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FELLOWSHIP FINAL REPORT

Adaptations to deep diving in seal lice, the exception to the rule that there are no marine insects

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REPORT INFO

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ABSTRACT

Insects are the most evolutionarily and ecologically successful group of living animals, being present in almost all possible mainland habitats; however, they are virtually absent in the ocean, which constitutes more than 99% of the Earth's biosphere. Only a few insect species can be found in the sea but they remain at the surface, in salt marshes, estuaries, or shallow waters. Remarkably, a group of 13 species manages to endure long immersion periods in the open sea, as well as deep dives, i.e., seal lice. During the evolutionary transition of pinnipeds from land to the ocean, echinophthiriid seal lice had to manage the gradual change to an amphibian lifestyle along with their hosts, some of which may spend more than 80% of the time submerged and performing extreme dives, some beyond 2000 m under the surface. These obligate and permanent ectoparasites have adapted to cope with hypoxia, high salinity, low temperature, and, in particular, conditions of huge hydrostatic pressures. A major remainig question is whether or not seal lice do breath underwater or, on the contrary they dramatically reduce their metabolism to spare oxygen when submerged. During the reported period, we investigated anatomical adaptations to prolongued immersion and also set up a method for measuring oxigen consumption in two media, air and water in small insects, both using state of the art methods.

1- Introduction

Insects are the most evolutionarily and ecologically successful group of living animals, being present in virtually all possible habitats, yet mostly absent in the ocean (Bradley *et al.* 2009). A few species are present in the sea, but remaining at the surface, salt marshes, estuaries, or shallow waters (Cheng 1976). Nevertheless, a group of 13 species manages to survive long immersion periods in the open sea and deep dives, i.e. seal lice (Leonardi and Palma 2012; Leonardi 2015).

Sucking lice (Phthiraptera: Anoplura) are obligatory hematophagous and permanent ectoparasites of mammals, living into the fur or among the hairs of their hosts. Among lice, the family Echinophthiriidae is peculiar in the sense that infests amphibious hosts, such as pinnipeds and the Northern river otter (Durden and Musser 1994). Pinnipeds are diving animals, i.e. most sea lions, fur seals, and phocids usually dive at depths of 150–200 meters (Stewarts 2009), but some species make deeper and longer dives, reaching 2100 meters under the surface (Costa *et al.* 2010; McIntyre *et al.* 2010). Moreover, pinnipeds remain with their bodies submerged from several weeks to several months (Teilmann *et al.* 2000; Stewart 2009). During the evolutionary transition of pinnipeds from land to the ocean, echinophthiriids lice had to manage the amphibian biology of their hosts, some of which may spend more than 80% of the time submerged and performing extreme dives.

One of the main and long-lasting interrogations concerning seal lice, was whether or not they would survive during the diving excursions of their hosts. The dominant idea was that they would not, infest seals only during their periods ashore. However, recent evidence (Leonardi *et al*. 2018) strongly suggested that seal lice could escort their host during their long diving excursion. Albeit the subject has been a matter of speculation for more than a century (Enderlein 1906; Scherf 1963; Leonardi and Lazzari 2014; Leonardi *et al*. 2020), the question about how echinophthiriids could survive in deep seawaters remains, however, fully open.

Recently, we demonstrated that experiments showed that the forms present in non-diving pups – i.e. eggs and first-instar nymphs – were unable to tolerate immersion in water while following instars and adults, all infesting diving hosts, supported weeks of submersion (Leonardi and Lazzari 2014). We also discovered that lice survival underwater, as well as their recovery once returned to the air, depends on the concentration of oxygen in the water, raising the possibility of an unknown capacity to breathe underwater (Leonardi and Lazzari 2014). Later on, to test the capacity of lice to survive host deep dives, we conducted a series of controlled experiments on lice infesting elephant seals. Lice supported hydrostatic pressures over 200 atm, equivalent to dives beyond 2000 meters below the surface (Leonardi *et al*. 2020).

The main goal of the project is unraveling the adaptations allowing seal lice to spend most of their life where any other known insect is not able to survive, i.e. the deep sea. This project should provide key information for understanding how to seal lice can tolerate the extreme conditions to which they are exposed by the particular biology of their hosts, i.e. extreme temperature variation between land and cold water, absence of gaseous oxygen, high salinity, and extreme hydrostatic pressure. In a broader sense, it should shed light on the reasons why insects have been so little successful in colonizing the ocean realm, which represents 99% of the biosphere of our planet.

The specific goals developed during my three months-stay were:

1- Determine anatomical and physiological adaptations for aquatic survival.

2- Analyze the particularities of the respiratory system of seal lice.

3- Set up a method for measuring oxygen consumption and metabolic rates in insects breathing air and underwater.

2- Experimental details

Micro-computed tomography: We examined *Lepidophthirus macrorhini* lice from Southern elephant seals using X-ray microtomography (micro-CT) in the *Centre Européen de Recherche et d'Enseignement des Géosciences de L'enviroment*, Aix-Marseille Université. The micro-CT uses a three-dimensional reconstruction of the sample to explore its anatomy, without requiring dissection or histology.

Respirometry methods: Provided that no method for rearing seal lice in the laboratory is at present available, the standardization of the methods was performed using insects of similar size easily available at the IRBI in high numbers, i.e. mosquito larvae. During the next seals' reproductive season, lice will be collected in order to perform the measures in the field.

Aerial and aquatic respiration were measured, by quantifying the oxygen consumption of insects using oxygen optode sensors (Pyroscience GmbH, Germany).

The consumption of oxygen dissolved in the air and the water was measured in groups of larvae and pupae of *Aedes aegypti* and *Aedes albopictus* maintained at different temperatures.

The data obtained were statistically analyzed using ANOVA, after confirmation of the adjustment to criteria of normality, symmetry, and homoscedasticity.

3- Results and discussion

1) *Morphological adaptations*

1.1) *Legs*

The legs of sucking lice present structural modifications as an adaptation for grasping to host hair fibers (Mullen and Durden, 2019). Particularly, echinophthiriid lice present robust legs, and the tarsal claws are modified into hooks (Leonardi *et al*. 2021). Leg muscles are strong and well-developed (Fig. 1). The oblique disposition of muscular fibers over a single central tendon does increase the force that the muscles apply in contraction (Chapman and Chapman 1998). Considering the long periods that seal lice have to remain attached to their host, and the hydrodynamic forces of diving, this constitutes one of the main adaptations of echinophthiriids to the strong dragging to which they are submitted by the powerful swimming of their hosts.

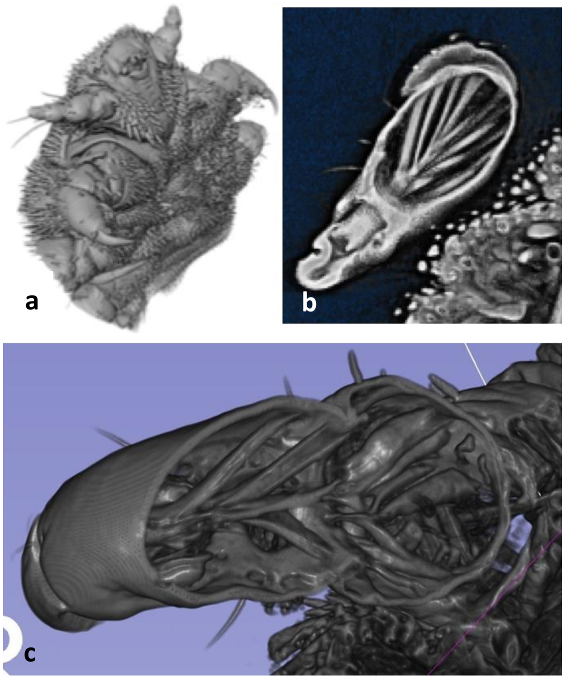


Figure 1- Images obtained by micro-computed tomography of an adult elephant seal louse, *Lepidophthirus macrorhini*. a- Photograph obtained by microCT, b- Leg muscles of L. macrorhini c- Detail of leg muscles in a 3D model made by processing the images with the free software 3D Slicer.

1.2) The respiratory system

Like most insects, lice possess a tracheal system, in which branches reach every organ and tissue. A striking particularity of seal lice is that the size and extension of big tracheae are more important than in other groups (Fig. 2b). This suggests that lice could store more air (i.e. more oxygen) in their body, which could be used during submersion.

On the other hand, as pointed out by Cheng (1976), the connection of the trachea with the exterior reveals complex closing structures forming chambers internally to the spiracles (Fig. 2a).

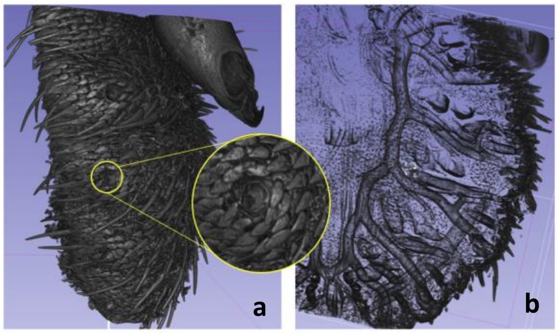
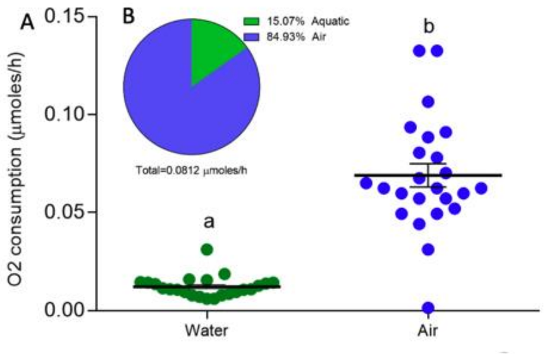


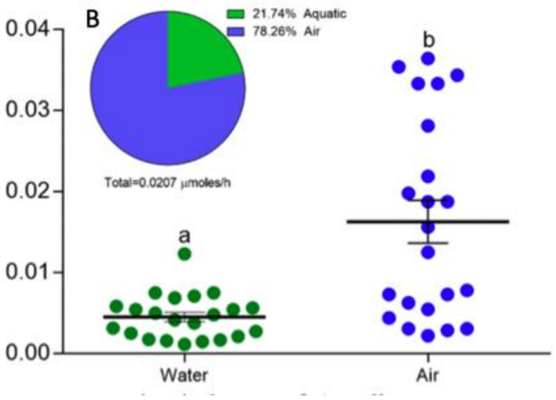
Figure 2- Details of the tracheal system of *Lepidophthirus macrorhini*, a- spiracle, b- the structure of tracheal tubes.

3) Oxygen consumption

We were able to set up a method for precisely measuring oxygen consumption in small insects in both, air and aquatic media. The validation of the methodology was performed by investigating some unknown aspects of breathing in mosquito larvae, such as whether or not are they able to get oxygen from the water and the effect of temperature on their metabolism. Both questions are identical to those that we prioritize to be analyzed in seal lice in Patagonia. Our experiments (Fig. 3) showed that the larvae that had contact with the air, even remaining on the surface of the water, obtained 15% of their oxygen from the water. Whereas when they were completely submerged, they were able to get all the oxygen they needed to survive. Temperature affected the respiration rate of both air and water, and relatively close Q10 values were calculated from measurements at 15°C, 25°C, and 35°C. When kept submerged in water at 25 °C, the larvae survived for several days, showing 50% mortality after 10 days, but some individuals remained alive for up to 30 days. Mortality increased when exposed to 35º, with 50% mortality after 5 days of treatment. At the time of my return, the larvae submerged at 15º were still alive, having been under that condition for more than a month. Interestingly, while the control larvae molted to pupae normally, the submerged individuals never molted. Although obtaining oxygen was not a limiting factor for survival, molting (or lack thereof) appears to be the critical factor responsible for the death of mosquito larvae.



Aedes aegypti



Aedes albopictus

Figure 3- Oxygen consumption from the air and the water of larvae of a- *Aedes albopictus* (mean and 95 % confidence intervals), and b- *A. albopictus*.

4- Conclusion

The development of this scholarship allowed me to familiarize myself with the experimental design in insect physiology, having gained experience in planning it, and collecting and analyzing data. These tools and skills will soon be applied in my study system, which will allow me to obtain fundamental information to understand the physiological processes involved in the underwater survival of pinniped lice.

My active participation in the planning and execution of experiments with mosquitoes also allowed me to get involved in a new system, which allows new questions to be raised, complementing my main line of work.

I consider that during my stay I have not only had the opportunity to train and acquire new tools, but also the possibility of actively discussing with a specialist of the level of Dr. Lazzari, which not only strengthens my current work but also enhances it and opens new lines. jobs that you would not otherwise have had access to. This stay effectively marks a turning point in my work, allowing me to approach the issue of lice adaptations to marine life from a new perspective.

5- Perspectives of future collaborations with the host laboratory

The Fellowship awarded to SL allowed the consolidation of a fruitful collaboration launched some years ago between the fellow and host laboratories. It provided an institutional frame that contributed to the success in obtaining support from the main French (CNRS) and Argentinean (CONICET, MinCyT) research councils. This support included not only funds for travel and equipment, but also co-supervised Ph.D. grants.

During the stay of SL in Tours, it was possible to collect more data and initiate more projects than possible to complete in just three months. So, the collaborative work continues at the distance, manuscripts are written and new questions arise. All these facts make the collaboration certainly maintained for many years.

The fellow and the host scientist are also planning the organization of an international workshop in the frame of the Le Studium fellowship in late 2022-early 2023.

6- Articles in preparation in the framework of the fellowship

In preparation:

Leonardi, M.S.; Lazzari, C. MicroCT reveals the native structure and functional morphology of the seal louse, *Lepidophthirus macrorhini*.

Álvarez Costa, A.; Leonardi, M.S.; Schilman, P.; Lazzari, C. Mosquito larvae breathe underwater, but pupae do not.

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