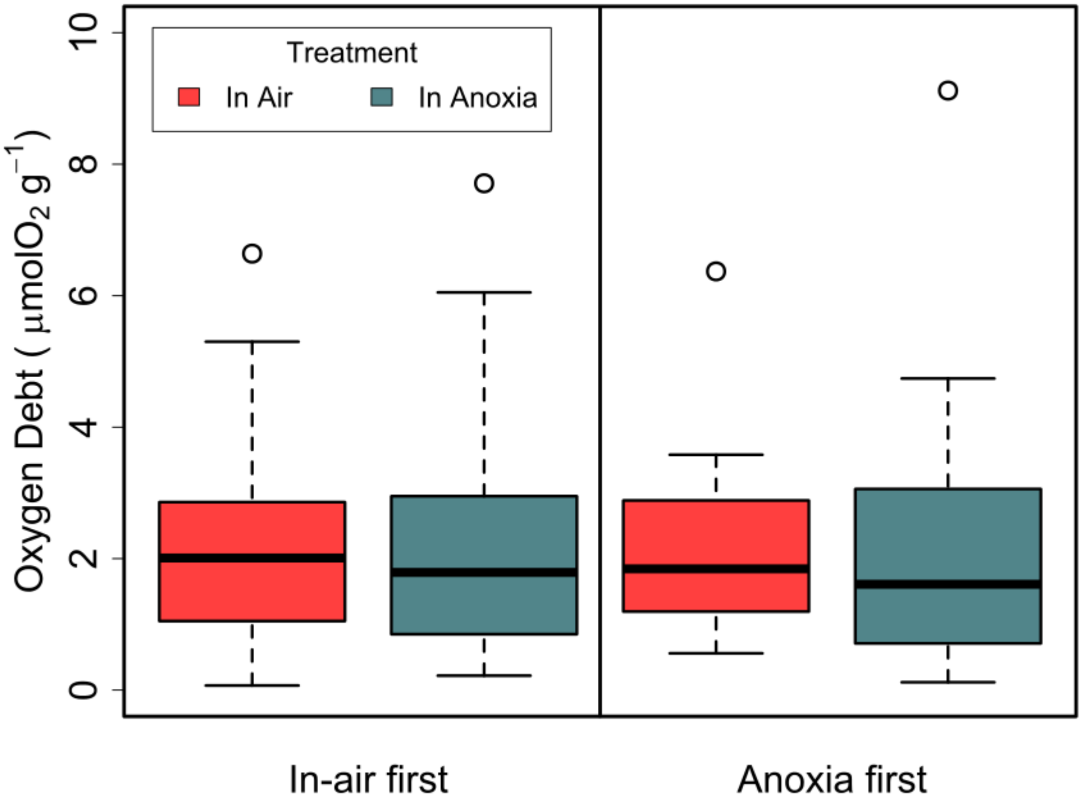

Figure 5. Effects of order of initial trail (in air or anoxic water) on oxygen debt magnitude. Means are not significantly different (Paired 2-way ANOVA on log-transformed data, df=28, Order of treatments F=0.018, p=0.893, Treatment F= 0.119, p=0.733, Order of treatment and treatment interaction F=0.456, p=0.505).

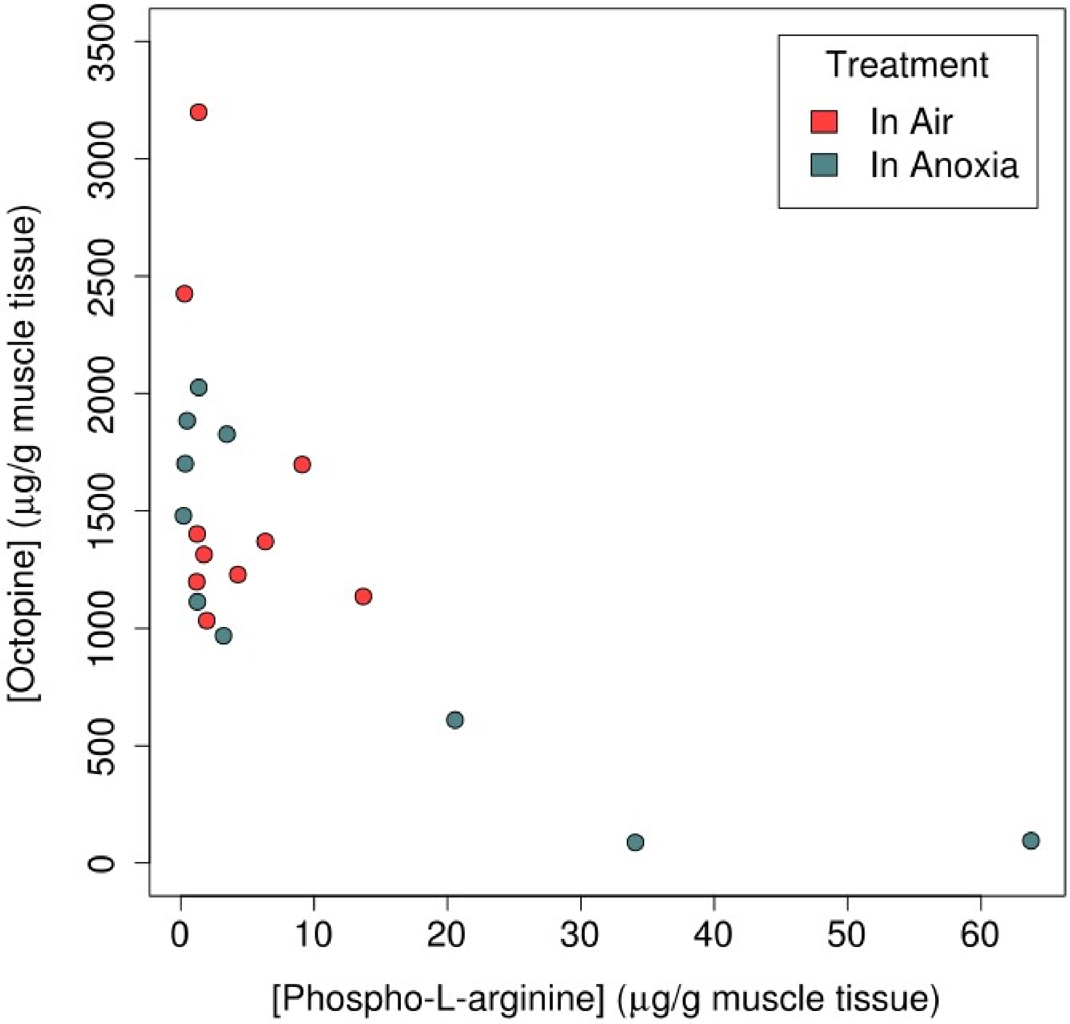

Figure 6. Comparison of octopine concentrations versus phospho-L-arginie concentrations in octopus arm muscle tissue following fifteen minute exposure to air or anoxic water.

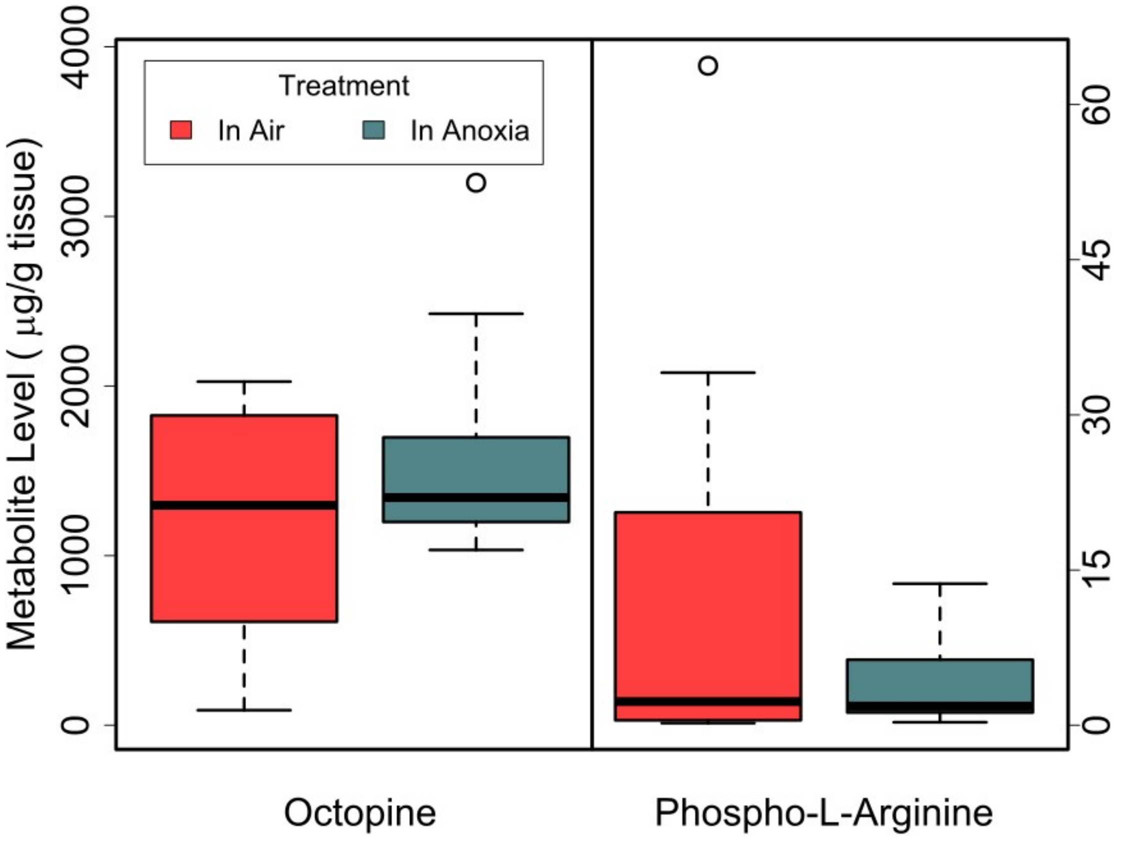

Figure 7. Concentrations of octopine and phospho-L-argine in arm tissue of Octopus rubescens following fifteen minute exposure to air or anoxic water. There are no significant differences between in air or anoxic water concentrations of these metabolites (PERMANOVA df=19, f-statistic=1.9215, p=0.2057)

Appendix I

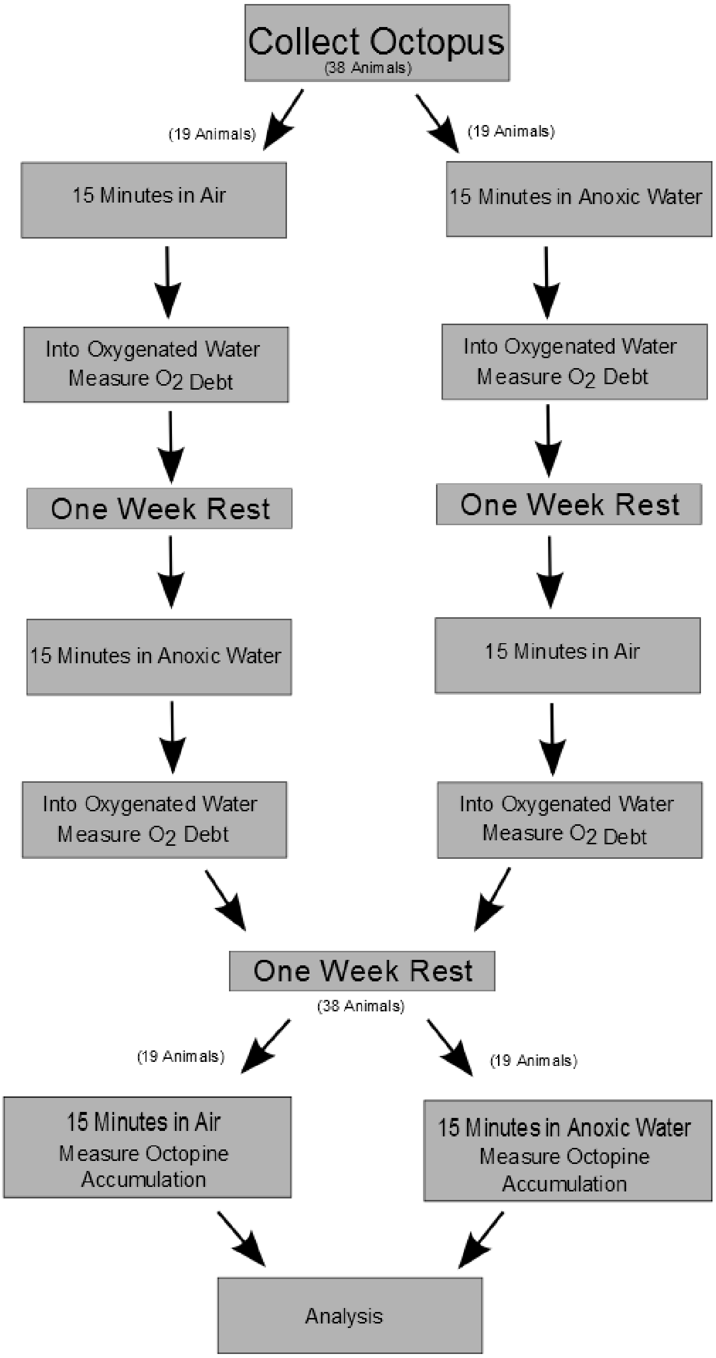

Figure A 1. Flowchart of procedure used in this experiment

Figure A 2. Picture of water bath used for in-air respirometry

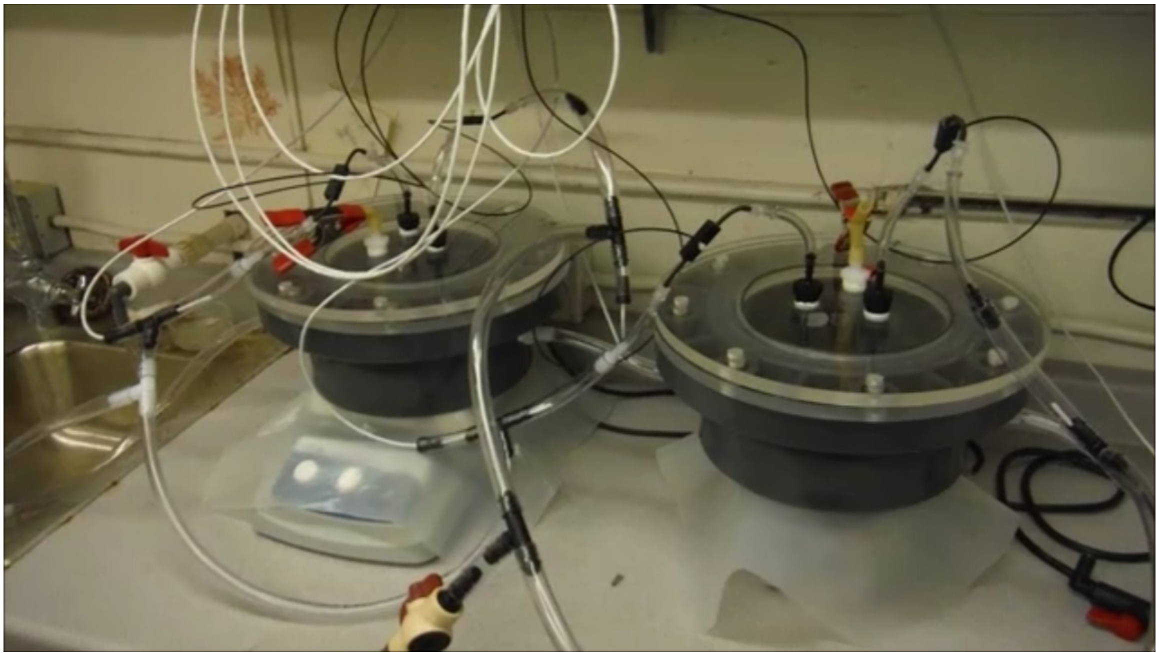

Figure A 3. Picture of flow through respiration system.

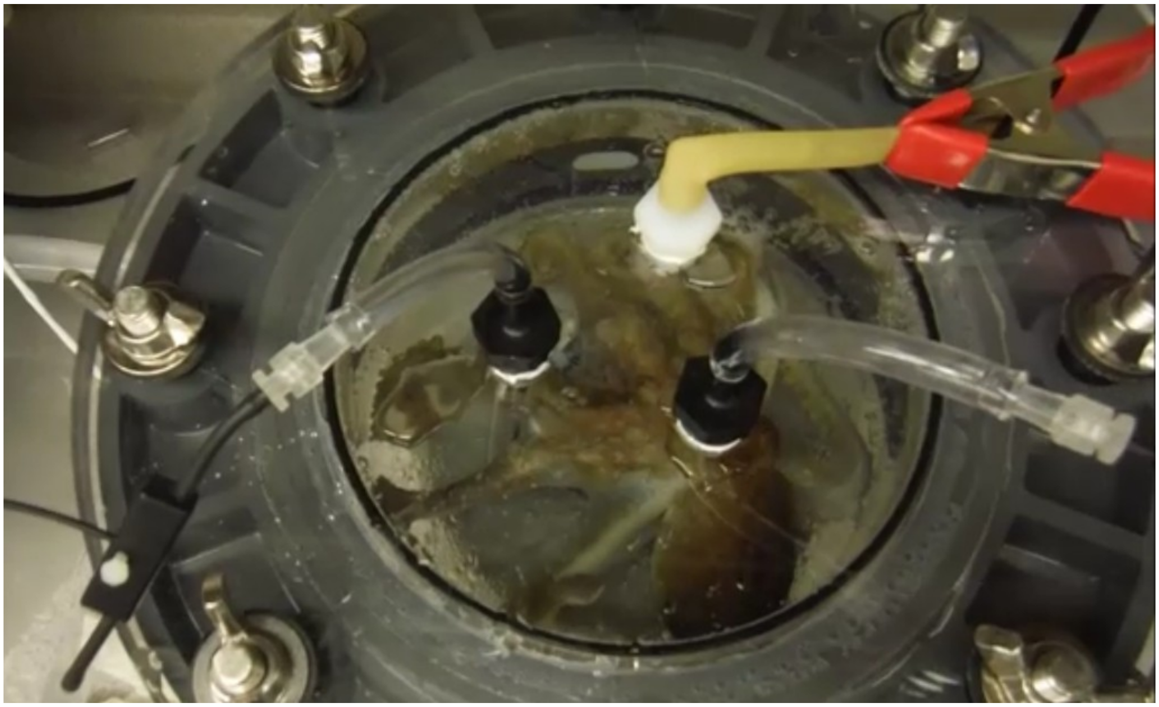

Figure A 4. Picture of octopus inside flow-through respirometer system.

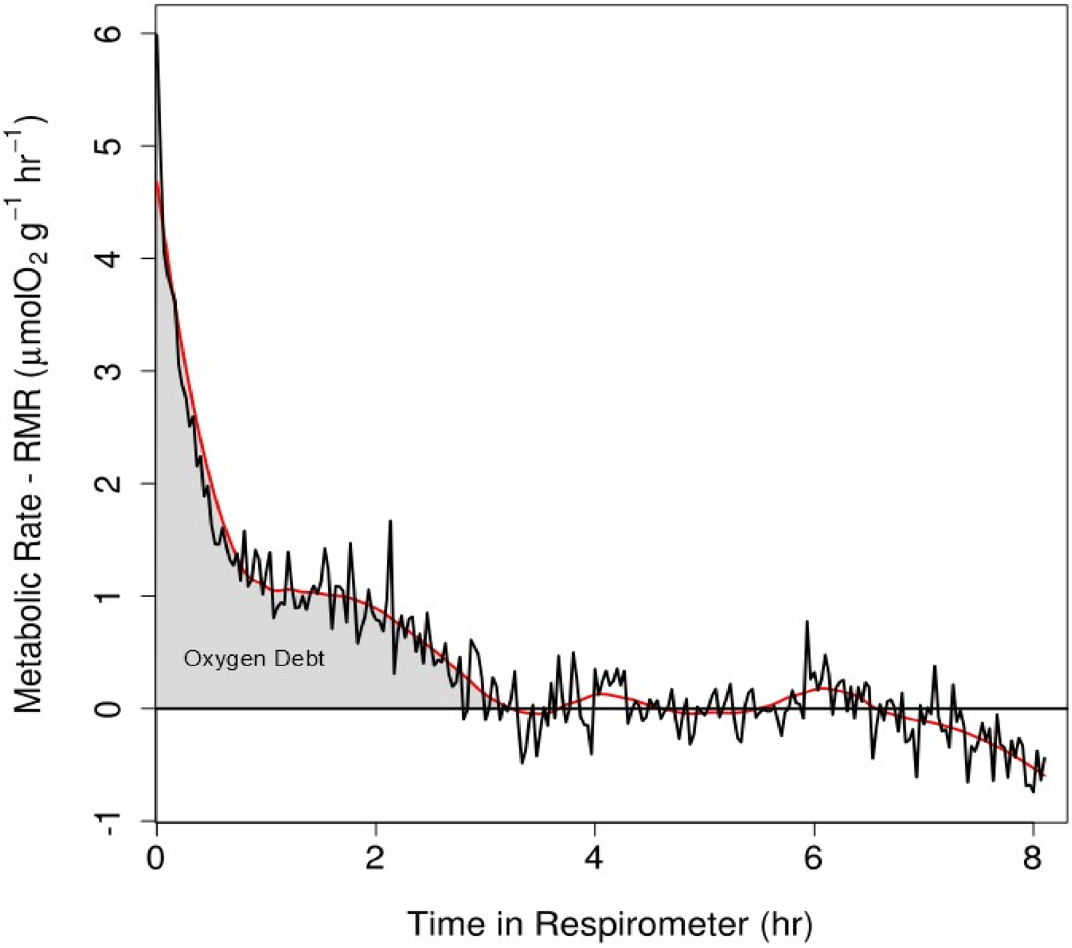

Figure A 5. Example of oxygen debt respirometry data. The black line represents the measured metabolic rate minus the routine metabolic rate measured previously for the same octopus. The red line represents the LOESS regression. The grey shaded region under the curve represents the oxygen debt.