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CRITICAL EVALUATION OF FIVE METHODS FOR QUANTIFYING CHEWING LICE (INSECTA: PHTHIRAPTERA)

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ABSTRACT: Five methods for estimating the abundance of chewing lice (Insecta: Phthiraptera) were tested. To evaluate the methods, feral pigeons (*Columba livia*) and 2 species of ischnoceran lice were used. The fraction of lice removed by each method was compared, and least squares linear regression was used to determine how well each method predicted total abundance. Total abundance was assessed in most cases using KOH dissolution. The 2 methods involving dead birds (body washing and post-mortem-ruffling) provided better results than 3 methods involving live birds (dust-ruffling, fumigation chambers, and visual examination). Body washing removed the largest fraction of lice (>82%) and was an extremely accurate predictor of total abundance ($r^2 = 0.99$). Post-mortem-ruffling was also an accurate predictor of total abundance ($r^2 \geq 0.88$), even though it removed a smaller proportion of lice (<70%) than body washing. Dust-ruffling and fumigation chambers removed even fewer lice, but were still reasonably accurate predictors of total abundance, except in the case of data sets restricted to birds with relatively few lice. Visual examination, the only method not requiring that lice be removed from the host, was an accurate predictor of louse abundance, except in the case of wing lice on lightly parasitized birds.

Chewing lice (Insecta: Phthiraptera) are parasitic insects found on virtually all bird species and many mammal species (Marshall 1981). Like the sucking lice (Anoplura), which are also members of Phthiraptera, chewing lice are permanent ectoparasites with a direct life cycle passed entirely on the body of the host. Louse eggs are glued to the host's feathers or hair. Hatched lice spend little, if any, time off the body of the host. Transmission to new hosts takes place primarily during periods of direct contact, such as between parent hosts and their offspring in the nest. Transmission also occurs during host copulation (Hillgarth, 1996) and between individuals of social species when they are in close contact (Rózsa et al., 1996). Lice are also known to disperse phoretically on hippoboscid flies (Corbet, 1956; Keirans, 1975), although the importance of this dispersal mechanism is largely unexplored (Clayton et al., in press). The close association of chewing lice and their hosts makes them unusually tractable for studies of host–parasite coadaptation (Clayton et al., 1999), cospeciation (Hafner et al., 1994), population genetics (Nadler et al., 1990), population ecology (Lee and Clayton, 1995), and host specificity (Tompkins and Clayton, 1999).

Many methods have been described for collecting and quantifying chewing lice. Marshall (1981) and Clayton and Walther (1997) reviewed the most commonly used methods. Walther and Clayton (1997) and Visnak and Dumbacher (1999) compared the efficiency of several methods and fumigants used for quantifying lice on live birds. However, a truly rigorous comparison of the efficiency of different methods has not been published. Such a comparison requires that the performance of each method be compared to a common standard in order to assess the fraction of lice removed by each method and how well each method predicts the total number of lice on a host, regardless of the size of the fraction removed. These 2 parameters are not necessarily correlated. It is possible for a method to remove a small fraction of the host's total lice, yet be a more accurate predictor of total abundance than another method that happens to remove a larger fraction of lice.

In this paper, we describe 5 methods for quantifying lice, and we present the results of a series of tests to determine how well each method performs. We have not attempted to cover all methods for collecting and quantifying chewing lice, much less other ectoparasites. However, we have tested all of the methods typically used to quantify the abundance (Bush et al., 1997) of chewing lice on birds. All 5 methods have been described previously in the literature (reviewed by Clayton and Walther, 1997). Two of the methods, body washing and post-mortem-ruffling, are used when quantifying the abundance of lice on dead hosts. The remaining 3 methods, dust-ruffling, fumigation chambers, and visual examination, are used to quantify the abundance of lice on live hosts. We evaluated the 5 methods by comparing how well each quantified 2 species of feather lice (Phthiraptera: Ischnocera) on feral pigeons (*Columba livia*), which are also known as Rock Doves. The 2 species of lice were *Columbicola columbae*, a ''wing'' louse found primarily on the host's wings and tail, and *Campanulotes bidentatus compar*, a ''body'' louse found primarily on the rump and other abdominal regions (see photos in Nelson and Murray, 1971; Clayton, 1991; Johnson and Clayton, in press).

MATERIALS AND METHODS

Each method was tested using a sample of feral pigeons that varied in louse abundance, but which did not exceed the variation observed in natural populations (Clayton et al., 1999). The birds were obtained at several locations in Salt Lake City, Utah, using walk-in traps baited with pigeon feed. Each bird was fitted with a numbered aluminum band, housed individually (1 to a cage), and provided water, food, and grit ad lib. We manipulated the abundance of lice on ca. 100 pigeons by impairing preening ability, which is the principle defense of birds against lice (Marshall, 1981). To create a range of louse abundance, preening was impaired for different time intervals, ranging from several weeks to several months. The sample of birds used to test each method was drawn randomly from this pool of 100 impaired pigeons. None of the birds was molting. Molt has been shown to alter the results of methods for quantifying lice (especially visual examination; see Discussion).

Preening was impaired using poultry bits, which are small C-shaped pieces of plastic that are inserted between the mandibles and crimped slightly in the nostrils to prevent dislodging, but without piercing the tissue. Bits create a 1.0–3.0-mm gap between the mandibles that blocks preening, leading to an increase in the louse population (Clayton, 1990; Clayton, 1991; Booth et al., 1993; Clayton and Tompkins, 1995; Clayton et al., 1999). Although bits interfered with preening, they did not interfere with feeding because the birds were fed grain that can be ingested easily by bitted birds. Prior studies of bitted birds have revealed no side effects of bits (e.g., Clayton and Tompkins [1995] showed that bits did not significantly alter the body mass of feral pigeons, nor their reproductive success).

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Determination of total abundance

The 5 methods were evaluated by checking their returns against a standard, which was the ''total'' number of lice on each of the 100 test birds (it is not actually feasible to recover 100% of the lice on a bird, see below). One way of determining the total number of lice is to examine every feather and remove the lice 1 by 1 (Ash, 1960; Nelson and Murray, 1971; Eveleigh and Threlfall, 1976; Doster et al., 1980). This method requires 7–10 hr per pigeon, making it vulnerable to error generated by investigator fatigue. A better approach is the dissolution method described by Hopkins (1949), which is analogous to burning down a haystack to find its needles. The method uses a solution of sodium or potassium hydroxide (KOH) to dissolve the skin and fur or feathers of an infested host, leaving behind the chitinous exoskeletons of ectoparasites. The exoskeletons are then filtered out of the solution and counted under a dissecting microscope. The dissolution method has been modified by a number of investigators, including Cook (1954), Ash (1960), Hilton (1970), Choe and Kim (1987), Lemke et al. (1988), and Clayton (1991). The method requires about 2 contact hours per bird spread over a period of 2 days. Several birds can be processed simultaneously.

Our KOH dissolution protocol was as follows. First, the wing feathers (primaries and secondaries) and tail feathers (rectrices) were pulled from the dead bird while holding it in an empty 19-L bucket to prevent lice from being lost. The skin (with attached body feathers) was carefully removed from the bird and placed in a 2-L beaker, along with the wing and tail feathers removed earlier. The contents of the bucket were then washed into the beaker, and the bucket was rinsed several times into the beaker with deionized water to remove lice adhering to its sides. The (white) bucket was searched carefully between rinses.

Next, trypsin (4× U.S.P. pancreatin) was added to the beaker to make a 0.5% solution, which was buffered to pH 7.5–8.3 using 0.2 M disodium phosphate. This solution was incubated for 24 hr at 37 C. The beaker was then placed on a stirring hot plate in a hood, and enough KOH was added to make a 5% solution (to control the reaction, a concentrated KOH solution was used, rather than KOH pellets or flakes dumped directly into the beaker). After bringing the solution to a boil, the heat was reduced and the mixture was allowed to simmer until all feathers and skin had dissolved (this required about 1 hr if a magnetic stirring rod was used to keep the solution well mixed). After cooling for another hour, xylene was added to break up the thin layer of fat on the surface of the solution. The beaker's contents were filtered through a #80-mesh (0.180 mm openings) stainless steel screen (Newark Wire Cloth Company, Newark, New Jersey). Contents of the screen were then washed into a buchner funnel lined with filter paper. The filtering process was sped up with vacuum suction. The paper was then spread over a 1-cm^2 numbered grid printed on a sheet of plastic, and all lice were counted under a dissecting microscope.

KOH dissolution is an elaborate procedure that is subject to investigator error. It is therefore unrealistic to expect 100% recovery of the lice from every bird (Clayton and Walther, 1997). The recovery rate was tested by checking it against known numbers of pigeon lice placed on louse-free, commercially bred Japanese Quail (*Coturnix japonica*). Quail were used to eliminate the possibility of extraneous pigeon lice that could bias the results. Ten quail were ''seeded'' with 50–250 lice per bird then run through the KOH dissolution protocol described above. Means of 97.1% (±1.2 SE) adult lice and 91.4% (±0.1) immature lice were recovered. Two hypotheses can explain why fewer adult lice (2.9%) than immature lice (8.6%) were lost during the procedure. First, Ash (1960) suggested that immature lice dissolve because their exoskeletons, which contain less chitin than adults, are less resistant to KOH dissolution. Second, the smaller body size of immatures presumably makes them more vulnerable to being lost through the screen (first instars only), splashing out of the beaker, or simply being overlooked.

To test the second hypothesis, that lice are lost, 10 sham dissolution trials (KOH omitted) were carried out using known numbers of lice added directly to the beaker, with no host tissue present. The sham trials recovered a mean of 97.3% (±0.8) adult lice and 89.4% (±1.5) immature lice. This recovery rate did not differ significantly from that of the 10 quail trials (adults: $t = 0.142$, $P = 0.89$; immatures: $t = -0.803$, $P = 0.43$). Thus, the lower yield of immature lice in the quail trials was apparently caused by investigator error, rather than by dissolution of lice. The few missing adult lice were presumably also caused by investigator error.

Although KOH dissolution is relatively accurate and efficient, it does have some drawbacks. First and foremost is the need to destroy the host's integument, which eliminates the possibility of saving an intact voucher specimen (although it is still possible to make a skeletal specimen). KOH dissolution also produces toxic, offensive-smelling fumes that must be ventilated in a fume hood. Dissolution was used to determine the number of lice remaining on birds after the application of the simpler methods described below.

Body washing

The first method tested was body washing, which was originally described by Lipovsky (1951) for use on birds and mammals. Steps in the procedure were as follows. First, the bird was immersed in a 3.8-L paint can containing a 1.0% solution of Palmolive Ultra Dishwashing Liquid® (Wicht and Crossley, 1983). The detergent, which serves as a wetting agent, was used in small quantity to prevent excessive foaming. The paint can had a rustproof lining that was caulked to eliminate any cracks and crevices in which lice could be lost. Next, the can was agitated on a mechanical paint shaker (Fleming Gray Limited, Ontario, Canada) for 10 min (wash cycle), as described by McGroarty and Dobson (1974).

Next, the can was opened and a stream of 95% EtOH was used to cut back the foam, which often contains suspended lice. The bird was then transferred to a second can, where it was immersed in water and shaken for another 10 min (rinse cycle). The bird was removed from the can and rinsed over a 19-L bucket with 95% EtOH to remove additional foam, then rinsed again with water to remove lice adhering to the plumage. The 2 paint cans (wash and rinse) were emptied into the bucket and rinsed several times with water into the bucket. The contents of the bucket were then strained through a #80-mesh screen (see above), transferred to filter paper, and counted as described under KOH dissolution.

Post-mortem-ruffling

The second method was post-mortem-ruffling, which removes lice from dead, fumigated birds through repeated bouts of feather ruffling (Clayton et al. 1992). The first step in the procedure was to fumigate each bird for 20 min in a 3.8-L Tupperware® container lined with a sheet of white paper. After fumigation, the paper was searched carefully for lice; a new sheet was used for each bird. The chamber contained several cotton balls soaked in ethyl acetate, which is toxic to arthropods but safe for human use (Fowler, 1984). (Ethyl acetate is relatively easy to obtain, even in remote locations, because it is the main ingredient in many fingernail polish removers.) At the end of the 20-min period, the bird was removed from the container and held over a cafeteria tray lined with a large sheet of white paper (new sheet for every bird). The bird's feathers were ruffled vigorously for 60 sec over the tray with careful attention to all feather tracts. At the end of the 1-min bout, lice on the paper were counted and removed with a jeweler's forceps or a fine brush dipped in alcohol (the brush was checked carefully between birds for parasites lodged between the bristles). The ruffling procedure was repeated twice. If no lice were found during any of the 3 bouts, the procedure was stopped. If any lice were recovered, then 60-sec bouts were continued until the number of lice collected during a single bout was <5% of the sum recovered during the first 3 bouts. An average of 7 bouts was required to reach the point of diminishing returns. The method required an average of 40 min per bird, including fumigation time and ruffling and searching time.

Dust-ruffling

Like the previous method, dust-ruffling removes lice from fumigated birds, but in this case, the birds are alive (Walther and Clayton 1997). The fumigant used was pyrethrin dust, a common insecticide that is available in a variety of commercial forms (Casida and Quistad 1995) and has been shown to have no effect on the growth rates or survival of nestling or adult pigeons (Clayton and Tompkins, 1995). The brand of dust used was Z3 Flea and Tick powder (Zema®, Research Triangle Park, North Carolina), which contains 0.1% pyrethrins and 1.0% piperonyl butoxide.

The first step in the method was to dust powder onto the bird and work it into the plumage with the fingers of 1 hand, while holding the

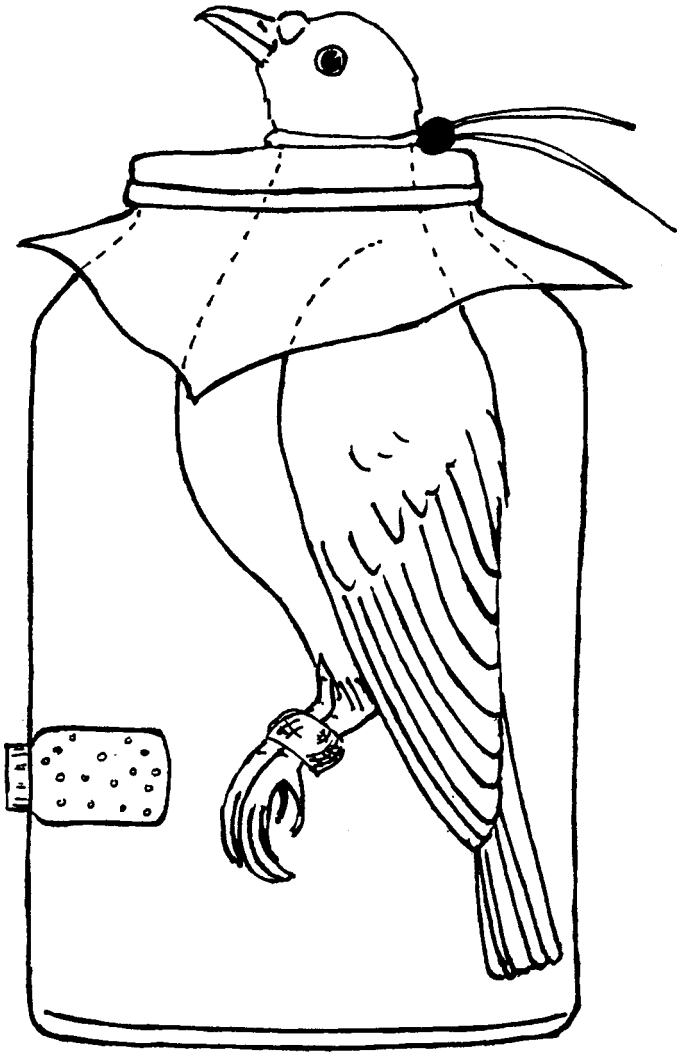

FIGURE 1. Fumigation chamber for the removal of lice from live birds (see text for details).

bird with the other hand over a cafeteria tray lined with paper. This step required about 5 min per bird. A paper mask was worn and care was taken to avoid getting dust in the bird's eyes or mouth. The bird's legs were immobilized at the start of the procedure using a strip of Velcro®. After distributing the dust through the plumage, the bird was ruffled for 60 sec with careful attention to all feather tracts. Because the dust is fast acting, there was no need to wait between the dusting and ruffling steps. From this point on, the procedure was similar to post-mortem-ruffling.

Fumigation chamber

The second method tested for live birds employed a fumigation chamber (Visnak and Dumbacher, 1999), or ''anaethesia jar'' (Walther and Clayton, 1997). The chamber, based on a design by R. Visnak (pers. comm.), consisted of a 1.9-L, wide-mouthed plastic jar fitted with a cloth (60/40 cotton/nylon blend) collar stretched over the opening and held in place with a heavy rubber band (Fig. 1). A screw-cap plastic bottle the size of a 35-mm film canister was glued to the wall of the chamber through a hole near its base. One end of the bottle protruded into the jar, while the other end, with a removable screw cap, remained outside the jar (Fig. 1). At the start of a trial, cotton balls soaked with ethyl acetate were placed in the bottle. The end of the bottle protruding into the jar had tiny holes to allow ethyl acetate fumes to fill the chamber. A piece of filter paper was placed on the bottom of the chamber to collect falling lice.

The collar had a 6-cm hole cut in the center with an adjustable drawstring stitched around the hole. The collar was secured to a bird by closing the drawstring gently. As in the case of dust-ruffling, the bird's legs were immobilized at the start of the procedure with a strip of Velcro. The bird was then lowered into the chamber and the collar stretched over the chamber's opening, leaving the bird's head outside the chamber (Fig. 1). Each bird suspended in this manner was fumigated for 20 min and was monitored closely for any sign of distress, in which case the drawstring was loosened slightly (2 of 24 cases). Following removal of the bird, the chamber was overturned on a sheet of white paper and tapped to make the filter paper drop out. Contrary to the advice of Walther and Clayton (1997), the birds' feathers were not ruffled. The inside of the chamber was wiped gently with tissue paper to remove adhering lice. The chamber was washed thoroughly with water between birds. Lice were counted with a $\times 2$ jeweler's headset.

Visual examination

Visual examination is the method of choice for longitudinal studies of louse demographics. Visual examination estimates the total number of parasites on a bird from timed visual counts of lice on various body regions. To prevent lice from shifting body regions prior to examination, birds were kept calm by placing them in a darkened room or paper bag for at least an hour. Each bird's legs were immobilized at the start of the procedure with a strip of Velcro.

Five body regions (Fig. 2) were examined under a bright light and with magnification from a $\times 2$ jeweler's headset. While holding the bird on its back in 1 hand, the other hand was used to extend 1 wing, and all lice on the undersurface of the wing's flight feathers (primaries and secondaries) were counted. The basal region of each feather was observed by deflecting the underwing covert with a forceps or by blowing on it. Examination of the wing feathers required <1 min, even in the case of heavily infested birds. Next, the tail was fanned out and lice on the underside of each tail feather were counted while deflecting the undertail coverts. Finally, a timed visual examination of feathers in 3 abdominal regions (Fig. 2) was carried out in the following sequence: adjacent to the keel (30 sec), back (30 sec), and rump (60 sec). Care was taken to avoid counting the same lice twice by examining feathers throughout each region. The relatively short time allocated to each region further ensures against repeat counts.

Data analysis

To assess the 5 methods described above, the number of lice removed or observed using each method was compared to the number of lice remaining on each test bird. The number of lice remaining on the bird was determined by KOH dissolution in the case of body washing, dust-ruffling, and the fumigation chamber method. In the case of post-mortem-ruffling, the last method tested, the number of lice remaining on the bird was determined by body washing, which by that point in the study was known to be an extremely accurate predictor of total abundance (see Results). Visual examination was tested by examining birds subsequently used to test the other 4 methods, then comparing the visual examination results to the total number of lice removed from the bird.

Data for wing and body lice were analyzed separately. We used 2 criteria to evaluate the performance of each method for each species of louse (i.e., percent lice removed or observed and how well the number of lice removed or observed predicted total abundance using least squares linear regression). Because the data showed the aggregated distribution typical of many parasites (Anderson and Gordon, 1982), all data were square root–transformed prior to regression analysis (log and natural log transformations gave similar results).

For each method, the complete data set was analyzed, as well as a more restricted data set. The latter was limited to birds with <300 lice of the species being analyzed, about an order of magnitude less than the highest abundance for either louse species. Restricting and reanalyzing the data in this way served 2 purposes. First, it tested the performance of each method when restricted to birds with the smaller louse populations typical of most wild birds (Clayton et al., 1992, 1999; Rózsa et al., 1996; Rózsa, 1997). Second, it eliminated the disproportionate leverage of individual birds with unusually large louse populations (e.g., the bird with highest abundance in Fig. 3a).

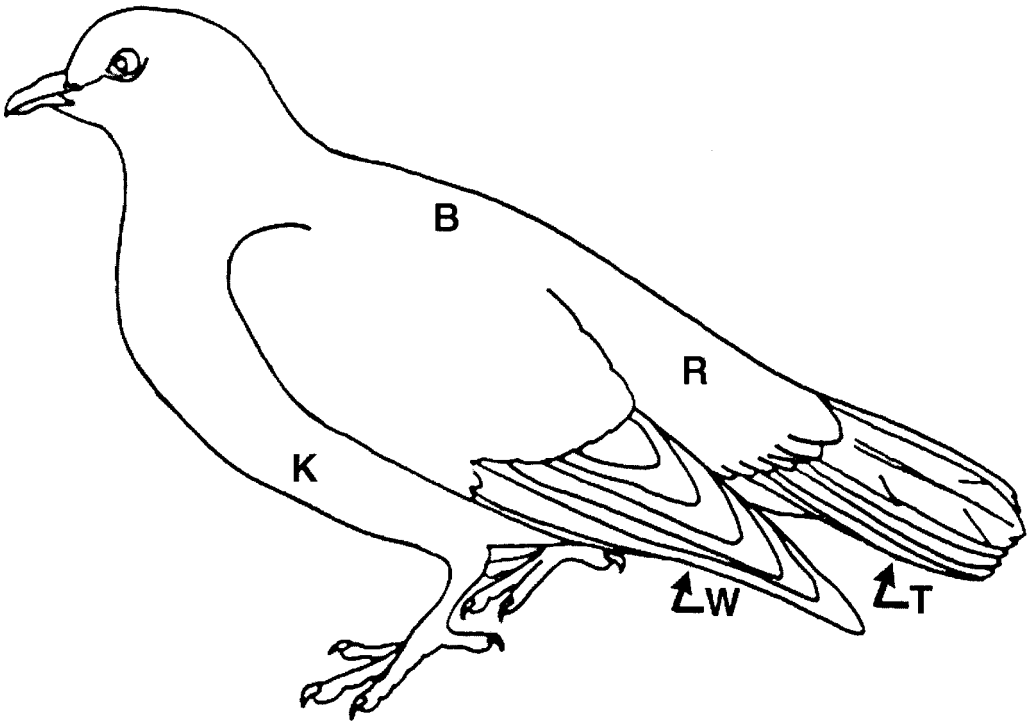

FIGURE 2. Regions visually examined for lice, according to the recommendations of Clayton (1991), and listed in the order in which they were examined: W, ventral surfaces of wing feathers (primaries and secondaries of 1 wing); T, ventral surfaces of tail feathers (rectrices); K, adjacent to keel (1 side only); B, back; R, rump.

RESULTS

Body washing

Body washing removed >82% of both wing and body lice (Table I). The 76.3% removal of body lice over the restricted data set was attributable to a single individual (bird with lowest abundance in Fig. 4a). When this individual was excluded, the percent removal increased to a mean of 88% for the remaining 5 birds. Body washing was an extremely accurate predictor of total abundance for wing lice (Fig. 3a), as well as body lice (Fig. 4a), over both the complete and restricted data sets (Table I).

Post-mortem-ruffling

Post-mortem-ruffling removed more wing lice than body lice. Using the 5% cutoff criterion (see Materials and Methods), this method removed >66% of wing lice but <43% of body lice (Table I). Despite this discrepancy, the method was an accurate predictor of total abundance for both wing and body lice over the complete and restricted data sets (Table I).

The 5% cutoff was based on the assumption that the most accurate index of relative abundance can be achieved by ruffling a bird to the point of diminishing returns (Clayton et al., 1992). This assumption was tested by reanalyzing a subset of the data limited to lice removed during the first 3 bouts of ruffling, regardless of the rate of return. The reanalysis thus simulated a method in which sampling is terminated after an arbitrary period of 3 min. As expected, the percent removal decreased for both species of lice (Table I); however, the method was still an accurate predictor of total abundance for both wing lice (Fig. 3b) and body lice (Fig. 4b) over the complete and restricted data sets (Table I).

Dust-ruffling

The results for dust-ruffling were somewhat similar to those for post-mortem-ruffling, although the percent removal and predictive power were even lower. Dust-ruffling removed >33% of wing lice, compared to <13% of body lice (Table I). Despite this discrepancy, the method was a reasonably good predictor of total abundance for both wing lice (Fig. 3c) and body lice (Fig. 4c) over the complete and restricted data sets (Table I).

Fumigation chamber

Like the previous 2 methods, the fumigation chamber removed more wing lice (>40%) than body lice (<22%). The chamber was a fair predictor of total abundance for both wing lice (Fig. 3d) and body lice (Fig. 4d) over the complete data sets. However, over the restricted data sets, the chamber was far less reliable for predicting either species of louse (Table I).

Visual examination

The mean percentage of wing lice observed during visual examination was 8.8% (±1.1 SE) of total abundance. Despite this small fraction, the method was a fair predictor of the total abundance of wing lice (Table II) over the complete data set. Interestingly, the number of lice observed on the wing (Fig. 3e) was a better predictor ($r^2 = 0.70$) than the sum of lice observed on both the wing and tail ($r^2 = 0.66$; Fig. 3f). A multivariate regression incorporating both regions ($r^2 = 0.73$) was superior

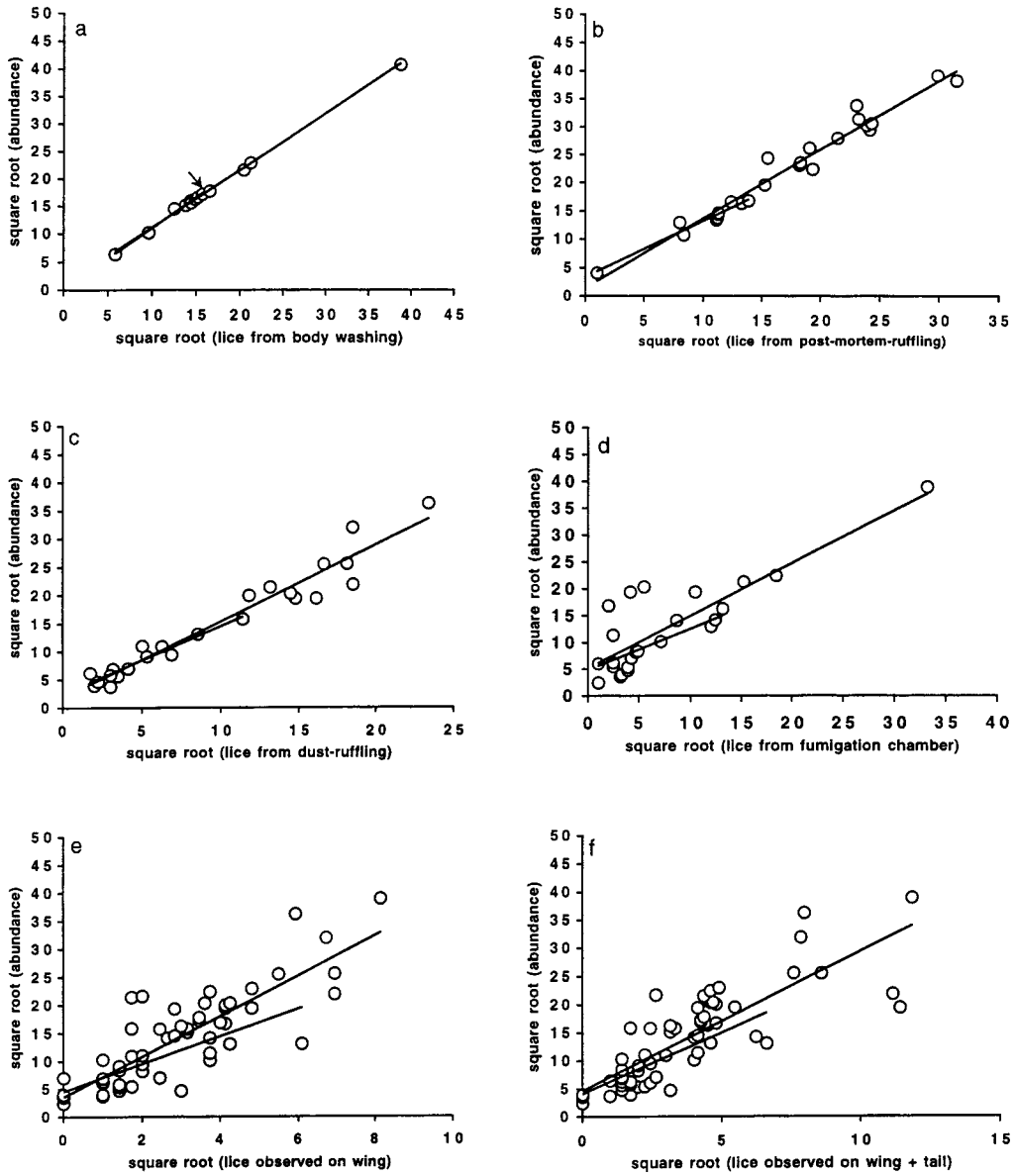

FIGURE 3. Relationship between total abundance of wing lice and the number of wing lice removed or observed using the 5 methods tested. Plots include best fit lines for the complete data set and the restricted data set (short segment). (**a**) Body washing; arrow indicates termination of short segment. (**b**) Post-mortem-ruffling (3-min cutoff). (**c**) Dust-ruffling. (**d**) Fumigation chamber. (**e**) Visual examination (wing). (**f**) Visual examination (wing + tail).

to the wing alone. Visual examination was less reliable for predicting the abundance of wing lice over the restricted data set (Table II).

The mean percentage of body lice observed during visual examination was 10.1% (±2.3 SE) of total abundance. As for wing lice, the method was a fair predictor of the total abundance of body lice over the complete data set (Table III). The number of lice observed on the rump (Fig. 4e) was not as good of a predictor (r^2 = 0.74) as the sum of the number observed on the rump, back, and keel (r^2 = 0.79; Fig. 4f). A multivariate regression incorporating all 3 regions was superior to any of the univariate regressions (r^2 = 0.87). Visual examination was also a good predictor of the abundance of body lice over the restricted data set (Table III).

DISCUSSION

The goal of this paper was to compare the performance of 5 methods for quantifying chewing lice. To this end, a series of tests was run using feral pigeons and their lice to determine the percentage of lice removed or observed by each method and how well the number of lice removed or observed predicted the total abundance of lice on birds.

Of the methods tested, body washing performed the best. It removed the greatest fraction of lice and was an extremely accurate predictor of louse abundance (Table I). Post-mortem-ruffling removed a lower fraction of lice, particularly in the case of body lice. However, post-mortem-ruffling was nearly as accurate at predicting total abundance as body washing, especially

TABLE I. Performance of 4 methods of lice removal.

Method	Complete data set				Restricted data set			
	n*	% Removal†	r^2‡	Regression equation§	n*	% Removal†	r^2‡	Regression equation§
Body washing								
Wing lice	14	83.7 (±1.0)	0.99	$y = (1.04\sqrt{x} + 0.85)^2$	10	82.1 (±1.1)	0.99	$y = (1.10\sqrt{x} + 0.09)^2$
Body lice	14	87.5 (±5.7)	0.99	$y = (0.98\sqrt{x} + 1.12)^2$	6	76.3 (±12.4)	0.99	$y = (0.81\sqrt{x} + 2.70)^2$
Post-mortem-ruffling								
5% cutoff								
Wing lice	24	69.7 (±2.8)	0.99	$y = (1.13\sqrt{x} + 0.98)^2$	10	66.2 (±6.5)	0.94	$y = (0.94\sqrt{x} + 2.98)^2$
Body lice	24	42.2 (±4.6)	0.98	$y = (1.14\sqrt{x} + 2.93)^2$	15	33.1 (±5.8)	0.90	$y = (1.25\sqrt{x} + 2.20)^2$
3-min cutoff								
Wing lice	24	57.2 (±2.8)	0.96	$y = (1.23\sqrt{x} + 1.37)^2$	10	54.7 (±6.1)	0.95	$y = (0.99\sqrt{x} + 3.33)^2$
Body lice	24	23.7 (±2.7)	0.96	$y = 1.70\sqrt{x} + 2.05)^2$	15	21.4 (±3.9)	0.88	$y = (1.49\sqrt{x} + 2.51)^2$
Dust-ruffling								
Wing lice	24	39.9 (±3.3)	0.94	$y = (1.36\sqrt{x} + 1.75)^2$	14	33.6 (±4.0)	0.88	$y = (1.22\sqrt{x} + 2.35)^2$
Body lice	24	12.4 (±3.5)	0.87	$y = (1.72\sqrt{x} + 2.22)^2$	20	9.5 (±3.7)	0.83	$y = (1.65\sqrt{x} + 1.96)^2$
Fumigation chamber								
Wing lice	24	40.5 (±5.6)	0.72	$y = (0.98\sqrt{x} + 5.16)^2$	18	41.1 (±6.5)	0.45	$y = (0.78\sqrt{x} + 4.75)^2$
Body lice	24	21.8 (±6.6)	0.75	$y = (1.59\sqrt{x} + 2.55)^2$	21	21.4 (±7.5)	0.55	$y = (1.46\sqrt{x} + 2.55)^2$

* Number of birds sampled.
† Mean (±1 SE) percentage of total abundance.
‡ Percent variation in total abundance explained by best fit regression line.
§ Best fit between x (number removed) and y (total abundance). $P < 0.0001$ in all cases. See Figures 3, 4.

over the complete data set (Table I). Dust-ruffling and the fumigation chamber removed an even smaller fraction of lice. Dust-ruffling was a good predictor of total abundance, whereas the fumigation chamber was only a fair predictor over the complete data set and a relatively poor predictor over the restricted data set, explaining only 45–55% of the variation in total abundance (Table I). Visual examination accounted for the smallest fraction of lice (≤10%) of any method. Nevertheless, it was a good predictor of the abundance of body lice (Table III) and a fair predictor of wing lice, at least over the complete data set (Table II).

Body washing removed the largest fraction of both wing and body lice; hence, it was the most reliable method at the louse infracommunity level (Bush et al., 1997). Thus, body washing is probably the most reliable of the 5 methods for assessing the species richness of avian louse communities. Watson and Amerson (1967) suggested that body washing can only be used on birds that are to be preserved in alcohol, skeletonized, or discarded. However, washing actually leaves birds in very good condition, with relatively little feather loss. The internal anatomy of washed birds in this study was in good enough shape to permit unhindered necropsies for internal parasites. Good-quality museum skins have been prepared in the lab of D.H.C. from several dozen species of washed birds, ranging in size from small-bodied passerines to large hawks. Washing can actually improve the quality of the skin by removing dirt and debris, enhancing the appearance of the completed skin.

Unlike KOH dissolution, which severely damages louse morphology, body washing causes no such damage. Washing does not affect the quality of lice for morphological or molecular studies; DNA can be extracted readily from washed lice (data not shown). The main drawback of body washing, aside from having to kill the host, is the need for a paint shaker, which can be expensive (US$1,000). Birds can also be washed manually using a jar (Lipovsky, 1951) or plastic bag (Clayton et al., 1992). However, the efficacy of manual washing (which is the only option under remote field conditions) has not been tested. Although body washing requires a little more time than some other methods (ca. 2 hr per bird), this is a small price to pay, in our opinion, given the method's impressive returns.

Like body washing, post-mortem-ruffling proved to be a reliable method. A major advantage of post-mortem-ruffling is its simplicity and portability. The materials required are minimal and easy to transport in the field, even to remote locations (Clayton et al., 1992). The method is also fast, particularly when the 3-min cutoff is used. This approach was nearly as accurate as the more laborious procedure of ruffling to the point of diminishing returns (Table I). A drawback of post-mortem-ruffling, aside from having to kill the host, is the need for a sheltered location for protection from wind. Feather loss can also be a problem, especially when attempting to ruffle birds that have been dead for more than a few hours. Freshly killed hosts are best. In addition to ruining the host specimen, loose feathers make finding lice on the collecting tray more difficult. The use of lightly colored paper on the tray makes it easier to find lice (Clayton and Walther, 1997).

Dust-ruffling has the advantage of not requiring that the host be killed, although it still kills the parasites. Although the efficiency of dust-ruffling to the point of diminishing returns was not compared to the use of an arbitrary cutoff, the latter would probably work as well for dust-ruffling as it did for post-mortem-ruffling. Like post-mortem-ruffling, dust-ruffling is simple and portable. However, it shares the disadvantage of feather loss and the need for a sheltered location. Species of birds with loosely attached plumage, such as doves, can lose quite a few feathers during this procedure. Dust-ruffling requires good bird-

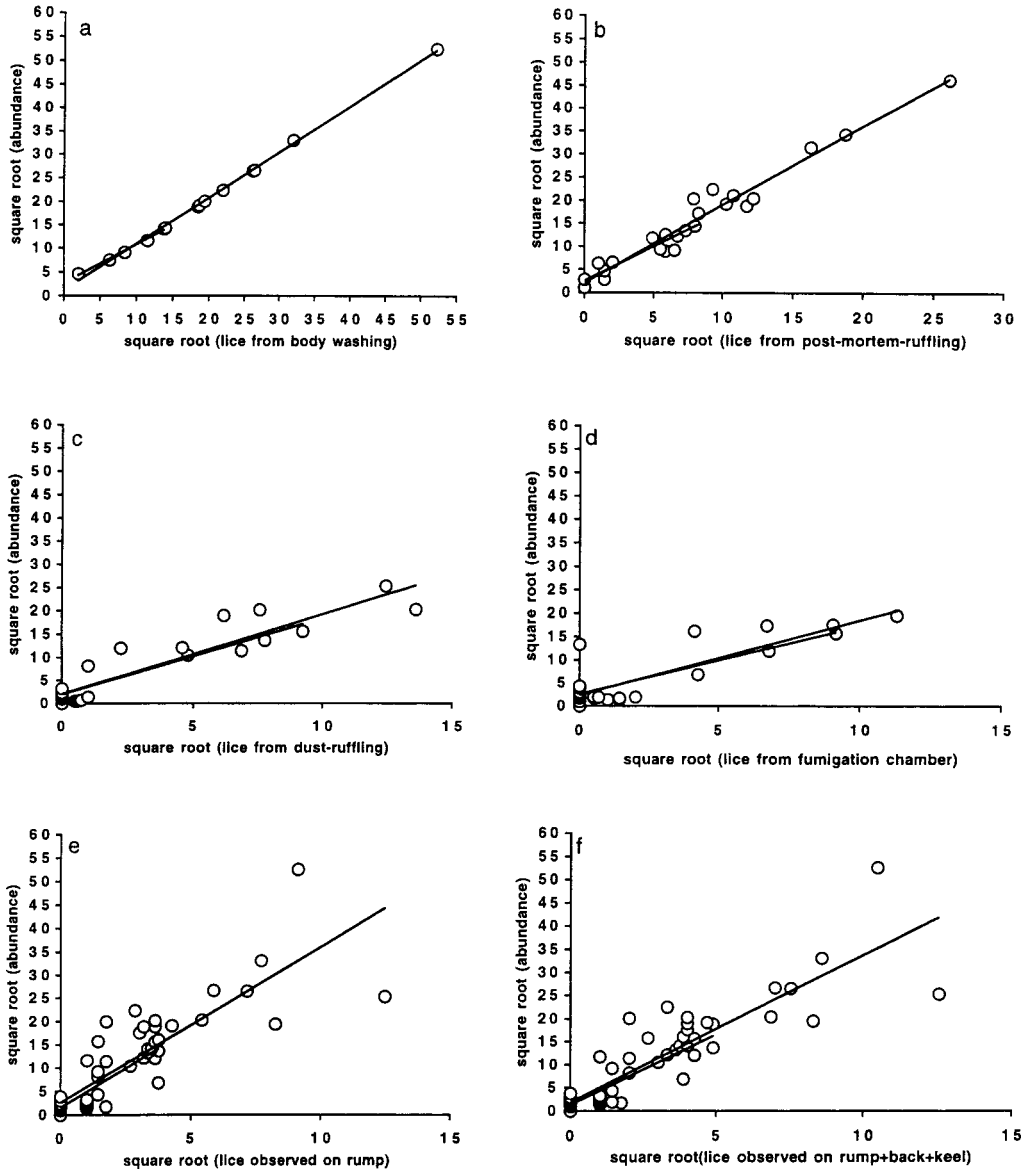

FIGURE 4. Relationship between total abundance of body lice and the number of body lice removed or observed using the 5 methods tested. Plots include best fit lines for the complete data set and the restricted data set (short segment). (**a**) Body washing. (**b**) Post-mortem-ruffling (3-min cutoff). (**c**) Dust-ruffling. (**d**) Fumigation chamber. (**e**) Visual examination (rump). (**f**) Visual examination (rump + back + keel).

TABLE II. Performance of visual examination for wing lice.

	Complete data set (n = 58 birds)		Restricted data set (n = 41 birds)	
Predictors	r^2*	Regression equation†	r^2*	Regression equation†
Univariate				
Wing	0.70	$y = (3.62\sqrt{x} + 3.54)^2$	0.53	$y = 2.47\sqrt{x} + 4.54)^2$
Tail	0.41	$y = (2.32\sqrt{x} + 9.04)^2$	0.21	$y = (1.85\sqrt{x} + 7.42)^2$
Wing + tail	0.66	$y = (2.48\sqrt{x} + 4.59)^2$	0.53	$y = (2.17\sqrt{x} + 4.12)^2$
Multivariate				
Wing, tail‡	0.73	$y = (3.05\sqrt{x_1} + 0.78\sqrt{x_2} + 3.53)^2$	0.54	$y = (2.29\sqrt{x_1} + 0.44\sqrt{x_2} + 4.38)^2$

* Percent variation in total abundance explained by best fit regression line.
† Best fit between x (number removed) and y (total abundance). $P < 0.0001$ in all cases. See Figures 3, 4.
‡ x_1 = wing, x_2 = tail.

TABLE III. Performance of visual examination for body lice.

Predictors	Complete data set (n = 58) r^2*	Complete data set (n = 58) Regression equation†	Restricted data set (n = 44) r^2*	Restricted data set (n = 44) Regression equation†
Univariate				
Rump	0.74	$y = (3.34\sqrt{x} + 2.59)^2$	0.74	$y = (3.36\sqrt{x} + 1.55)^2$
Back	0.72	$y = (7.45\sqrt{x} + 2.74)^2$	0.64	$y = (5.31\sqrt{x} + 2.53)^2$
Keel	0.49	$y = (11.26\sqrt{x} + 6.67)^2$	0.13	$y = (6.72\sqrt{x} + 4.80)^2$
Rump + back + keel	0.79	$y = (3.18\sqrt{x} + 1.87)^2$	0.78	$y = (3.05\sqrt{x} + 1.28)^2$
Multivariate				
Rump, back, keel‡	0.87	$y = (1.82\sqrt{x_1} + 3.78\sqrt{x_2} + 2.36\sqrt{x_3} + 1.62)^2$	0.80	$y = (2.35\sqrt{x_1} + 2.35\sqrt{x_2} - 0.30\sqrt{x_3} + 1.47)^2$

* Percent variation in total abundance explained by best fit regression line.
† Best fit between x (number removed) and y (total abundance). $P < 0.0001$ in all cases. See Figures 3, 4.
‡ x_1 = rump, x_2 = back, x_3 = keel.

handling skills to prevent undue feather loss and wing flaps, which can scatter lice far and wide. It is important to avoid getting dust in the eyes or mouth of the bird during the dusting step. The hands-on nature of dust-ruffling makes it impossible for a single person to ruffle more than 1 bird at a time (cf. fumigation chamber). Walther and Clayton (1997) noted that additional lice can be recovered from birds by putting them in a bag for at least 30 min *after* the dust-ruffling procedure.

Like dust-ruffling, fumigation chambers do not require the host to be killed. The method is also relatively ''hands off'', allowing several birds to be fumigated at once (e.g., Brown and Brown, 1996, fig. 2.8). Another advantage is that feather loss is minimized unless the bird is ruffled after fumigation (see below). A sheltered site is still required when transferring lice from fumigation chambers to vials. The principle disadvantage of this method is that it misses the head, making it desirable to add a visual search of the head, which reduces standardization and efficiency. The method also requires fumigation chambers, which can be bulky to transport in the field.

The most novel feature of our chamber design is the cloth collar (Fig. 1). The collar's drawstring allows it to be adjusted quickly if the bird shows any signs of distress. It is important to provide adequate ventilation because fumes can diffuse through the collar and affect the bird (and researcher). Most birds, especially small-bodied species, do very well in fumigation chambers. However, heavy-bodied species with delicate necks, such as White-tipped Doves (*Leptotila verreauxi*), should not be suspended in collars. When fumigating such species, the chamber can be turned on its side, allowing the bird to stand inside the jar. Although lice do not fall onto the filter paper in this position, they are recovered from the side of the jar after the bird has been removed.

Although the fumigation chamber removed a higher proportion of lice than dust-ruffling, it was still not very good at predicting the abundance of lice on birds with small infestations (Table I). This can probably be overcome by ruffling birds over a collecting surface after fumigation in the chamber. The addition of ruffling nearly doubled the number of lice recovered from birds in an earlier study (Walther and Clayton, 1997, fig. 2a). Although the addition of ruffling requires time, this is a small price to pay in exchange for more accurate data.

Ethyl acetate was used as a fumigant because it is safe and easy to obtain (see Materials and Methods). Ethyl acetate is less likely than chloroform to make birds drowsy or sick. On the other hand, chloroform is less flammable than ethyl acetate (or ether), and it detaches 76% of lice from feathers, compared to only 33% for ethyl acetate (Visnak and Dumbacher, 1999). Chloroform also detaches lice in a mere 5 min, reducing the handling time of birds. It may thus be the fumigant of choice under certain conditions, particularly when dealing with lightly parasitized birds.

CO_2 can be used in fumigation chambers to remove live lice from birds (e.g., Clayton et al., in press). CO_2 is even faster than chloroform, although it removes a significantly smaller percentage of the lice (Visnak and Dumbacher, 1999). It is critical to ruffle birds after CO_2 fumigation because ruffling provides up to an order of magnitude more lice (data not shown). Visnak and Dumbacher (1999) recommend CO_2 as the fumigant of choice for fumigation chambers because of its fast knockdown time. However, because CO_2 detaches <22% of lice on a bird, compared to the >75% in the case of chloroform, the latter may often be the best choice. Although lice removed by CO_2 can be revived and starved to eliminate host material in the gut, simplifying amplification of DNA for molecular studies (Visnak and Dumbacher, 1999), primers are now available that amplify DNA even from well-fed lice (data not shown).

Visual examination is the method of choice for estimating the abundance of lice in longitudinal studies where neither the parasites nor the host can be harmed. Like Lemke and Collison (1985), who visually estimated northern fowl mite populations on chickens, we found that visual examination accounts for only a small fraction of the lice on a pigeon. Nevertheless, the method predicts louse abundance with a degree of accuracy (Tables II, III). It accounts for a higher fraction of lice on birds with short plumage, such as swifts (Walther and Clayton, 1997).

The accuracy of visual examination depends on the microhabitat distribution of lice. It is important to examine the same regions in the same sequence to collect data that can be compared across individual hosts. It is also important to keep in mind that the results of visual examination can vary considerably among even closely related species. For example, *Columbicola passerinae* is found mainly on the head of its host,

the Common Ground-dove (*Columbina passerina*) (data not shown), despite being a close relative of *C. columbae*, the feral pigeon wing louse. Any attempt to quantify the abundance of *C. passerinae* by only examining the wings and tails of the host would be doomed to failure. When using visual examination, it is also important to note whether birds are in molt because lice avoid molt by hiding in the shafts of newly emerging feathers, where they are not visible (Moyer et al., in press).

Visual examination requires little equipment and can be done in any location. Good bird-handling skills are needed to help keep track of which regions have already been examined. Visual examination can estimate the abundance of other avian ectoparasites (Clayton and Walther, 1997). However, it is not a reliable method in the case of highly mobile parasites, such as hippoboscid flies or fleas, that can abandon the host or can move about the body of the host so rapidly that the same individuals are likely to be counted repeatedly.

Tables I–III provide equations for the 5 methods tested that can be used in conjunction with new data sets to predict total abundance. Strictly speaking, these equations are applicable only to the pigeon–louse system tested. Furthermore, they should be used only for birds with louse populations that are within the range of those tested. With caution, however, the equations may work for other bird–louse systems.

The 5 methods tested varied substantially in the fraction of lice removed from the host. For example, body washing removed >87% of body lice, whereas dust-ruffling removed <13% of body lice. Percent removal is an important consideration for studies needing to maximize the number of lice collected, such as when working with small-bodied or other host species that tend to have very few lice to begin with. The methods tested were generally better at removing wing lice than body lice, presumably because wing lice are on the extremities and are more easily dislodged. The methods were also better at predicting wing louse abundance, although the difference between wing and body lice in this regard was less pronounced (Table I).

Accuracy is not the only criterion when choosing a method. Logisitics and fate of the host and parasites are also important factors. Figure 5 summarizes these criteria in the form of a decision tree. In cases where longitudinal monitoring of louse populations is required, visual examination is the method of choice because it does not harm the lice. If the lice can be killed, the next most relevant question is whether the host can also be killed. When this is not an option (e.g., in long-term studies of host biology), then fumigation chambers or dust-ruffling are the methods of choice. The 2 methods involving dead birds provide better results than the 3 live-host methods. Post-mortem-ruffling is the method of choice under field conditions or when resources are not available to purchase a paint shaker. When possible, however, body washing provides the best returns and predictive power of any method, and it is somewhat easier on the host specimen than post-mortem-ruffling, at least in terms of reducing feather loss.

The 5 methods tested can also be used to quantify chewing lice on mammals. The simple structure of hair, compared to feathers, means that combing can also be an effective means of removing lice from live or dead mammals. The density of the hair usually makes it necessary to use a fine comb, toothbrush, or grout brush for efficient combing. General reviews of methods for quantifying lice and other ectoparasites of mammals are provided by Ignoffo (1958), Marshall (1981), Gardner (1996), and Southwood and Henderson (2000). Discussions of particular methods for mammals can be found in Henry and McKeever (1971) for body washing, Ulmanen and Myllymäki (1971) for post-mortem-ruffling, Cyprich et al. (1985) for fumigation chambers, and Barnard and Morrison (1985) for visual examination. KOH dissolution has also been used by several authors to quantify mammal lice (e.g., Kim, 1972).

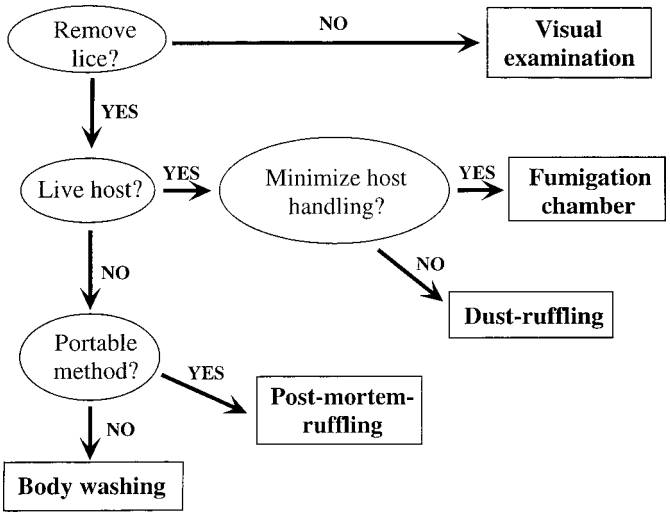

FIGURE 5. Decision tree for choosing among 5 methods for quantifying chewing lice.

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