Journal of Aquatic Animal Health 17:345–352, 2005

DOI: 10.1577/H05-006.1

[Communication]

Case Report: Outbreak of Bumper Car Disease Caused by *Anophryoides haemophila* in a Lobster Holding Facility in Nova Scotia, Canada

SPENCER J. GREENWOOD*

AVC Lobster Science Centre and Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

BÉATRICE M. DESPRÉS

AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

RICHARD J. CAWTHORN

AVC Lobster Science Centre and Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

JEAN LAVALLÉE

AVC Lobster Science Centre, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

DAVID B. GROMAN

Aquatic Diagnostic Services and Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island C1A 4P3, Canada

ADRIAN DESBARATS

Aquatic Solutions, Post Office Box 372, Barrington Passage, Nova Scotia B0W 1G0, Canada

Abstract.—This case report provides pathologic and confirmatory molecular characterization of an outbreak of bumper car disease caused by the scuticociliate *Anophryoides haemophila* in American lobster *Homarus americanus*, from a commercial holding facility in Nova Scotia, Canada. Although sporadically and anecdotally reported to be present in Atlantic Canada, this is the first report detailing observations from a natural outbreak of bumper car disease by gross and microscopic pathology and its positive identification by small subunit rRNA gene sequencing. Randomly amplified polymorphic DNA analysis confirmed that the genotype of the present outbreak isolate is identical to the original isolate of *A. haemophila* reported from Maine lobsters in 1993.

Bumper car disease, caused by the scuticociliate *Anophryoides haemophila* was first recognized in American lobsters *Homarus americanus* during the early 1970s (D. E. Aiken, J. B. Sochasky, and P. G. Wells, International Council for the Exploration of the Sea, unpublished data). The significance of this pathogen to the long-term, cold-water (<5°C) storage of lobsters has been well recognized (Aiken et al. 1973; Loughlin et al. 1993; Cawthorn 1997) and many intervention strategies have been devised to lessen the impact of disease (Loughlin et al. 1993; Novotny et al. 1996; Cribb et al. 1999). *Anophryoides haemophila* is known to exist endemically in lobsters and due vigilance is recommended in commercial holding facilities for this continual source of potential economic losses (Cawthorn et al. 1996; Lavallée et al. 2001; Aiken et al., unpublished data).

Experimental infections of *A. haemophila* in lobsters that followed pathology sequentially indicate that the disease progresses in two phases. During the initial phase of infection (weeks 1–4 after in-

* Corresponding author: sgreenwood@upei.ca

Received February 7, 2005; accepted June 17, 2005
Published online November 4, 2005

fection), there are no observable clinical signs and ciliates are absent from the general circulation. Ciliates replicate locally in the vesicular spaces and associated tissues of the gills by binary fission (Athanassopoulou et al. 2004). The second phase of disease occurs as ciliates spread systemically, via the hemolymph, to the connective tissue of other organs, which results in localized hemocyte granulomas and encapsulated parasite lesions in the gills and associated connective tissue 4–9 weeks after infection (Athanassopoulou et al. 2004). Systemic invasion is concurrent with multiplication of the ciliates, disappearance of reserve inclusion cells, and marked hemocytopenia. Lobsters succumb to the disease 4–14 weeks after infection in cold waters. The time course of lesion appearance and time to death decreases with an increasing size of the parasite inoculations (Athanassopoulou et al. 2004). The terminal pathology of experimental infections of *A. haemophila* correlates with observations from laboratory and natural mortalities (Sherburne and Bean 1991; Cawthorn 1997).

In March 2004, a commercial holding facility in Nova Scotia, Canada, reported daily mortality rates that increased from 0.5–1% to 5–10% over a period of approximately 1 week as lobsters were taken out of a short-term holding system for live shipping. The lobsters, caught in autumn from Nova Scotia, were maintained within individual compartments in a flow-through system for a period of 2–3 months. The lobsters were then moved to a short-term holding system for 2–3 weeks until being packed for live shipping. During short-term holding, the lobster were contained in conventional communal crates. Water temperatures in the long-term holding system ranged from −0.7°C to 5°C, ammonia levels were less than 3 mg/L, and dissolved oxygen levels were typically recorded at more than 90% saturation. Short-term holding system water temperatures ranged from 5°C to 8°C; ammonia levels and dissolved oxygen were similar to those in the long-term holding system, although some areas of the short-term holding system had dissolved oxygen levels as low as 60% saturation. Other than the increased mortality rates, lobsters displayed no clinical signs of disease. Examination of hemolymph wet mounts by microscopy, however, revealed low numbers of ciliates morphologically similar to *A. haemophila* in 11 of 13 lobsters.

Mortality within the long-term holding system was negligible (<2%), well within the normal limits for segregated storage facilities. Severe mortality was not observed until the lobsters were moved to the short-term holding system.

Outbreaks of bumper car disease continue to be reported sporadically, and often only anecdotally, from lobster-holding facilities on the Atlantic coast of North America. This new outbreak afforded the opportunity to isolate the causative agent and investigate whether intraspecific differentiation exists between isolates of *A. haemophila* responsible for causing disease in lobsters on the east coast of North America. This is the first report documenting the description of lesions from an outbreak and the direct genetic characterization of *A. haemophila* isolates from two temporally and spatially separated outbreaks.

Methods

Lobster assessment.—Total hemolymph protein concentrations were determined by refractometry (Atago Brix N20E, with manual temperature compensation) at the holding facility. Lobsters were considered to have low total hemolymph protein when concentrations were less than 30 g/L. Hemolymph was assessed by light microscopy under low magnification (4× with 10× objective) for semiquantitative hemocyte and ciliate parasite numbers. Hemocyte numbers were evaluated based on ''percent coverage of the field of view'': absent = no observed hemocytes, low = 25–50% coverage of field, normal = 75–100% coverage of field. Ciliate numbers were evaluated with a relative scale: low = fewer than 5 ciliates/field, moderate = 5–20 ciliates/field, and high = more than 20 ciliates/field.

Lobster necropsy.—Twelve moribund or recently dead lobsters were examined for gross external and internal lesions. Hemolymph was collected aseptically from the ventral sinus from moribund lobsters and cultured in phenyl ethyl alcohol broth at 28°C and on trypticase soy agar with 2% NaCl at 22°C for 96 h. Direct microscopic examination of hemolymph wet mounts confirmed the presence of numerous ciliates. Lobsters were killed with potassium chloride, 100 mg/100 g of body weight (Battison et al. 2000).

Histopathology.—Tissues (gills, heart, muscle, hepatopancreas, gonads, renal/antennal glands, and intestine) were fixed in 10% solutions of neutral buffered formalin in seawater (30 g/L) and processed for routine histology. Samples were stained with hematoxylin and eosin and examined via direct light microscopy at Aquatic Diagnostic Services, Atlantic Veterinary College (Charlottetown, Prince Edward Island, Canada).

Ciliate isolation.—Hemolymph (100 μL) from moribund lobsters was placed into 10-mL modified marine axenic medium (ATCC 1651 MA medium; Messick and Small 1996) composed of artificial seawater supplemented with vitamins (RPMI 1640; Sigma-Aldrich, Ltd., Oakville, Ontario, Canada), 10% fetal bovine serum, and penicillin and streptomycin (100 U/mL and 100 μg/mL, respectively) at 4°C for 24 h. This allowed sufficient time for contaminating cell debris to settle and for hemocytes to attach to the plasticware. The Nova Scotia 2004 isolate was transferred to fresh media and axenic clonal cultures were established by serial passage. An *A. haemophila* isolate from a Maine lobster pound in 1993 has been maintained in cell culture with occasional passage through lobsters at the AVC Lobster Science Centre and was used for comparison (Cawthorn et al. 1996).

Genomic DNA extraction.—Genomic DNA was extracted from axenic clonal cultures (from Maine in 1993 and Nova Scotia in 2004) by using the GenElute Mammalian Genomic DNA Miniprep kit (Sigma-Aldrich, Ltd.). DNA concentration and quality were assessed spectrophotometrically and by electrophoretic separation in a 0.8% agarose gel containing 0.5 μg/mL of ethidium bromide (Sambrook et al. 1989).

Amplification and sequencing of small subunit rRNA genes.—The small subunit rRNA (SSU rRNA) gene was amplified with the universal eukaryotic Medlin A forward primer (5′-AACCTG GTTGATCCTGCCAGT-3′) and Medlin B reverse primer (5′-TGATCCTTCTGCAGGTTCACCTA C-3′; Medlin et al. 1988). Approximately 10–50 ng of DNA was used in a 100-μL reaction containing 20 pmol of primers Medlin A and Medlin B with the following reagents (Fermentas International Inc., Burlington, Ontario, Canada): 200 μM of each deoxynucleotide triphosphate (dNTP; A,G, C, and T), 1.5 mM $MgCl_2$, 1× polymerase chain reaction (PCR) buffer (10 mM Tris, pH 8.8, 50 mM KCl, and 0.08% Nonidet P40), and 1.25 U of *Taq* DNA polymerase. A negative control of molecular biology–grade water (Sigma-Aldrich, Ltd.) was included in each run. Amplification was carried out in a MJ Research PTC-200 thermal cycler (MJ Research, Inc., Waltham, Massachusetts) with an initial denaturization at 94°C for 1 min, followed by 25 cycles consisting of denaturization at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1.5 min. Final extension was at 72°C for 10 min. Amplified DNA was purified by using a GenElute PCR clean-up kit (Sigma-Aldrich, Ltd.) and separated by electrophoresis in an 0.8% agarose gel containing ethidium bromide, 0.5 μg/mL. Direct sequencing of PCR products was performed on an ABI Prism 377 sequencer by using Big-Dye terminators (Applied Biosystems, Inc., Foster City, California) at the Guelph Molecular Supercentre (University of Guelph, Ontario, Canada). Forward (Medlin A, 300F, 690F, 1055F) and reverse (300R, 690R, 1055R, Medlin B) sequencing primers were used on each template (Elwood et al. 1985).

To ensure homogeneous single-species sequence information, all chromatograms were examined to determine whether only single peaks and therefore single-species DNA was amplified and sequenced (Drancourt et al. 2000). Sequences were screened for the presence of chimeric sequences with the programs Chimera Check (Cole et al. 2003) and Bellerophon (Hugenholtz and Huber 2003). The SSU rRNA sequences were aligned with the published *A. haemophila* Maine 1993 isolate (GenBank accession number U51554; Ragan et al. 1996) and with ciliate sequences from GenBank by using both Bio-Edit (Hall 1999) and Clustal X (Thompson et al. 1997).

Randomly amplified polymorphic DNA analysis.—Randomly amplified polymorphic DNA (RAPD) analysis was performed with Ready-To-Go RAPD Analysis Beads (Amersham Biosciences Corp., Piscataway, New Jersey). A 25-μL reaction solution consisting of 25 pmol of the selected arbitrary primer, 50 ng of DNA (Nova Scotia 2004 or Maine 1993 isolate), and molecular biology–grade water was added to the beads, which contained AmpliTaq DNA polymerase and Stoffel fragment, dNTPs (0.4 mM each), bovine serum albumin (2.5 μg), and buffer (3 mM $MgCl_2$, 30 mM KCl and 10 mM Tris (pH 8.3). Amplification was performed as described above: an initial denaturization at 95°C for 5 min; followed by 45 cycles consisting of denaturization at 95°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min; and a final extension at 72°C for 5 min. The RAPD products were electrophoresed in a 1.5% agarose gel containing ethidium bromide (0.5 μg/mL), and RAPD fragment sizes were determined with a DNA molecular weight marker, GeneRuler 100 bp DNA Ladder Plus (Fermentas International, Inc.). All agarose gels were visualized under UV light and images captured by using GeneSnap 4.0 (Syngene, Frederick, Maryland). Images of three independent experiments for each primer–DNA template combination ($n = 3$) were assessed.

Results and Discussion

Pathological Findings

Clinical observations.—Two phases of bumper car disease were identified in lobsters from the holding facility. In phase-1, lobsters displayed no clinical signs of disease, weakness, or external injury. Hemolymph total protein concentrations were normal for lobsters caught in autumn (40–80 g/L) but the hemolymph appeared opaque. Light microscopic evaluation of hemolymph wet mounts revealed normal hemocyte numbers and low numbers of ciliates. In phase-2, lobsters appeared weak to moribund and hemolymph was clear, with protein levels typically less than 30 g/L. In this phase, evaluation of hemolymph wet mounts by light microscopy indicated low to absent hemocyte numbers and moderate to high numbers of ciliates. Hemolymph often displayed a scintillating characteristic when held to the light.

Bacteriology.—All cultures from moribund phase-2 lobsters were negative for *Aerococcus viridans* and *Vibrio* spp.

Histopathology.—The primary morphological changes for moribund phase-2 lobsters included high numbers of ciliates in all hemolymph sinuses and low numbers of hemocytes. The agent was morphologically similar to *A. haemophila* (Cawthorn et al. 1996). The highest density of ciliates was found between the cuticle of gill lamellae and the underlying vasculature (Figure 1A, B, and C). Aggregations of hemocytes have formed granulomas within the center of gill filaments (Figure 1A and B). The heart lumen and endocardium were heavily colonized by both ciliates and a mixed rod-form bacterial population, with evidence of myofiber degeneration (Figure 1D). The subcuticular space of the eyestalk contained many ciliates (Figure 1E). Ciliates were also found associated within the vascular sinuses of the nerve cord (not shown). The intestinal lumen contained sloughed mucosal cell debris that was heavily colonized by a mixed rod-form bacterial population (not shown). The morphological diagnoses included (1) systemic ciliate parasitemia, classified as subacute (in terms of lesion age) and marked (in terms of severity); and (2) necrotizing bacterial enteropathy and hepatopancreatopathy (subacute, diffuse [in terms of distribution], marked).

Molecular Findings

Anophryoides haemophila has been the only scuticociliate shown to be a pathogen of American lobsters. Other species of scuticociliates, however, are known to infect crustaceans on the east coast of North America (Morado and Small 1995), and because ciliates vary in their degrees of morphological plasticity, we deemed it prudent to determine the SSU rRNA sequence to confirm the species identity of the isolated ciliate. The SSU rRNA gene sequence from the Nova Scotia 2004 ciliate was identical to the sequence of the Maine 1993 isolate of *A. haemophila* (Ragan et al. 1996) and confirms the presumptive diagnosis made with light microscopy.

To perform RAPD analysis of the Nova Scotia 2004 and Maine 1993 isolates, we used six arbitrary primers (A–F; Figure 2). The reproducibility of the results was verified by repeating all RAPD reactions for each isolate at least three times. The RAPD profiles for the *A. haemophila* isolates from Nova Scotia 2004 and Maine 1993 were identical (Figure 2), although a few minor bands (indicated by arrows on Figure 2) varied in intensity between isolates. These faint bands appeared in either one or both isolates during the three replicates of this experiment. These results reinforce the need for sufficient replicates in RAPD experiments in order to determine true genotype differences from variable band intensity (Welsh and McClelland 1990; Williams et al. 1990; Soll 2000).

The presence of identical RAPD profiles between ciliate isolates separated geographically has been observed previously in the freshwater *Paramecium aurelia* complex, where identical DNA fingerprints were found regardless of the extreme geographic distribution (e.g., Japan versus the USA for *P. primaurelia*; Stoeck and Schmidt 1998). A similar relationship was detected between strains of the free-living marine hypotrichs *Uronychia setigera* and *U. binucleata* associated with shellfish-, shrimp- and crab-farming sites in coastal China (Chen et al. 2003). Biogeographical variability in RAPD genotype profiles has however, frequently been observed among strains of ciliates, such as *Euplotes* (Kusch and Heckmann 1996), *Stentor coeruleus* (Kusch 1998), *Paramecium novaurelia* (Stoeck et al. 2000), *Gonostomum affine* (Foissner et al. 2001), and *Paramecium jenningsi* (Skotarczak et al. 2004).

Although we are limited by the sporadic occurrence and reporting of this important lobster parasite, the genetic characterization of the two *A. haemophila* isolates clearly demonstrated that a combination of SSU rRNA gene sequencing and RAPD analysis is useful for determining the species identity and genotypic relatedness of the outbreak pathogens. In the present case we do not know the

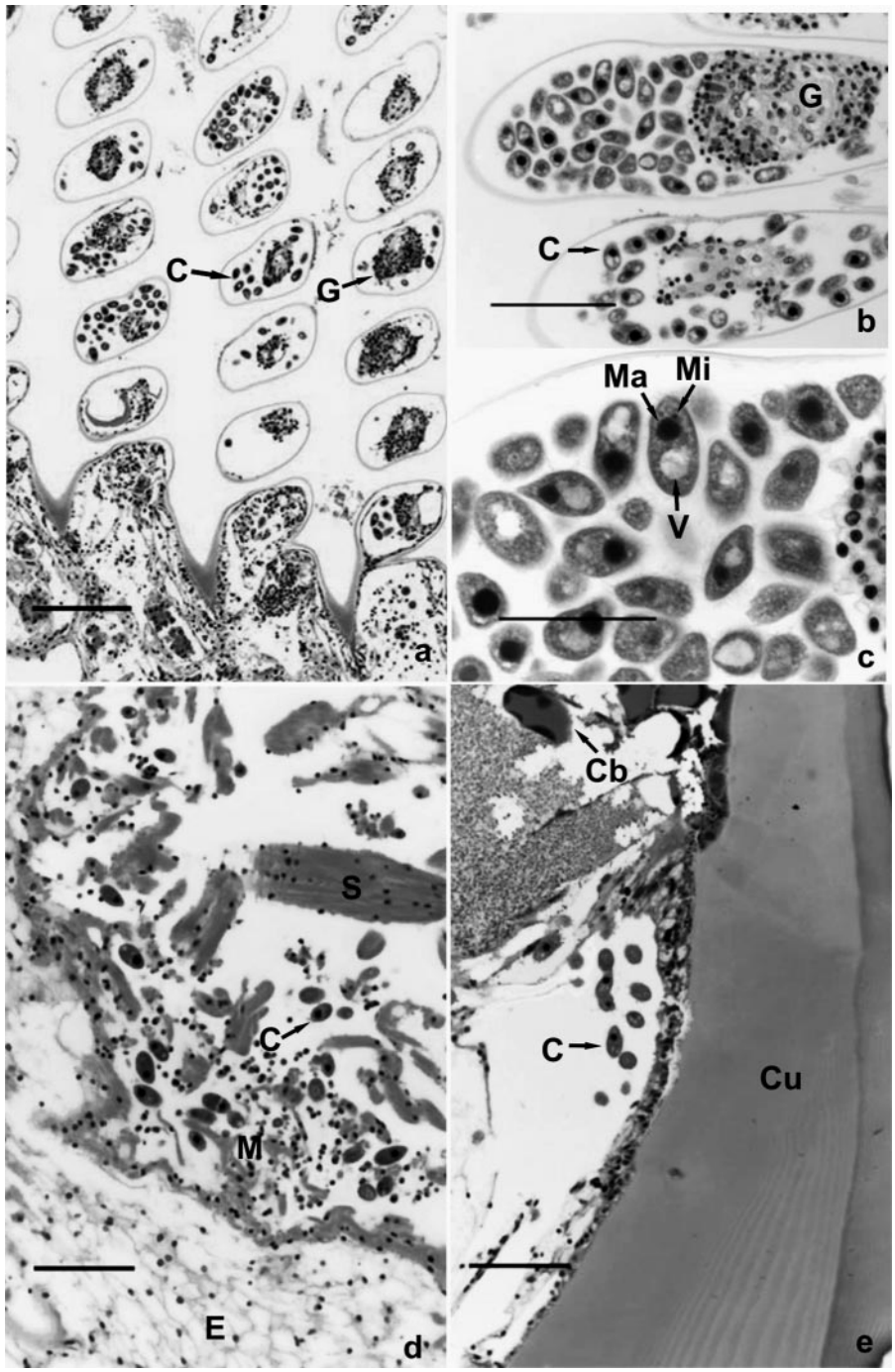

FIGURE 1.—Histopathology of American lobsters infected with *Anophryoides haemophila*. Panel **(a)** shows a transverse section of gill filaments indicating ciliates (C) and a hemocyte granuloma (G); bar = 200 μm. Panel **(b)** shows an oblique section of gill filament indicating ciliates and a hemocyte granuloma; bar = 100 μm. Panel **(c)** shows an oblique gill filament section indicating a macronucleus (Ma), micronucleus (Mi), and vacuole (V) of ciliate; bar = 50 μm. Panel **(d)** shows a transverse section of heart with ciliates, epicardium (E), spongiosum (S), and area of myofiber degeneration (M); bar = 100 μm. Panel **(e)** shows a sagittal section through eyestalk with ciliates within subcuticular space, crystalline bodies (Cb), and a cuticle (Cu); bar = 100 μm. Slides stained with hematoxylin and eosin are from phase-2 lobsters (see Results and Discussion section).

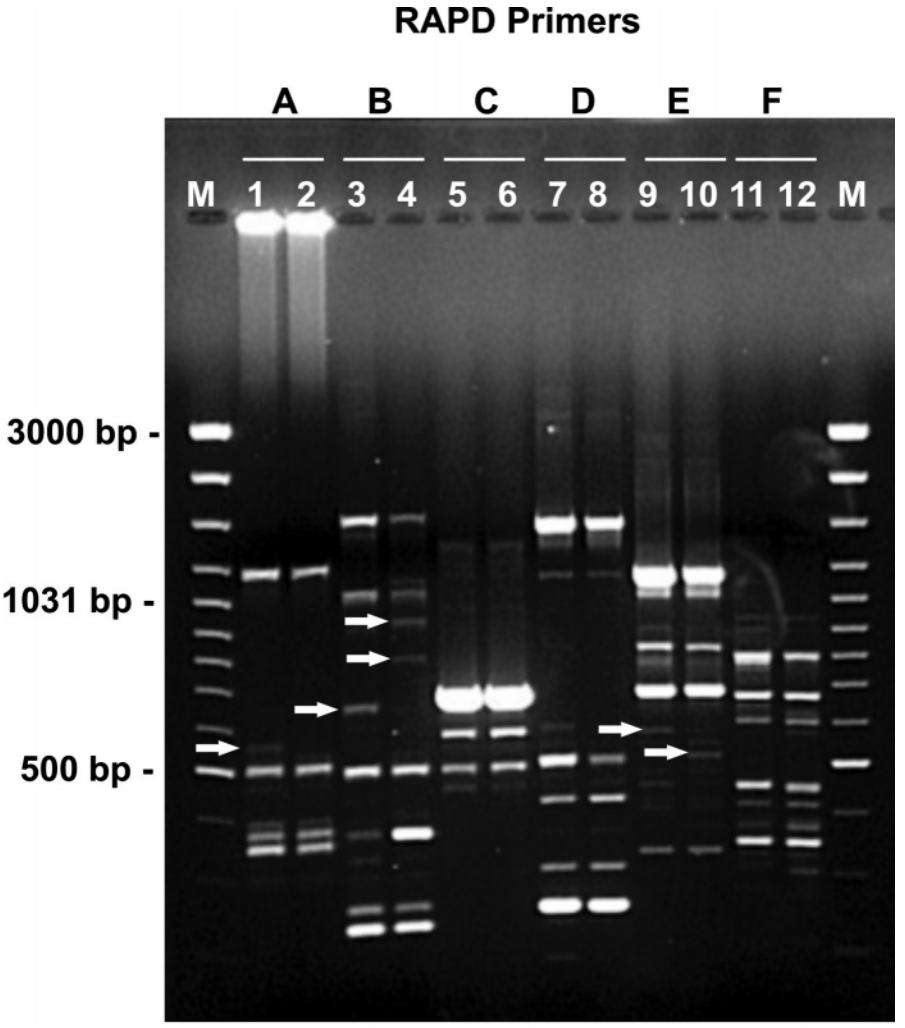

FIGURE 2.—Agarose gel electrophoresis of randomly amplified polymorphic DNA profiles for *Anophryoides haemophila* obtained with six primers (A–F). Samples of the Maine 1993 isolate (lanes 1, 3, 5, 7, 9, and 11) are compared with the Nova Scotia 2004 isolate (lanes 2, 4, 6, 8, 10, and 12). Molecular weight markers are in the lanes labeled M. Sizes of specific bands in base pairs (bp) are noted on the left. Minor bands that vary in intensity between isolates are indicated by arrows (see Results and Discussion section).

source of the outbreak; however, like *Paramecium primaurelia*, *Uronychia setigera*, and *U. binucleata*, isolates of *A. haemophila* appear to be quite stable over large geographic areas. The stability of microbes over large geographic areas has been explained by the shear abundance of organisms being able to sustain their continuous global dispersal (Finlay 2002). The temporal stability of the *A. haemophila* genotype pattern, however, parallels the observations of the causative agent of gaffkemia in lobsters, *Aerococcus viridans* var. *homari* (Greenwood et al. 2005). This may be a general feature of long-established host–pathogen relationships that involve lobsters in the relatively stable marine environment. Alternatively, the appearance of the same pathogen genotype in different geographic areas may indicate that the movement of lobsters (natural dispersal/migration or importation from other regions for processing) leads to the movement of their pathogens. Further studies of the interactions between lobsters and their pathogens will, we hope, provide these answers. Continued collaboration with scientists and prompt reporting of disease outbreaks by the commercial lobster industry will in-

crease the likelihood of understanding and preventing future disease outbreaks within lobster-holding facilities.

Acknowledgments

The authors thank A. Battison for constructive comments on the manuscript. Funding for the Canadian Lobster Health Research Project delivered by the AVC Lobster Science Centre represents a consortium of private sector companies, fishermen's organizations, and First Nations and provincial and federal government agencies, including the Atlantic Innovation Fund through the Atlantic Canada Opportunities Agency.

References

Athanassopoulou, F., D. Speare, R. J. Cawthorn, R. MacMillan, and B. Després. 2004. Pathology of *Anophryoides haemophila* (Scuticociliatida: Orchitophryidae), parasite of American lobster *Homarus americanus* kept under experimental conditions. Aquaculture 236:103–117.

Battison, A., R. MacMillan, A. MacKenzie, P. Rose, R. Cawthorn, and B. Horney. 2000. Use of injectable potassium chloride for euthanasia of American lobsters (*Homarus americanus*). Comparative Medicine 50:545–550.

Cawthorn, R. J., D. H. Lynn, B. Després, R. MacMillan, R. Maloney, M. Loughlin, and R. Bayer. 1996. Description of *Anophryoides haemophila* n. sp. (Scuticociliatida: Orchitophryidae), a pathogen of American lobsters *Homarus americanus*. Diseases of Aquatic Organisms 24:143–148.

Cawthorn, R. J. 1997. Overview of ''bumper car'' disease: impact on the North American lobster fishery. International Journal for Parasitology 27:167–172.

Chen, Z. G., W. B. Song, and A. Warren. 2003. Species separation and identification of *Uronychia* spp. (Hypotrichia: Ciliophora) using RAPD fingerprinting and ARDRA riboprinting. Acta Protozoologica 42: 83–90.

Cole, J. R., B. Chai, T. L. Marsh, R. J. Farris, Q. Wang, S. A. Kulam, S. Chandra, D. M. McGarrell, T. M. Schmidt, G. M. Garrity, and J. M. Tiedje. 2003. The Ribosomal Database Project (RDP-II): previewing a new autoaligner that allows regular updates and the new prokaryotic taxonomy. Nucleic Acids Research 31:442–443.

Cribb, A. E., B. Després, and R. J. Cawthorn. 1999. Tetrazolium-based cytotoxicity assay to determine anti-protozoal activity against the scuticociliate *Anophryoides haemophila*. Diseases of Aquatic Organisms 35:213–219.

Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J. P. Gayral, and D. Raoult. 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. Journal of Clinical Microbiology 38:3623–3630.

Elwood, H. J., G. J. Olsen, and M. L. Sogin. 1985. The small subunit ribosomal RNA gene sequences from the hypotrichous ciliates *Oxytricha nova* and *Stylonychia pustulata*. Molecular Biology and Evolution 2:399–410.

Finlay, B. J. 2002. Global dispersal of free-living microbial eukaryote species. Science 296:1061–1063.

Foissner, W., T. Stoeck, H. Schmidt, and H. Berger. 2001. Biogeographical differences in a common soil ciliate, *Gonostomum affine* (Stein), as revealed by morphological and RAPD-fingerprint analysis. Acta Protozoologica 40:83–97.

Greenwood, S. J., I. R. Keith, B. M. Després, and R. J. Cawthorn. 2005. Genetic characterization of the lobster pathogen *Aerococcus viridans* var. *homari* determined by 16S rRNA gene sequence and randomly amplified polymorphic DNA analyses. Diseases of Aquatic Organisms 63:237–246.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95–98.

Hugenholtz, P., and T. Huber. 2003. Chimeric 16S rDNA sequences of diverse origin are accumulating in the public databases. International Journal of Systematic and Evolutionary Microbiology 53:289–293.

Kusch, J. 1998. Local and temporal distribution of different genotypes of pond-dwelling *Stentor coeruleus*. Protist 149:147–154.

Kusch, J., and K. Heckmann. 1996. Population structure of *Euplotes* ciliates revealed by RAPD fingerprinting. Ecoscience 3:378–384.

Lavallée, J., K. L. Hammell, E. S. Spangler, and R. J. Cawthorn. 2001. Estimated prevalence of *Aerococcus viridans* and *Anophryoides haemophila* in American lobsters *Homarus americanus* freshly captured in the waters of Prince Edward Island, Canada. Diseases of Aquatic Organisms 46:231–236.

Loughlin, M. B., R. C. Bayer, and D. L. Prince. 1993. Ciliated protozoan disease in lobster, *Homarus americanus*. Connecticut Sea Grant College Program, CT-SG-93-05, Groton.

Medlin, L., H. J. Elwood, S. Stickel, and M. L. Sogin. 1988. The characterization of enzymatically amplified eukaryotic 16S-like rRNA coding regions. Gene 71:491–499.

Messick, G., and E. B. Small. 1996. *Mesanophrys chesapeakensis* n. sp.: a histophagous ciliate in the blue crab, *Callinectes sapidus*, and associated histopathology. Invertebrate Biology 115:1–12.

Morado, J. F., and E. B. Small. 1995. Ciliate parasites and related diseases of Crustacea: a review. Reviews in Fisheries Science 3:275–354.

Novotny, M. J., R. J. Cawthorn, and B. Després. 1996. In vitro effects of chemotherapeutants on the lobster parasite *Anophryoides haemophila*. Diseases of Aquatic Organisms 24:233–237.

Ragan, M. A., R. J. Cawthorn, B. Després, C. A. Murphy, R. K. Singh, M. B. Loughlin, and R. C. Bayer. 1996. The lobster parasite *Anophryoides haemophila* (Scuticociliatida: Orchitophryidae): nuclear 18S rDNA sequence, phylogeny, and detection using oligonucleotide primers. Journal of Eukaryotic Microbiology 43:341–346.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sherburne, S. W., and L. L. Bean. 1991. Mortalities of impounded and feral Maine lobsters, *Homarus americanus* H. Milne Edwards, 1837, caused by the protozoan ciliate *Mugardia* (formerly *Anophrys* = *Paranophrys*), with initial prevalence data from ten locations along the Maine Coast and one offshore area. Journal of Shellfish Research 10:315–326.

Skotarczak, B., E. Przybos, B. Wodecka, and A. Maciejewska. 2004. Sibling species within *Paramecium jenningsi* revealed by RAPD. Acta Protozoologica 43:29–35.

Soll, D. R. 2000. The ins and outs of DNA fingerprinting the infectious fungi. Clinical Microbiology Reviews 13:332–370.

Stoeck, T., and H. J. Schmidt. 1998. Fast and accurate identification of European species of the *Paramecium aurelia* complex by RAPD fingerprints. Microbial Ecology 35:311–317.

Stoeck, T., E. Przybos, J. Kusch, and H. J. Schmidt. 2000. Intraspecies differentiation and level of inbreeding of different sibling species of the *Paramecium aurelia* complex. Acta Protozoologica 39: 15–22.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL_X–Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18:7213–7218.

Williams, J. G., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18:6531–6535.