

NOTE

Estimated prevalence of *Aerococcus viridans* and *Anophryoides haemophila* in American lobsters *Homarus americanus* freshly captured in the waters of Prince Edward Island, Canada

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ABSTRACT: The Canadian lobster industry holds lobsters *Homarus americanus* in captivity for various periods to supply markets with live product year-round. Mortality during holding results in considerable losses, estimated at 10 to 15% yr⁻¹ by the industry. This study examined the prevalence of *Anophryoides haemophila* and *Aerococcus viridans*, causative agents of 'bumper car' disease and gaffkemia, respectively, in lobsters freshly captured in the waters of Prince Edward Island during the spring and fall fishing seasons of 1997. A total of 116 lobsters were sampled in the spring, and 138 in the fall. *A. haemophila* was not detected in the spring, while the prevalence was 0.72% in the fall with a 95% confidence interval (CI) of 0.02 to 3.97% and an overall prevalence of 0.39% (95% CI: 0.01 to 2.17%). The prevalence of *A. viridans* was estimated at 6.9% (95% CI: 3.0 to 13.14%) in the spring, 5.8% in the fall (95% CI: 2.54 to 11.10%), and 6.30% overall (95% CI: 3.64 to 10.03%). Because of the reduced interest in food of diseased lobsters, and compromised metabolism in the case of gaffkemia, these prevalence estimates are likely underestimates of the true prevalence of gaffkemia and 'bumper car' disease in the wild populations of lobster around Prince Edward Island.

KEY WORDS: Lobster · *Homarus americanus* · Disease · *Anophryoides haemophila* · *Aerococcus viridans* · Gaffkemia · 'Bumper car' disease

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The American lobster *Homarus americanus* fishery has been one of the most stable fisheries in Canada. While Canadian landings reached a record peak in 1991 with more than 48 493 metric tonnes (mt), in 1998 they were stable at 41 165 mt (Fisheries and Oceans Canada, Statistical Services Unit, Policy Sector. Canadian Landings Information, updated 11 January 2001. Available at: www.dfo-mpo.gc.ca/communic/statistics/landings/S1998pqe.htm). To supply the market with

live product year-round, the lobster industry maintains live lobsters in different forms of containment for various periods. However, significant mortality can occur during holding, resulting in substantial financial losses. Pre-processing mortality has been estimated by the industry to average 10 to 15% yr⁻¹ (Cawthorn 1997), which conservatively represents annual losses of \$35 to 50 million (US). In confinement situations, including lobster pounds, the likelihood of infectious disease outbreaks is usually higher than in wild environments, especially when many lobsters from various sources are held. Although many mortality problems can be attributed to non-infectious causes, infectious diseases may contribute to losses during holding.

Gaffkemia, caused by the bacterium *Aerococcus viridans* var. *homari*, is probably the most important infectious disease of impounded American lobsters, causing major economic impact (Martin & Hose 1995). Because of the lack of exoenzymes and, thus, lack of specific invasive powers, *A. viridans* requires open wounds or ruptured exoskeleton to infect lobsters (Stewart 1975).

Ciliates are commonly found in invertebrates, including the edible crab *Cancer pagurus* (Bang et al. 1972), the Dungeness crab *C. magister* (Armstrong et al. 1981, Sparks et al. 1982), marine isopods (Hibbits & Sparks 1983), the Pacific oyster *Crassostrea gigas* (Bower & Meyer 1993), and the American lobster (Sherburne & Bean 1991, Cawthorn et al. 1996). 'Bumper car' disease, or ciliate disease, is caused by the scuticociliate *Anophryoides haemophila* (Cawthorn et al. 1996), and is another potential cause of lobster mortality, especially during winter impoundment. This disease eventually leads to depletion of hemocytes, resulting in significantly reduced clotting ability (Cawthorn 1997). Lobsters can become infected by transmission of the ciliates through open wounds often

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inflicted during and shortly after ecdysis, and possibly through the thin cuticle lining the gill epithelium (Cawthorn 1997).

A third disease of economic significance that affects American lobsters in captivity is shell disease, also called rust disease, black spot or brown spot disease (review by Getchell 1989). Shell disease is an external infection apparently caused by various opportunistic pathogens (Prince et al. 1993), resulting in degradation of the chitin component of the exoskeleton and melanisation of the affected site (Getchell 1989, Vogan et al. 1999).

Both gaffkemia and 'bumper car' disease affect wild populations of lobsters in Atlantic Canada, with levels of *Aerococcus viridans*-infected lobsters ranging from 0 to $\leq 40\%$ (Stewart et al. 1966, Vachon et al. 1981, Keith et al. 1992), and levels of *Anophryoides haemophila* infection approaching 20% (Aiken et al. 1973, Cawthorn et al. 1996). Until recently, shell disease appeared to cause significant problems mostly in lobsters landed in the southwestern part of Nova Scotia, Canada (Getchell 1989). However, significant increases in the prevalence of shell disease over the last 3 yr in wild-caught lobsters in the eastern part of the Long Island Sound, USA, have been documented (Castro & Angell 2000, Connecticut Sea Grant Extension 2000). The prevalence of shell disease in Prince Edward Island lobsters is usually very low, with only anecdotal reports of isolated cases.

The objective of this study was to estimate the prevalences of the causative agents of 'bumper car' disease, *Anophryoides haemophila*, and gaffkemia, *Aerococcus viridans*, in freshly caught lobsters from the waters of Prince Edward Island, Canada. The prevalence of shell disease was also subjectively estimated.

Materials and methods. Source of lobsters: Thirty-nine fishing ports on Prince Edward Island with more than 10 vessels per port actively fishing lobsters were identified in the federally regulated spring Lobster Fishing Areas (LFAs) 24, 26a and 26b. A random list of 5 ports was computer generated using Minitab®, version 10.1 software (Minitab Incorporated, State College, Pennsylvania, USA, 1994). Visits to each selected port enabled investigators to identify 2 boats per port on which fishers were willing to participate in the study. An average of 12 market-sized lobsters (carapace length [CL] > 81 mm) per boat for a total of 116 lobsters were purchased at the wharves directly from fishers on June 26–27, 1997. A similar protocol was carried out in the fall for LFA 25, with an average purchase of 14 lobsters per boat for a total of 138 lobsters obtained directly from fishers on September 4–5, 1997.

Sampling: Lobster body weight recorded in kilograms, carapace length measured in millimetres from

the caudal end of the eye socket to the caudal extremity of the dorsal carapace, sex, shell score (from 0 to 5 depending on the severity of the lesions), and overall physical condition index were recorded for each lobster according to industry standards. The overall physical condition index was a combination of a subjective assessment of the liveliness of the lobsters and their physical appearance, lobsters either had a normal physical condition index or a downgraded index. Lobsters were classified as downgraded, if they met at least one of the following criteria: dead, weak, open wound(s) or active lesion(s), missing claw(s), missing leg(s), damaged claw(s), damaged leg(s), missing antenna(e), broken rostrum, damaged body or tail, or damaged antenna(e). A frequency distribution was generated for each criteria, and because no obvious distribution pattern was seen, it was collapsed into a dichotomous variable.

Lobsters were examined for evidence of *Aerococcus viridans* (causative agent of gaffkemia) infection, by hemolymph sampling conducted directly at the wharves. Using a 3 ml syringe with a 23-gauge needle, we aseptically removed 1.5 ml of hemolymph from the ventral sinus after swabbing the surface with 70% alcohol. The presumptive phenylethylalcohol (PEA) broth test was used for *A. viridans* isolation (Stewart et al. 1966) by adding 0.5 ml of hemolymph to 4.5 ml of PEA broth, and vigorously shaking and incubating at 28°C for 96 h. Duplicate PEA broth cultures were inoculated for each lobster. Suspicious broth culture tubes were identified by the typical colour change of the broth from purple to green and then yellow, and confirmatory testing for the presence of *A. viridans* (tetrad-forming Gram-positive cocci) was performed using Gram stain and microscopic examination. Following hemolymph sampling, each lobster was euthanised with benzocaine (15 ml of stock solution l⁻¹ of saltwater; stock solution = 100 g l⁻¹ of ethanol). The left side of the carapace was cut open and removed, exposing underlying tissues. Samples of gills, hepatopancreas, tail muscle, heart and gonad were collected and fixed in a solution of 1G4F fixative (1% glutaraldehyde, 4% formaldehyde). After 48 h, tissues were paraffinized and mounted on slides. An immunofluorescence technique was employed on tissue sections to detect the presence of *Anophryoides haemophila* ciliates. The slides were de-paraffinized by soaking in consecutive solutions of xylene (10 min), 100% ethanol (10 min), 95% ethanol (5 min), 70% ethanol (5 min) and distilled water. The tissue on the slides was outlined with a hydrophobic barrier pen. Samples were incubated with the primary antibody (undiluted monoclonal antibody supernatant) for 40 min, then rinsed and soaked in PBS for 15 min. Slides were incubated in the dark with the secondary antibody, fluorescein-labelled

Table 1. *Homarus americanus*. Summary results and mean values (standard deviation) of the physical and physiological parameters of American lobsters sampled during the 1997 spring and fall sampling periods in Prince Edward Island. Significant differences ($p < 0.05$) between the spring and fall seasons are represented by different superscripts

Parameter	Sampling season		Overall
	Spring	Fall	
n	116	138	254
Gender			
Female	56.9%	44.1%	50.0%
Male	43.1%	55.9%	50.0%
Ratio female/male	1.32 ^a	0.79 ^b	1.00
Body weight (kg)	0.58 ^a (0.2)	0.47 ^b (0.1)	0.51 (0.1)
Carapace length (mm)	88.9 ^a (7.8)	84.6 ^b (3.2)	86.6 (6.1)
Downgraded (%) ¹	12.9 (0.3)	14.5 (0.4)	13.8 (0.4)

¹Downgraded lobsters consisted of lobsters with open wound(s) or active lesion(s), missing claw(s), missing leg(s), damaged claw(s), damaged leg(s), missing antenna(e), broken rostrum, damaged body or tail, or damaged antenna(e), and dead or weak lobsters

rabbit anti-mouse IgG, M, A diluted 1/100 in PBS with 1 drop of Evans Blue counter stain (per 1 to 2 ml of conjugate preparation) for 30 min. The samples were then rinsed and soaked in PBS for 15 min in the dark. The slides were cover slipped with Fluorescent Antibody (FA) mounting fluid, and kept in the dark until used. Positive structures stained bright green with an ultra-violet light source (Zeiss Standard 16 microscope, Jena, Germany).

Statistical analysis: Data were transferred to a spreadsheet (Quattro[®] Pro version 7, Corel Corporation Limited, Ottawa, Ontario, Canada, 1996). A random sample of 60 records was examined and manually checked for data entry errors. Error checking for outliers and data description were conducted by examining descriptive statistics including means, medians, standard deviations, and minima and maxima for each continuous variable. The dataset was transferred into the statistical software STATA[™] version 5.0 (Stata Corporation, College Station, Texas, USA, 1996) for further analysis. For all analyses, differences among groups were considered significant when $p < 0.05$.

Results. Every fisher approached in both sampling periods agreed to cooperate, and therefore, the participation rate was 100%. Monetary constraints and the difference in mean lobster

weight resulted in different sample sizes for the 2 seasons. Overall, 254 lobsters were sampled for both fishing seasons. There were significantly more female lobsters assessed in the spring season than in the fall season, while the overall ratio was exactly 1:1. The overall averages were 0.51 kg for weight, 86.6 mm for CL (Table 1).

Prevalence of disease causative agents: The prevalence of *Aerococcus viridans* in freshly caught lobsters was estimated to be 6.9% (95% confidence interval [CI]: 3.0 to 13.1%) in the spring season, and 5.8% (95% CI: 2.5 to 11.1%) for the early part of the fall season (Table 2). The overall prevalence of *A. viridans* for both seasons combined was estimated at 6.3% (95% CI: 3.6 to 10.0%). No significant difference in prevalence was found between the 2 seasons. Only 1 lobster was positive by immunofluorescent assay (IFA) testing for the presence of *Anophryoides haemophila* in the fall season, while no positives were observed in the spring season (Table 2). Therefore, the prevalence in freshly caught lobsters in the spring was estimated at 0% (1-sided 97.5% CI: 0 to 3.1%), 0.7% (95% CI: <0.1 to 4.0%) in the fall, and 0.4% (95% CI: <0.1 to 2.2%) for both seasons combined. There were no statistical differences between sexes or seasons.

All lobsters assessed for external lesions of shell disease were given a score of '0' for both sampling periods. Therefore, the estimated prevalence of shell disease for the spring and the fall seasons in freshly caught lobsters was 0% (1-sided 97.5% CI: 0 to 1.4%) (Table 2).

Association with downgraded lobsters: The overall condition index, after dichotomized into 'downgraded' or 'not downgraded' (normal) was not significantly associated with lobster body weight and CL in the

Table 2. Prevalence of *Aerococcus viridans* (causative agent of gaffkemia), *Anophryoides haemophila* (causative agent of 'bumper car' disease) and shell disease in freshly caught American lobsters *Homarus americanus* sampled during the 1997 spring and fall sampling periods in Prince Edward Island. No significant differences were found between seasons

Season	Cases	<i>A. viridans</i>	<i>A. haemophila</i>	Shell disease
Spring (n = 116)	No. of positive	8	0	0
	Prevalence (%) (95% CI)	6.90 (3.02–13.14)	0 (0–3.13) ¹	0 (0–3.13) ¹
Fall (n = 138)	No. of positive	8	1	0
	Prevalence (%) (95% CI)	5.80 (2.54–11.10)	0.72 (0.02–3.97)	0 (0–2.64) ¹
Overall (n = 254)	No. of positive	16	1	0
	Prevalence (%) (95% CI)	6.30 (3.64–10.03)	0.39 (0.01–2.17)	0 (0–1.44) ¹

¹One-sided 97.5% confidence interval

spring or fall sampling periods. Although infection levels with *Aerococcus viridans* were consistently higher in 'downgraded' lobsters compared to 'normal' lobsters, no statistically significant associations were established (Table 3).

Discussion. Gaffkemia is a disease endemic to lobster populations of North America, and has also been reported in European waters (Alderman 1996). Prevalences of *Aerococcus viridans* infection in freshly caught lobsters in Atlantic Canada have been estimated between 0 and 40% (Stewart et al. 1966, Keith et al. 1992) and between 0 and 22% off the coast of Maine (Vachon et al. 1981, Huang & Bayer 1989). In this study both spring and fall sampling periods were conducted over 2 consecutive days, and may not reflect the true prevalence of gaffkemia in freshly caught lobsters during the 2 fishing seasons. This sampling protocol was necessary to get a point estimate of prevalence that would not be affected by changing water temperature over a longer period. The overall prevalence of *A. viridans*-infected lobsters caught from the waters of Prince Edward Island for the 1997 spring and fall fishing seasons was 6.3%.

Stewart et al. (1972) reported that lobsters infected with *Aerococcus viridans* only ate the food presented to them on the second post-infection and then only sparingly. Furthermore, they suggested that an infected lobster would not be attracted by the bait in fishing traps except possibly during the earlier stages of the infection. As intensity of the infection with *A. viridans* increases, the lobster's reserve in glycogen and

the levels of adenosine triphosphate (ATP) in the hepatopancreas, tail muscle and heart decline significantly (Stewart & Arie 1973, Stewart 1975, Johnson et al. 1980). The relative lethargic state observed in recently infected lobsters and which lasts throughout the course of the infection, is most likely because of these decreasing levels of glycogen and ATP. These compromised metabolic processes coupled with the possible food aversion of infected lobsters will result in gaffkemic lobsters being fished at a lower rate than non-gaffkemic lobsters. Therefore, the true prevalence of lobsters infected with *A. viridans* in the wild population must be higher than those estimated in fished lobsters. This is likely the case with the *A. viridans* infection prevalences reported herein.

In the fall, only 1 lobster was positive for the presence of the ciliate *Anophryoides haemophila*. In the spring, no lobsters infected with *A. haemophila* were observed. These results were surprising because earlier studies documented prevalences of *A. haemophila* in lobsters from Prince Edward Island waters of 17.8% (Cawthorn et al. 1996), and up to 20% in New Brunswick (Aiken et al. 1973). The most likely explanation was that the true prevalence was indeed low, and that the lobster population sampled here was different from those sampled in studies reported earlier. Low prevalence could also reflect environmental factors, such as increasing seawater temperatures, or a seasonal cycle of *A. haemophila* in wild lobsters. Perhaps the indirect fluorescent test had a low sensitivity in a population with these characteristics.

No evidence of shell disease was found during this study. Shell disease is a significant problem in lobster holding facilities throughout the Canadian Maritime provinces and the state of Maine, USA. In live-holding situations, this disease is seen almost exclusively during the winter months and early spring and is believed to have a multi-factorial aetiology (Prince et al. 1993). Until recently, shell disease appeared to be restricted mostly to lobsters landed in southwest Nova Scotia in late November to early January, and to a lesser degree from the Jonesport area in Maine (Getchell 1989). However, wild-caught lobsters from Eastern Long Island Sound, Massachusetts, Rhode Island and Connecticut have had significant levels of shell disease, with prevalences ranging from approximately 20 to 70% (Castro & Angell 2000, Connecticut Sea Grant Extension 2000) and

Table 3. Proportion (frequency) of American lobsters *Homarus americanus* positive for *Anophryoides haemophila* (causative agent of 'bumper car' disease) and *Aerococcus viridans* (causative agent of gaffkemia), according to the overall condition of the lobsters assessed during the 1997 spring and fall sampling periods in Prince Edward Island. p-values for comparisons between 'normal' and 'downgraded' were obtained by chi-squared tests

Pathogen	Season	Overall condition		Exact p-value (Fisher)
		Normal	Downgraded ¹	
<i>A. haemophila</i> (IFAT) ²	Spring	0% (0/101)	0% (0/15)	–
	Fall	0% (0/118)	5.0% (1/20)	0.145
	Overall	0% (0/219)	2.9% (1/35)	0.138
<i>A. viridans</i> (PEA broth + Gram staining) ³	Spring	5.9% (6/101)	13.3% (2/15)	0.276
	Fall	5.1% (6/118)	10.0% (2/20)	0.327
	Overall	5.5% (12/219)	11.4% (4/35)	0.163

¹Downgraded lobsters consisted of lobsters with open wound(s) or active lesion(s), missing claw(s), missing leg(s), damaged claw(s), damaged leg(s), missing antenna(e), broken rostrum, damaged body or tail, or damaged antenna(e), and dead or weak lobsters

²Fluorescent testing methods using monoclonal antibodies (IFAT)

³Culture on phenylethylalcohol broth (PEA broth), with confirmation by identification of Gram-positive cocci under microscopic examination

associated decreased landings estimated at 50 % (Connecticut Sea Grant Extension 2000). The prevalence of shell disease in lobsters landed in Prince Edward Island waters from June to October was expected to be extremely low, since cases of shell disease have rarely been reported. The absence of shell disease in Prince Edward Island lobsters during this study was confirmed.

Anorexia is a clinical sign well documented in many diseased animals, terrestrial or aquatic, including crustaceans (Southgate 1993, Ulda & Buchmann 1996, Mikolajczyk & O'Reilly 2000, Smith 2000). Consequently, this behaviour in wild population of lobsters is conceivable among diseased individuals. However, moribund and dead lobsters inside fishing traps have been anecdotally reported by New England fishers (Gillis & Cawthorn 2000). Therefore, it is possible that diseased lobsters enter fishing traps, even if they are not feeding, conceivably looking for shelters. If bait attractiveness and palatability are highly correlated, perhaps lobsters with shell disease or *Anophryoides haemophila*-infected lobsters are not fished at similar rates as non-infected lobsters. Thus, sampling bias may be present, resulting in an underestimation of the true shell disease and 'bumper car' infection prevalence in wild lobsters.

In summary, less than 7 % of the lobsters examined were positive for *Aerococcus viridans* while only 1 lobster out of 254 (0.4 %) was infected with *Anophryoides haemophila*. However, these 2 prevalences are likely underestimates of the true infections prevalence in wild lobster populations of lobster around Prince Edward Island.

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