

Population dynamics of *Antarctophthirus microchir* (Anoplura: Echinophthiriidae) in pups from South American sea lion, *Otaria flavescens*, in Northern Patagonia

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SUMMARY

We analysed population dynamics of the louse *Antarctophthirus microchir* in pups of the South American sea lion, *Otaria flavescens*, at the Punta León rookery (Argentina) over a period of 2 years. A total of 136 pups were aged and marked at the beginning of the lactation period ashore, then sampled for lice at different times within 30 days. Sampling was restricted to the chest and belly, two sites where lice were especially abundant. This concentration on ventral areas might protect lice from thermal stress in the austral summer. Infestation patterns in pups ≤ 3 days old suggested that the potential for transmission increased from first nymphs to adults. Population trends of each instar with pup age, based on standardised values of abundance, were conserved between years, reflecting the basic dynamics of recruitment and reproduction. However, trends based on log-transformed abundances varied significantly between years; apparently, environmental conditions affected growth of lice populations differently each year. Stage-based deterministic models for population growth of *A. microchir* suggested generation times from 18 to 23 days. Accordingly, only 2 lice generations might be produced before pups start going to the sea. Shortening the cycle to accommodate a third generation might be risky, whereas a 2-generation cycle might at least result in larger females producing higher numbers of viable offspring.

Key words: Anoplura, Echinophthiriidae, life cycle, trade-off, South American sea lion.

INTRODUCTION

Sucking lice of the family Echinophthiriidae represent an integral element of the parasite fauna of pinnipeds, with records worldwide in species from all pinniped genera (Murray, 1976). Echinophthiriids are peculiar among the Anoplura by their morphological, physiological, behavioural and/or ecological adaptations to the amphibious life of their hosts (Kim, 1971; Murray, 1976; Mehlhorn *et al.* 2002), being among the few insects that have been able to adapt to the marine environment (Leidenberger *et al.* 2007).

Studies on ecology and life cycles of echinophthiriids flourished in the decades 1960–70. Some studies focused on lice from 2 phocid species, i.e., *Lepidophthirus macrorhini* on the Southern elephant seal, *Mirounga leonina* (Murray, 1958, 1964, 1967; Murray and Nicholls, 1965) and *Antarctophthirus*

ogmorhini on the Weddell seal, *Leptonychotes weddelli* (Murray, 1964; Murray *et al.* 1965). Other studies focused on 2 lice species, i.e., *Antarctophthirus callorhini* and *Proechinophthirus fluctus*, infesting the Northern fur seal, *Callorhinus ursinus* (Otaridae) (Kim, 1971, 1972, 1975). One of the major findings of these studies was that the life cycle of echinophthiriids adjusts precisely to that of their hosts because reproduction, and possibly transmission, of lice can only occur when hosts are on land. As a consequence, the number of lice generations per year is constrained by the duration of haul out periods of their hosts.

Considering the methodological problems inherent to working with pinnipeds, the above studies are of exceptional value because they combined a diverse array of analyses including field counts of lice, *in vitro* observations, experimental infections and even the follow-up of natural infections in confined hosts (see references above). However, a missing element of all these studies was the lack of replicated data about the actual population dynamics. Field counts of lice on single occasions can convey only a static picture of population dynamics, and the follow-up of infections on single, or few

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caged or restrained hosts (Murray and Nicholls, 1965; Kim, 1975) may have a limited value to infer population patterns.

In this paper we investigate, for the first time, the population dynamics of an echinophthiriid species based on replicated sampling of its hosts under natural conditions. Pups of the South American sea lion, *Otaria flavescens*, from a rookery in Patagonia (Argentina), were periodically sampled for the louse *Antarctophthirus microchir* throughout the lactation period ashore over 2 consecutive years. The main methodological advantages of this approach were that individual hosts were uninfested at the beginning of the study, the age of infestations was known, and host disturbance was kept at a minimum. Also, population inferences could be based on statistical analysis with replication. Pups were chosen because they are a key population group for lice reproduction, similarly as in other echinophthiriids (Murray and Nichols, 1965; Murray *et al.* 1965; Kim, 1975).

The population dynamics of *A. microchir* in sea lions from Patagonia is also interesting for ecological reasons. The cycle develops in a temperate region (20 °C on average in the austral summer, see Yorio *et al.* 1995, and references therein), unlike the other species of echinophthiriid thus far investigated (the cycle develops under a range of temperatures from -2 °C to 10 °C, see Murray and Nichols, 1965; Murray *et al.* 1965; Kim, 1975). Temperature is a key determinant for the survival and reproduction of anoplurans, and for the length of egg incubation time (Murray, 1987). In fact, echinophthiriids from cold regions have developed ecological strategies and physiological adaptations to withstand low environmental temperatures (Murray, 1976). On the other hand, the period in which *A. microchir* can reproduce on pups is short (pups are about 30 days continuously on land; see below). This scenario resembles that of *A. ogmorhini* on the Weddell seal and *L. macrorhini* on Southern elephant seals, but contrasts with that of *A. callorhini* and *P. fluctus* on Northern fur seals (over 10 weeks on land) (see Murray and Nichols, 1965; Murray *et al.* 1965; Kim, 1975). Could this combination of a temperate climate and a short period for reproduction lead to shorter generation times in *A. microchir* compared with other echinophthiriids?

This study addresses 2 specific goals. First, we describe the population dynamics of *A. microchir* identifying key features that influence transmission patterns and changes in population structure with pup age. Second, we develop simple models of population growth and compare them with empirical data to investigate the duration of the life cycle and, therefore, the number of land generations of *A. microchir*. These results are discussed in relation to the selective pressures that may act upon the reproductive schedule of this species.

Reproductive biology of the South American sea lion

The South American sea lion is distributed along the Atlantic and Pacific coasts of South America, from 29°S in Brazil to 4°S in Peru; in Argentina, more than 100 colonies have been recorded (Dans *et al.* 2004). The main events of the reproductive biology of *O. flavescens* in Argentina have been summarized by Cappozzo (2002). Adult males and females arrive at the breeding rookeries during the first half of December. Pregnant females give birth 2–3 days after arrival, and copulation occurs on land 6 days after parturition. Mothers stay with their pups for 2–3 days more and then go to forage offshore for 1–4 days; each foraging trip is followed by 2 days of nursing bouts on land. When pups are alone, they tend to stay in close congregations whose size increases as the reproductive season goes on. At about 30 days of age, pups begin to moult the pelage and go to sea for the first time (Campagna, 1985). Lactation continues for 8–10 months.

MATERIALS AND METHODS

Data collection

This study was carried out throughout 2 consecutive years (2007–2008) in the sea lion rookery of Punta León, Chubut Province, Argentina (63°03'S, 47°43'W). The reproductive season on land begins by mid-December and ends at the beginning of February. In both years, the study began the 3rd of January (approximately 1 week after the first births were observed), and ended the 2nd of February.

Within the first 3 days of study, pups were randomly selected, captured with a noose pole and individually marked with a bleaching solution. The total numbers of pups marked in 2007 and 2008 were 73 (39 male, 34 female) and 63 (35 male, 28 female), respectively. Marked pups were then randomly selected for lice sampling within days 1st–6th (20 pups in 2007 and 20 in 2008), 19th–20th (20 and 21 pups), and 27th–30th (33 and 22 pups) after marking. Initial age of marked pups was determined by examining the degree of umbilicus healing (E.A. Crespo, manuscript in preparation). Based on this method, the age (in days) can confidently be determined in pups from 1 to 3 days old; however, in older pups up to 8 days old, errors of up to 2 days may occur in age determination. For instance, a pup considered as being 4 days old could actually be up to 6 days old, or vice versa. The range of initial age for pups of 2007 and 2008 were 1–8 and 1–7 days, respectively; mean values did not significantly differ between the years (2 ± 2 days in both cases; *t*-test with log-transformed values, $P > 0.05$). In both years, over 70.0% of the pups were ≤ 3 days old.

In a preliminary survey of lice during 2 previous years, we had examined the naked parts of the body of approximately 80 pups to collect lice, based on

information about habitat selection from other species of *Antarctophthirus* (see Murray *et al.* 1965; Kim, 1975). However, lice had rarely been found on these locations, but were particularly abundant on the chest and belly. Since pup manipulation had to be very quick (about 3 min) to minimize stress, we sampled only these sites in 2007 and 2008. To collect the lice, each pup was restrained by 2 people and a third person (always the same to minimize biases) combed the pelage with fine-tooth combs of the type used for treating human pediculosis. Inter-teeth width was narrow enough (300 μm) to avoid differential sampling of instars (the mean length \times width of the smallest instar was 980 \times 450 μm , see below). Combing stopped when no more lice were collected. All collected lice from each individual pup, and the comb, were put in a Ziploc[®] bag with 96% (v/v) ethanol. Eggs were not considered in further analyses. Mothers of manipulated pups readily accepted and nursed them, and all manipulated pups survived the study period.

Lice were classified into nymphal stages and male and female adults following Ferris (1934, 1951) and Kim (1987). Adults are distinguished from nymphs by the presence of sexual characters: females (average length \times maximum width: 2780 \times 1640 μm , $n=20$) have a group of setae surrounding the genital opening, and males (2480 \times 1260 μm , $n=20$) have pseudopenis. Nymphs 1 (hereafter referred to as N1, 980 \times 450 μm , $n=18$) are differentiated from other nymph stages by smaller size, absence of scales and the absence of ventral spines or hairs on thorax. Nymphs 2 (N2, 1510 \times 830 μm , $n=32$) and 3 (N3, 1870 \times 1070 μm , $n=22$) are distinguished by size and by arrangement of occipital apophyses (parallel in N2 and converging at apex in N3) (S. Leonardi, manuscript in preparation).

Infestation patterns

Five pups from 2007, but none of 2008, were > 30 days old at the time of lice sampling. These pups were excluded in comparisons between years because they start going to the sea and this may strongly affect lice populations (see the Discussion section).

Infestation parameters were estimated following Bush *et al.* (1997) and Rószka *et al.* (2000). Sterne's exact 95% confidence interval (CI) was calculated for prevalence (Reiczigel, 2003) and overall sex ratio (no. males/total population). Bootstrap 95% CIs using 10 000 replications were estimated for mean intensity and mean abundance (Rószka *et al.* 2000). Prevalence and sex ratio were compared between years with Fisher's exact tests, and population structure with a non-parametric MANOVA based on the Bray-Curtis similarity index (Anderson, 2001). Significance of the F value was computed by permutation of group membership with 10 000 replicates. Inter-year comparisons of mean abundance of specific instars

were carried out with bootstrap-based *t*-tests (Rószka *et al.* 2000).

To investigate transmission patterns, we selected pups 1–3 days old in which infestations were assumed to have originated only by transmission. Prevalence, mean abundance and population structure were compared between years as described above. In addition, we tested, for each year separately, whether there was a significant trend of increase in the abundance from less developed (N1) to most developed (adult) instars using Page's tests (Conover, 1999).

In infested pups, changes of log₁₀-transformed values of the number of N1, N2, N3 and adults of *A. microchir* with pup age were described, for each year, using robust local nonparametric regression models (LOESS). This procedure is suitable to discern systematic structure when variables exhibit complex nonlinear relationships (see Cleveland and Devlin, 1988; Jacoby, 2000, for details). The degree of polynomial and the choice of an optimal fraction of data for each local fitting (the bandwidth) are the most critical factors to balance the risk of under- and overfitting in LOESS (Loader, 1999; Schucany, 2004). To select the most suitable combination of these parameters we examined (i) cross-validation plots (Loader, 1999); (ii) residual plots after varying the degree of the polynomial (1 and 2) and the bandwidth (0.4 to 0.8) (Loader, 1999; Jacoby, 2000), and (iii) stability of trends after constructing confidence bands using 1000 bootstrapped regression curves for each combination of parameters (Efron, 2005). In all cases, the best fitting was obtained with linear regression (polynomial degree=1) and a bandwidth=0.5. For each selected LOESS curves we set point-wise 95% confidence intervals following Loader (1999).

We compared LOESS regressions between years based on an approximate F test:

$$F = \frac{(\text{RSS}_{\text{combined}} - \text{RSS}_{\text{separate}}) / (\text{df}_{\text{combined}} - \text{df}_{\text{separate}})}{\text{RSS}_{\text{separate}} / \text{df}_{\text{separate}}}$$

where $\text{RSS}_{\text{combined}}$ and $\text{df}_{\text{combined}}$ are, respectively, the residual sum of squares and the equivalent degrees of freedom for the LOESS fit using pooled data from both years; and $\text{RSS}_{\text{separate}}$ and $\text{df}_{\text{separate}}$ are, respectively, the summations of the residual sum of squares, and equivalent degrees of freedom for the LOESS regressions calculated separately for each year (see Cleveland and Devlin, 1988; Loader, 1999 for details). The degrees of freedom associated to this F test are $\text{df}_{\text{combined}} - \text{df}_{\text{separate}}$ (numerator) and $\text{df}_{\text{separate}}$ (denominator).

Population projection models

The most likely range of generation times (egg to egg) of *A. microchir* was investigated by developing

Table 1. Life-history data from species of the Echinophthiriidae and other Anoplura

(Duration is expressed in days, and fecundity as eggs/day. Daily probability of survival was calculated from experimental reports of the number of survivors in a sample after a known period. For instance, Murray and Nichols (1965) found that, from an initial sample of 70 N1s + N2s, 20 had become adults after 6 days. Accordingly, the combined daily probability of survival for N1 and N2 was 0.815 since $70 \times 0.815^6 \cong 20$.)

Taxon	Duration of stage [Daily probability of survival]					Fecundity [Preov. period]	Generation time
	Egg	N1	N2	N3	Adult		
Echinophthiriidae*							
<i>Lepidophthirus macrorhini</i>	5–10 [0.985–0.990]	3–4 [?]	3–4 [0.815]	3–4 [0.815]	> 28 [0.975]	6.5–8.6 [?]	21
<i>Antarctophthirus callorhini</i> †	7 [?]	2–3 [?]	4 [?]	4 [?]	? [?]	8–9 [1]	18–20
<i>Antarctophthirus ogmorhini</i>	> 7 [?]	? [?]	? [?]	? [?]	> 28 [?]	6–10 [?]	21–28
Other Anoplura	5–25 [0.926–0.983]	3–9 [0.856–0.993]	3–7 [0.856–0.993]	3–10 [0.856–0.993]	10–45 [0.983]	1–9	13–43

* Sources of data: *L. macrorhini*: Murray and Nicholls (1965); *A. callorhini*: Kim (1972, 1975); *A. ogmorhini* (Murray *et al.* 1965). Information about the duration of stages, fecundity and life cycles of other Anoplura were obtained from Evans and Smith (1952) for *Pediculus humanus*; Murray (1961) for *Polyplax serrata*, and the reviews by Kim (1987) and Price and Graham (1997), which included relevant information for 5 species of *Haematopinus*, 6 of *Linognathus*, 1 of *Solenopotes* and 1 of *Pthirus*. Information from daily survival rates was complete only for *Pediculus humanus* (Evans and Smith, 1952); partial data for specific stages were obtained from *P. serrata* (Murray, 1961); *Haematopinus tuberculata* (Chaudhuri and Kumar, 1961); *H. suis* (Florence, 1921), *H. eurysternus* (Craufurd-Benson, 1941) and *Linognathus pedalis* (Scott, 1950). Information on survival data are more abundant from chewing lice, and values are in the range considered here (see references in Price and Graham, 1997).

† Duration of stages is assumed to be similar in *Proechinophthirus fluctus* (Kim, 1975).

deterministic matrix models of population growth based on life tables that assumed a reasonably realistic set of values (see Murray and Gordon, 1969, for a similar procedure). This probably is the best (and arguably the only) option when many life-history data are missing and difficult to obtain, which is the case for the vast majority of species of Anoplura (see Kim, 1987).

To perform the analysis we focused on the subset of pups ≤ 15 days old because, in this host group, the regression between \log_{10} -transformed population size of lice and pup age was linear, so were approximately the regressions theoretically obtained from models. Therefore, a comparison of slopes was feasible and served to ascertain possible generation times: shorter generation times should result in steeper slopes and vice versa.

Life-history data (partial, except for *Pediculus humanus*) were available from 18 anopluran species, including 3 echinophthiriids and 15 species from other families (Table 1). A set of potential life tables for *A. microchir* was constructed as follows. Concerning the duration of stages, we selected a range of values for the egg (5–10 days), N1 (2–3 days), N2 (3–4 days) N3 (3–4 days), pre-ovoposition period (1 day) and adult (>15 days) based on information from other echinophthiriids (Table 1). It is important to note that combining these data we could construct generation times from 14 days (which allows accommodating 3 lice generations on pups and is

close to the shortest value known for an anopluran, see Murray, 1987) to 23 days (which allows accommodating only 2 generations and is relatively close to the time limit for *A. microchir* to reproduce on pups).

We obtained 30 types of models by combining duration values of non-adult stages (see Fig. 3). Next, for each type of model, we considered 5 realistic values of daily survival rate (0.80, 0.85, 0.90 and 0.95 and 0.99) for each stage, and 5 fecundity values (6, 7, 8, 9 and 10 eggs), based on information from echinophthiriids and other anoplurans (Table 1). Accordingly, there were $5 \times 5 \times 5 \times 5 \times 5 = 3125$ possibilities of combining these values, of which we randomly selected 100 to construct life tables. Since there were 30 types of models of stage duration, we eventually produce a total of $30 \times 100 = 3000$ individual life tables.

For each life table, the theoretical population growth during the first 15 days was calculated by assuming a constant daily rate of mother-to-pup transmission of 2 N1s, 6 N2s, 6 N3s and 7 adults during the first 10 days; this proportion was selected based on overall infestation data from pups ≤ 3 days old (see the Results section). Population data were then \log_{10} -transformed to achieve linearity with pup age, and the slope of minimum-squares regression was calculated. Average slopes for each of the 30 combinations of generation time were calculated based on the 100 replicas, and 95% confidence

Table 2. Infection parameters of *Antarctophthirus microchir* collected from the chest and belly of pups of *Otaria flavescens* from Patagonia, Argentina

(Data from 2007 (upper row, $n=68$ pups*) and 2008 (lower row, $n=63$). N1–N3: Nymphs 1–3. CI: Confidence Interval.)

Instar	Prevalence (%) (95% CI)	Intensity		Mean abundance (95% CI)
		Mean (95% CI)	Median	
N1	69.1 (57.2–79.3)	22.9 (16.1–32.1)	11.0	15.8 (10.8–22.9)
	69.8 (57.2–80.4)	11.6 (7.4–19.6)	5.0	8.1 (5.0–14.1)
N2	79.4 (67.8–87.7)	30.6 (22.7–42.6)	18.5	24.3 (17.7–35.4)
	85.7 (74.8–92.6)	30.3 (19.8–51.0)	12.0	26.0 (16.4–44.4)
N3	77.9 (66.3–86.4)	15.8 (11.5–23.7)	9.0	12.3 (8.7–18.5)
	87.3 (76.4–94.0)	15.4 (11.1–23.2)	11.0	13.4 (9.6–21.2)
Adult	77.9 (66.3–86.4)	7.4 (5.8–9.7)	5.0	5.8 (4.4–7.7)
	93.7 (84.4–97.8)	10.3 (7.8–14.6)	6.0	9.7 (7.2–13.5)
Total	86.8 (76.6–93.1)	67.1 (51.0–89.8)	44.0	58.2 (43.4–78.2)
	95.2 (86.8–98.7)	60.0 (42.8–92.1)	35.5	57.1 (40.4–87.3)

* Five pups of the original sample were excluded from calculations because they were >30 days old (see the text for details).

intervals (CI) were set based on the normal theory. Then, we compared theoretical slopes with those empirically obtained.

Most statistical analyses were carried out with SPSS v. 15.0 and SYSTAT 12.0. Models were developed with Excel spreadsheets. Other specific analyses were performed with free software: the program 'Past' for nonparametric MANOVAS; the Locfit procedure of the R package to estimate and compare LOESS regressions, and Quantitative Parasitology v. 3.0 (Reiczigel and Rózsa, 2005) to estimate infection parameters and conduct pair-wise comparisons with the bootstrap t -test.

RESULTS

The great majority of pups examined (124 out of 136) were infected with *A. microchir* and prevalence did not differ significantly between years (Fisher exact test, $P=0.131$) (Table 2). Of the 12 uninfected pups, 10 (9 from 2007 and 1 from 2008) were 1 day old, and 2 pups from 2008 were 2 and 3 days old, respectively. Mean intensity of *A. microchir* did not significantly differ between years (bootstrap t -test, $t=0.465$, $P=0.654$); the range of intensity was 1–382 in 2007 and 1–466 in 2008. However, the comparison of overall population structure was close to significance (nonparametric MANOVA, $F=2.098$, $P=0.066$). Individual comparisons between instars indicated that the mean abundance of both N2 and N3 was very similar between years, but pups collected in 2007 harboured significantly more N1s (bootstrap t -test, $t=2.151$, $P=0.038$) whereas those collected in 2008 harboured more adult lice ($t=2.072$, $P=0.039$) (Table 2). The adult sex ratio in the overall sample of *A. microchir* did not depart significantly from 1:1

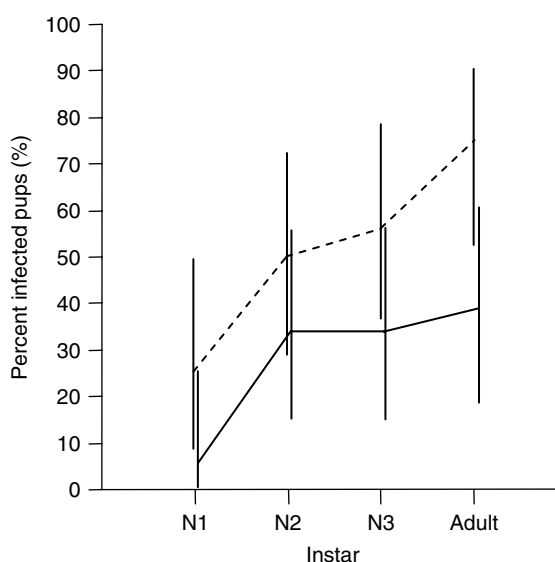


Fig. 1. Prevalence of instars (N1–N3, nymphs 1–3) of the louse *Antarctophthirus microchir* in 1 to 3-day-old pups of the South American sea lion, *Otaria flavescens*. Solid and broken lines connect values for the sample of 2007 ($n=18$) and 2008 ($n=16$), respectively. Vertical bars are the 95% confidence intervals.

and did not differ between years (in 2007: 0.459 [95% CI: 0.409–0.509]; in 2008: 0.470 [0.430–0.510]; Fisher exact test, $P=0.795$).

A total of 34 pups (18 and 16 from 2007 and 2008, respectively) were ≤ 3 days old. In this subsample, the prevalence of all instars of *A. microchir* was consistently higher in pups collected in 2008 (Fig. 1); the difference was significant for adults (Fisher exact test, $P=0.02$). Overall population structure also differed between years (non-parametric MANOVA,

$F = 3.132$, $P = 0.036$); univariate comparisons indicated that mean abundance of all stages was higher in 2008, but only that of adults was found to be statistically significant ($t = 2.05$, $P = 0.036$). Within each year, prevalence differed significantly among instars (Cochran test; in 2007, $Q = 8.5$, 3 D.F., $P = 0.037$; in 2008, $Q = 13.55$, $P = 0.004$), with values increasing from N1 to adult (Fig. 1). Using intensity data, this trend was found to be significant in both years (Page test; in 2007, $L = 242$, $P < 0.01$; in 2008, $L = 350.2$, $P < 0.01$).

LOESS regressions of log-transformed intensity of instars of *A. microchir* on pup age are shown in Fig. 2. There were significant differences of regression lines between years for all instars, but not for total intensity (Table 3). The largest differences occurred in the case of N1s and, particularly, adults (Fig. 2B, D; Table 3). The number of N1 in young pups (≤ 10 days old) was similar in both years, but increased more in older pups collected in 2007 than in 2008 (Fig. 2B). In contrast, the number of adult lice was consistently higher in pups from 2008, except for pups approximately 28–30 days old (Fig. 2D). In spite of these numerical differences, regressions with standardized log-transformed values of intensity did not differ between years for any instar (Table 3), suggesting that a basic population trend was conserved.

Five pups in the sample from 2007 were > 30 days old when examined for lice. When we compared mean abundance of each instar between this subsample and that of pups 28–30 days old ($n = 7$), we did not find significant differences in the case of N2, N3 and adults (bootstrap t -test, all $P > 0.22$), but we did in the case of N1 ($t = -2.574$, $P = 0.0240$) although the samples to be compared were small. The number of N1 sharply decreased in pups > 30 days old (Fig. 2B).

The slopes of generation times considering different combinations of life-history data for *A. microchir* are shown in Fig. 3. Variability of slopes was heavily dependent on egg duration (incubation time), with minor changes related to the duration of the nymphal stages (Fig. 3). This is not surprising because incubation time determines when the second generation of lice will appear within the 15-day period, thus strongly affecting the slope of population growth (nymphs plus adults). The empirical slopes of population growth were similar in 2007 and 2008 i.e. 0.76 (95% CI: 0.50–0.100) and 0.73 (0.56–0.88), respectively. An ANCOVA indicated that the two slopes did not significantly differ from each other ($F_{(1,63)} = 0.040$, $P = 0.842$) and, therefore, a single empirical regression was obtained from pooled data. The comparison of the empirical and the theoretical slopes of population growth suggested a range of generation times from 18 to 23 days (which was the maximum established by default), for a minimum incubation time of 9 days (Fig. 3). In fact,

an upward change in the trend of increase of N1 in both years occurred in pups 9 days old or older (Fig. 2B, G), which probably is indicative that lice from a new generation are being recruited in pups ≥ 9 days old.

In Fig. 4, we provide 2 examples of population projection of each instar for pups ≤ 30 days old for generation times of 18 and 23 days. The only additional assumption was that adults have a life span > 30 days. Note that the basic shape of projections agrees well with empirical trends (Fig. 2G–J), and was not substantially altered by assuming that additional events of vertical or horizontal (pup to pup) transmission occur in pups > 10 days old (data not shown).

DISCUSSION

This study tracks, for the first time, the population dynamics of a species of echinophthiriid using replicate samples under natural conditions. However, the study relies on lice sampling of only a part of the host's body. Thompson *et al.* (1998) faced a similar logistic problem and counted individuals of *Echinophthirius horridus* only on the dorsal surface of one hind flipper of harbour seals. These authors justified their decision based on previous evidence showing that this was the principal site of infestation. Our sampling strategy was justified on similar grounds, i.e., individuals of *A. microchir* were particularly abundant on the chest and belly of sea lion pups. However, this observation is in contrast to the reports from other species of *Antarctophthirus* indicating a preference for bare areas of the body (Murray and Nicholls, 1965; Murray *et al.* 1965; Kim, 1972, 1975). Apparently, these thermoregulatory areas provide suitable temperature conditions for the activity and reproduction of lice in cold climates (Murray and Nicholls, 1965). However, *A. microchir* faces the opposite problem: at Punta León, average air temperatures in January are about 20 °C, with maximum temperatures sometimes reaching 38 °C (Yorio *et al.* 1995, and references therein). Therefore, direct solar radiation on pups produces thermal stress and likely lethal conditions for lice on bare areas, whereas ventral areas with pelage may provide maximal protection. In any event, our sampling strategy assumes that the preference for ventral body areas is similar for all instars. Although this assumption might be valid, we dealt with the absolute number, and not with their relative proportion, of each instar per host to alleviate potential biases of differential habitat selection among instars.

As noted above, *A. microchir* has a window of time of about 1 month to reproduce on newborn pups. Afterwards, pups go to the sea and start moulting, and both events likely kill all lice eggs (see Murray, 1976, 1987). Our data also suggest that the N1 might

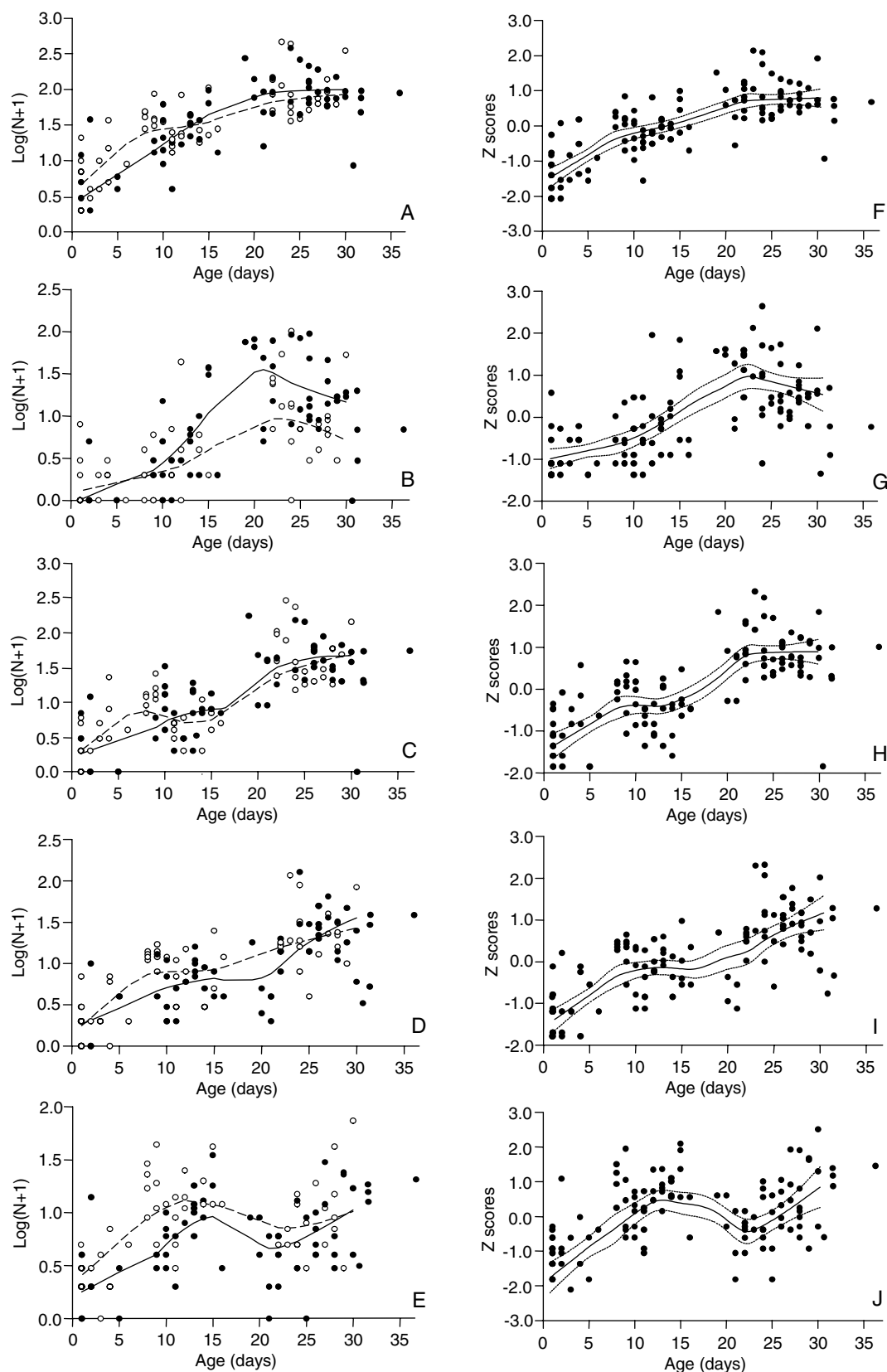


Fig. 2. LOESS regression models for \log_{10} -transformed values, and standardized \log_{10} -transformed values, of the number of each instar and total intensity of the louse *Antarctophthirus microchir* in pups of South American sea lion, *Otaria flavescens*. Pups > 30 days old were excluded in the calculations. A–E: Regressions on \log_{10} -transformed values. Black points, solid lines: sample of 2007; open points, broken lines: sample of 2008. F–I: Regressions on standardized \log_{10} -transformed values with pooled data from both years. Dotted lines: point-wise 95% confidence intervals for the LOESS curve. (A, F). Total population; (B, G). First nymph (N1); (C, H) Second nymph (N2); (D, I). Third nymph (N3); (E, J) Adult.

Table 3. Inter-year comparison of LOESS regression curves of the raw, and standardized, log-transformed number of each instar or total intensity of the louse *Antarctophthirus microchir* on age of pups from South American sea lion, *Otaria flavescens* (see Fig. 2)

(The equivalent degrees of freedom, which were the same in all F tests (D.F.₁ = 3·94 and D.F.₂ = 111·26), were rounded to the nearest integer to calculate P-values.)

Instar	Log-transformed intensity		Z-scores of log-transformed intensity	
	F	P	F	P
N1	3·545	0·014	0·993	0·535
N2	2·846	0·027	1·859	0·123
N3	3·096	0·029	2·077	0·089
Adult	4·857	0·001	1·973	0·103
Total	1·334	0·262	1·992	0·101

be affected negatively by the first immersions because its abundance, but not that of other instars, sharply decreased in pups > 30 days old. Interestingly, the N1 of *A. microchir* lacks, as other species of *Antarctophthirus* (Kim, 1971; M. S. Leonardi, unpublished data), the specialized abdominal scales that assist more developed instars in obtaining oxygen underwater (Kim, 1971; see also Murray, 1976, Mehlhorn *et al.* 2002). In other words, the N1 would depend on atmospheric air for respiration. Kim (1975) suggested that this was the reason why the N1 of *A. callorhini* favours the underfur layer of pelage of the Northern fur seal, where an air blanket is formed during immersions. However, the pelage of the South American sea lion wets completely when submerged.

Vertical transmission of *A. microchir* starts shortly after the pup's birth and is very effective: most 1-day-old pups already harboured lice and uninfected pups were all ≤ 3 days old. Likewise, Kim (1972, 1975) reported infestation with *A. callorhini* in pups of Northern fur seal as early as 7 h after parturition. However, instars of *A. microchir* seem to differ in their potential for transmission. Each year, we found a consistent pattern of increase in numbers from the N1 to the adult in pups ≤ 3 days old, which might probably result from corresponding differences in the potential for transmission of each instar. In a series of experiments with human head lice, Takano-Lee *et al.* (2005) demonstrated that travelling speed and dispersal ability were minimal in the N1 and increased in more advanced instars. This hypothesis is also compatible with previous studies on echinophthiriids (Murray and Nicholls, 1965; Kim, 1972, 1975), which reported a relative rarity or absence of N1 in newborn pups.

Our results suggest that the shape of population increase for each instar of *A. microchir* with pup age is

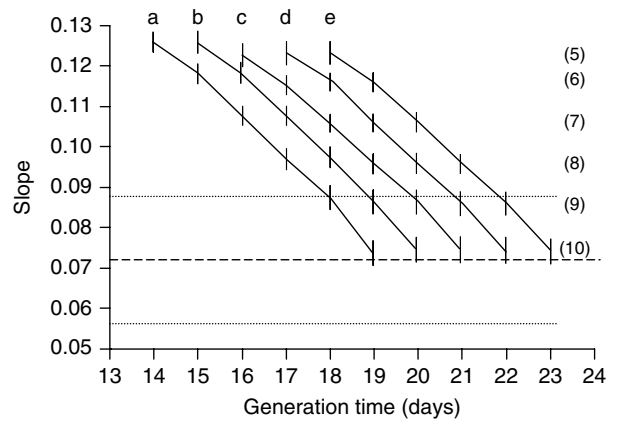


Fig. 3. Average slopes of the theoretical regressions between the log₁₀-transformed population size of *Antarctophthirus microchir* and the age of sea lion pups, for pups ≤ 15 days old. A total of 30 different combinations of stage duration were considered; the right numerical column identifies the egg incubation time, and top letters identify the duration of the N1, N2 and N3 (a: 2,2 and 3 days; b: 3,3,3; c: 2,4,4; d: 3,4,4; e: 4,4,4); a constant pre-ovoposition period of 1 day was assumed for all combinations. For instance, the rightmost combination in the row at the bottom assumes an incubation time of 10 days, and 4, 4 and 4 days of duration for the N1, N2 and N3, respectively, plus a pre-ovoposition period of 1 day; therefore, the generation time (egg to egg) for this combination is 23 days. For each of the 30 combinations of stage duration, 100 regression models were obtained by varying the daily fecundity rate, and survival rate for each stage (see the text for details). Vertical bars represent the 95% CIs of average slopes. The broken line represent the empirical slope observed for *A. microchir*, with its 95% CI limits (dotted lines).

conserved between years. Simple models of population growth suggest that this basic pattern would primarily reflect the dynamics of recruitment and reproduction: recruitment would depend mainly on vertical (mother to pup) transmission (Leidenberger *et al.* 2007), particularly during the first 10 days post-partum, whereas reproduction would be continuous on land (Murray, 1976) and so at least a second generation of lice would be produced (see also below). However, the specific trend of change in abundance differed between years for all instars of *A. microchir*. This is not surprising as populations of lice are strongly affected by a number of factors that often produce remarkable short-term changes in population structure (see Murray, 1987, and Price and Graham, 1997, for references). Environmental factors are particularly relevant in our case. Newborn pups apparently acquired more lice, particularly adults, from their mothers in 2008. However, although the number of adult lice remained higher on older pups, the number of N1 strongly declined compared to 2007. In fact, the decline affected all instars but was sequential with pup age (LOESS regression lines cross at later pup ages from N1s to adults; see

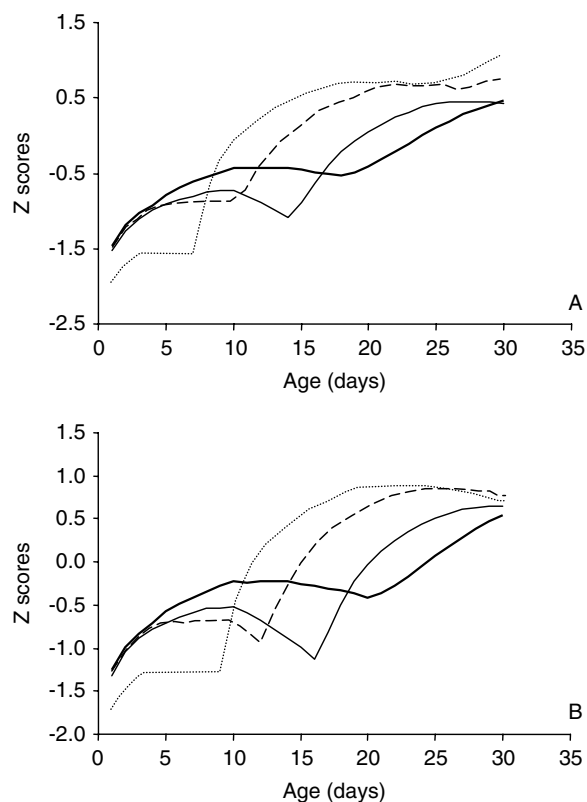


Fig. 4. Two examples of theoretical population projection (standardized \log_{10} -transformed abundance) of the different instars of the louse *Antarctophthirus microchir* in sea lion pups 1–30 days old. Only mother-to-pup transmission during the first 10 days was considered, with daily ‘packets’ of 2 N1s, 6 N2s, 6 N3s and 7 adults. Dotted line: Nymph 1. Broken line: Nymph 2. Thin line: Nymph 3. Thick line: Adult. A. Generation time: 18 days. Duration of stages (in days): Egg: 6; N1: 3; N2: 4; N3: 4; adult: > 30. The pre-ovoposition period was 1 day. B. Generation time: 23 days. Duration of stages (in days): Egg: 10; N1: 4; N2: 4; N3: 4; adult: > 30. The pre-ovoposition period was 1 day. In both examples, daily survival rates were 0.900 for all stages, and daily fecundity rate was 8 eggs/female (see text for details).

Fig. 2). It would appear that some factor induced a lower reproduction rate or a higher mortality of eggs, probably 10 days after the sampling period had begun. Environmental conditions (e.g., temperature, solar radiation, relative humidity), but not host-related factors (e.g., population density, changes in pelage or grooming behaviour) can change idiosyncratically in a matter of days. We suspect that relative humidity, which has a specific impact upon lice eggs (see Murray, 1963), may account at least partly for the differences that we observed. At Punta León, rainy days are regularly scattered throughout January every year, but the duration of rainy periods and the amount of rainfall may vary unpredictably among years (Yorio *et al.* 1995, and references therein).

We used a number of combinations of realistic values of survival probability, stage duration and fecundity to model the population growth of

A. microchir and so ascertain its possible generation time. The range obtained was 18–23 days, which is congruent with data from other echinophthiriids (note, however, that the upper limit could increase if we assume a longer duration of nymphal stages, see Table 1). What makes our result particularly robust is that slopes of population growth for a given combination of stage durations varied little, regardless of the values of survival rate and fecundity. However, the minimum generation time obtained depends on a relatively long egg incubation time (≤ 9 days). Since incubation time can vary widely according to small changes of microhabitat temperature (see Price and Graham, 1997), could shorter generation times be expected in better years?

To answer this question, we should consider the potential selective pressures acting upon the tight reproductive schedule of *A. microchir*. This species might accommodate 3 generations at best during the lactation period of pups ashore, but further regular reproduction is unlikely in other host age classes because they do not stay continuously on land enough time (see Campagna and Le Boeuf, 1988) for newly laid eggs to develop into N2 (as discussed above, the N1 probably requires also aerial conditions). For comparison, consider the 6–12 generations per year of a typical terrestrial louse, the hog louse, *Haematopinus suis* (Price and Graham, 1997). Having such strict temporal limits for reproduction, *A. microchir* may confront a trade-off between generation time and fecundity (Stearns, 1992; Gemmill *et al.* 1999). A short generation time (e.g., 14 days) would potentially allow accommodation of a third generation of lice before pups go to the sea, but with the clear risk that unfavourable environmental conditions at any time (e.g., low temperature) may delay development until it is too late. In contrast, a long generation time (e.g., 23 days) would prevent the possibility of producing a third generation of lice, but parental individuals may produce more eggs if a prolonged development increases adult size (Gemmill *et al.* 1999). Ascertaining whether this trade-off is actually shaping the life cycle of *A. microchir*, and that from other echinophthiriids, could be investigated in the future through comparative analysis with other anoplurans.

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