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Maureen K. Purcell a, Anthony L. Murray a, Anna Elz b, Linda K. Park b, Susan V. Marcquenski c, James R. Winton a, Stewart W. Alcorn d, Ronald J. Pascho a & Diane G. Elliott a

a Western Fisheries Research Center, U.S. Geological Survey, 6505 Northeast 65th Street, Seattle, Washington, 98115, USA
b Northwest Fisheries Science Center, National Marine Fisheries Service, 2725 Montlake Boulevard East, Seattle, Washington, 98112, USA
c Wisconsin Department of Natural Resources, 101 South Webster Street, Madison, Wisconsin, 53707, USA
d School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, Washington, 98195-5020, USA

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Decreased Mortality of Lake Michigan Chinook Salmon after Bacterial Kidney Disease Challenge: Evidence for Pathogen-Driven Selection?

MAUREEN K. PURCELL* AND ANTHONY L. MURRAY
U.S. Geological Survey, Western Fisheries Research Center, 6505 Northeast 65th Street, Seattle, Washington 98115, USA

ANNA ELZ AND LINDA K. PARK
National Marine Fisheries Service, Northwest Fisheries Science Center, 2725 Montlake Boulevard East, Seattle, Washington 98112, USA

SUSAN V. MARQUENSKI
Wisconsin Department of Natural Resources, 101 South Webster Street, Madison, Wisconsin 53707, USA

JAMES R. WINTON
U.S. Geological Survey, Western Fisheries Research Center, 6505 Northeast 65th Street, Seattle, Washington 98115, USA

STEWART W. ALCORN
School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, Washington 98195-5020, USA

RONALD J. PASCHO AND DIANE G. ELLIOTT
U.S. Geological Survey, Western Fisheries Research Center, 6505 Northeast 65th Street, Seattle, Washington 98115, USA

Abstract.—In the late 1960s, Chinook salmon Oncorhynchus tshawytscha from the Green River, Washington, were successfully introduced into Lake Michigan. During spring from 1988 to 1992, large fish die-offs affecting Chinook salmon occurred in the lake. Multiple ecological factors probably contributed to the severity of the fish kills, but the only disease agent found regularly was Renibacterium salmoninarum, the causative agent of bacterial kidney disease. In this study, survival after challenge by R. salmoninarum was compared between two Chinook salmon stocks: a Lake Michigan stock from Wisconsin (WI) and the progenitor stock from the Green River. We found that the WI stock had significantly greater survival than the Green River stock. Next, the WI and Green River stocks were exposed to the marine pathogen Listonella anguillarum (formerly Vibrio anguillarum), one of the causative agents of vibriosis; survival after this challenge was significantly poorer for the WI stock than for the Green River stock. A close genetic relationship between the Green River and WI stocks was confirmed by analyzing 13 microsatellite loci. These results collectively suggest that disease susceptibility of Lake Michigan Chinook salmon has diverged from that of the source population, possibly in response to pathogen-driven selection.

Species introduced into new environments may experience altered or novel selective pressures. Studies of such transplants can provide insight into the processes of adaptation and may help to predict how a species will react evolutionarily to changes within its native range. The commercial and recreational value of Pacific salmon Oncorhynchus spp. has led to the deliberate and successful introduction of members of this genus into waters well beyond the natural ranges. The introduction of Chinook salmon O. tshawytscha into Lake Michigan in the late 1960s was partly intended as a method to control an invasive species, the alewive Alosa pseudoharengus (Holey et al. 1998). The documented source of gametes used by the Michigan Department of Natural Resources (MDNR) for the introduction was a fall-run (ocean-type) Chinook

* Corresponding author: mpurcell@usgs.gov
1 Present address: Hollister-Stier Laboratories, 3525 North Regal Street, Spokane, Washington 99207-5788, USA
2 Present address: Washington Department of Fish and Wildlife, 600 Capitol Way North, Olympia, Washington 98501-1091, USA

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salmon stock from the Green River, Washington (Holey et al. 1998; Weeder et al. 2005); the Green River stock is part of the Puget Sound evolutionarily significant unit (ESU; Myers et al. 1998). Chinook salmon eggs were transferred from MDNR to the Wisconsin Department of Natural Resources (WDNR) to establish runs in that state as well (Holey et al. 1998). Chinook salmon density increased in Lake Michigan during the 1970s and 1980s, supporting a high-value and popular recreational fishery. Peak Chinook salmon harvest was recorded during the summers of 1985–1987. However, the size and condition of harvested fish declined during that time, partly because their forage base, the alewife stock, was reduced (Hansen 1986). Starting in the spring of 1988, high mortality was observed among Chinook salmon; thousands of dead or moribund fish representing all year-classes appeared on the lake surface or were washed onto beaches (Holey et al. 1998). The only disease agent regularly associated with these mortality events (although with varying severity) was *Renibacterium salmoninarum*, a Gram-positive bacterium that causes a chronic disease and is one of the most significant pathogens of salmonid fishes worldwide (Wiens and Kaattari 1999). Chinook salmon are more susceptible to BKD than are other salmonid species (Fryer and Lannan 1993), although in Lake Michigan the severity of the BKD epizootic may have been complicated by additional stressors, such as poor nutrition and parasitic infections (Holey et al. 1998).

The present study was prompted by observations made during experiments with Chinook salmon obtained from Wisconsin (hereafter, WI stock). Using standard *R. salmoninarum* challenge methodology, we observed much lower mortality in the WI stock than in a West Coast Chinook salmon stock. To investigate this further, we designed several pilot studies and a full-scale laboratory challenge experiment to compare several cohorts of the WI stock with three different Pacific Northwest stocks, including the progenitor stock from the Green River. We also investigated the relative susceptibility of the WI and Green River stocks to the marine pathogen *Listonella anguillarum* (formerly *Vibrio anguillarum*; Austin and Austin 1999).

Finally, we assessed the genetic diversity of the contemporary WI stock and confirmed its relationship to Pacific Northwest populations by using microsatellite markers. The results are discussed in the context of our understanding of how the host–pathogen relationship may have been altered in Lake Michigan.

### Methods

#### Population genetic analysis

The collection of DNA samples and the typing of 13 microsatellite loci for the West Coast stocks listed in Table 1 were part of a multi-agency effort that resulted in a standardized, coastwide genetic baseline for Chinook salmon (Moran et al. 2005). The DNA from WI stock adults returning to the Strawberry Creek weir in 2003 and 2005 (Table 1) was extracted from ethanol-preserved fin or kidney tissue either manually (Qiagen 96-Well DNeasy Kit) or by use of a BioRobot 8000 (Qiagen) according to the manufacturer’s instructions. The DNA was quantified with a fluorometer and was diluted to 5 ng/µL before amplification by polymerase chain reaction (PCR). The DNA samples were assayed for allelic variation in the same 13 microsatellite loci that were used by Moran et al. (2005) to establish the coastwide genetic baseline. The PCR amplifications were performed in a 10-µL final reaction volume containing 0.5 units of *Taq* polymerase (Promega; enzyme number 2.7.7.7, IUBMB 1992), 1× reaction buffer (Promega), 1.75

### Table 1.—Description of 13 Chinook salmon populations used in an analysis of microsatellite loci to determine genetic relationships among an introduced Lake Michigan stock from Wisconsin (WI); its source population from the Green River, Washington (WA); and other populations from Puget Sound (N = north, S = south) and the Columbia River basin. Fish were collected as adults during the given return years (sampled run: Fa = fall, Sp = spring, Su = summer; sample origin: H = hatchery stock, W = wild or naturally spawning stock).

<table>
<thead>
<tr>
<th>Population</th>
<th>Region</th>
<th>Run</th>
<th>Origin</th>
<th>Year</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry Creek weir (WI)</td>
<td>Lake Michigan</td>
<td>Fa</td>
<td>H</td>
<td>2003</td>
<td>48</td>
</tr>
<tr>
<td>Green River (Soos Creek, WA)</td>
<td>S. Puget Sound</td>
<td>Fa</td>
<td>H</td>
<td>2004</td>
<td>53</td>
</tr>
<tr>
<td>Nooksack River (WA)</td>
<td>N. Puget Sound</td>
<td>Sp</td>
<td>H</td>
<td>1999</td>
<td>40</td>
</tr>
<tr>
<td>Stillaguamish River (WA)</td>
<td>N. Puget Sound</td>
<td>Su</td>
<td>H, W</td>
<td>1996</td>
<td>85</td>
</tr>
<tr>
<td>Skagit Hatchery (WA)</td>
<td>N. Puget Sound</td>
<td>Su</td>
<td>H</td>
<td>1994</td>
<td>81</td>
</tr>
<tr>
<td>Cowitz River (WA)</td>
<td>Lower Columbia River</td>
<td>Fa</td>
<td>H</td>
<td>2004</td>
<td>142</td>
</tr>
<tr>
<td>McKenzie River (OR)</td>
<td>Willamette River</td>
<td>Sp</td>
<td>H</td>
<td>2002</td>
<td>43</td>
</tr>
<tr>
<td>Queets River (WA)</td>
<td>Coastal WA</td>
<td>Fa</td>
<td>H</td>
<td>1996</td>
<td>50</td>
</tr>
<tr>
<td>Methow River (WA)</td>
<td>Upper Columbia River</td>
<td>Su-Fa</td>
<td>W</td>
<td>1994</td>
<td>84</td>
</tr>
<tr>
<td>Carson National Fish Hatchery (WA)</td>
<td>Middle and Upper Columbia River</td>
<td>Sp</td>
<td>H</td>
<td>2004</td>
<td>95</td>
</tr>
</tbody>
</table>
mM of MgCl$_2$ (Promega), 200 mM of deoxynucleotide triphosphates (Promega), 0.4 mM of each primer pair, 1× bovine serum albumin reagent (New England BioLabs, Inc.), and 15 ng of DNA. The PCR products were run on an Applied Biosystems 3100 Genetic Analyzer. GeneScan software version 3.7 was used to interpret raw fluorescence readings and to estimate relative fragment mobility. Genotyper version 3.7 (Applied Biosystems) was used to standardize genotypes as described by Moran et al. (2005).

Summary statistics for genotype diversity were calculated with FSTAT version 2.9.3 (Goudet 1995) and Genetix version 4.0.5 (Belkhir et al. 2004). The software GENEPOP version 3.4 (Raymond and Rousset 1995) was used to test for deviations from Hardy–Weinberg equilibrium (HWE). All $P$-values were adjusted by Bonferroni correction. Pairwise values of the genetic differentiation index $F_{ST}$ were calculated with adjusted significance based on the permutation process in FSTAT. To summarize genetic relationships among samples (Table 1), we constructed a neighbor-joining (NJ) dendrogram from Nei’s genetic distances $D_{ij}$ (Nei et al. 1983) between samples and assessed our results via bootstrap resampling (1,000 iterations) of the loci with the program Populations (Langella 1999). When multiple years of data were available from a single population, analyses were performed separately for each year and then repeated with the years pooled. Because overall results were similar, data from any given population were pooled across years for ease of presentation.

Obtaining specific pathogen-free Chinook salmon.—Returning adult Chinook salmon representing various stocks and years were used in this study, including the Abernathy fall run (sampled in 1999; U.S. Fish and Wildlife Service, Abernathy Fish Technology Center, Abernathy, Washington) and the Green River fall run (sampled in 2003 and 2005; Washington Department Fish and Wildlife, Soos Creek Hatchery, Rainier Complex, Auburn). Samples of the WI fall run were taken from the Root River Hatchery in 1999 and from the Strawberry Creek weir in 2003 and 2005 (i.e., the same stock was sampled at the two sites). Renibacterium salmoninarum is transmitted both horizontally and vertically, from infected adult females to their eggs (Evelyn et al. 1986). Vertical transmission requires that adults be screened for the presence of bacterial antigen, and only the progeny of adults with negative or low antigen levels are used in laboratory research (Evelyn et al. 1986; Pascho et al. 1991). This procedure is effective in minimizing the vertical transmission to progeny but cannot guarantee that all fish are specific pathogen free (SPF). Kidney tissue of males and females and ovarian fluids from females were tested by double polyclonal antibody enzyme-linked immunosorbent assay (ELISA II; Pascho et al. 1991). Gametes from parents with negative or low ELISA II values were used to create progeny. The cutoffs for ELISA II categories are based on absorbance values above established negative kidney and ovarian fluid samples used during the assay; samples were considered positive if the absorbance value (optical density [OD]) was two SDs above the mean value of the negative control, whereas the low category included any OD that exceeded the negative cutoff but was less than 0.2.

Immediately after fertilization, eggs of the WI stock were hardened in 750 mg of thiamine/L of water (to ameliorate thiamine deficiency) and were disinfected with iodophor (Winton 2001). Eggs of West Coast stocks were disinfected but not treated with thiamine. Before fertilization or at the eyed stage, all eggs were transferred to the Western Fisheries Research Center (WFRC), Seattle, Washington. The eggs were hatched and fish were reared in SPF, ultraviolet-treated, sand-filtered, 12°C freshwater; a natural photoperiod was used, and fish were fed a semimoist, pelleted commercial diet (BioProducts, Warrenton, Oregon). Progeny representing full-sibling families were pooled and used for all experiments. The number of pooled families varied for each cohort and ranged from 4 to 19. Fish representing a spring-run (stream-type) stock of Chinook salmon from Carson National Fish Hatchery (Carson NFH; U.S. Fish and Wildlife Service, Wind River, Washington) were also included in the 2003 experiment. These fish were obtained directly from the hatchery, and parents were not screened by WFRC for _R. salmoninarum_. However, females are routinely screened at Carson NFH with the same ELISA II methodology, and gametes from females with medium and high levels of _R. salmoninarum_ antigen are destroyed. A sample of progeny fish ($n = 30$) representing the three stocks used in the 2003 experiment was confirmed as being negative for the _R. salmoninarum_ antigen based on ELISA II conducted before the challenge, and no clinical signs of BKD were observed in the stock fish or mock-challenged controls.

Bacterial isolates and culture.—Two _R. salmoninarum_ isolates were used in this study: ATCC (American Type Culture Collection) 33209 (type strain isolated from a Chinook salmon collected in the Mackenzie River, Oregon) and GL64 (isolated from the spleen of an adult Lake Michigan Chinook salmon in 1990). Bacteria were cultured in kidney disease medium-2 (KDM-2) broth (Evelyn 1977) supplemented with 0.05% (weight/volume [w/v]) cysteine HCl, 10% (volume/volume [v/v]) fetal bovine serum, and 1.5%
(v/v) nurse medium containing filter-sterilized supernatant from *R. salmoninarum* cultures (Evelyn et al. 1990). Bacteria were cultured at 15°C for 7–10 d. Before use in the challenge, cultures were centrifuged at 5,000 × gravity for 20 min, and bacterial pellets were resuspended in sterile diluent (1 × phosphate-buffered saline [PBS]–peptone, 0.1% w/v; adjusted to a pH of 7.4). Bacterial cultures were enumerated by a membrane-filtration fluorescent antibody test (FAT) described by Elliott and Barila (1987). Serial dilutions of bacterial cultures were plated onto KDM-2 agar prepared as described above but with the addition of 1.5% (w/v) agar. Final challenge doses were reported as the number of colony-forming units (CFU), which was determined by plate counts.

*Listonella anguillarum* strain 775 (ATCC 68544) was cultured at 17°C in tryptic soy broth supplemented with 1.5% (w/v) sodium chloride until the OD reading at 630 nm reached about 0.8. Serial dilutions of cultures were plated onto tryptic soy agar (TSA) supplemented with 1.5% sodium chloride; the final challenge dose was reported in CFU per milliliter.

**Preliminary *R. salmoninarum* strains: 1999 and 2003 cohorts.**—Abernathy and WI fish from the 1999 cohort were challenged with *R. salmoninarum* isolates ATCC 33209 and GL64. Fish (average weight = 10 g) were anesthetized with tricaine methanesulfonate (MS-222; Argent Laboratories, Inc.) at 100 μg of MS-222/mL of water and were injected intraperitoneally (IP) with either 100 μL of diluent containing 5.0 × 10^5 CFU of the GL64 strain or 100 μL of diluent containing 6.0 × 10^5 CFU of the ATCC 33209 strain. After 50 fish/stock were injected, treatment groups were placed into separate 276-L tanks supplied with 12°C freshwater. Fish were monitored daily until 160 d postinjection. Fish were monitored daily for mortality until 160 d postinjection.

The relative postchallenge survival of Carson NFH, Green River, and WI fish from the 2003 cohort was assessed in a manner similar to that described above. Groups of fish (average weight = 10 g) were IP injected with either 100 μL of diluent (N = 20 fish/stock) or 100 μL of diluent containing 1.0 × 10^6 CFU of *R. salmoninarum* ATCC 33209 strain (N = 50 fish/stock). These groups were held in 276-L tanks at 12°C for 160 d postchallenge and were monitored daily for mortality. *R. salmoninarum* infection in 37 dead fish was confirmed by visualizing the bacterium in kidney tissue imprints with a direct FAT (dFAT; Pascho et al. 1991).

**Challenges with *R. salmoninarum* and *L. anguillarum*: 2005 cohort.**—Postchallenge survival of Green River and WI fish from the 2005 cohort was assessed in a manner similar to that used in preliminary studies, but with several modifications. Groups of 50 fish (average weight = 10 g) from each stock were IP injected with 100 μL of diluent and were held as mock-challenged controls in separate 276-L tanks. To account for tank effects, three replicate groups of fish (50 fish/replicate) from each stock (total N = 300 fish) were injected with 100 μL of diluent containing 9.5 × 10^5 CFU of *R. salmoninarum* strain ATCC 33209. All replicate challenged groups were held in separate 276-L tanks at a constant temperature of 12°C. Fish were monitored daily until 160 d postinjection; BKD was confirmed by dFAT as the cause of death for 30 fish, and *R. salmoninarum* was successfully cultured from 16 dead individuals.

Chinook salmon from the 2005 cohort were also challenged by waterborne exposure to *L. anguillarum* as described by Murray et al. (2003). A small preliminary challenge was used to determine the dose required to achieve 70% mortality in a 14-d period. Before the challenge, the fish (~10 g) were allowed to acclimate to 15°C. Eighty WI fish were apportioned equally to four replicate buckets (20 fish/bucket) that were provided with oxygen and that contained 6 L of static freshwater supplemented with 0.85% (w/v) sodium chloride. One bucket received 30 mL of culture medium only, whereas the other three buckets received 30 mL of medium containing 5.6 × 10^7 CFU/mL of *L. anguillarum*. An equal number of Green River fish was exposed simultaneously in an identical experiment. After the fish were exposed to the medium or bacterial suspension for 15 min, fish from each bucket were placed in separate 137-L tanks that received flowing 15°C freshwater. Fish were monitored daily for 14 d postchallenge. Kidney tissue from five dead fish was streaked onto TSA supplemented with 1.5% sodium chloride to confirm cause of death, and *L. anguillarum* was successfully isolated.

**Statistical analysis of challenge data.**—All statistical analyses were performed using the Statistical Package for the Social Sciences version 11.5. Because the preliminary challenges did not include replicate tanks, differences in final mortality between groups were assessed by Fisher’s exact test. Replicate tanks were included in the experimental design for the 2005 cohort, and the cumulative percent survival for each replicate tank was determined. Percent survival values were arcsine, square-root transformed and differences between the stocks were evaluated with an unpaired t-test. The significance level used in all tests was 0.05.

**Results**

**Population Genetic Analysis**

To assess the genetic relationship between the WI stock (Strawberry Creek adults returning in 2003 and 2005) and its progenitor, the Green River stock (Soos Creek adults returning in 1998, 2003, and 2004), we
analyzed 13 microsatellite loci (Table 1). Significant departures from HWE were observed for Sxa408 in the WI 2005 sample and for Ots201b in the Green River 1998 sample after Bonferroni correction. The two departures did not follow a trend and no other departures were observed, which suggested all populations were in HWE as measured by these 13 loci. Because pairwise $F_{ST}$ tests indicated no significant differences between years within a given population, data were combined to simplify presentation. The differences between years within a given population, these differences were not significant.

Higher in the Green River stock (values across loci for the combined years were slightly higher in the Green River stock ($H_0 = 0.83; N_r = 15.1$) than in the WI stock ($H_0 = 0.80; N_r = 13.4$), although these differences were not significant.

The average $F_{ST}$ (Wright 1931) for the entire standardized coastwide Chinook salmon data set was 0.071 (Moran et al. 2005). The pairwise $F_{ST}$ value between the WI and Green River stocks was 0.0075, indicating that a small but significant subdivision occurred ($P = 0.0001$). However, when analyzed with other representative populations across the species’ range by use of a neighbor-joining dendrogram of Nei’s $D_A$, the WI and Green River stocks were grouped together in a single node with 100% bootstrap support (Figure 1); moreover, these populations clustered separately from eight other populations, including those found in the northern range of the Puget Sound ESU (Nooksack, Stillaguamish, and Skagit rivers). The Abernathy stock used in the 1999 challenges is part of the lower Columbia River ESU and is represented by the Cowlitz River sample in Figure 1 and Table 1. The Carson NFH stock, used in the 2003 experiments, does not belong to any ESU but is most genetically related to fish from the Upper Columbia and Snake River basins (Figure 1; Table 1; Drake et al. 2003).

**Preliminary R. salmoninarum Challenges: 1999 and 2003 Cohorts**

After challenge with *R. salmoninarum*, WI fish from the 1999 cohort had significantly greater survival and delayed mean days to death (MDD) than the Abernathy stock, irrespective of whether the GL64 or ATCC 33209 isolate was used (Figure 2a). However, the ATCC 33209 strain was more pathogenic than the GL64 strain in both stocks. Total survival of WI fish was 82% (MDD = 107) after challenge with the GL64 isolate and 54% (MDD = 94) after challenge with the ATCC 33209 isolate. In contrast, Abernathy fish had only 28% survival (MDD = 84) when challenged with GL64 and 16% survival (MDD = 80) when challenged with ATCC 33209 (Figure 2a). Significant differences in final mortality were observed between the two stocks for groups challenged with either ATCC 33209 or GL64 (Fisher’s exact test: $P < 0.001$ for both).

Fish from the 2003 cohort were IP injected with ATCC 33209, and survival was compared with that of the mock-challenged control groups. Low mortality was observed in the mock-challenged control groups; these fish were negative for BKD but had clinical signs of fin rot (etiology unknown). Total survival in the mock-challenged groups was 85% for the WI stock, 95% for the Green River stock, and 95% for the Carson NFH stock (Figure 2b). Total survival in the ATCC 33209 challenged groups was 0% for the Carson NFH stock (MDD = 62), 30% for the Green River stock (MDD = 81), and 80% for the WI stock (MDD = 87; Figure 2b). Final mortality of the WI stock differed significantly from that of the Green River and Carson NFH stocks (Fisher’s exact test: $P < 0.001$ for both).

**Renibacterium salmoninarum and Listonella anguillarum challenges: 2005 cohort**

The *R. salmoninarum*-challenged WI fish of the 2005 cohort exhibited tail rot (etiology unknown), which was first observed at 103 d postchallenge. No mortality was observed in mock-challenged controls throughout the 160-d challenge period. Several fish from the WI group were euthanized, and it is not clear, after this time point, if the natural mortality was due to BKD, tail rot, or mixed etiology. Because tail rot possibly confounded the experiment after 103 d postchallenge, the challenge results were considered reliable only up until 100 d postchallenge. These data are presented in Figure 3a. Total survival (mean ± SD) in the *R. salmoninarum*-challenged groups (3 replicate tanks/stock) at 100 d postchallenge was 41.2 ± 5.1% for the WI stock (MDD = 66) and 21.2 ± 5.6% for the Green River stock (MDD = 58). Percent survival at 100 d postchallenge differed significantly between the WI and Green River stocks (unpaired t-test: $P = 0.013$). At 160 d postchallenge (despite the tail rot that affected only the WI stock), total survival was greater for the WI stock (25.6 ± 11.3%) than for the Green River stock (15.7 ± 3.4%).

In contrast to *R. salmoninarum* challenge results, the *L. anguillarum* challenge produced lower survival in the WI stock than in the Green River stock. As shown in Figure 3b, total survival (mean ± SD) in the WI stock at 14 d postchallenge was 11 ± 4% (MDD = 5.4), whereas that in the Green River stock was 32 ± 6% (MDD = 5.8); cumulative percent survival was significantly different between stocks (unpaired t-test: $P = 0.008$). No mortality was observed in the mock-challenged control groups.
The documented source of Chinook salmon in Lake Michigan is the Green River stock (Weeder et al. 2005). A previous allozyme study found strong support for the Green River stock as the predominant founding population for several Lake Michigan stocks, although there was a slight reduction in the mean number of alleles in Lake Michigan fish, possibly the result of founding effects (Weeder et al. 2005). Because no WI Chinook salmon were included in the allozyme study by Weeder et al. (2005), we used microsatellite markers to examine the WI stock. Our results are in agreement with results obtained for other Lake Michigan stocks (Weeder et al. 2005), supporting the close relationship between the WI and Green River stocks. A small but significant divergence between the contemporary stocks was observed, but the pairwise $F_{ST}$ value for the WI and Green River stocks was very low and approximated values observed between stocks.
within the same watershed (Moran et al. 2005; Neville et al. 2006). Our analysis revealed a slight, nonsignificant reduction in \( N_r \) in the WI stock relative to the Green River stock, but overall these two stocks have maintained similarly high levels of genetic diversity as measured by neutral genetic markers.

Disease resistance varies both within and among salmonid populations (Zinn et al. 1977; Winter et al.
In this study, we demonstrated that the WI stock had significantly higher survival in laboratory challenges with *R. salmoninarum* than did the Green River stock. Additionally, the WI stock had higher survival than other Pacific Northwest stocks (Abernathy and Carson NFH), which indicates that the divergence occurred in

1980; Wertheimer and Winton 1982; Beacham and Evelyn 1992; Ibarra et al. 1994; Bower et al. 1995; Dalgaard et al. 2003;); this is also true of BKD resistance (Withler and Evelyn 1990; Hard et al. 2006). In this study, we demonstrated that the WI stock had

![Graph A](image1)

**FIGURE 3.**—Percent survival (mean ± SD) of Chinook salmon from the 2005 cohort after (A) intraperitoneal injection with diluent (mock challenge) or with *Renibacterium salmoninarum* strain ATCC (American Type Culture Collection) 33209 (9.5 × 10^5 colony-forming units [CFU]/fish) and (B) waterborne exposure to culture medium (mock challenge) or to *Listonella anguillarum* strain 775 (6.0 × 10^5 CFU/mL). Open diamonds represent mock-challenged fish from the Strawberry Creek, Wisconsin (WI), stock; black diamonds represent bacterially challenged WI fish. Open squares represent mock-challenged fish from the Green River, Washington, stock; black squares represent bacterially challenged Green River fish (3 tanks/bacterial treatment; 50 fish/tank).
the WI stock. This pattern was evident regardless of whether a Great Lakes or a Pacific Northwest strain of \textit{R. salmoninarum} was used. In our study, we did not assess heritability of BKD resistance; thus, we cannot rule out the possibility that an environmental factor contributed to the differential survival. However, fish were transferred to the laboratory facility as eggs and were reared in SPF water in a common environment before challenge occurred. Previous studies indicate a relatively high heritability of BKD resistance in Chinook salmon, suggesting additive genetic variation exists for this trait; such variation has a strong evolutionary potential of responding to selection (Beacham and Evelyn 1992; Hard et al. 2006). It is unclear whether the WI stock is resistant to \textit{R. salmoninarum} (i.e., limiting the infection such as by limiting bacterial replication) or is tolerating the pathogen by compensating for or reducing pathological damage. Ongoing WFCR studies are aimed at understanding the mechanistic basis of the lower mortality in the WI stock; these studies are examining \textit{R. salmoninarum} persistence after challenge, host immune responses, and histopathological damage.

We hypothesize that the greater survival of the WI stock after \textit{R. salmoninarum} challenge may result from pathogen-driven selection. If true, these findings raise the question of why pathogen-driven selection has not resulted in increased resistance in Pacific Northwest stocks, as \textit{R. salmoninarum} probably has been present in the region over a long evolutionary period. Although the prevalence of \textit{R. salmoninarum} in juvenile Chinook salmon has been reported to reach 100\% in the Columbia River basin (Elliott et al. 1997) and 64\% in Puget Sound (Rhodes et al. 2006), fairly low infection intensities were observed in the majority of sampled fish. The magnitude of direct or indirect, delayed mortality after ocean entry that can be attributed to \textit{R. salmoninarum} infection is uncertain (Pascho et al. 1991; Elliott et al. 1997). The Lake Michigan BKD epizootics impacted all life stages of Chinook salmon over 4 years, and the loss of prespawning adults could apply particularly strong selective forces to the stock. In the Pacific Northwest, many of the returning Chinook salmon that are infected with \textit{R. salmoninarum} do successfully spawn—although some hatcheries cull the eggs from highly infected females (Pascho et al. 1991; Elliott et al. 1997). In Lake Michigan, the collapse of the alewife population probably produced an additional stressor that exacerbated the disease process and contributed to the severity of the BKD epizootics (Hansen and Holey 2002).

Various factors can affect \textit{R. salmoninarum} laboratory challenges, and the among-year variation in survival observed in our studies may reflect this. Although the progeny of different year-classes may have had varying susceptibility to BKD, we cannot draw this conclusion from our data, because challenge conditions varied slightly across years (i.e., fish density, fish age, and photoperiod [differing by month of challenge]). Results for WI fish (2005 cohort) challenged with \textit{R. salmoninarum} were confounded by tail rot after 100 d postinjection; no tail rot or mortality was observed in the corresponding mock-challenged controls. Although some of the WI fish that died after 100 d had clinical signs of tail rot, all dead fish had clinical signs of BKD. Tail rot can expose the muscle cells directly to the environment, compromising osmotic regulation. Thus, there is a potential synergistic effect of tail rot and \textit{R. salmoninarum} on kidney function. \textit{Renibacterium salmoninarum} possesses a virulence factor with immunosuppressive properties (Wiens and Kaattari 1999), making fish with BKD more susceptible to opportunistic pathogens. Tail rot probably contributed to the overall higher mortality observed in the 2005 challenges of the WI stock, but survival in that stock was still greater than survival of the Green River stock.

The bacterium \textit{L. anguillarum} is typically encountered when Chinook salmon enter the marine environment; thus, the WI stock and other Lake Michigan stocks are not exposed to this pathogen. This leads to the intuitive prediction that relaxed selection may increase the WI stock’s susceptibility to \textit{L. anguillarum} and, indeed, our results support that prediction. Additional studies should be done to confirm this finding, because the \textit{L. anguillarum} challenge was applied to a single cohort. However, the \textit{R. salmoninarum} challenge results for WI fish of the 2005 cohort provide no support for the hypothesis that a nonspecific or generalized enhancement of resistance to bacterial infection contributed to the lower mortality in WI fish relative to Green River fish. Although we observed reverse patterns of susceptibility to \textit{R. salmoninarum} and \textit{L. anguillarum}, it is not appropriate to conclude from these data that susceptibility of Chinook salmon to the two pathogens is genetically correlated. However, strong selection for immunity against one pathogen may result in increased susceptibility to other pathogens. The WI stock may be a useful model for further study of immunological tradeoffs in disease resistance.

Our focus was a single stock from WI; study of other populations could strengthen the hypotheses developed herein. To date, there has not been a systematic screening of BKD susceptibility in other Chinook salmon stocks within Lake Michigan or the other Great Lakes. Such studies are difficult because of the need to
produce large numbers of size- and age-matched SPF fish; also, *R. salmoninarum* challenges are laborious and lengthy and require specialized laboratory facilities. Alternatively, development of genetic markers associated with fitness traits of interest may facilitate rapid measurement of trait frequencies in a population and increase our understanding of the relationship between neutral and adaptive genetic variation (Carvalho et al. 2002). The WI and Green River stocks may constitute a suitable model system with which to develop and validate potential surrogate markers of disease resistance for population-level surveys. In summary, the introduced Chinook salmon stock maintained by the WDNR exhibits neutral genetic variation similar to levels observed in the progenitor stock; however, since its founding approximately 13 generations ago, the WI stock has diverged from the source stock in the ability to resist or tolerate *R. salmoninarum*. Our results underscore the adaptive evolutionary potential of a diverse salmonid population to respond to changes in disease ecology.

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