vicinity of the intake pipe and gaining entry via the incoming unfiltered seawater. *Loma salmonae* has caused significant economic problems in chinook salmon sea-pen culture in British Columbia (Ramsay et al., 2002). Consequently, the infection might represent a major pathogen to cod culture, not only in hatcheries but also in grow-out sea cages based on observations of morbidity and mortality from the present study.

Presence of xenomas in all 41 Atlantic cod originating from a cod-ranching facility suggests that the fish probably acquired the infection on site. Shaw et al. (1998) reported that transmission of *Loma salmonae* by spores, fed per os, developed within 24 hr when compared to other methods, such as cohabitation. These authors also noted that autoinfection from spores released at the initial site occurred and that uninfected fish acquired the infection after spores were released into the water. Ramsay et al. (2002) reported that xenoma formation was detected at week 5 and persisted in coho and chinook salmon up to week 9. It appears likely, then, that the caged cod, which were held together for almost 1 yr, acquired the infection of *Loma branchialis* by cohabitation, as noted previously in species of salmonids (Ramsay et al., 2002). Recently, Rodriquez-Tovar et al. (2003) provided ultrastructural evidence of autoinfection with *Loma* sp. in the gills of Atlantic cod. The results from the present and other studies support the view that autoinfection was responsible for secondary infection in hosts infected with *Loma* spp.

Prevention of *Loma branchialis* in the hatchery system at the OSC appears to be unlikely unless the filtration system is geared to remove spores 4–7 μm in diameter. Fumagillin has been used to control *Loma salmonae*, but it has not been approved for use in aquaculture. An alternative approach to control the infection was used by Sanchez et al. (2001a), who reported that rainbow trout previously infected naturally with a low-virulence variant of *Loma salmonae* exhibited reduced numbers of xenomas following challenge with spores fed per os. In contrast, more xenomas were observed in naive controls. This low-virulence variant originated after several serial passages through brook trout (*Salvelinus fontinalis* (Mitchill)) (Sanchez et al., 2001b). Consequently, experimental trials to produce a low-virulence variant of *Loma branchialis*, which eventually could be incorporated in the feed, appears to be a feasible alternative to drug therapy. Additionally, Beaman et al. (1999) reported temperature as a factor to control development of spores of *Loma salmonae*. Rainbow trout held at 5 and 21 C failed to develop xenomas, probably because of interruption of their development. Cod are adapted to live at low temperatures (Scott and Scott, 1988). Therefore, cultivation at 5 to 6 C might be appropriate to control development of the spores of *Loma branchialis* entering the seawater supply.

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Detection of Interspecific Competition in Parasite Communities

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ABSTRACT: Matrices of correlation coefficients between the abundances or intensities of all pairs of helminth species, across all individual hosts in a sample, are regularly used to detect possible cases of interspecific competition in parasite communities. In these matrices, however, the range of possible values that any correlation coefficient can take is not −1 to 1, contrary to what is generally assumed. The number and magnitude of other correlation coefficients in a matrix will constrain the values that any given correlation can achieve. This property of matrices, and of inter-related natural variables, is explained and illustrated with 2 examples from real helminth communities. As a rule, the pres-

TABLE I. Matrices of pairwise correlations, and their possible ranges, between the abundance or intensity of infection of 3 helminth species in either the bank vole, *Clethrionomys glareolus*, or the trout, *Salmo trutta.**

	Species A	Species B	Species C
Bank vole (abundance)			
Species A	—	−0.160	−0.320
Species B	$-0.901 \leq r_{AB} \leq 0.453$	—	0.700
Species C	$-0.609 \leq r_{AC} \leq 0.385$	$-0.884 \leq r_{BC} \leq 0.986$	—
Trout (intensity)			
Species A	—	−0.530	−0.150
Species B	$-0.909 \leq r_{AB} \leq 0.992$	—	−0.276
Species C	$-0.669 \leq r_{AC} \leq 0.961$	$-0.759 \leq r_{BC} \leq 0.918$	—

*Values above the diagonal are the correlation coefficients observed; the upper and lower bounds of the possible range of values for each coefficient, given the other 2, are shown below the diagonal. In bank vole, species A, B, and C are, respectively, *Heligmosomum mixtum, Mastophorus muris,* and *Capillaria* sp; in trout they are *Acanthocephalus anguillae, Echinorhynchus truttae,* and *Cyathocephalus truncatus* (data from Haukisalmi and Henttonen, 1993; Dezfuli et al., 2001).

ence of many negative correlations in a matrix raises the lower value that any of them can possibly achieve. This has important but previously overlooked implications for the interpretation of correlation coefficients, and the detection of competition in natural parasite communities.

Experimental studies have presented clear-cut evidence of interspecific interactions between helminth species in their definitive host. In such experiments, parasites of 1 species typically establish in different regions of the gut when they share the host with a second helminth species than when they occur alone, thus minimizing their spatial overlap with the potential competitor (e.g., Holmes, 1961; Patrick, 1991). Other experiments have demonstrated marked reductions in the abundance of 1 helminth species in concurrent infections with a second species, relative to the abundance it achieves in single-species infections (Dash, 1981; Holland, 1984). Although this type of evidence is convincing, it is not easily reconciled with field data (Poulin, 2001). Natural helminth communities consist of several potentially interacting species, and interactions among these species can involve direct as well as indirect effects; this is in sharp contrast with the simple 2-species systems typical of laboratory studies.

The most common way to detect interspecific competition or other forms of interactions in natural helminth communities has been to build a matrix of correlation coefficients between the abundances or intensities of all pairs of helminth species, across all individual hosts in a sample (see Moore and Simberloff 1990; Lotz and Font, 1991; Haukisalmi and Henttonen, 1993; Dezfuli et al., 2001). It is then standard practice to infer competition, or facilitation, or other interaction from the statistically significant coefficients in the matrix. This allows only weak inference, but is often the only approach possible. Recently, Brown et al. (2004) discussed the problems associated with inference from correlation matrices. As they point out, when some pairwise relationships in the matrix are negative, the magnitude of the correlation coefficients may be mathematically constrained. For example, consider a parasite community consisting of 3 helminth species, A, B, and C. Let's assume that we can set up 3 simpler communities, each consisting of a different pair of helminth species, and that we demonstrate very strong competition in all pairs, such that the correlation coefficients between the abundances or intensities of the species in each pair (r_{AB}, r_{AC} and r_{BC}) are all close to −1. Now that we know there are direct negative interactions between all pairs, what would be the outcome of placing all 3 species in the same system? When all 3 species are present, the 3 correlation coefficients cannot be −1 because the range of possible values that a coefficient can take is now constrained by the values of the other coefficients. If 2 of the values approach −1, the third must approach 1. If all 3 coefficients are negative and of similar magnitude, it can be shown that the lowest value they can achieve is −0.5, even if it reaches −1 when only 2 species are involved. The more species in the system, the more pronounced the constraints. Brown et al. (2004) show that if there are 11 competing species in a system, and if all pairwise correlation coefficients among them are negative and roughly equal, the lowest value they can achieve would be −0.1. In complex systems, detecting significant negative interactions becomes impossible. Here, I discuss the implications of the paper by Brown et al. (2004) and illustrate them using data from helminth communities.

Constraints on the magnitude of correlation coefficients arise from a property of correlation matrices known as the positive semidefinite condition (Rao, 1973; Brown et al. 2004). Simply put, in a symmetric $n \times n$ matrix, where n is the number of species, all eigenvalues of the correlation matrix must be non-negative. Other, more formal definitions, are provided by Rao (1973). Consider the following correlation matrix, in which each coefficient represents the pairwise correlation between the abundances of 3 helminth species, A, B, and C:

$$R_3 = \begin{pmatrix} 1 & r_{AB} & r_{AC} \\ r_{AB} & 1 & r_{BC} \\ r_{AC} & r_{BC} & 1 \end{pmatrix}.$$

Entries above the diagonal are the same as those below, hence the symmetric nature of the matrix. Under the constraints imposed by the positive semidefinite condition, once 2 coefficients are known, only a certain range of values is possible for the third one (i.e., the third coefficient cannot take any value between −1 and 1, which are the usual bounds for a single correlation). Thus, if r_{AC} and r_{BC} are given, then we can compute the lower and upper bounds of the possible range for r_{AB}:

$$\text{Lower bound} = r_{AC}r_{BC} - \sqrt{1 - r_{AC}^2 - r_{BC}^2 + r_{AC}^2 r_{BC}^2}$$

$$\text{Upper bound} = r_{AC}r_{BC} + \sqrt{1 - r_{AC}^2 - r_{BC}^2 + r_{AC}^2 r_{BC}^2}.$$

Brown et al. (2004) provide further details. What matters here are the implications of this limited range. If all pairwise correlations in a matrix are negative, they cannot all be very strong. If 2 pairwise correlations are strongly positive, the third cannot be strongly negative. An examination of a couple of real examples will help to illustrate the importance of these constraints.

The bank vole, *Clethrionomys glareolus*, from Pallasjärvi in Lapland, Finland, is host to a few gastrointestinal helminth species, including the nematodes *Heligmosomum mixtum*, *Mastophorus muris*, and *Capillaria* sp. (Haukisalmi and Henttonen, 1993). Whereas there is a strong and significant positive correlation between the abundances of *M. muris* and *Capillaria*, both of these species correlate negatively and nonsignificantly with *H. mixtum* (Table I). The statistical significance of correlation coefficients is assessed independently for each coefficient; whether the coefficient is part of a matrix of pairwise correlations or whether it is a single isolated value does not affect the test of significance. In nature, associations between pairs of species do not exist in an ecological vacuum, but instead they are just one of many interactions within a complex network. If we use the other correlation coefficients in the 3-species matrix to compute the lower and upper bounds of the range of values that each correlation can take in the context of this network, we can see how the correlations influence each other (Table I). The correlation between *H. mixtum* and *Capillaria* stands at −0.32; although not weak, it fails a test of significance and cannot be taken as evidence of some form of competition between these species. However, given that the lowest value it could possibly achieve is not −1 but −0.61, because of the other correlations in the matrix, can we dismiss the possibility of a negative interaction between these 2 species? I would hesitate to do so.

Another example is provided by the helminth community of brown trout, *Salmo trutta*, from the San Giorgio stream in northern Italy (Dezfuli et al., 2001). Among individual hosts, pairwise correlations between the intensities of the cestode *Cyathocephalus truncatus* and the acanthocephalans *Acanthocephalus anguillae* and *Echinorhynchus truttae* are all negative, although only the correlation between the latter 2 species is statistically significant (Table I). The correlation between *C. truncatus* and *E. truttae* is -0.276 and almost significant ($P < 0.10$). Again, however, the other correlations constrain the range of values that the *C. truncatus-E. truttae* correlation can take: its lowest possible value is not -1, but -0.76. Should this change our view of the possibility of competition between these species? I suggest it should.

For the sake of simplicity, I considered only 3 species in each of the above examples; in fact, both helminth communities include additional species. Including these species would have reinforced the constraints in the correlation matrices, and thus reinforced the point I want to make. And this point is simply that correlation coefficients are not independent of each other, and must be interpreted with greater caution. Standard statistical tests do not take into account the constraints limiting the range of values of one coefficient exerted by the other coefficients in the matrix.

Are these mathematical constraints nothing but statistical artifacts, or do they also reflect limitations acting on the associations between species in complex systems? The constraints affect the possible ways in which real variables can be interrelated (Brown et al., 2004). With 3 coexisting and competing species, it is simply impossible for the abundances of all 3 pairs to be strongly negatively correlated: if we have correlations of -1 between species A and B, and between species A and C, then the correlation between B and C just cannot also be strongly negative. This is not a mathematical artifact; it is plain common sense. Indirect and direct effects are balanced in such 3-way interactions. Thus, the limited range of values that correlation coefficients can take in multispecies scenarios reflects real phenomena and the mechanisms that produce them. In some situations, the use of partial correlations may help uncover the true underlying relationship between any 2 species. Partial correlations hold the influence of a third species mathematically constant while the association between the first 2 species is evaluated. However, this approach is not without pitfalls of its own, and is not a general solution to the problem (see Carnes and Slade, 1988; Brown et al., 2004).

Competition is a negative interaction, and the negative correlations that competition generates in species-by-species matrices create problems for the ecologist trying to detect significant interactions. If all species in a system are competing, then the absolute magnitude of correlation coefficients among their abundances necessarily decreases with an increasing number of species. The more species in a helminth community, the weaker the evidence for direct pairwise competition is likely to be. In many helminth communities, positive associations appear to outnumber negative ones in correlation matrices (Bush and Holmes, 1986; Lotz and Font, 1994; Holmstad and Skorping, 1998). For instance, among the 16 most common species in the helminth community of lesser scaup ducks, *Aythya affinis*, a total of 120 pairwise correlations were computed on intensity data by Bush and Holmes (1986). All of these were positive, and 31 were statistically significant. In communities in which negative relationships are rare, constraints on the magnitude of correlation coefficients are relaxed. It must be pointed out, however, that the excess of positive correlations in several studies may lead to an underestimation of the importance of competition. Two factors can contribute to an apparent excess of positive correlations. First, the constraints on the lower bound of the range of possible values that a correlation coefficient can take, imposed by the positive semidefinite condition, can *weaken* any existing negative correlation between any 2 species. Second, the methods used to detect nonrandom associations between the occurrence or abundance of 2 species are more sensitive to positive associations than to negative ones (Haukisalmi and Henttonen, 1998). In other words, these methods are more likely to detect a positive covariance than a negative one of comparable absolute magnitude (also see Appendix IV in Vickery and Poulin, 2002). These different processes combine to make it difficult to detect interspecific competition in complex natural communities.

The use of measures of overall association among the parasite species in a community can overcome some of the problems arising from the interdependence among pairwise associations. The overall association among all species in the community can be assessed by averaging all pairwise associations, or by considering their frequency distribution (Gotelli and Graves, 1996; Haukisalmi and Henttonen, 1998). For instance, the observed distribution of pairwise associations can be compared to the distribution expected from some null model of random associations (Gotelli and Graves, 1996). This approach can tell us whether positive or negative associations are more or less important in determining the structure of the parasite community as a whole than what we might expect from chance alone. It will not, however, tell us whether there exists a significant association between any given pair of species; this can only be answered using pairwise correlations subject to the pitfalls explained above.

In the end, it will be difficult to replace simple experimental situations in which 2 species are allowed to compete as a source of strong evidence that competition exists. Correlations between species abundances in natural systems are not easy to detect, primarily because of the substantial sampling heterogeneity resulting from the distribution of parasite populations (see Haukisalmi and Henttonen, 1998). When such correlations are found, they do not necessarily imply causation. However, because this is usually the only approach that can be taken to understand the functioning of complex natural systems, it would be advisable to consider the interdependence that arises in multivariate correlation analyses and the constraints it imposes on the values of the coefficients.

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The First Record of *Dirofilaria immitis* Infection in a Humboldt Penguin, *Spheniscus humboldti*

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ABSTRACT: *Dirofilaria immitis* infection is an important parastic disease in many mammals, especially canids, but has not been reported in bird hosts. Filarial worms were isolated from the lumen of the right atrium of the heart and the connective tissue of the lung of a captive female Humboldt penguin, *Spheniscus humboldti*, that died at a zoo in Japan. One of these worms was observed morphologically and identified as *D. immitis* by features such as 4 pairs of cephalic papillae, 1 pair of cervical papillae, esophagus divided into 2 regions, 4 pairs of pre-anal papillae, 5 pairs of post-anal papillae, and unequal spicules. In addition, the partial DNA sequence (234 bp) of mitochondrial COⅠ gene of the filarial worm was identical to that of *D. immitis*. This is the first report of *D. immitis* infection in a bird.

Dirofilaria immitis infection is one of the most important parasitic diseases in canids, especially domestic dogs. The filarial species has also been detected in many other mammals such as cats, bears, seals, ferrets, and humans, though the species does not always mature in these hosts (Kamiya et al., 1977; Ohishi, 1986; Murata et al., 2003). However, *D. immitis* has not been found in avian hosts. We detected *D. immitis* in the lumen of the heart of a captive female Humboldt penguin, *Spheniscus humboldti*, that died at a zoo in Japan. This is the first reported bird case of *D. immitis* infection.

The female penguin was born at a zoo in Aichi Prefecture on 25 June 1997 and transferred to Akita Municipal Omoriyama Zoo, Akita Prefecture on 8 April 2003; at that time, the body weight was 2.94 kg. On May 6, the penguin was found to be physically weakened and was immediately isolated. Body weight and temperature were 2.72 kg and 34.8 C, respectively. Although antibiotics (oxytetracycline, 300 mg and pyrimethamine, 0.2 ml) were administered intramuscularly, and the bird was force-fed its favorite food, physical condition did not improve and the penguin died on May 7. The penguin was necropsied. Congestion was observed in the heart, lung and kidney. The liver was brittle and dark greenish-brown. A few filarial worms were found in the lumen of the right atrium of the heart and the subserous connective tissue of the lung. Localized necrotic lesions were found macroscopically in the heart. These lesions were confirmed by histological examination to be necrotic and thromboembolic myocarditis. Neither microfilariae nor the adult female antigen using Solo Step™ CH (Heska, Fort Collins, Colorado) were detected in fluid collected from the frozen lung and liver. One male worm obtained from the lumen of the heart was fixed in 5% formalin, cleared in lactophenol solution, and then observed under light microscopy.

Genomic DNA was extracted using an E.Z.N.A.Mollusc DNA Kit (Omega Bio-tek, Inc., Doraville, Georgia) from the male worm examined morphologically and from *D. immitis* obtained from a dog and a raccoon dog in Iwate Prefecture. Fragments (about 250 bp) of DNA coding mitochondrial cytochrome oxidase Ⅰ (COⅠ) gene were amplified by nested PCR. Primer sets of Di3 (5′-TGGCTTTTCCTCGTGTTAATGC-3′), Di4 (5′-AAGTCCCCAATACAGCAATCC-3′), of Di5 (5′-CTTTGAGTGTAGAGGGTC-3′), and Di6 (5′-ATTACCCCCCTTATTAGC-3′) that were designed on the basis of the mitochondrial DNA sequence of *D. immitis* (accession number AJ537512) deposited in the GenBank were used for the first PCR and second PCR, respectively. The amplification was done at 94 C for 1.5 min, 40 cycles at 94 C for 1.5 min, 53 C for 1.5 min, and 72 C for 2 min, and then 72 C for 10 min, with a final volume of 25 µl containing 12.5 pmol of each primer, 0.4 mM 4×dNTPs, 1.25 U Taq polymerase (Promega Corporation, Madison, Wisconsin), 1×Taq polymerase buffer, and 1.5 mM $MgCl_2$. The PCR products were sequenced using Di5 and Di6 and a Bigdye Terminater V 3.0 Cycle sequencing Kit (Applied Biosystems, Tokyo, Japan) on an automated sequencer ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Each sample was sequenced twice.

The male worm was 105 mm in length and 0.58 mm in maximum width. Four pairs of cephalic papillae and a pair of small cervical papillae were located in the anterior region. (Figs. 1, 2). The esophagus was 1.28 mm long and divided into the anterior muscular and the posterior glandular parts (Fig. 1). The distances from the nerve ring and the cervical papillae to the anterior end were 0.368 mm and 0.648 mm, respectively. The body surface had short and low longitudinal ridges (Fig. 3). The tail was spirally coiled and rounded at the tip (Fig. 4). The tail length was 0.11 mm. Four pairs of pre-anal papillae were digitiform. Five pairs of post-anal papillae were relatively small (Figs. 5, 6). The first pair was immediately behind the anus, and the second pair was pedunculate and larger than the other 4 pairs. A pair of phasmids was present at the tail tip. The spicules were unequal and dissimilar (Fig. 4); the left was slender with a sharp tip and 0.37 mm long, and the right was short and wide with a blunt tip and 0.19 mm long.

Filarial worms such as those belonging to *Sarconema*, *Parachandlerella*, *Splendidofilaria*, and *Cardiofilaria* that have been detected in birds not only principally parasitize the cardiac muscle and connective tissue rather than the lumen of the heart (Yamaguti, 1961; Itagaki, 1997; MacNeil, 1995), but are also remarkably different from *D. immitis* in body size and morphological features. *Dirofilaria* contains more than 30 species (Yamaguti, 1961; Uni, 1978), and the 3 species, *D. immitis*, *D. ursi*, and *D. repens*, are distributed throughout Japan (Yamaguti, 1935; Uni, 1978, 1983; Ohishi, 1986, 1995). Morphometric comparison of the present worm with males of the three species is shown in Table I. *Dirofilaria ursi* has been detected in black and brown bears and is morphologically characterized as having body dilatation in the posterior region of the esophagues, no cervical papillae, and a larger left spicule (0.50–0.61 mm) than those of *D. immitis* (Yamaguti, 1935; Uni, 1978, 1983). In contrast, *D. repens* is 48–70 mm long, 0.37–0.45 mm wide, and 66–85 µm in distance between the anus and tail tip; it has 5–6 pairs of pre-anal papillae, 2–4 pairs of post-anal papillae, and a left spicule of 0.465–0.59 mm in length (Ohishi, 1995). On the basis of these morphological features, the present worm was identified as *D. immitis*.

The 234 bp sequences determined using the primer Di6 were compared among samples. The sequence of the present worm was identical to those of *D. immitis* from a dog and a raccoon dog (GeneBank™