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Uncovering deep mysteries: The underwater life of an amphibious louse

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ABSTRACT

Despite the incredible success of insects in colonizing almost every habitat, they remain virtually absent in one major environment – the open sea. A variety of hypotheses have been raised to explain why just a few insect species are present in the ocean, but none of them appears to be fully explanatory. Lice belonging to the family Echinophthiriidae are ectoparasites on different species of pinnipeds and river otters, i.e. they have amphibious hosts, who regularly perform long excursions into the open sea reaching depths of hundreds of meters (thousands of feets). Consequently, lice must be able to support not only changes in their surrounding media, but also extreme variations in hydrostatic pressure as well as breathing in a low oxygen atmosphere. In order to shed some light on the way lice can survive during the diving excursions of their hosts, we have performed a series of experiments to test the survival capability of different instars of *Antarctophthirus microchir* (Phthiraptera: Anoplura) from South American sea lions *Otaria flavescens*, when submerged into seawater. These experiments were aimed at analyzing: (a) immersion tolerance along the louse life; (b) lice's ability to obtain oxygen from seawater; (c) physiological responses and mechanisms involved in survival underwater. Our 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 usually found in diving hosts, supported it very well. Furthermore, as long as the level of oxygen dissolved in water was higher, the lice survival capability underwater increased, and the recovery period after returning to air declined. These results are discussed in relation to host ecology, host exploitation and lice functional morphology.

1. Introduction

Insects are the most evolutionarily and ecologically successful group of living animals, being present in virtually all possible habitats (Bradley et al., 2009). Despite this, few species have colonized the ocean and the vast majority of marine insects live closely associated with the sea surface, salt marshes, estuaries, or shallow waters (Cheng, 1976). Much has been argued about the reasons why insects have not successfully colonized the ocean despite their success in land (Ruxton and Humphries, 2008).

Maddrell (1998) postulated that insects failed in colonizing the marine realm because they would be poor competitors. Accordingly, crustaceans have successfully evolved thanks to the development of strategies to avoid predators, such as transparency, and on this point, insects would have failed. Besides, this author suggested that the insect tracheal system would not be able to support elevated hydrostatic pressure and, in consequence, insects could not survive beyond the first tens of meters of the water column (Maddrell, 1998, and references therein). Yet, some insects manage to survive underwater at great depths and during long immersion periods, e.g. lice.

Sucking lice (Phthiraptera: Anoplura) are obligatory hematophagous insects and permanent ectoparasites of mammals, living into the fur or among the hairs of their hosts. Among sucking lice, those belonging to the family Echinophthiriidae are peculiar in the sense that they infect amphibious hosts, such as pinnipeds (sea lions, walruses, true seals) and river otters (Durden and Musser, 1994). As a consequence, echinophthiriids must be capable of dealing with the challenges of the marine realm. Along the evolutionary time echinophthiriids have developed unique morphological adaptations to cope with the amphibious lifestyle of their hosts. All species possess prehensile tibio-tarsal claws in the second and third pairs of legs adapted to firmly grasping onto hairs and abdominal spiracles with a specialized closing device that preserves atmospheric air and prevents water entering the body during the host's

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immersions. Their membranous abdomen has been proposed as a possible surface for gas exchange underwater (Kim, 1975).

Pinnipeds are also diving animals, i.e. most sea lions, fur seals, and phocids usually dive at depths of 150–200 meters (Stewart, 2009). Some species of pinnipeds make deeper and longer dives to reach their prey, e.g. adult southern elephant seal has been recorded to dive up to 1468 meters (Campagna et al., 1999). Moreover, pinnipeds can have their bodies submerged from several weeks to several months (Teilmann et al., 1999; Stewart, 2009). Therefore, during the evolutionary transition of pinnipeds from land to the ocean, echinophthiriids lice have managed to continue exploiting these hosts, some of which may spend more than 80% of the time swimming and performing extreme dives.

The question about how do echinophthiriids survive in deep seawaters remains fully open, albeit the subject has been matter of speculation since more than a century (Enderlein, 1906; Scherf, 1963). Thus, even though there has been collected many data on the biology of pinnipeds and on some aspects of the ecological association with their parasitic partners (see Leonardi and Palma, 2013), the diving physiology of these lice remains unexplained. Considering the above stated, this work is aimed at describing the response of *Antarctophthirus microchir* from South American sea lions, *Otaria flavescens* to immersion, and examining the underwater survival of lice submitted to different conditions of temperature and oxygen in water.

2. Materials and methods

2.1. Lice

The samples were taken in Punta León rookery (43°04′S, 64°29′W) during the breeding season 2010/11. Nits and lice were collected from *O. flavescens* pups, which were captured with a noose pole and restrained by two people. A third person collected the lice using a fine-tooth comb commonly used for treating human pediculosis (for details see Leonardi, 2014).

Lice were taken from the field to the laboratory immersed in seawater. In order to verify the viability of lice, they were taken out of water and exposed to air at room temperature (ca. 25 °C/77 °F). Those individuals who responded to this change moving their legs and/or antennae were selected for experiments. Eggs were transported separately in plastic tubes. The experiments were carried out no longer than 3 h after the collection.

Different instars and experimental conditions are depicted in Fig. 1. Eggs are usually laid on the back of pups that do not go into the sea. Those of other echinophthiriid louse species do not survive immersion (Murray and Nicholls, 1965). Pups can get wet during high tides, but their back usually dries fast (Leonardi et al., 2012a). First-instar nymphs (N1) usually occupy the back of the host after hatching and slowly migrate to the belly; the number decreases when pups start swimming (Aznar et al., 2009). N1 are the only instar lacking of scales over their body. Second – (N2) and third-instar nymphs (N3) and adults are found together in the ventral side of the host body, even at ages when hosts start swimming (Leonardi et al., 2012a). So, three categories of experimental subjects were established according to their association with the host: eggs, N1 and N2 + N3 + Adults.

2.2. Eggs tolerance

Considering that the exposure of eggs to seawater during their development is reduced because they are laid on young pups, we tested their tolerance to submersion and to different temperatures. To discriminate between these effects, we chose temperatures close to the natural exposure limits (0° and 35 °C/32 °F and 95 °F).

We collected 177 louse eggs from infested sea lion pups. According to their morphological features, eggs were classified as *viable* or *non-viable* following the criteria proposed by Mougabure Cueto et al. (2006), which also apply to *A. microchir*. A total of 84 eggs (47.5%) were considered viable and subjected to the following treatments (Fig. 1): (i) immersion in seawater at 0 °C/32 °F ($n = 21$) and 35 °C/95 °F ($n = 21$) and then exposed to air at 35 °C/95 °F; (ii) exposure to cold air (0 °C/32 °F) during 14 ($n = 9$) and 25 days ($n = 12$) and then transferred to 35 °C/95 °F; (iii) incubated at 35 °C/95 °F in air ($n = 21$). During each treatment, each egg was observed daily and categorized as live or dead.

2.3. Submersion tolerance of first-instar nymphs

Three groups of 25 N1 were kept submerged in fresh seawater at 10 °C/50 °F (normal water temperature in the area during the study season) for 24, 48 and 72 h, respectively. After the corresponding immersion period, the insects were individually placed on a filter paper and observed with the aid of a stereomicroscope at different times, i.e. 0, 5, 15, 30, 45, 60, 90, 120, 150 and 180 min. At every stage, lice were categorized as *mobile* (with the ability to walk and/or to move the antennae) or *immobile* (not able to walk and/or to move the antennae). If after 180 min a louse still did not show any sign of recovering, it was considered as dead.

2.4. Immersion and recovery of diving instars

Groups of 18 lice including second- and third-instar nymphs and adults were placed into glass vials (5 ml) and submerged during 1–15 days in seawater with different oxygen saturations (i.e. $n = 18 \times 2$ conditions $\times$ 15 time periods, $N = 540$). To test oxygen dependence, trials were conducted into 1-liter identical aquaria on different conditions each. Half of the lice were placed in seawater constantly bubbled with ambient air by a pump, here indicated as "normoxic". The other half was kept into seawater previously boiled for 15 min and without aeration, here called "hypoxic". Both aquaria were closed so that equilibrium with atmospheric air would not be reached. Experiments were conducted at 10 °C/50 °F, approximately the mean temperature of seawater at Punta León latitude during the reproductive season of sea lions. The survival was determined following the same criterion that was used with the first-instar nymphs. In addition, the recovery time, i.e.

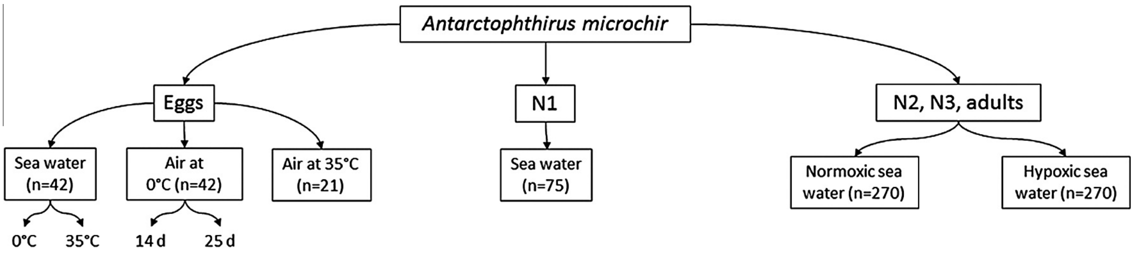

Fig. 1. Summary of the experiments conducted with *Antarctophthirus microchir*.