steranes involved the coupling of 5β-cholanyl chloride with either bromomagnesium diisopropylcuprate [$(i\text{-}Pr)_2CuMgBr$] or isopropylmagnesium bromide (i-PrMgBr) in the presence of vanadium trichloride (*11*) to produce the ketones **6**. The Wittig reaction of **6** with the ylid derived from *n*-propyl triphenylphosphonium bromide [$(Ph_3PCH_2CH_2CH_3)Br$] furnished the olefins (**7**) as an *E-Z* mixture. Hydrogenation of these olefins with 10% Pd on C provided the desired alkanes (**1bA** and **1aA**) as a diastereomeric mixture at C-24. Significant impurities (3 to 5%) of 5β and 5α-24-methylcholestane were detected by GCMS analysis of the 5β and 5α-24-*n*-propylcholestane products, respectively, which were generated in a retroene reaction during the catalytic hydrogenation step. {A retroene process is the reverse of the "ene synthesis" [W. Oppolzer and V. Snieckus, *Angew. Chem. Int. Ed. Engl.* **17**, 476 (1978)] in which a hydrocarbon RH formally adds to an olefin.} They were removed by preparative, nonaqueous, reversed-phase HPLC. No attempt was made to resolve 24*R* and 24*S* isomers because the isolated C_{30}-sterane should be a mixture of both isomers. Epimerization of the C-24 position occurs early in sediment diagenesis (*12*). The synthetic route to the isopropylcholestanes (**1bD** and **1aD**) involved addition of i-PrMgBr to 5β or 5α methyl cholanate to produce principally the tertiary alcohol **8**. Dehydration of **8** with phosphorus oxychloride led to a mixture of olefins that was hydrogenated at high pressure to give impure samples of **1bD** and **1aD**. The principal difficulty with this route was that the competitive reduction of the intermediate ketone **6** by i-PrMgBr led to a small but significant amount of a secondary alcohol that cochromatographed with **8**. This contaminant led ultimately to 5β and 5α-cholestane, which were removed by HPLC.

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Effects of Fish and Plankton on Lake Temperature and Mixing Depth

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A comparative study of small temperate lakes (<20 square kilometers) indicates that the mixing depth or epilimnion is directly related to light penetration measured as Secchi depth. Clearer lakes have deeper mixing depths. This relation is the result of greater penetration of incident solar radiation in lakes and enclosures with high water clarity. Data show that light penetration is largely a function of size distribution and biomass of algae as indicated by a relation between the index of plankton size distribution (slope) and Secchi depth. Larger or steeper slopes (indicative of communities dominated by small plankton) are associated with shallower Secchi depth. In lakes with high abundances of planktivorous fish, water clarity or light penetration is reduced because large zooplankton, which feed on small algae, are reduced by fish predation. The net effect is a shallower mixing depth, lower metalimnetic temperature and lower heat content in the water column. Consequently, the biomass and size distribution of plankton can change the thermal structure and heat content of small lakes by modifying light penetration.

THERMAL STRATIFICATION OF THE water column occurs in most temperate lakes during late spring and summer (*1*). The epilimnion or upper mixed layer, with a fairly uniform temperature, is separated from the cooler bottom layer or hypolimnion by a middle layer or metalimnion where temperature drops rapidly with depth. The mixing depth of a lake has a fundamental influence on the physical, chemical, and biological processes that take place in the water column (*2–5*).

Wind-induced transport of heat to deeper strata has been considered more important in determining the mixing depth than the direct absorption of solar energy (*3, 6*). In this report, we show that water clarity, associated with the biomass and size distribution of plankton (algae and zooplankton), can influence the mixing depth and heat content of small lakes. Physical characteristics of lakes, such as light and temperature, have a profound influence on their biology, but the effect of organisms on lake physics has been uncertain and generally thought to be of secondary importance.

Solar irradiance may be reflected at the surface, backscattered by planktonic particles to the atmosphere, and absorbed as heat by water. The balance between the two losses and heat absorption determines the heat content of the surface mixed layer. Secchi depth, an index of light penetration, can be as shallow as 0.5 m or less in hypereutrophic and highly colored (brown-water) lakes (*7*) or as deep as 20 m in clear lakes, such as Lake Tahoe (*8*). The deeper the Secchi depth, the greater the absorption of solar radiation by the water, and the smaller the loss of solar energy to the atmosphere (*9*).

Algae have a major influence on water clarity and attenuation of light in noncolored lakes (*7, 10*) and in oceans (*11*). Light is both scattered and absorbed by algae, and the effects depend on their size distribution and biomass. Smaller algae (pico- and nanoplankton, 0.2 to 20 μm) have greater absorption and scattering per unit mass than larger algae (>20 μm) because their surface area per unit mass is larger (*12*). Fish predation on large zooplankton, which graze mostly on small algae, is a strong determinant of the biomass and size distribution of algae (*13*). This effect has been supported by many experimental and empirical studies where low abundance of planktivorous fish was associated with high abundance of large zooplankton, low biomass of algae, and improved water clarity (*13–15*). Therefore fish predation, and the resulting size distribution and biomass of plankton, might influence thermal structure of small lakes by changing water clarity where wind-induced transport of solar heating to deeper water is less important.

We performed a series of manipulation experiments in large enclosures situated in Lake St. George, Toronto, Ontario (*16*). During May through August, 1986 and 1987, we collected data on the size distribution and biomass of the entire plankton community, from bacteria to zooplankton, measured as particulate phosphorus (*PP*) (*17*). We also measured size distribution and abundance of *Daphnia*, algal biomass (chlo-

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rophyll a) in two size categories (1.2 to 20 μm and >20 μm), and water clarity. Water temperature was measured at each meter depth, and the depth of epilimnion or mixing depth (*1*) and heat content (*18*) were calculated. To test our hypothesis further, we collected similar data from Lake St. George itself (4.3 ha, maximum depth 15 m) and Haynes Lake (2.1 ha, maximum depth 16 m) which had contrasting abundances of planktivorous fish. Similar data were also collected from 27 small Ontario lakes [surface area = 0.12 to 17 km^2, fetch (*19*) <10 km, maximum depth >10 m, and oligotrophic to hypereutrophic] during August 1988, to search for a relation between water clarity and mixing depth.

The effects of fish predation on plankton communities and associated water clarity were similar in the enclosures and in the lakes (Figs. 1 and 2). *Daphnia* were larger and more abundant in the enclosures (control) without fish and in the lake (Haynes Lake) with few planktivorous fish. These large *Daphnia* were associated with reduced biomasses of algae, especially algae <20 μm, reduced biomass (*PP*) of pico- and nanoplankton (0.2 to 20 μm), and smaller or shallower index (slope) of plankton size distribution (*17*). The net effect was a significant (paired *t* tests done separately for enclosures and lakes, for all dates together for the whole season or each month, $P < 0.01$) increase in water clarity (Figs. 1 and 2). Among the enclosures with and without fish, Secchi depths were not significantly different only during May (Fig. 2A). Our observation that fish can change water clarity by changing the size distribution and biomass of plankton is consistent with many earlier studies where changes in the abundance of planktivorous fish changed water clarity (*13–15*).

Temperature at the surface was higher in the enclosures without fish on all four dates (Fig. 2). In all of the enclosures, mixing depths were poorly defined in May. As the season progressed, mixing depth increased in the enclosures without fish, but not in the enclosures with fish (Fig. 2A). The water temperature at specific depths in the metalimnion (4 to 8 m) was 3° to 8°C higher in the enclosures without fish.

Independent observations from two lakes also support the concept that fish, by changing size distribution and biomass of algae and associated water clarity, can influence the thermal structure of small lakes (Fig. 2B). In May 1987, temperature profiles were similar for both lakes, but as the summer progressed, these two lakes exhibited increasingly different temperature profiles. Mixing depths were deeper in Haynes Lake ($P < 0.05$, for four weekly measurements from each month). In July and August, temperature at specific depths in the metalimnion (4 to 8 m) were 6° to 13°C higher in Haynes Lake than in Lake St. George.

Fish also influenced the heat content of enclosures and lakes (Fig. 2, A and B). Enclosures without fish had higher ($P < 0.001$, for eight measurements per treatment per month) heat content than the enclosures with fish. Similarly, the heat content was higher ($P < 0.005$, for four measurements per month) in Haynes Lake than in Lake St. George during May through August. Haynes Lake gained heat continuously during this period, while Lake St. George gained heat only during spring and early summer, and lost heat after July. If wind-induced transport of heat associated with lake morphometry was the principal force in regulating the mixing depth of these lakes, then Lake St. George should have had a deeper mixing depth and greater heat content than Haynes Lake because its fetch is twice as long as for Haynes Lake, but the opposite was observed.

Our suggestion that water clarity is a function of biomass and size distribution of

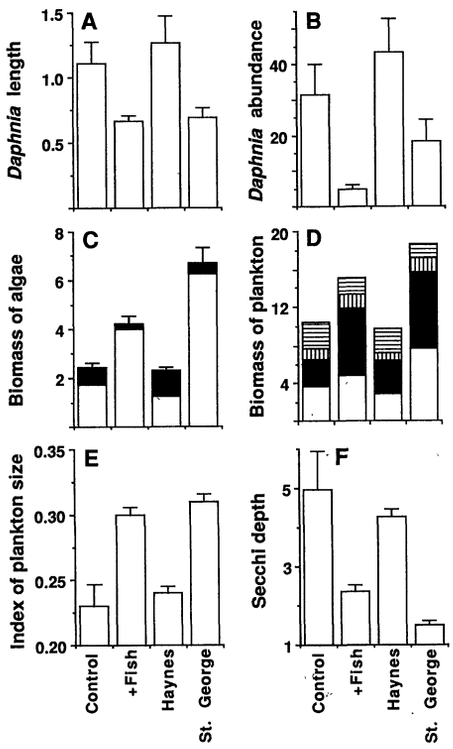

Fig. 1. Seasonal means from weekly samples of (**A**) length of *Daphnia* in millimeters; (**B**) abundance of *Daphnia* (individuals per liter); (**C**) biomass of algae (micrograms of chlorophyll a per liter); algae <20 μm, clear; algae >20 μm, dark; (**D**) biomass of plankton (micrograms of particulate phosphorus per liter) in size classes of 0.2 to 1 μm, clear; >1 to 20 μm, dark; >20 to 200 μm, vertical lines; and >200 μm, horizontal lines; (**E**) index (slope) of plankton size distribution (*17*); and (**F**) Secchi depth (in meters). Error bars are 1 SE of the mean, based on weekly samples for (A), (B), (C), and (F), and on biweekly samples for (D) and (E). In (C), error bars are for total biomass. For clarity of illustration, error bars are not shown in (D), but error bars in (E) are indicative of variation in (D). The abundances of planktivorous fish were 0 and 8952 fish per hectare in the control and +Fish enclosures, and 98 and 5500 fish per hectare in Haynes Lake and Lake St. George, respectively.

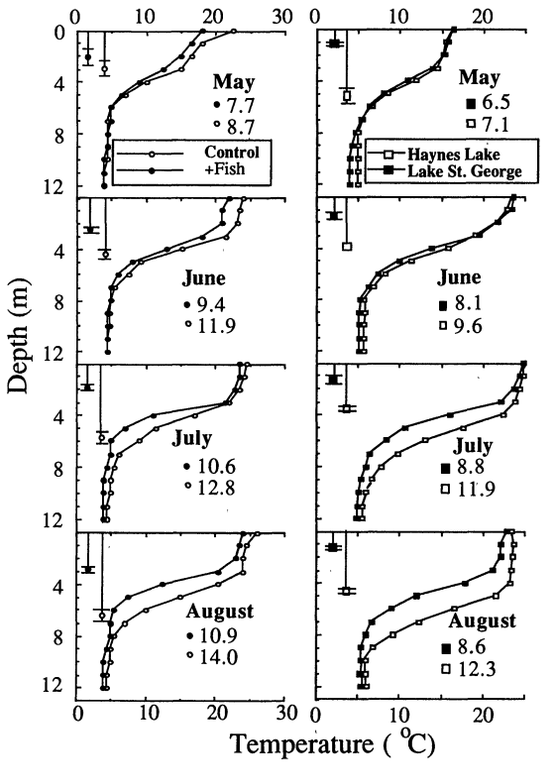

Fig. 2. Monthly means (from weekly samples) of Secchi depth and temperature profiles for (**left**) enclosures without fish (control) and with fish, and (**right**) for lakes with low (Haynes Lake) and high (Lake St. George) abundances of planktivorous fish. Secchi depths (in meters) are illustrated by vertical lines in upper left of each panel; error bars are 1 SE calculated from four measurements per month (from each lake and from each of the two replicate enclosures with and without fish). Numbers beneath the month on each panel are average monthly heat content (in kilocalories per square centimeter) (*18*).

plankton (slope), and that high water clarity causes deeper mixing depth is also supported by empirical relations between the index of plankton size distribution and water clarity [Fig. 3A; $S = 15.11 \pm 0.89 - (42.55 \pm 3.43)$ slope, SE = 0.96; S, Secchi depth] and between water clarity and mixing depth [Fig. 3B; $E = 1.36 + (0.76 \pm 0.049)\ S$, SE = 0.623; E = epilimnion] for 27 Ontario lakes. Data from our experimental enclosures and from the two lakes with high and low abundance of planktivorous fish conform to these relations. For the lakes of this study, log fetch (the conventional predictor of mixing depth that has typically been used) was not a good predictor of mixing depth (Fig. 3, inset); 38 lakes from Ontario (*4, 6, 20*) and Wisconsin (*7*) studied by others also conform to the Secchi depth–mixing depth relation. Furthermore, an increase in water clarity following experimental acidification of Lohi Lake (*20*), one of the Ontario lakes, was associated with an increase in metalimnetic temperature, hypolimnetic heating rate, and mixing depth. Decreased water clarity following nutrient enrichment of lakes has been associated with shallower mixing depth and lower heat content (*21*).

Why have others obtained good correlations between mixing depth and fetch (*3*), while we found a poor relation for small Ontario lakes? The answer may lie in the range of size or fetch of lakes used in their models. If lakes are small (<10 km fetch) the prediction of mixing depth from fetch is poor (*4, 6*). The strength of the relation between fetch and mixing depth (*3*) may be mainly because of data from large lakes, which tend to be clear, and more affected by wind. Gorham and Boyce (*5*) showed that a single model for predicting mixing depth from fetch cannot be used for both small (<25 km^2) and large lakes, and concluded that small and large lakes behave differently.

In the absence of wind effects, the depth of light penetration sets the lower limits of mixing depth. A good example would be the mixing depths in small, deep lakes with high water clarity. For example, in Crawford Lake, a small (0.2 ha) clear (Secchi depth 7 to 9 m) lake near Burlington, Ontario, where wind effect is negligible, mixing depth is roughly three times as great (*22*) as would be predicted from its fetch (*3*). The importance of water clarity in influencing mixing depth can also be illustrated by comparison of Lake Tahoe (*23*), Nevada (500 km^2, 27 km fetch), with Lake Simcoe (*24*), Ontario (736 km^2, 30 km fetch). Lake Tahoe, one of the clearest (Secchi depth, 20 to 42 m) lakes in the world, has a mixing depth of 18 to 20 m compared to 8 to 10 m for Lake Simcoe (Secchi depth, 3 to 5 m).

We conclude that among small lakes the biomass and size-distribution of plankton, which is largely determined by fish predation, can strongly influence the water temperature and mixing depth by modifying the underwater light environment. Lakes with similar size, but greater water clarity, will have deeper mixing depths and greater heat content. That organisms in lakes can influence their physical environment, such that reduced algal biomass and primary productivity will increase both light penetration and temperature, and vice versa, may contribute to ecosystem homeostasis.

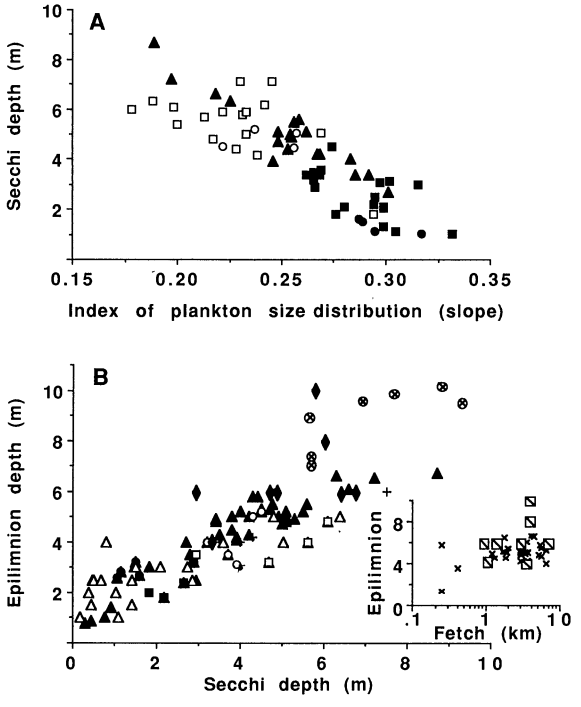

Fig. 3. (**A**) Relation between slope (index of plankton size distribution) and Secchi depth for enclosures with and without fish, the two lakes with low (Haynes Lake) and high (Lake St. George) abundances of fish, and 20 Ontario lakes. (**B**) Relation between water clarity (Secchi depth) and epilimnion or mixing depth for 27 survey lakes, Haynes Lake, Lake St. George, and enclosures with and without fish. Data from literature for Ontario (*4, 6, 20*) and Wisconsin (*7*) lakes are illustrated in the plot, but not used in regression model. Secchi depths were not available for Wisconsin lakes, therefore the attenuation coefficients of light were used on the basis of an available model (*25*) to predict Secchi depths. We used attenuation coefficient to predict Secchi depth because these two variables are strongly related, with an intercept of 0 and a slope of 1. Symbols are: ○ control enclosures, ● +Fish enclosures, □ Haynes Lake, ■ Lake St. George, ▲ survey lakes, + data from (*4*), ◆ data from (*6*), △ data from (*7*), and ○ data from (*20*). Inset is the relation between fetch and mixing depth for survey lakes (x) and from literature (*6*) ⊠.

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16. Four enclosures, made of impervious polyvinyl impregnated nylon (Felfab, Hamilton, Ontario), were used for our experiments. They were large (15 m deep, 8 m diameter), and open at the air-water and water-sediment interfaces. Enclosure walls are thin (0.25 mm), therefore may not have any insulating effects other than as barriers to diffusion. The top of enclosure walls were suspended from foam collars, and the bottom of the walls were buried into the sediment with steel chains. Two enclosures (+Fish) were stocked with planktivorous fish (1-year-old yellow perch, 8952 fish per hectare with mean wet weight 2.92 g per fish). Two (control) enclosures had no fish [see (*14*) for details].
17. Replicate concentrations of *PP* were determined for different size classes following filter fractionation. Cumulative proportions of *PP* retained on each filter

(pore sizes of 0.2, 1, 3, 12, 20, 41, 200, and 400 μm) were plotted against the logarithm of filter size. The slope of this linear relation was used as the index of plankton size distribution [see (*14*) for details].
18. Heat content of lakes was calculated for each date from volume-weighted mean temperature for each meter depth stratum [G. A. Cole, *Textbook of Limnology* (Mosby, London, 1979)]. Temperature measured at the center of the enclosures was used for the calculation.
19. Fetch is the distance in a water body over which wind can blow uninterrupted by land (*3*). It is equal to the square root of surface area.
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Phenotypic Switching in Mycoplasmas: Phase Variation of Diverse Surface Lipoproteins

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The ability of some microorganisms to rapidly alter the expression and structure of surface components reflects an important strategy for adaptation to changing environments, including those encountered by infectious agents within respective host organisms. *Mycoplasma hyorhinis*, a wall-less prokaryotic pathogen of the class Mollicutes, is shown to undergo high-frequency phase transitions in colony morphology and opacity and in the expression of diverse lipid-modified, cell-surface protein antigens. These proteins spontaneously vary in size, contain highly repetitive structures, and are oriented with their carboxyl-terminal region external to the membrane. Thus, mycoplasma membrane lipoproteins generate microbial surface diversity and may be part of a complex system that controls interactions of these organisms with their hosts.

SEVERAL MICROBIAL SPECIES SHOW reversible, high-frequency changes in phenotype, including oscillating expression (phase variation) or antigenic diversification of surface macromolecules (*1, 2*). In many instances, components associated with phase variation in bacteria are controlled by complex regulatory systems that affect both physiologic processes and virulence of the organisms (*1*).

We have investigated the presence of antigenic and phase variation in *Mycoplasma hyorhinis*, one of over 70 species in the genus *Mycoplasma*. Like several members of this group, *M. hyorhinis* is an animal pathogen. It causes rhinitis, polyserositis, and chronic experimental arthritis in its natural swine host (*3, 4*). This agent is also a common contaminant of tissue cell cultures where it propagates as a cell-surface parasite (*5*). Isolates of *M. hyorhinis* differ with respect to pathogenic potential (*4*), the ability to adsorb to cells in vitro (*6*), requirements for independent growth in agar medium (*7*), and antigenic characteristics (*8*), including marked differences in the expression and size of lipid-modified, integral membrane protein antigens at the cell surface that are recognized by monoclonal antibodies (*9*). It is important to determine whether phenotypic differences within this species arise from accumulated mutational events specifying particular characteristics of an isolate or, rather, reflect intrinsic, heritable mechanisms for generating diversity within populations in order to understand the interactions of this species, and perhaps several other pathogenic mycoplasmas, with their respective hosts. For this reason, we analyzed *M. hyorhinis* strain SK76, an arthritogenic swine pathogen (*10*) obtained as a multiply filter-cloned isolate, for its ability to diversify phenotypically during independent, cell-free growth in vitro.

Early and late (over 100) passage broth cultures of *M. hyorhinis* showed consistent heterogeneity in colony morphology when plated on standard agar medium (Fig. 1, A to D). A stable proportion of three "morphotypes" (indicated as a percentage of the colonies plated from filtered broth culture) was independent of the number of passages: a small-diameter S morphotype with a prominent center (98.6%), an intermediate-size M morphotype with a flat appearance and rough surface (1.3%), and a large-diameter L morphotype with a hemispherical rounded shape and smooth surface (0.1%). Several rounds of single colony isolation and filter cloning yielded purified cultures of each morphotype (Fig. 1, A to C), which were easily distinguished when plated as a mixture (Fig. 1D). However, close examination of subcloned populations ("lineages"), derived from serial rounds of single colony isolations, showed switching of each morphotype to both of the other morphotypes. Further analysis of lineages derived from "switched" progeny showed additional switching, either reverting to the original or again generating alternate phenotypes. Oscillating morphotypic switches of L, S, and M morphotypes were quantitatively monitored in several lineages (n) with the following ranges of switch frequencies (fraction of switched phenotype per cell per generation) (*11*): S → L [(0.1 to 43) × 10^{-4}, $n = 6$]; L → S [(0.7 to 2.9) × 10^{-4}, $n = 5$]; S → M [(0.7 to 64) × 10^{-4}, $n = 10$]; M → S [(1.0 to 6.2) × 10^{-4}, $n = 8$]; L → M (0.2 × 10^{-4}, $n = 1$); and M → L [(1.3 to 4.8) × 10^{-4}, $n = 3$]. Mean frequencies for these transitions ranged from 0.2×10^{-4} to 14.4×10^{-4}. Extremely high switch frequencies ($\sim 3 \times 10^{-2}$, the maximum measurable) were apparent in some lineages, where virtually every colony plated showed one morphotype spawning "sectors," subsequently shown to represent a switch to a different morphotype (Fig. 1, inset).

A second phenotype showing diversity in *M. hyorhinis* populations was colony opacity. Subcloned lineages of each morphotype showed heterogeneity in this feature, which was therefore not linked to colony morphology. Lineages of L variants (the most easily observed morphotype) generated oscillating transparent (tr) or opaque (op) populations (Fig. 1, E to G) with a high frequency of switching with respect to this characteristic: tr → op [(0.3 to 74) × 10^{-4}, $n = 8$] and op → tr [(3.2 to 93) × 10^{-4}, $n = 17$]. In several additional lineages, sectoring was observed in all colonies plated. The ability to undergo reversible high frequency, independent switching of colony morphotype, and opacity was a heritable feature of the organism.

An additional phenotypic instability was identified in subcloned *M. hyorhinis* populations by immunostaining colonies with the monoclonal antibody (MAb) F192C17a to assess expression of corresponding epitope-bearing lipoproteins (*9*). Unlike the uniform immunostaining of colonies obtained with polyclonal antibodies to *M. hyorhinis* or the MAb AB3C to p70 (*12*), a strain-invariant surface protein of this species (*9*), the staining obtained with MAb F192C17a was

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