

# Lousy mums: patterns of vertical transmission of an amphibious louse

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**Abstract** In this study, we document patterns of vertical transmission of the amphibious louse *Antarctophthirus microchir* (Echinophthiriidae) in pups of South American sea lion, *Otaria flavescens*, from Patagonia. Vertical transmission is fundamental for the long-term stability of *A. microchir* populations because only pups stay long enough (1 month) on land for the louse to reproduce. A total of 72 pups  $\leq 7$  days old from a single rookery were captured and examined for lice. Infection parameters and population structure of *A. microchir* did not differ among pups collected at the beginning, middle, and end of the reproductive season, suggesting that patterns of early vertical transmission are not affected by the increase of rookery size during this period. Over 60 % of 1-day-old pups were infected with *A. microchir*, and recruitment increased in pups up to 3 days old and then leveled off. In 1-day-old pups, significantly more adults than nymphs were found, but the pattern was reversed in older pups. The number of first-stage nymphs was significantly smaller than that of second- and third-stage nymphs, as it was the number of males vs. females, particularly in 1-day-old pups. Three non-exclusive hypotheses could account for these patterns, i.e., recruitment merely reflects the population structure of *A. microchir* is cows; the relative ability of lice to pass from cows onto pups increases in advanced instars; and/or natural selection favors transmission of adults, especially females, because they accrue greater

fitness. The importance of latter hypothesis should not be underestimated in a species with a tight reproductive schedule.

## Introduction

Lice (order Phthiraptera) are the only insects that have become obligate and permanent parasites throughout their entire life cycles, living as ectoparasites on the host's surface (Kim 1985; Bush et al. 2001). Being wingless insects that cannot survive for prolonged periods off their hosts, lice have limited opportunities for dispersal, and transmission from one host to another largely depends on physical contact between hosts (Demastes et al. 1998; Galloway 2012; Toloza et al. 2009). Such direct transmission can be potentially either vertical or horizontal. Vertical transmission involves the movement of lice from a parent onto its offspring, whereas horizontal transmission occurs between siblings or during mating (de Brooke 2010). The ecological and evolutionary consequences of each mode of transmission are potentially profound for both lice and their hosts and involve, inter alia, the evolution of virulence (e.g., Clayton and Tompkins 1994; Darolova et al. 2001; de Brooke 2010), patterns of co-speciation (e.g., Demastes et al. 1998; Vas et al. 2012 and references therein), or sexual selection (e.g., Lee and Clayton 1995).

Rather surprisingly, there are very few studies that have collected accurate data on patterns of vertical or horizontal transmission of lice, and most of them involve birds (see Lee and Clayton 1995; Galloway 2012 and references therein). However, these studies represent a basic, yet fundamental step to understand the selective pressures that act on transmission according to host's biology (de Brooke 2010). For instance, horizontal transmission is fundamental to account for epidemiological patterns of the head louse, *Pediculus humanus capitis*, in modern societies (Toloza et al. 2009; Rózsa and Apari 2012). Head-to-head transmission is largely restricted to children to the extent that pediculosis is considered as a pediatric pandemic (Rózsa and Apari 2012). In contrast,

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vertical transmission has been suggested to play a prominent role in lice species specific to hosts with limited physical contacts, e.g., swifts (Lee and Clayton 1995) or pocket gophers (Demastes et al. 1998). Also, the developmental stages and sexes that are predominantly transmitted depend on the trade-offs each stage experiences which, in turn, are linked to host's biology. For instance, adult females of the sucking louse *Haemodipsus setoni* are the stage predominantly found in young cottontail rabbits, *Sylvilagus floridanus* from Canada. Galloway (2012) suggested that fertilized females can readily establish founder populations in a habitat (the young rabbit) relatively free from competitors and with smaller pressures associated to grooming or immunity. In contrast, individuals of the chewing louse *Dennyus hirundinis* have been observed to pass from parent swifts onto nestlings mainly as nymphs, which apparently became adults and reproduced in synchrony with the feathering process in nestlings (Lee and Clayton 1995).

In this study, we document, for the first time, patterns of vertical transmission of an amphibious louse based on an extensive host sampling in a natural scenario. Lice infecting diving hosts are especially interesting because they likely experience adverse conditions for survival and transmission compared to their strictly terrestrial counterparts (Felső and Rózsa 2006, 2007). Our study focuses on a species of sucking lice of the family Echinophthiriidae, *Antarctophthirus microchir* (Trouessart and Neumann 1888). The Echinophthiriidae is the only family whose species have adapted to survive on pinnipeds (Durden and Musser 1994). Adaptation to marine conditions drove important changes in the morphology of these lice (Mehlhorn et al. 2002; Leonardi et al. 2012a). Echinophthiriids have stout spines that are apparently used to fix a layer of host's sebum that offers protection against low water temperatures (Mehlhorn et al. 2002), and most species also possess small scales that are postulated to function as a plastron that traps bubbles of air during immersions (Mehlhorn et al. 2002; Leonardi et al. 2012a). Marine conditions also imposed severe constrictions in the life cycle of many species because the egg of anoplurans cannot survive underwater for long periods, i.e., days (Murray 1976). In particular, the echinophthiriids infesting pinnipeds with a wettable pelage (sea lions, walruses, and true seals) have a strict dependence on land for reproduction, and the number of generations that they can produce per year is constrained by the duration of haul out periods of their hosts (Murray and Nicholls 1965; Murray et al. 1965; Aznar et al. 2009; Leonardi et al. 2012b).

We recently suggested that similar time constraints may operate upon the reproductive schedule of *A. microchir* on South American sea lions, *Otaria flavescens*, in Patagonia, Argentina (Fig. 1). Reproduction of this louse species is mostly dependent on sea lion pups because pups stay continuously on land for longer periods than any other age group (Aznar et al. 2009). In pups, *A. microchir* has a time window of just 1 month to reproduce because, afterwards, pups go to

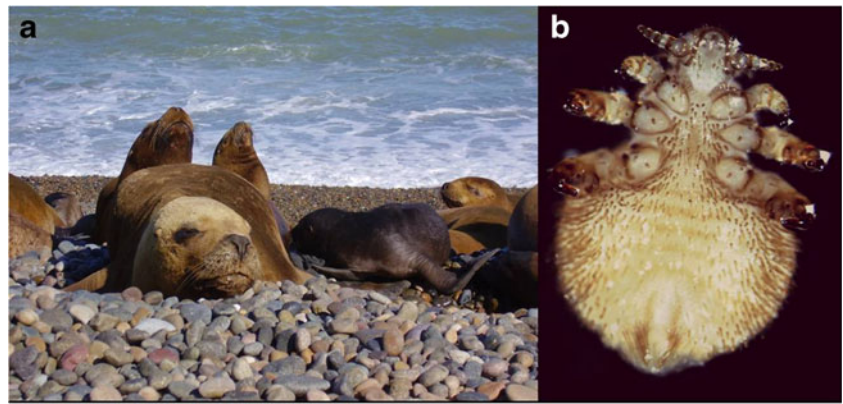
sea and start molting (Aznar et al. 2009). Stage-based deterministic models for population growth of *A. microchir* suggest generation times from 18 to 23 days, with a period of egg development of 7–9 days and a duration time of 3–4 days for each of the three nymphal stages, namely, N1, N2, and N3. Accordingly, only one to two lice generations can be produced per year (Aznar et al. 2009).

Transmission of echinophthiriid lice among pinnipeds is only possible during the periods of host's hauling out (Kim 1975; Leidenberger et al. 2007). Opportunities for lice to spread are affected by this behavior (Murray and Nicholls 1965; Murray et al. 1965; Kim 1972, 1975). In general, the major transmission of Arctic and Antarctic lice species occurs from cows to pups during nursing, since pups are infected few hours after birth (Kim 1972). Horizontal transmission among pups seems to be important in species where pups form close congregations (Kim 1972), whereas transmission among adults may play a minor role (Kim 1975).

Given this tight reproductive schedule and the strong dependence on pups for reproduction, vertical transmission during the first week of life becomes a fundamental process to understand population dynamics of *A. microchir*. Pregnant females of South American sea lion give birth to one pup 2–3 days after arrival to the breeding rookery. Mothers stay with their pups for 8–9 days and then go to forage offshore for 1–4 days; each foraging trip is followed by 2 days of nursing bouts on land (Campagna and Le Boeuf 1988; Werner and Campagna 1995; Cappozzo and Perrin 2009). Thus, there are many opportunities for cow-to-pup transmission, but the lice transmitted late to pups (i.e., after their first 8–9 days of life) would have less time for their eggs to be laid and hatch before the pups start going to the sea (see above).

Lice populations in pups  $\leq 7$  days old are made up only by recruitment from their mothers (Aznar et al. 2009; see above), and recruitment patterns could theoretically depend on three non-exclusive factors. First, population structure of lice in pups might grossly be transferred from that in their mothers (Kim 1975). This is a sort of “null” hypothesis: one would expect that certain instars are more represented in pups simply because they are more abundant on their mothers. Second, Takano-Lee et al. (2005) experimentally demonstrated that traveling speed, thus dispersal ability, of *P. humanus capitis* was minimal in nymph 1 and increased in more advanced instars. Apparently, transmission efficiency would increase as lice develop simply because of an improvement (maturation) of locomotor performance. If a similar phenomenon occurs in *A. microchir*, more adults than nymphs would be transferred from cows onto pups and more nymphs 2 and 3 than nymph 1. Third, natural selection should also favor the transmission of certain instars. In particular, adult lice would accrue more reproductive benefits than nymphs because they need shorter periods to produce offspring; early transmitted adults could accommodate even two generations within 1 month (Aznar

**Fig. 1** **a** South American sea lion (*O. flavescens*) cow nursing her newborn pup. **b** Photograph of the amphibious louse *A. microchir* from South American sea lion



et al. 2009). In addition, among adults, reproductive benefits would be more apparent for female lice because males have opportunities to inseminate females on the cow and, therefore, they would not need to pass to the pup. In summary, we can predict that adult lice should be transmitted to sea lion pups significantly more than nymphs and females more than males.

The specific goal of this study is therefore to describe the patterns of vertical transmission of *A. microchir* to pups of South American sea lion to shed light on the influence of the above three factors in determining transmission patterns. Ideally, this would require a comparison of population structure of *A. microchir* between mothers and pups. Unfortunately, this approach was considered unrealistic because adult females of South American sea lion weigh 100–150 kg (Cappozzo and Perrin 2009) and cannot be manipulated unless they are sedated, a procedure that is unadvisable during the reproductive period for ethical and logistic reasons. Therefore, sampling of *A. microchir* was restricted to pups. We investigated how the colonizing lice population in pups up to 1-week old was built over time and tested whether population structure conformed to the predictions made above, i.e., that pups should harbor more adults than nymphs and more females than males. Although we lacked information about the source population in the cows, the overall evidence was informative and allowed us to better understand this exceptional host–parasite system.

## Materials and methods

### Sampling design

This study was carried out during the South American sea lion breeding seasons 2009–2010 at the rookery of Punta León, Chubut Province, Argentina (43°04'S, 64°29'W). A total of 72 South American sea lion pups  $\leq 7$  days old were captured and examined for lice. The age of pups at the moment of capture was determined by examining the degree of umbilicus healing (see Aznar et al. 2009). For logistic reasons, the pups were captured randomly at the beginning

(the first week of January,  $n=21$ ), middle (after 14 January,  $n=33$ ), and end (during the last week of January,  $n=18$ ) of the reproductive season. The age of pups increased from the first to the third sample (Table 1) because the likelihood of finding newborn pups is lower as the breeding season goes on.

To collect the lice, each sea lion pup was restrained by two people and a third person (always the same to minimize biases) combed the pelage of the back, the chest, and the belly with fine-tooth combs of the type used for treating human pediculosis (see details in Aznar et al. 2009; Leonardi et al. 2012a). Combing stopped when no more lice were collected. Previous studies on population dynamics (Aznar et al. 2009) and habitat selection (Leonardi et al. 2012a) of *A. microchir* indicate that this combing procedure provides a reliable index of population structure and abundance of *A. microchir*. The mean manipulation time ( $\pm$ S.D.) per pup in a random sample of 29 pups was 4 min and 19 sec  $\pm$  36 sec. Manipulation included measuring, weighing, tagging, and combing the pup. Combing took about half of the whole manipulation time. Once pups were released, their mothers readily accepted and nursed them, and all manipulated pups survived during the study period. All collected lice from each individual pup, and the comb, were put in a Ziploc bag with 96 % (v/v) ethanol. Once in the laboratory, lice specimens were classified into nymph 1 (N1), nymph 2 (N2), nymph 3 (N3), and adult male (M) or adult female (F) stages following the morphological criteria given by Leonardi et al. (2009).

### Statistical analyses

We firstly examined whether sampling period (beginning, middle, and end of reproductive season) had significant effects on infection parameters and population structure of *A. microchir*. Prevalence was compared between sampling periods with Fisher's exact test and intensity with a Kruskal–Wallis test. Also, a permutational analysis of covariance (PERMANCOVA) based on a similarity matrix (Anderson et al. 2008) was used to investigate the effect of sampling period and pup age on the abundance of N1, N2, N3, M, and F specimens per pup. Uninfected pups were removed for this

**Table 1** Frequency of ages from 1 to 7 days old and mean age of samples of pups of South American sea lion, *Otaria flavescens*, collected at the beginning, the middle, and the end of the reproductive season at PuntaLeón rookery, Argentina in 2010 (see the text for details). Infection parameters for the louse *Antarctophthirus microchir* (with 95 % confidence intervals in parentheses) for each pup sample are also provided

Period	Number	Pup age (days)							Mean (SD)	Infection parameters	
		Frequency								Prevalence (95 % CI)	Mean intensity (95 % CI)
		1	2	3	4	5	6	7			
Beginning	21	13	3	1	2	2	–	–	1.9 (1.4)	85.7 (64.6–96.0)	4.4 (2.8–7.1)
Middle	33	11	5	6	4	6	–	1	2.8 (1.7)	75.8 (57.7–88.3)	9.0 (6.0–13.5)
End	18	4	3	2	3	4	1	1	3.4 (1.9)	83.3 (58.6–95.3)	20.1 (10.0–35.9)
Total	72	28	11	9	9	12	1	2	2.7 (1.7)	80.6 (69.6–88.4)	10.5 (7.3–15.7)

analysis. Sampling period was treated as a fixed factor, and pup age was used as a covariate. The main goal to include pup age was to test the interaction term “sampling period” × “pup age”; in this way, we could investigate whether the pattern of lice recruitment differed between sampling periods. The interaction term was not significant (see the “Results”) and was removed to increase the sensitivity of the analysis and to correctly interpret main effects (Engqvist 2005). To build the models, raw intensity data for each stage were firstly square root transformed, and then, a Bray–Curtis similarity matrix between infracommunities was obtained. Pseudo-*F* statistics under a true null hypothesis were obtained by using a permutation procedure, i.e., group labels were randomly shuffled onto different sample units, and this procedure was repeated 10,000 times, according to the procedure described by Anderson et al. (2008) in models including covariates. Our design was unbalanced and, therefore, we used a type III sum of squares to ensure a complete independence of all effects being tested (Anderson et al. 2008).

No significant effects of sampling period (beginning, middle, and end of reproductive season) on infection parameters and population structure of *A. microchir* were found (see the “Results”) and, therefore, we used the whole sample of pups to increase statistical power in the detection of patterns of vertical transmission. The overall pattern of recruitment in relation to pup age was examined with a one-way ANOVA with polynomial contrasts. The number of *A. microchir* per pup (including uninfected pups) was used as the dependent variable after a  $\log_{10}+1$  transformation. Age was considered as an ordered factor with five levels, i.e., ages 1 to 5; the latter also included three 6- and 7-day-old pups (Table 1). An examination of residuals indicated that the ANOVA model was satisfactory.

The remaining comparisons were carried out with nonparametric tests because the distribution of abundances of all developmental stages was strongly non-normal even after a  $\log_{10}+1$  transformation. The number of N1, N2, N3, and adults was compared with a Friedman test with pair-wise post hoc comparisons (Conover 1999). This test was repeated using only pups  $\leq 3$  days old to minimize changes of developmental

stages that may occur on the pup. Three days is the minimum estimated duration of nymphal stages of *A. microchir* (Aznar et al. 2009; see the “Introduction”). The number of adults vs. nymphs and adult females vs. adult males was compared with Wilcoxon tests. We also calculated the difference between the number of adults vs. nymphs, and females vs. males, and examined whether these differences changed monotonically with pups’ age using Spearman correlations.

The 95 % confidence interval (CI) for prevalence was calculated with Sterne’s exact method (Reiczigel 2003) and for mean values of intensity with the bias-corrected and accelerated bootstrap method using 20,000 replications (Rózsa et al. 2000).

The package Permanova+ for Primer v.6 (Clarke and Warwick 2001) was used to carry out the PERMANCOVAS and the free software Quantitative Parasitology v. 3 (Reiczigel and Rózsa 2005) to set 95 % CIs and the statistical package SPSS v. 19 for the remaining analyses. Statistical significance was set at  $p < 0.05$ . In multiple comparisons, *p* values were also corrected by the sequential Bonferroni procedure (Rice 1989).

## Results

Infection parameters of *A. microchir* collected from newborn pups at the beginning, the middle, and the end of the reproductive season are shown in Table 1. Neither the prevalence (Fisher’s test,  $p = 0.743$ ) nor the median intensity (Kruskal–Wallis test,  $\chi^2 = 4.642$ , 2 *df*,  $p = 0.098$ ) of *A. microchir* differed among sampling periods. Note, however, that the age of sampled pups increased from the beginning to the end of the season (Table 1); indeed, there was a significant difference in the median age of pups among sampling periods (K–W test,  $\chi^2 = 9.733$ , 2 *df*,  $p = 0.008$ ). As noted in “Material and methods,” this heterogeneity was caused by the increasing difficulty to finding 1-day-old pups as the reproductive season went on. The increase of age of pups was paralleled by an increase of mean intensities of *A. microchir* (Table 1).

PERMANCOVA models that investigate changes in population structure of *A. microchir* between sampling periods are shown in Table 2. In the full model, pup age was a highly significant predictor of changes in population structure, but neither sampling period nor the interaction “sampling period  $\times$  pup age” was. No significant changes in population structure were detected in simpler models involving sampling period as a predictor (Table 2). Therefore, we assumed that sampling period did not influence population structure and pooled data for subsequent analyses.

Seventeen out of 28 one-day-old pups were already infected with *A. microchir* (prevalence (95 % CI), 60.7 % (40.9–77.1)). The mean intensity (95 % CI) in these 17 pups was just 2.2 (1.5–3.6) lice per pup. The abundance of *A. microchir* increased with pup age (Fig. 2). However, both the linear ( $F_{(1, 67)}=36.831$ ,  $p<0.0001$ ) and the quadratic ( $F_{(1, 67)}=11.980$ ,  $p=0.001$ ) terms of polynomial contrasts were highly significant, indicating that recruitment increased in pups up to 3 days old but then leveled off (Fig. 2).

When nymphs were considered as a single group, their number was higher than that of adults in the overall sample of infected pups (Fig. 3). Overall, this difference was significant (Wilcoxon test,  $Z=-1.999$ ,  $n=58$ ,  $p=0.045$ ), but it was not consistent among pups of different age. In 1-day-old pups, more adults than nymphs were found (Fig. 3), and the difference was highly significant (Wilcoxon test,  $Z=2.675$ ,  $n=17$ ,  $p=0.007$ ). Also, the difference between nymphs and adults increased significantly with age (Spearman correlation,  $r=0.457$ ,  $n=58$ ,  $p<0.001$ ; Fig. 3).

There were significant differences in the number of N1, N2, N3, and adults of *A. microchir* in infected pups (Friedman

test,  $\chi^2=43.601$ , 3 *df*,  $n=58$ ,  $p<0.001$ ). The post hoc comparison revealed significant differences ( $p<0.05$ ) between adults and the other stages and between N2 and N3 vs. N1. The same result was obtained when only infected pups  $\leq 3$  days old ( $n=36$ ) were considered. The pattern of recruitment with age of each developmental stage in pups  $\leq 3$  days old indicated that (a) the differences between stages were generally conserved, and (b) the pattern of recruitment was similar in all stages except for N1 specimens, which were recruited in lesser amount (Fig. 4).

In pups harboring adult lice, the number of females was significantly higher than that of males (Wilcoxon test,  $Z=2.076$ ,  $n=52$ ,  $p=0.035$ ) (Fig. 5). This difference was not consistent in pups of different ages (Fig. 5), but was clearly conserved in 1-day-old pups (Wilcoxon test,  $Z=2.639$ ,  $n=16$ ,  $p=0.008$ ). The difference between the number of females and males did not change monotonically with the age of the pup (Spearman correlation,  $r=-0.039$ ,  $n=52$ ,  $p=0.785$ ; Fig. 5).

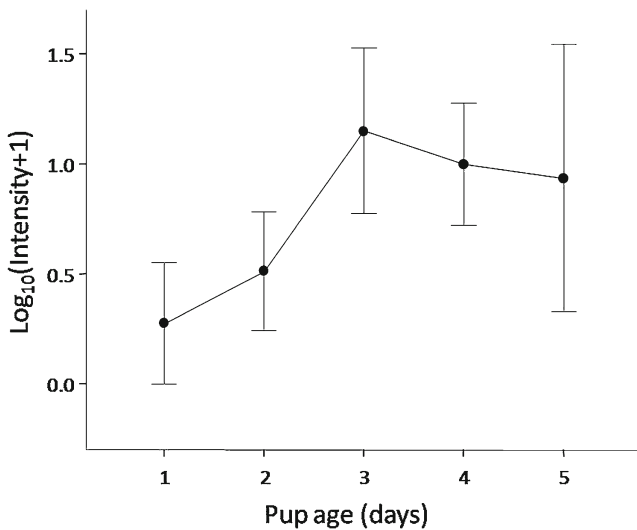
## Discussion

Previous studies on echinophthiriids that reported information about transmission patterns dealt with a reduced number of free-living, captive, or restrained adult seals, and/or restrained pups, that could be examined for long periods, i.e., 45–60 min (Murray and Nicholls 1965; Murray et al. 1965; Kim 1972, 1975). Our study benefits from data obtained from a much larger host sample size studied under natural conditions, but the handling time of pups of South American sea lion had to be very short ( $<5$  min), which precluded any attempt to collect whole louse populations. Partial censuses of lice populations are now commonly used in studies dealing with large or delicate hosts, but have still allowed making valid inferences about population structure and transmission dynamics in many host–lice systems (see, e.g., Thompson et al. 1998 for lice from seals, and Darolova et al. 2001 for lice from bee-eaters). In the case of sea lion pups, combing of pelage has proved to be a very useful method to study a variety of ecological aspects about *A. microchir*, including population dynamics and microhabitat selection (Aznar et al. 2009; Leonardi et al. 2012b). In a recent paper, Gallardo et al. (2013) reported differences of commercial combs in removing different instars of *P. humanus capitis*. However, we consider that combing was reliable to address the goals of the present study because inter-teeth width of combs was narrow enough (300  $\mu\text{m}$ ) to minimize differential sampling of developmental stages (mean length and width of the smallest nymphal instar are 980 and 450  $\mu\text{m}$ , respectively; see Leonardi et al. 2009).

Host density is known to positively affect transmission rate of directly transmitted parasites (see Anderson and May 1978; May and Anderson 1978; Arneberg et al. 1998). In the

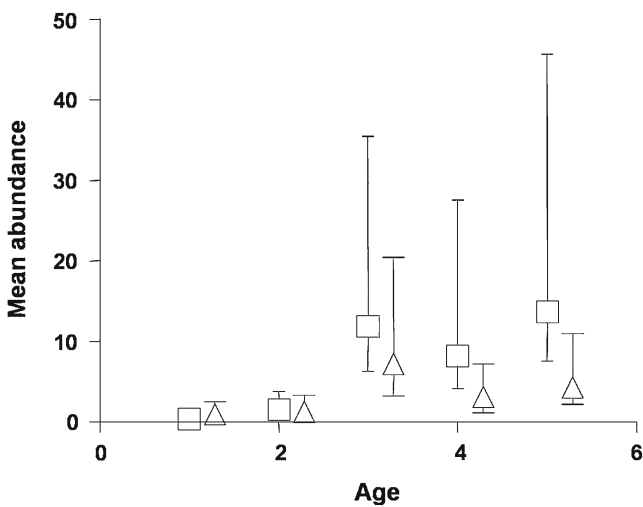
**Table 2** Alternative models of permutational multivariate analysis of covariance (PERMANCOVA) that compare the effect of sampling period and age of pups of South American sea lion, *Otaria flavescens*, on the number of nymph stages 1, 2, and 3 and adult females and males of the louse *Antarctophthirus microchir*

Source of variation	Degrees of freedom	Sum of squares	Pseudo- <i>F</i>	<i>p</i> value
<b>Model 1</b>				
SP	2	1,087.9	0.276	0.9376
Age	1	13,547	6.880	0.0003
SP $\times$ age	2	1,970.4	0.500	0.8091
Residual	52	102,398.4		
<b>Model 2</b>				
SP	2	1,076.8	0.279	0.0002
Age	1	13,364	6.915	0.9356
Residual	54			
<b>Model 3</b>				
SP	2	4,105.6	0.959	0.4553
Residual	55	117,727.5		

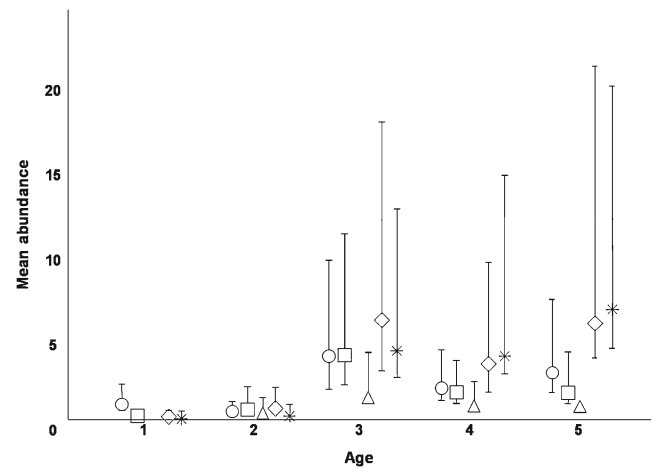


**Fig. 2** Log10-transformed values of abundance of the louse *A. microchir* in 1- to 5-day-old pups of the South American sea lion, *O. flavescens*. Vertical bars are the 95 % confidence intervals

case of lice, this factor has been shown to operate at several spatial scales. For instance, Rukke et al. (2011) found that, in Norway, the prevalence of head lice increased significantly in more densely populated municipalities. At a smaller scale, Hoi et al. (1998) reported an increase of the infestation rate of *Meropoeus meropis* in bee-eaters as colony size increased. The factor more frequently invoked to account for this pattern is that physical contacts between individuals increase in denser populations. In this context, the size of rookeries of South American sea lion can increase by tenfold during the reproductive period (Campagna 1985; Campagna and Le Boeuf 1988) and, therefore, one could expect a potential

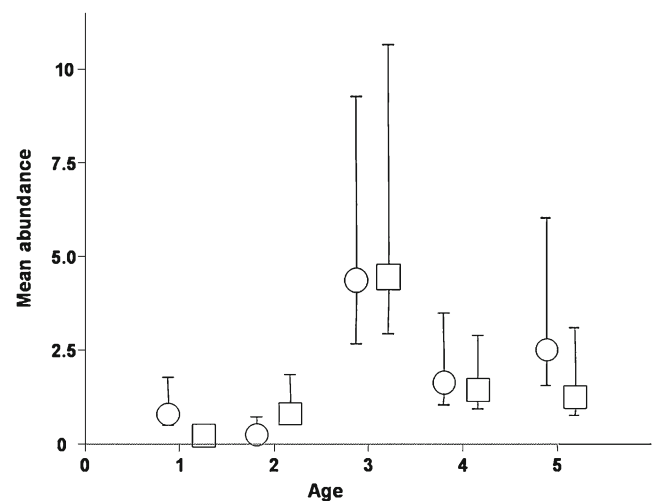


**Fig. 3** Mean abundance of adults and nymphs of *A. microchir* in 1- to 5-day-old pups of the South American sea lion, *O. flavescens*. Triangle: adults, square: nymphs (N1 to N3). Vertical bars are the 95 % confidence intervals



**Fig. 4** Mean abundance of the louse *A. microchir* in 1- to 5-day-old pups of the South American sea lion, *O. flavescens*. Vertical bars are the 95 % confidence intervals. Circle: females, square: males, triangle: nymph 1, rhomb: nymph 2, asterisk: nymph 3. Vertical bars are the 95 % confidence intervals

increase of opportunities for horizontal transmission of *A. microchir* through physical contacts between individuals in the rookery throughout the reproductive season. However, our results indicate that this potential process has no significant effect on patterns of early vertical transmission. Pups  $\leq 7$  days old that were born at different moments during the reproductive season had lice populations with similar size and structure. This could be explained, at least in part, by the fact that young pups spend most of the time in close contact with its mother and their mobility is the lowest of all the nursing period (Rivas and Trimbles 2009), thus reducing the opportunities of contacts with other individuals in the rookery.



**Fig. 5** Mean abundance of adults of *A. microchir* in 1- to 5-day-old pups of the South American sea lion, *O. flavescens*. Triangle: females, square: males. Vertical bars are the 95 % confidence intervals

Our results indicate that cow-to-pup transmission is fast; over 60 % of 1-day-old pups were already infected with *A. microchir*. It is important to stress that this figure is clearly conservative because we did not carry out a complete census of lice populations. Under controlled conditions, Kim (1972, 1975) reported infestation with *Antarctophthirus callorhini* in pups of northern fur seal, *Callorhinus ursinus*, as early as 7 h after parturition (i.e., soon after the coat had dried up). In any event, recruitment of *A. microchir* to pups  $\leq 7$  days old is best described by a curvilinear trend, with the maximum increase occurring within the first 3 days. Surprisingly, there are very few studies that actually measure vertical transmission of terrestrial lice for comparison. Studies on lice from altricial birds are not comparable because infestations are usually delayed until feathers emerge in nestlings (Clayton and Tompkins 1994; Lee and Clayton 1995). We are aware of a single study dealing with transmission rate from a louse species from mammals. Using artificial human “heads” connected by a hair bridge, Takano-Lee et al. (2005) measured the transmission of head lice from infested to uninfested heads. Even at low lice densities ( $n \leq 30$ ), inter-head movement was detected as early as 5 min and was evident 1 h after the heads were connected. This would suggest that the rate of vertical transmission observed in *A. microchir* and other echinophthiriids is not particularly fast.

Not all instars or sexes were equally represented in the populations of *A. microchir* from pups  $\leq 7$  days old, which are made up only by recruitment (Aznar et al. 2009). As noted in the “Introduction,” three non-exclusive hypotheses could account for the observed patterns of vertical transmission, i.e., recruitment merely reflects that the population structure of *A. microchir* is cows (hereafter referred to hypothesis 1 or H1); the relative ability of louse individuals to pass from cows onto pups increases in advanced instars (H2); and natural selection favors transmission of adults, especially females, because they accrue greater fitness (H3). In what follows, we discuss patterns of recruitment in the light of these hypotheses.

The number of nymphs 1 was significantly lower than that of any other instar, particularly as pups got older. This pattern is compatible with H2 and H3, i.e., nymph 1 possibly has lower dispersal ability because of relative lack of maturation in crawling ability (see the experiments by Takano-Lee et al. 2005 for the case of head lice) and the lowest reproductive value (thus, they experience less selective pressures to infect pups). Nevertheless, nymphs could also be comparatively scarce in cows (H1). Pregnant cows arrive to the beach after a long feeding trip and, therefore, they should not carry viable eggs of *A. microchir* from which nymph 1 could be recruited (the minimum incubation time of newly laid eggs is ca. 9 days; see Aznar et al. 2009); many nymph 1 could also have molted at sea to nymph 2. Furthermore, it has been speculated that nymph 1 has a higher dependence on aerial

oxygen to breathe; thus, they would be negatively affected by immersions (Aznar et al. 2009; Leonardi et al. 2009, 2012a, 2012b). Accordingly, pregnant cows should harbor mainly nymphs 2 and 3 and adult lice upon arrival to the beach. A similar scarcity or even absence of nymph 1 of other echinophthiriid species has also been reported on pups from northern fur seal and southern elephant seal (Murray and Nicholls 1965; Murray et al. 1965; Kim 1972, 1975), strongly suggesting that at least some of these processes consistently operate also in related host–louse systems.

We found a significantly higher abundance of nymphs vs. adults, and the difference increased in older pups; 1-day-old pups, however, had significantly more adults than nymphs. This finding is particularly interesting because other studies that compare population structure of lice harbored by parents and their offspring usually pool data from several ages in the latter (see, e.g., Rust 1974; Galloway 2012), thus potentially masking subtle temporal changes in the transmission process. In other echinophthiriid species, reports of patterns of vertical transmission suffer from this problem and offer contrasting evidence: *Antarctophthirus ogmorhini* in Weddell seal pups (Murray et al. 1965), *Lepidophthirus macrorhini* in southern elephant seals (Murray and Nicholls 1965), and *Proechinophthirus fluctus* in northern fur seal (Kim 1972, 1975) appear to be transferred mainly as adults, but *A. callorhini* from the northern fur seal seems to be transferred mainly as nymph 2 (Kim 1972, 1975). None of the above hypotheses (H1–H3) can, by itself, account for the overall pattern found in *A. microchir*. However, it seems plausible that adult lice, having higher dispersal ability than the nymphs (according to H1 and/or H2), would firstly appear in pups, with nymphs soon outnumbering adults in older pups because their pool in mothers is higher. Note that the later hypothesis only assumes that probability of parent-to-offspring transmission of each developmental stage is positively influenced by its density in mothers.

Finally, we observed the higher overall abundance of females vs. males, which is compatible with H1 and H3; H2 makes no specific prediction in this respect. With regard to H1, in a comprehensive study of adult sex ratios in parasitic insects, Marshall (1981) found that 62 % of 359 populations examined were female-biased, 7 % were male-biased, and 30 % did not significantly depart from unity. It is important to remind that sex ratios of lice are prone to sampling error, particularly when samples are small (Szczykutowicz et al. 2006). Apparently, adult females have generally a longer life span than males in many parasitic insects (Marshall 1981), and this may explain why most lice populations are female-biased. Data from other echinophthiriid species offer contrasting evidence regarding sex ratios. In adult hosts, the sex ratio did not depart from unity in *A. callorhini* but was slightly female-biased in *P. fluctus* (Kim 1972). In the case of *A. microchir*, it is interesting to note that differences between

males and females were uneven among pups of different age, with pups 1 and 4 days old having the highest difference between female and male lice. Apparently then, females would infect pups more than males right from the beginning, which conforms particularly to H3. Note, however, that an important requisite of this hypothesis is that female lice are able to recognize pups as hosts. This does not seem to be unrealistic for an ectoparasitic arthropod. For instance, Christe et al. (2007) experimentally demonstrated that a mite species from bats was able to discriminate between male and female hosts and preferentially infested females because they were the most profitable sex (see also Khokhlova et al. 2011).

In summary, young pups ( $\leq 7$  days old) of South American sea lion acquire most, if not all, individuals of *A. microchir* from their mothers, and our study suggests that this process seems to be unaffected by the moment when pups are born in the reproductive colony. In addition, patterns of vertical transmission are compatible with the three hypotheses, but evidence suggests that the importance of selective forces in shaping transmission strategies in a species with a tight reproductive schedule should not be underestimated. The long-term stability of populations of *A. microchir* depends on vertical transmission, and lice have a very small window of time to reproduce on pups (Aznar et al. 2009). Therefore, instars with a putatively higher reproductive value should be transferred to pups in higher amounts. This conclusion agrees with the limited evidence obtained from other host–louse systems that are particularly amenable to measure vertical transmission (typically birds and their chewing lice), which confirm the adaptive schedules of lice according to the ecology of their hosts (see, e.g., Lee and Clayton 1995; Darolova et al. 2001; de Brooke 2010). Nevertheless, to shed more light on the transmission of *A. microchir* to sea lion pups, data about population structure of lice in pregnant cows are fundamental. Thus, every single fresh carcass of adult sea lion that is available in the future will be invaluable in this respect (Aznar et al. 2010).

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