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Epidemiological investigation of *Renibacterium salmoninarum* in three *Oncorhynchus* spp. in Michigan from 2001 to 2010

Mohamed Faisal[a,b,*], Carolyn Schulz[b], Alaa Eissa[c], Travis Brenden[b,d], Andrew Winters[b], Gary Whelan[e], Martha Wolgamood[f], Edward Eisch[g], Jan VanAmberg[h]

[a] *Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI 48824, USA*
[b] *Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA*
[c] *Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt*
[d] *Quantitative Fisheries Center, Michigan State University, East Lansing, MI 48824, USA*
[e] *Fisheries Division, Department of Natural Resources, Lansing, MI 48917, USA*
[f] *Wolf Lake State Fish Hatchery, 34270 County Road 652, Mattawan, MI 49071, USA*
[g] *Platte River State Fish Hatchery, 15210 U.S. 31, Beulah, MI 49617, USA*
[h] *Thompson State Fish Hatchery, 944 S. State Highway M149, Manistique, MI 49854, USA*

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ABSTRACT

Bacterial kidney disease (BKD) has caused mortalities and chronic infections in wild and farm-raised salmonids throughout the world. In the Laurentian Great Lakes of North America, BKD was associated with several large-scale mortality events of *Oncorhynchus* spp. throughout the 1980s and 1990s. In response to these mortality events, the state of Michigan implemented several enhanced biosecurity measures to limit the occurrence of BKD in state-operated hatcheries and gamete-collection weirs. The objectives of this study were to assess if infection levels (prevalence and intensity) of *Renibacterium salmoninarum*, the causative agent of BKD, have changed in broodstock and pre-stocking fingerlings of three feral *Oncorhynchus* spp. (Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), and steelhead (*O. mykiss*)) over a decade, following the implementation of the enhanced biosecurity measures. Between 2001 and 2010, a total of 3,530 broodstock salmonids collected from lakes Huron and Michigan tributaries during spawning runs and 4,294 propagated pre-stocking salmonid fingerlings collected from three state of Michigan fish hatcheries were tested for the presence of *R. salmoninarum* antigens using the enzyme-linked immunosorbent assay. Substantial declines in the overall prevalence of the bacterium were detected in each of the examined broodstocks. Most propagated pre-stocking fingerlings also exhibited substantial declines in *R. salmoninarum* prevalence. Prevalence was typically higher in Chinook salmon from Lake Michigan than from Lake Huron; prevalence was also generally higher in the Hinchenbrooke strain of coho salmon than in the Michigan-adapted strain. For most strains and stocks examined, intensity of *R. salmoninarum* infection was found to have declined. Although there were declines in the potential for shedding the bacteria for both male and female Chinook and coho salmon, overall shedding rates were generally low (<15%) except for Hinchenbrooke coho salmon strain, which had shedding prevalences in excess of 50% at the beginning of the study. This study provides evidence that enhanced biosecurity measures at culture facilities and collection sites are capable of severely curtailing disease infection in wild populations even at the scale of Lake Michigan fisheries.

* Corresponding author at: Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI 48824, USA. Tel.: +1 517 884 2019; fax: +1 517 432 2310.
E-mail address: faisal@cvm.msu.edu (M. Faisal).

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1. Introduction

The Laurentian Great Lakes (LGL) support a diverse fish community, including Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*O. kisutch*), and steelhead (*O. mykiss*; the migratory strain of rainbow trout), which are both recreationally and ecologically important. Even though these three species have supported valuable sport fisheries for a number of decades, they are not native to the LGL and were initially introduced to exert predatory pressure on and reduce densities of non-native pelagic prey fishes (mainly alewives, *Alosa pseudoharengus*), and further expand LGL sportfishing opportunities (Keller et al., 1990; Holey et al., 1998; Hansen and Holey, 2002). The stocking attempts proved to be successful and high quality recreational fisheries quickly became established (Hansen and Holey, 2002; Tanner and Tody, 2002).

The successful introduction of Chinook salmon, coho salmon, and steelhead to the LGL and the popular sport fisheries that resulted, led the State of Michigan to develop a Pacific salmonid rearing program in several state-operated fish hatcheries (Dexter and O'Neal, 2004). Chinook salmon, coho salmon, and steelhead are propagated annually using egg and milt samples collected at gamete-collection weirs operated by the Michigan Department of Natural Resources (MDNR) on several LGL tributaries (Dexter and O'Neal, 2004). Fish are raised in the hatcheries until they are between 6 and 18 months of age (depending on the species) and are stocked at several locations in the LGL, as well as in a number of LGL streams in Michigan (Dexter and O'Neal, 2004).

Among the pathogens that *Oncorhynchus* spp. are susceptible to is the Gram-positive diplobacillus *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD), which is transmitted both horizontally and vertically and hence extremely difficult to control. BKD can take the form of chronic and acute infections and is characterized by the formation of granulomas in kidneys and other visceral organs (Fryer and Sanders, 1981). The disease was first documented in Michigan hatcheries in 1955 (Allison, 1958) and has since spread to all of the Great Lakes, as well as several inland lakes and rivers in the LGL region (Allison, 1958; MacLean and Yoder, 1967; Holey et al., 1998; Beyerle and Hnath, 2002; Eissa et al., 2006; Nuhfer, 2006; Faisal et al., 2010).

BKD has been associated with several mortality events of *Oncorhynchus* spp. in the LGL (MacLean and Yoder, 1967; Holey et al., 1998). MacLean and Yoder (1967) documented the presence of *R. salmoninarum* in dead coho salmon from lakes Michigan and Superior. During the period from 1988 to 1992, annual mortality events of Chinook salmon in Lake Michigan occurred, which was attributed at least in part to a BKD epidemic (Holey et al., 1998). These die-offs had drastic effects on the Lake Michigan Chinook salmon fishery, with the recreational fishery yield declining approximately four-fold between the mid and late 1980s (Hansen and Holey, 2002).

In response to the BKD epidemic, the MDNR initiated a number of enhanced biosecurity practices at state-operated hatcheries and gamete-collection weirs to limit the occurrence and spread of BKD (Table 1). The MDNR expanded its biosecurity practices to include clinical inspections, culling, egg disinfection, hardening the eggs in water containing the antibiotic erythromycin, regular screening of propagated fish, and treatment with antibiotics, such as erythromycin. While erythromycin is not yet fully approved by the U.S. Food and Drug Administration to treat BKD, limited use of the antibiotic is allowed as an investigational new animal drug exemption.

The objectives of this study were to assess if infection levels (prevalence and intensity) of *R. salmoninarum* in broodstock and pre-stocking fingerlings from three feral *Oncorhynchus* spp. during the decade 2001–2010, have changed with the enhanced biosecurity practices. An additional objective of the study was to assess the role of shedding *R. salmoninarum* in the gametes of the broodstock on the overall prevalence and intensity of *R. salmoninarum* in pre-stocking fingerlings.

2. Materials and methods

2.1. Fish collection

Between 2001 and 2010, a total of 3,530 feral, spawning Chinook and coho salmon and steelhead were collected from MDNR gamete-collection weirs in Michigan, USA (Fig. 1; Table 2). Ages of the fish ranged from approximately 3–5 years. For this study, the five species/stocks/strains of fish were designated based on the location of collection (or source of gametes) and fish species or strain: Chinook salmon from the Little Manistee River Weir (LMRW-CHS), Chinook salmon from the Swan River Weir (SRW-CHS), the Hinchenbrooke strain of coho salmon from the Platte River Weir (HB-COS), the Michigan-adapted strain of coho salmon from the Platte River Weir (MI-COS), and steelhead from the Little Manistee River Weir (LMRW-STT;

Table 1
Enhanced biosecurity measures to control Bacterial Kidney Disease that have been implemented at Michigan Department of Natural Resources gamete-collecting weirs and hatchery facilities.

Biosecurity measure	Description
Clinical inspection	Examination for disease signs such as hemorrhages, exophthalmia, congested internal organs, and granulomas
Culling	Euthanasia of any individuals exhibiting the above signs
Egg disinfection	Disinfecting the external surface of the eggs to reduce the amount of bacteria
Hardening eggs in erythromycin-laden water	Water hardening the eggs (a necessary step for fertilization) in the antibiotic erythromycin, which *Renibacterium salmoninarum* is susceptible to
Regular screening	Frequent testing of propagated fish to determine if infection prevalence or mortality exceeded 0.05%
Antibiotic treatment	If prevalence indeed exceeded 0.05%, treatment with antibiotics under the investigational new animal drug exemption (INAD), as chosen by the antibiotic disc diffusion test

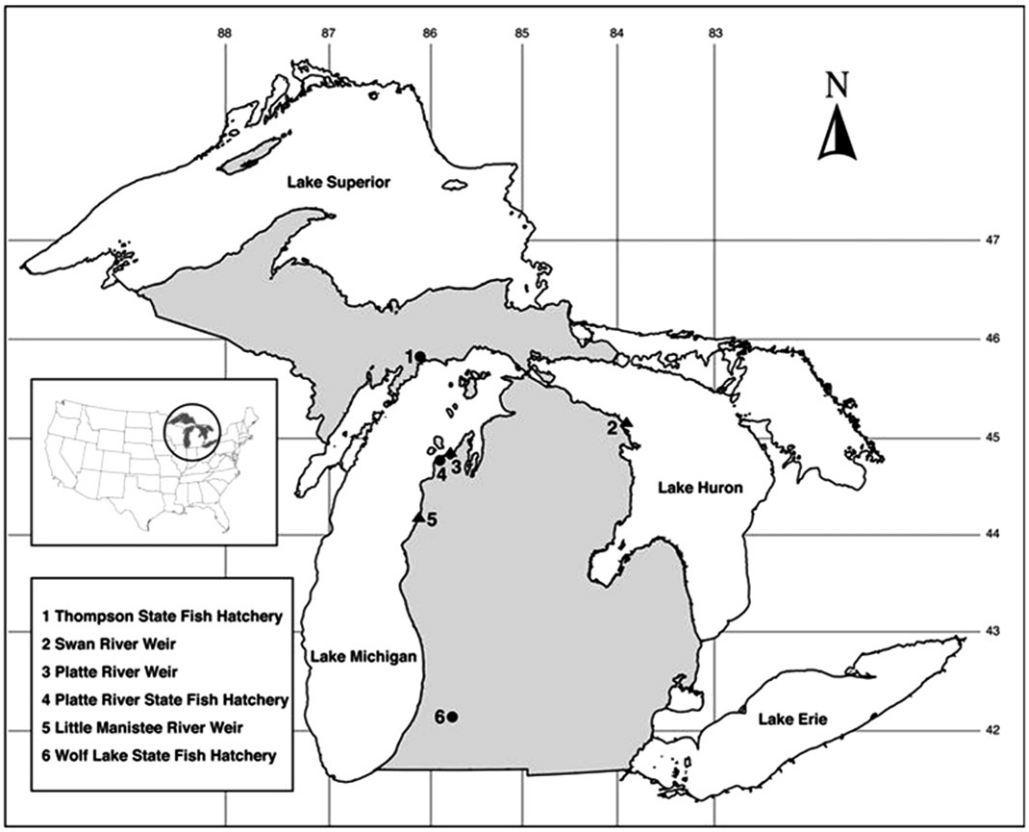

Fig. 1. The Michigan Department of Natural Resources state fish hatcheries and gamete-collecting weirs where Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) and steelhead (*O. mykiss*) were collected from 2001 to 2010: Little Manistee River Weir (44°11′51.66″N, 86°11′38.99″W), Platte River Weir and State Fish Hatchery (44°39′48.88″N, 85°56′13.20″W), Swan River Weir (45°24′10.09″N, 83°44′5.52″W), Thompson State Fish Hatchery (45°57′16.07″N, 86°15′29.36″W), and Wolf Lake State Fish Hatchery (42°17′40.14″N, 85°47′2.29″W).

Table 2). Chinook and coho salmon were sampled during the months of September and October of every year, while steelhead were sampled in April of every year. Overall, male and female fish were collected in roughly equal proportions, although sex ratios did vary across the sampling periods.

In addition to the broodstock, 4,294 propagated prestocking fingerlings were evaluated for *R. salmoninarum* (Table 3). Fish were between 6 and 18 months of age at time of sampling. Chinook salmon fingerlings were propagated at the Platte River State Fish Hatchery (PRSFH), the Thompson State Fish Hatchery (TSFH), and the Wolf Lake State Fish Hatchery (WLSFH). However, prevalence declines were not assessed for LMRW-CHS at the TSFH because there was only one year of data collection. Coho salmon fingerlings were only propagated at the PRSFH. Steelhead fingerlings were propagated at the TSFH and WLSFH (Fig. 1). The environmental conditions at the hatcheries (water temperature, dissolved oxygen levels, fish densities, etc.) were monitored and remained optimal for fish rearing, while reducing the risk of disease.

The number of fish sampled varied depending on the availability of fish returning to spawn at the gamete-collection weirs and the number of fish available from MDNR hatcheries (Tables 2 and 3). Additionally, sacrificing large groups of fish (i.e., at least 60 fish) is required to provide a 95% confidence of detecting the pathogen, with an assumed minimum incidence of 5% of the disease (Hnath, 1993; Ossiander and Wedemeyer, 1973).

A description of the enhanced biosecurity measures implemented by the MDNR at gamete-collecting weirs and hatchery facilities is provided in Table 1.

2.2. Sample collection

2.2.1. Broodstock

Length, weight, and sex of all fish collected by MDNR personnel at gamete collection weirs were recorded. Each fish was also thoroughly examined both externally and internally for clinical signs of BKD or other diseases. The gross pathology that was observed included exophthalmia, ascites, granulomas in the kidneys, and swelling and congestion of hematopoietic organs. Samples (<5 g) of kidneys and spleens were removed in the field and stored on ice in whirlpaks (VWR International, West Chester, PA), as recommended by the American Fisheries Society Bluebook (2010) and the World Organization for Animal Health (OIE, 2006), and frozen at −20 °C until processing. Additionally, approximately 5 ml of ovarian fluid or milt were collected from the same feral fish, stored on ice in 15 ml centrifuge tubes (Denville Scientific, Inc., Metuchen, NJ), and frozen at −20 °C once returned to the laboratory. Samples were

Table 2
The number of spawning *Oncorhynchus* spp. analyzed for the presence of *R. salmoninarum* antigens from 2001 to 2010. Hinchenbrooke coho salmon were not collected after 2007. ND = no data collected.

Year	Chinook salmon						Coho salmon						Steelhead		
	Little Manistee River Weir (LMRW-CHS)			Swan River Weir (SRW-CHS)			Michigan-adapted (MI-COS)			Hinchenbrooke (HB-COS)			Little Manistee River Weir (LMRW-STT)		
	# of Females	# of Males	TOTAL	# of Females	# of Males	TOTAL	# of Females	# of Males	TOTAL	# of Females	# of Males	TOTAL	# of Females	# of Males	TOTAL
2001	30	30	60	ND	ND	ND	19	19	38	25	24	49	ND	ND	ND
2002	30	30	60	30	29	59	83	82	165	28	28	56	ND	ND	ND
2003	30	30	60	17	17	34	30	30	60	24	24	48	ND	ND	ND
2004	60	60	120	560	0	560	58	59	117	60	60	120	30	30	60
2005	30	30	60	30	30	60	31	30	61	31	30	61	31	31	62
2006	30	30	60	30	30	60	30	30	60	30	30	60	30	30	60
2007	30	30	60	30	30	60	30	30	60	30	30	60	30	30	60
2008	30	30	60	30	30	60	30	30	60	ND	ND	ND	30	30	60
2009	50	50	100	50	50	100	50	50	100	ND	ND	ND	30	30	60
2010	50	50	100	50	50	100	50	50	100	ND	ND	ND	30	30	60
TOTAL	372	371	740	827	266	1093	411	410	821	228	226	454	211	211	422

frozen at −20 °C for no longer than 30 days before being processed by laboratory personnel.

2.2.2. Pre-stocking fingerlings

Propagated fish were collected within the hatcheries and euthanized with an overdose (250 mg/L) of Tricaine Methanesulfonate (MS-222, Argent Chemicals, Redmond, WA). Lengths and weights of each fish were recorded. Each fingerling was examined both externally and internally for clinical signs of BKD infection. The gross pathology that was observed included exophthalmia, ascites, granulomas in the kidneys, and swelling and congestion of hematopoietic organs. The entire kidney and a sample of spleen were aseptically removed, stored in whirlpaks, and frozen at −20 °C until processing. Samples were frozen at −20 °C for no longer than 30 days before being processed by laboratory personnel.

2.3. Sample processing

Kidney and spleen tissue samples were diluted 1:4 (w:v) with Hank's Balanced Salt Solution (HBSS, Sigma–Aldrich, St. Louis, MO) and homogenized on high speed for 2 min with a Biomaster Stomacher (Wolf Laboratories Limited, Pocklington, York, UK) as described by Faisal et al. (2009). Gamete samples were also diluted 1:4 (w:v) with HBSS and vortexed on high speed for approximately 30 s. Each homogenized kidney and spleen tissue sample and gamete sample were then tested for the presence of *R. salmoninarum* using the quantitative enzyme-linked immunosorbent assay (Q-ELISA).

2.4. Quantitative-enzyme linked immunosorbent assay procedure

The general Q-ELISA protocol outlined in Pascho and Mulcahy (1987), with modifications recommended by Gudmundsdottir et al. (1993) and Olea et al. (1993), was used to assess *R. salmoninarum* antigens in all sampled fishes. Homogenized spleen, kidney, and gamete samples (250 μl) were aliquoted into 1.5-ml microcentrifuge tubes (DOT Scientific, Inc., Burton, MI) containing 250 μl of Phosphate Buffered Saline with Tween-20 (PBS-T20; Sigma) and 5% goat serum (Sigma) and 50 μl of CitriSolv (Fisher Scientific, Pittsburgh, PA). The purpose of the CitriSolv solvent was to dissolve and remove liquids from the aqueous supernatant (Gudmundsdottir et al., 1993), while the introduction of 5% goat serum was to increase the sensitivity of the assay (Olea et al., 1993). Additionally, Pascho and Mulcahy (1987) demonstrated the excellent specificity of the procedure by showing that the Q-ELISA did not react with antigens from 11 species of bacteria, including the common fish pathogens *Aeromonas salmonicida*, *Vibrio anguillarum*, and *Yersinia ruckeri*. Samples were vortexed for approximately 10 s, heated at 100 °C for 15 min, and then centrifuged at 14,000 rpm for 10 min. The aqueous supernatant of each sample was used for Q-ELISA testing. The positive–negative cut-off absorbance for the samples was 0.10 (Meyers et al., 1993). Samples that tested positive were assigned the following antigen levels: low (0.10–0.199), medium (0.20–0.999), and high (≥1.00), as

Table 3
The number of propagated *Oncorhynchus* spp. reared in state fish hatcheries and tested for *Renibacterium salmoninarum* antigens prior to stocking. State fish hatchery facilities included the Platte River State Fish Hatchery (PRSFH), the Thompson State Fish Hatchery (TSFH), and the Wolf Lake State Fish Hatchery (WLSFH). *Species/strains were not reared at these locations at that year.

Year	Chinook salmon						Coho salmon		Steelhead	
	Little Manistee River Weir			Swan River Weir			Platte River Weir		Little Manistee River Weir	
	PRSFH	TSFH	WLSFH	PRSFH	TSFH	WLSFH	HB-PRSFH	MI-PRSFH	TSFH	WLSFH
2002	65	*	60	*	*	*	*	*	*	*
2003	60	*	60	60	60	*	60	60	*	*
2004	60	*	60	60	60	60	60	60	*	*
2005	60	*	60	60	60	60	120	120	60	60
2006	60	*	60	60	39	60	60	60	30	92
2007	60	*	60	60	60	60	59	60	119	159
2008	60	58	11	*	*	*	*	60	141	137
2009	60	*	60	60	60	*	*	60	126	120
2010	60	*	60	*	59	*	*	60	120	139
TOTAL	545	58	491	360	398	240	359	540	596	707

recommended by Meyers et al. (1993) and Pascho et al. (1998). Each assay included two negative controls (a negative fish kidney and spleen sample and a dilution buffer) and two positive controls (a positive kidney and spleen sample and the standards supplied with the kit).

Broodstock specimens were separated into one of four infection categories based upon Q-ELISA results from the kidney/spleen and gamete samples: (1) individuals that were negative for *R. salmoninarum* in the kidney and spleen sample and the gamete sample (KS−/G−); (2) individuals that were positive for *R. salmoninarum* in the kidney and spleen sample and negative in the gamete sample (KS+/G−); (3) individuals that were negative for *R. salmoninarum* in the kidney and spleen sample and positive in the gamete sample (KS−/G+); and (4) individuals that were positive for *R. salmoninarum* in the kidney and spleen sample and the gamete sample (KS+/G+). Individuals that were positive for *R. salmoninarum* in the gamete sample were considered to be potential shedders of the bacterium.

2.5. Data analyses

Logistic regression was used to evaluate how *R. salmoninarum* prevalence in broodstock and propagated pre-stocking fingerlings had changed over time. Logistic regression was also used to evaluate how prevalence of broodstock shedding had changed over time. For each response variable, a series of models that differed with respect to model intercepts and slopes were fit to observed infection or shedder data. A complete listing and description of the models is presented in Table A.1 of the Appendix. For assessing *R. salmoninarum* prevalence in broodstock, the most complex model had species/stock/strain-specific intercepts and slopes. For assessing *R. salmoninarum* prevalence in pre-stocking fingerlings, the most complex model had species/stock/strain/hatchery-specific intercepts and slopes. For assessing shedding prevalence in broodstock, the model complex model had species/stock/strain/sex-specific intercepts and slopes. For each fitted model, Akaike information criterion (AIC) was calculated to evaluate model goodness of fit (a low AIC value indicates a parsimonious model that provides a good fit to the data; Burnham and Anderson, 2002).

Changes in rates of *R. salmoninarum* infection intensities over time for both broodstock and propagated pre-stocking fingerlings were evaluated using multinomial logistic regression. Multinomial logistic regression was also used to assess changes in the different shedding categories (i.e., gamete− and gamete+) over time. We used multinomial logistic regression models rather than cumulative logit models because generally the data did not meet the proportional odds assumption necessary for a cumulative logit model (Agresti, 2007). When fitting the multinomial logistic regression models to infection intensities, we used the negative infection category (Q-ELISA < 0.10) as the reference category. When fitting the multinomial logistic regression models to the shedder categories, we used KS−/G− as the reference category. Thus, the multinomial regression models for the infection intensity levels fit models that evaluated how the log-odds of the low, medium, and high infection intensity levels changed relative to that of non-infected category. For the shedding data, the multinomial logistic regression models evaluated how the log-odds of the KS+/G−, KS−/G+, KS+/G+ shedding categories changed relative to the KS−/G− shedding category. Unlike the model selection process that was used when fitting the *R. salmoninarum* prevalence data, intensity and shedding category models were fit individually to each species/stock/strain (broodstock intensity), species/stock/strain/hatchery (propagated pre-stocking fingerling intensity), and species/stock/strain/sex (broodstock shedding) combination to simplify analysis and facilitate interpretation. All logistic and multinomial logistic regression models were fit in SAS using PROC GLIMMIX (SAS Institute Inc, 2010).

To determine if there was any association in *R. salmoninarum* prevalence between broodstock and progeny, we conducted Pearson correlation analyses on the calculated broodstock and pre-stocking fingerling prevalences. Correlation analyses were only conducted on Chinook salmon and coho salmon as there was insufficient data for steelhead for this analysis. For Chinook salmon, there was a 1 year time lag between the broodstock and pre-stocking

fingerling comparisons as this species is reared as spring fingerling then released. Thus, Chinook salmon broodstock prevalence in 2007 was compared to pre-stocking fingerling prevalence in 2008. For Coho salmon, there was a 2-year time lag as fish of this species as this species is reared as yearlings then released. Thus, coho salmon brood stock prevalence in 2007 was compared to pre-stocking fingerling prevalence in 2009. Correlation analyses were conducted in SAS using PROC CORR (SAS Institute Inc, 2010).

3. Results

3.1. Renibacterium salmoninarum infection prevalence and intensity in salmon broodstocks

When data were combined for the five stocks, the overall prevalence of *R. salmoninarum* was 22.1% (SE = 0.7%). Coho salmon had the greatest overall prevalence of *R. salmoninarum* at 34.0% (SE = 1.3%), followed by Chinook salmon at 18.0% (SE = 0.9%) and steelhead at 3.8% (SE = 0.9%). For individual stocks, HB-COS had the greatest overall prevalence of *R. salmoninarum* at 48.2% (SE = 2.3%), followed by LMRW-CHS at 27.3% (SE = 1.6%), MI-COS at 26.2% (SE = 1.5%), SRW-CHS at 11.7% (SE = 0.9%), and LMRW-STT at 3.8% (SE = 0.9%).

The decline of *R. salmoninarum* prevalence in all five stocks was supported by the calculated AIC values, which showed that the models with the poorest fit were those that assumed prevalences had remained constant over time. These models had much greater AIC values than models that allowed prevalence to change over time. For all fitted models where infection was assumed to change over time, the estimated slope parameters describing the linear (on a logit scale) change in infection prevalence per year ranged from −0.678 to −0.901. In all cases, these slopes were significantly different from 0 at *P*-values less than 0.0001, providing strong indication that *R. salmoninarum* rate of infection had indeed declined over time. The four best-performing models had nearly equal AIC values, suggesting that each of these models would be almost equally useful for describing declines of *R. salmoninarum* infection data. The model with the lowest AIC value had species/stock/strain-specific intercepts and species-specific slopes, but it performed only slightly better than the model with species-specific intercepts and species/stock/strain-specific slopes. The next best performing model had species/stock/strain-specific intercepts and a common slope, but it again performed only slightly better than the model with a common intercept and species/stock/strain-specific slopes. Additionally, the model with species/stock/strain-specific intercepts and slopes had an AIC difference of within 3, suggesting that there was at least some support for this model based on the observed data.

Because there were several models with at least some support for being the best model based on observed *R. salmoninarum* prevalence data, we chose to use AIC model averaging based on AIC weights to calculate a weighted-average of model parameters (intercepts and slopes) from those models. Only parameter estimates from those models with AIC differences of 3.0 or smaller were included in the model averaging (Burnham and Anderson, 2002). The model-averaged logistic regression slopes that were calculated for each of the species/stocks/strain combinations were −0.718 (SE = 0.055) for LMRW-CHS, −0.719 (SE = 0.058) for SRW-CHS, −0.804 (SE = 0.063) for HB-COS, −0.796(SE = 0.058) for MI-COS, and −0.775 (SE = 0.141) for LMRW-STT. Based on these model-averaged slopes, *R. salmoninarum* prevalence was predicted to have declined at a rate of approximately 51% per year for LMRW-CHS (Fig. 2A) and SRW-CHS (Fig. 2B). The predicted prevalence of *R. salmoninarum* infection declined at a rate of approximately 55% per year for HB-COS (Fig. 2C) and MI-COS (Fig. 2D). Lastly, the *R. salmoninarum* predicted prevalence declined at a rate of approximately 54% per year for LMRW-STT (Fig. 2E). Regardless of sampling year, HB-COS had the greatest predicted *R. salmoninarum* prevalence of the species/stocks, strains, followed by LMRW-CHS, and MI-COS. SRW-CHS and LMRW-STT had approximately equal *R. salmoninarum* prevalences during those years where prevalence data were available for both stocks (Fig. 2A–E).

Infection rates of the different intensity levels generally decreased for all species relative to non-infected fish (Fig. 2F–J), the only exceptions being medium intensity infection rate for SRW-CHS (Fig. 2G) and medium and high infection rates for LMRW-STT (Fig. 2J). The multinomial log-odds for having a low, medium, or high infection intensity level versus not being infected declined by 3.14–10.80 log-odds units per year depending on the stock and intensity level. In the case of LMRW-CHS, predicted low, medium, and high intensity infection prevalences declined to less than 0.1% during the sampling period (Fig. 2F). For SRW-CHS, the predicted low and high intensity infection prevalences also declined to less than 0.1% during this same time period (Fig. 2G). There were also declines in the predicted low, medium, and high intensity infection prevalences for HB-COS (Fig. 2H). For MI-COS, the predicted low and medium intensity infection rates declined to less than 1% in 2010 (Fig. 2I), while the high intensity infection prevalences for MI-COS initially increased 2003; however, since then, the high infection prevalences have declined to a rate of 1.2% in 2010 (Fig. 2I). For LMRW-STT, low intensity infection prevalences also declined throughout the study period (Fig. 2J).

3.2. Renibacterium salmoninarum infection prevalence and intensity in propagated pre-stocking salmon fingerlings

When data were combined for the five stocks over the entire study period, the overall prevalence of *R. salmoninarum* was 15.0% (SE = 0.5%). For individual species, coho salmon had the greatest overall prevalence of *R. salmoninarum* at 20.0% (SE = 1.3%), followed by Chinook salmon at 18.1% (SE = 0.8%) and steelhead at 6.7% (SE = 0.7%). For individual stocks, HB-COS had the greatest overall prevalence of *R. salmoninarum* at 28.1% (SE = 2.4%), followed by LMRW-CHS at 24.2% (SE = 1.3%), MI-COS at 14.6% (SE = 1.5%), SRW-CHS at 11.4% (SE = 1.0%), and LMRW-STT at 6.7% (SE = 0.7%). When categorized by hatchery,

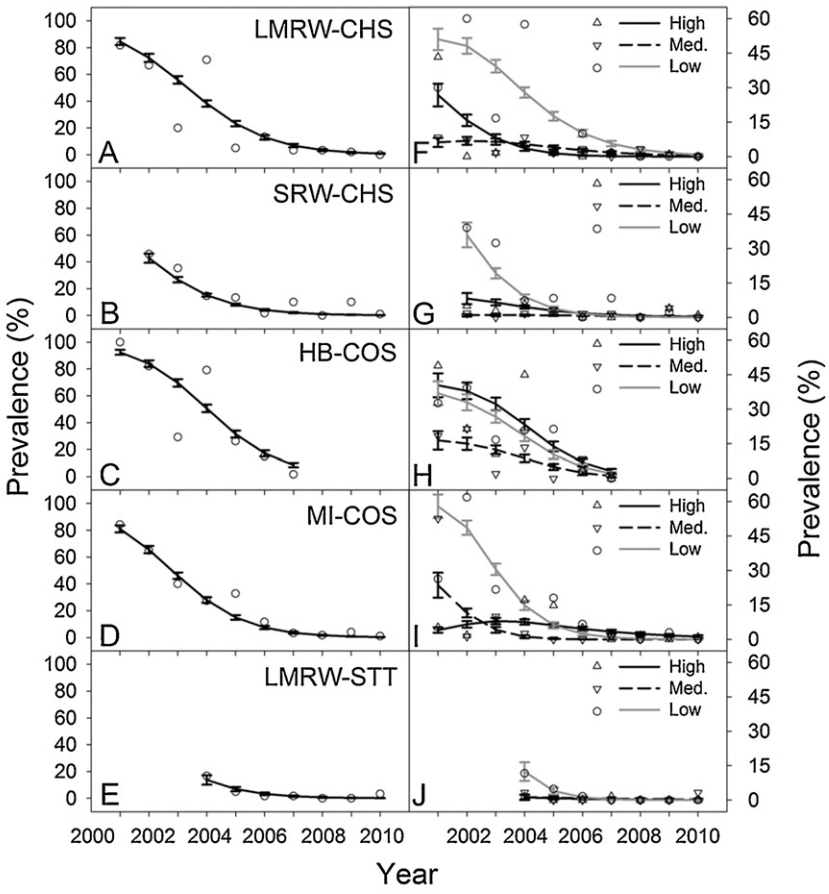

Fig. 2. (A–E) The prevalence of *R. salmoninarum* in Chinook salmon (*Oncorhynchus tshawytscha*) broodstock from the Little Manistee River Weir (LMRW-CHS) and the Swan River Weir (SRW-CHS), Hinchenbrooke coho salmon (*O. kisutch*) broodstock (HB-COS) and Michigan-adapted coho salmon broodstock (MI-COS) from the Platte River Weir, and steelhead (*O. mykiss*) broodstock from the Little Manistee River Weir (LMRW-STT) from 2001 to 2010. (F–J) The low, medium, and high intensity levels of infection of *R. salmoninarum* in LMRW-CHS, SRW-CHS, HB-COS, MI-COS, and LMRW-STT from 2001 to 2010. The lines represent the logistic regression predicted prevalence and intensity levels of infection, while open circles and triangles denote the observed prevalence and intensity levels of infection.

LMRW-CHS WLSFH had an overall prevalence of 32.4% (SE = 2.1%), whereas LMRW-CHS PRSFH had an overall prevalence of 19.4% (SE = 1.7%) and LMRW-CHS TSFH had an overall prevalence of 0% (SE = 0.0%). Conversely, SRW-CHS WLSFH had an overall prevalence of 13.3% (SE = 2.2%), whereas SRW-CHS PRSFH had an overall prevalence of 18.3% (SE = 2.1%) and SRW-CHS TSFH had an overall prevalence of 4.0% (SE = 0.9%). Steelhead propagated at the TSFH and WLSFH had overall prevalences of 7.9% (SE = 0.01%) and 5.8% (SE = 0.01%), respectively.

Like the broodstock analysis, based on calculated AIC values, the models with the poorest fit to observed *R. salmoninarum* infection data for the propagated pre-stocking fingerlings were those that assumed infection rates had remained constant over time. Unlike the results from the broodstock analysis, however, for the propagated pre-stocking fingerlings there was a single model that was picked by the AIC model selection criteria as having vastly superior performance compared to the other models. The model with the lowest AIC value had species/stock/strain/hatchery specific model intercepts and slopes. Therefore, the best performing model demonstrated that there was a considerable difference among the species and strains of fish, as well as the hatcheries where the fish were propagated. The next best performing model had stocks/hatchery specific intercepts and species/stock specific slopes, but this AIC difference for this model was greater than 90, indicating that there was very little empirical support for this model (Burnham and Anderson, 2002).

Analysis demonstrated that the *R. salmoninarum* infection rate in propagated fish had indeed declined over time, since for most of the stock/hatchery combinations, the logistic regression slopes were negative and were significantly different from 0 at *P*-values < 0.0001 (Fig. 3A–E). The only exceptions to this were for LMRW-CHS from the TSFH for which a slope could not be calculated because there was only 1 year of data collected from this hatchery, and for SRW-CHS from the TSFH which had a positive slope indicating that *R. salmoninarum* infection was increasing over time (Fig. 3E). For the other stock/hatchery combinations, estimated slopes equaled −1.295 (SE = 0.135) for LMRW-CHS PRSFH, −1.241 (SE = 0.116) for LMRW-CHS WLSFH, −1.325 (SE = 0.176) for SRW-CHS PRSFH, −1.196 (SE = 0.252) for SRW-CHS WLSFH, −0.745 (SE = 0.111) for HB-COS PSFH,

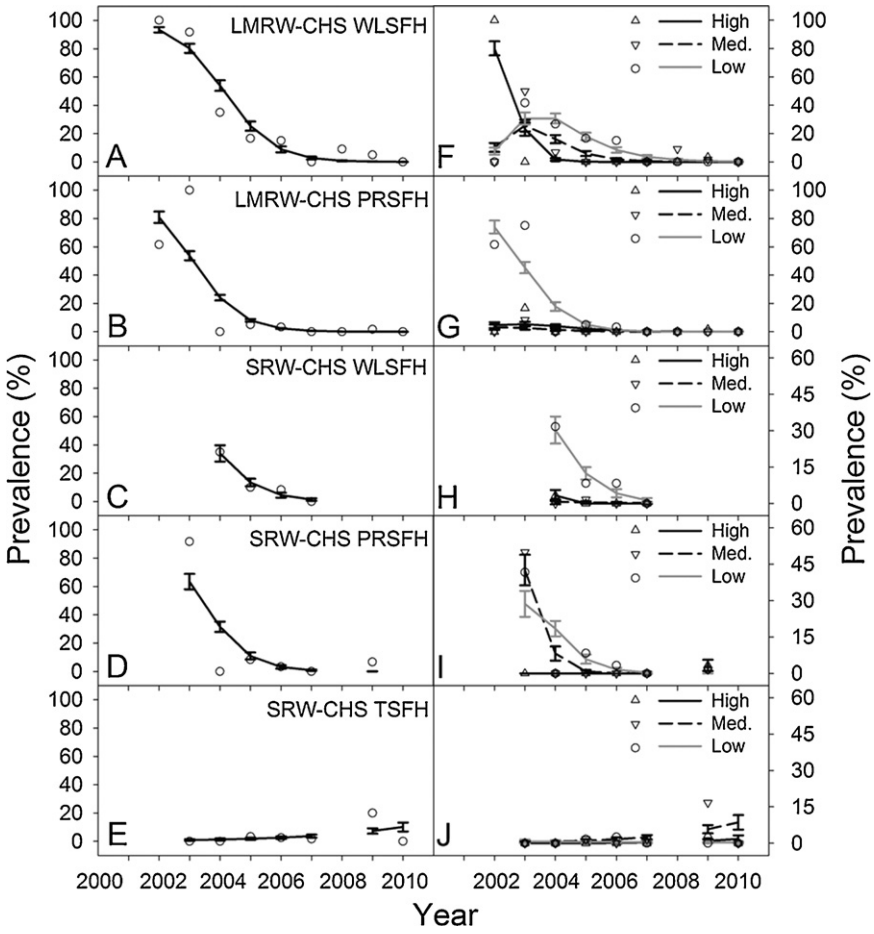

Fig. 3. (A–E) The prevalence of *R. salmoninarum* in Chinook salmon (*Oncorhynchus tshawytscha*) pre-stocking fingerlings propagated at the Wolf Lake State Fish Hatchery (WLSFH), the Platte River State Fish Hatchery (PRSFH), and the Thompson State Fish Hatchery (TSFH) from 2002 to 2010. Fingerlings are the progeny of broodstock spawned at the Little Manistee River Weir (LMRW) and the Swan River Weir (SRW). (F–J) The low, medium, and high intensity levels of infection of *R. salmoninarum* in LMRW-CHS from WLSFH and PRSFH and SRW-CHS from the WLSFH, PRSFH, and TSFH from 2002 to 2010. The lines represent the logistic regression predicted prevalence and intensity levels of infection, while open circles and triangles denote the observed prevalence and intensity levels of infection.

−0.730 (SE = 0.096) for MI-COS PRSFH, −2.079 (SE = 0.000) for LMRW-STT WLSFH, and −0.317 (SE = 0.0967) for LMRW-STT TSFH.

Based on these estimated slopes, *R. salmoninarum* prevalences in propagated pre-stocking fingerlings were predicted to decline by between 52% to 87% per year for each of these species/stock/strain/hatchery combinations. In terms of predicted *R. salmoninarum* prevalences, prevalence of *R. salmoninarum* for LMRW-CHS from the WLSFH was predicted to have declined to less than 0.1% in 2010 (Fig. 3A). LMRW-CHS from the PRSFH also experienced a major decline in *R. salmoninarum* prevalence (Fig. 3B). For SRW-CHS from the WLSFH, prevalences were predicted to have declined to 1.4% (Fig. 3C), whereas the predicted prevalence of *R. salmoninarum* in SRW-CHS from the PRSFH declined to less than 0.1% (Fig. 3D).

In terms of infection intensity, there were significant declines in rates of infection at all intensity levels using non-infected as the baseline for LMRW-CHS at both PRSFH and WLSFH (Fig. 3F–J). For LMRW-CHS at the WLFSH, predicted rates of infection at low and medium intensity levels initially increased from 2002 to 2003; however, since 2003, the predicted prevalence of low and medium intensity infection levels has declined to less than 0.1% (Fig. 3F). Predicted prevalence of high intensity infection levels for LMRW-CHS at the WLSFH declined overall from 2002 to 2010 (Fig. 3F). The predicted rates of infection at low, medium, and high intensities for LMRW-CHS from the PRSFH declined to less than 0.1% (Fig. 3G). For SRW-CHS, significant declines in rates of infection for low intensity levels were detected at the WLSFH (Fig. 3H), as well as for low and medium intensity levels at the PRSFH (Fig. 3I). Additionally, the predicted prevalence for medium intensity levels of infection for SRW-CHS at the PRSFH declined to less than 0.1% (Fig. 3I). For SRW-CHS from the TSFH, there was a significant increase in prevalence at medium intensity levels from 2002 to 2010 (Fig. 3J).

Coho salmon fingerlings propagated at the PRSFH also saw a substantial decline in the prevalence of *R. salmoninarum* infections from 2003 to 2010. For HB-COS and

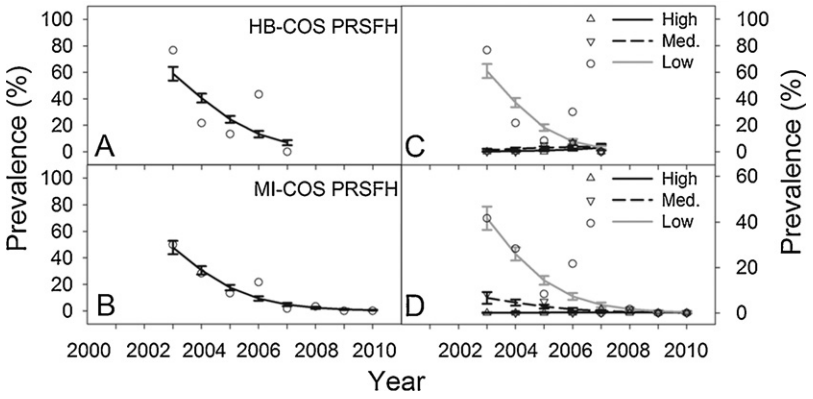

Fig. 4. (A and B) The prevalence of *R. salmoninarum* in the Hinchenbrook strain (HB-COS) of coho salmon (*Oncorhynchus kisutch*) pre-stocking fingerlings and the Michigan-adapted strain of coho salmon (MI-COS) propagated at the Platte River State Fish Hatchery (PRSFH) from 2003 to 2010. (C and D) The low, medium, and high intensity levels of infection of *R. salmoninarum* in HB-COS and MI-COS propagated at the PRSFH from 2003 to 2010. The lines represent the logistic regression predicted prevalence and intensity levels of infection, while open circles and triangles denote the observed prevalence and intensity levels of infection.

MI-COS from the PRSFH, prevalence was predicted to have declined throughout the study period (Fig. 4A and B). In regards to infection intensity for coho salmon, significant decreases in low rates of infection were found for both Hinchenbrooke and Michigan-adapted strains (Fig. 4C and D). Also, a significant decrease in medium rate of infection was found for the MI-COS (Fig. 4D).

Moreover, the prevalence infection rates of *R. salmoninarum* in steelhead pre-stocking fingerlings also decreased considerably from 2005 to 2010. For LMRW-STT from the WLSFH, predicted prevalence declined to less than 0.1% in 2010 (Fig. 5A); while for LMRW-STT from the TSFH the predicted prevalence declined to 4.0% in 2010 (Fig. 5B). For LMRW-STT, significant decreases in prevalence at low intensity levels were observed for both WLSFH and TSFH (Fig. 5C and D).

3.3. *Renibacterium salmoninarum* in gametes of spawning broodstock

Across all species/stocks/strains throughout the study period, the overall prevalence of broodstock that had *R. salmoninarum* antigens in ovarian fluid and milt was 6.2% (SE = 0.5%). By species, the overall prevalence of broodstock with positive gametes was 11.1% (SE = 1.1%) for coho salmon, 4.3% (SE = 0.6%) for Chinook salmon, and 0.7% (SE = 0.5%) for steelhead. By species/stock/strain, the overall prevalence of gamete positive broodstock was 3.6% (SE = 0.8%) for LMRW-CHS, 4.9% (SE = 0.8%) for SRW-CHS, 23.6% (SE = 2.4%) for HB-COS, and 4.3% (SE = 0.9%) for MI-COS. When calculated by sex, the overall prevalence of broodstock that were shedding the bacteria in gametes was 6.5% (SE = 0.7%) for females and 5.9% (SE = 0.7%) for males.

Based on the calculated AIC values, there were two models that had some support based on observed shedding data. The model with the lowest AIC value had species/stock/strain/sex-specific intercepts and slopes. The next best performing model had species/stock/strain/sex-specific model intercepts and species/stock/strain-specific slopes. As with the broodstock prevalence analysis, we used model averaging based on AIC weights to average the parameter estimates from these two models. The calculated model-averaged slopes equaled −0.515 (SE = 0.236) for female LMRW-CHS, −0.983 (SE = 0.237) for male LMRW-CHS, −0.384 (SE = 0.208) for female SRW-CHS, −0.093 (SE = 0.179) for male SRW-CHS, −2.165 (SE = 0.001) for female HB-COS, −2.132 (SE = 0.025) for male HB-COS, −0.379 (SE = 0.123) for female MI-COS, −0.430 (SE = 0.213) for male MI-COS, 0.088 (SE = 0.113) for female LMRW-STT, and 0.210 (SE = 0.018) for male LMRW-STT. Based on model-averaged slopes, gamete shedding prevalences were predicted to have declined by between 40% and 63% per year for LMRW-CHS (Fig. 6A) and 9% and 32% per year for SRW-CHS (Fig. 6B). Additionally, the predicted decline in gamete shedding prevalence for HB-COS was approximately 88% per year (Fig. 6C) and between 32% and 35% per year for MI-COS (Fig. 6D). For LMRW-STT, shedding prevalence was predicted to have increased by between 9% and 23% (Fig. 6E). In terms of predicted gamete shedding prevalences, for most species/stocks/strains shedding prevalence in gametes was generally less than 15% throughout the course of the study. The one exception to this was HB-COS where both females and males had gamete shedding prevalences of between 50% and 60% at the beginning of this study. However, by 2007, gamete shedding prevalences of both sexes had declined to less than 1% (Fig. 6C). Although there were clear differences between sexes in predicted shedding prevalences for some of the examined broodstock, results were inconsistent as to whether males or females had higher shedding prevalences.

In terms of the different shedding categories, significant declines in shedding categories when compared to the KS−/G− shedding category were detected for some of the species/stock/strain/sex combinations. For HB-COS, significant declines in the KS+/G−, KS+/G+, and KS−/G+ categories were found for both sexes. For female LMRW-CHS, SRW-CHS, and MI-COS, significant declines in the KS+/G− and KS+/G+ categories were detected, while for male LMRW-CHS, SRW-CHS, and MI-COS, only significant declines in the

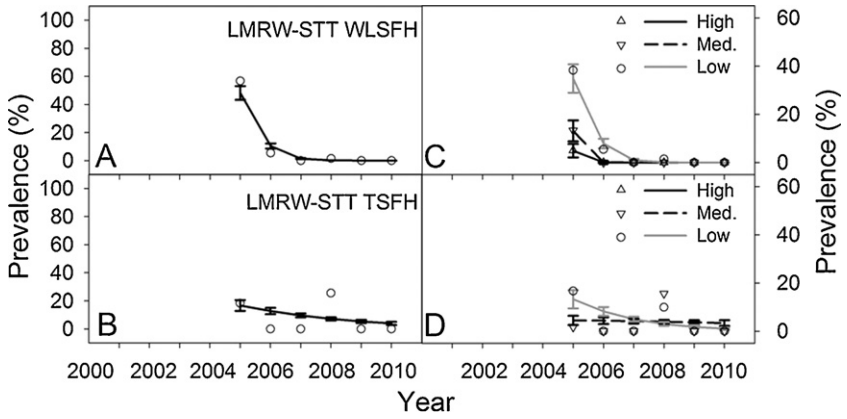

Fig. 5. (A and B) The prevalence of *R. salmoninarum* in steelhead (*Oncorhynchus mykiss*) pre-stocking fingerlings (LMRW-STT) propagated at the Wolf Lake State Fish Hatchery (WLSFH) and the Thompson State Fish Hatchery (TSFH) from 2005 to 2010. (C and D) The low, medium, and high intensity levels of infection of *R. salmoninarum* in LMRW-STT propagated at the WLSFH and TSFH from 2005 to 2010. The lines represent the logistic regression predicted prevalence and intensity levels of infection, while open circles and triangles denote the observed prevalence and intensity levels of infection.

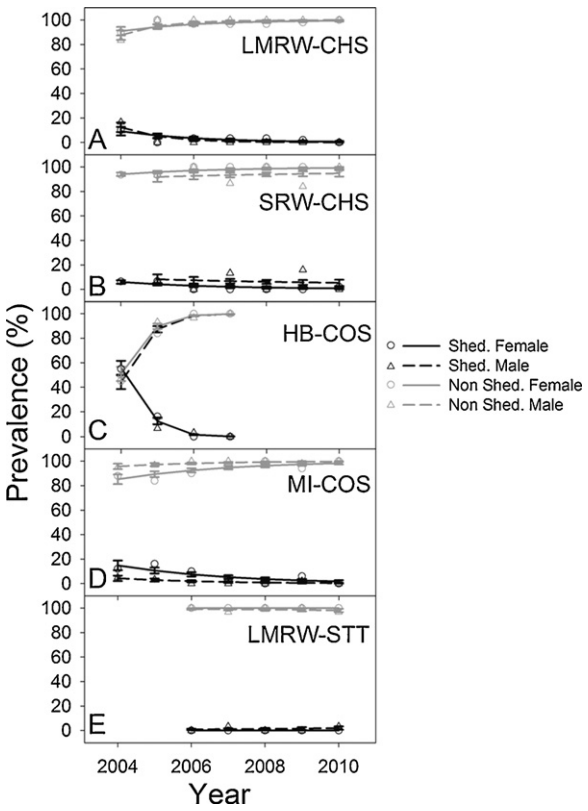

Fig. 6. The prevalence of male and female fish that may be shedding *R. salmoninarum* and are not shedding *R. salmoninarum* for (A) Chinook salmon (*Oncorhynchus tshawytscha*) broodstock from the Little Manistee River Weir; (B) Chinook salmon broodstock from the Swan River Weir; (C) Hinchenbrooke coho salmon (*O. kisutch*) broodstock from the Platte River Weir; (D) Michigan-adapted coho salmon broodstock from the Platte River Weir; (F) and steelhead (*O. mykiss*) broodstock from the Little Manistee River Weir. The lines represent the logistic regression predicted prevalence and intensity levels of infection, while open circles and triangles denote the observed prevalence and intensity levels of infection.

KS+/G− category were detected. For LMRW-STT, no significant declines in shedding rates for any of the shedding categories were detected. The largest predicted changes in shedding category prevalences were in the KS+/G− category for female and male LMRW-CHS, which were predicted to have declined from between 40% and 60% in 2004 to less than 3% by 2007 (Fig. 6A). The other large predicted change in shedding prevalences were in the KS+/G+ category for female and male HB-COS, which were predicted to have declined from between 40% and 55% in 2004 to less than 5% in 2005 (Fig. 6C).

3.4. Relationship between *R. salmoninarum* prevalence in broodstock and progeny

Based on the correlation analyses conducted, no significant association between broodstock and progeny prevalences in Chinook salmon ($r = 0.220$; P-value = 0.517) was detected. There was, however, a statistically significant positive association found between broodstock and progeny prevalences in coho salmon ($r = 0.890$; P-value = 0.0073). A strong positive, albeit not statistically significant, association was also found between broodstock and progeny prevalences in steelhead ($r = 0.697$; P-value = 0.0549).

4. Discussion

The findings of this study demonstrate that the intensity and prevalence of *R. salmoninarum* infections have decreased during the course of the last decade; not only in the feral broodstock, but also in hatchery settings. This correlated with the implementation of enhanced biosecurity practices in the state fish hatcheries. In this study, we opted to use the Q-ELISA as the only diagnostic tool to assess *R. salmoninarum* presence in fish tissues. In a previous study, it was demonstrated that Q-ELISA values were commensurate with disease progression in feral stocks (Faisal and Eissa, 2009).

There are several likely reasons why *R. salmoninarum* has rapidly declined in an area where it has been endemic

for over half a century. As fish are exposed to *R. salmoninarum*, it is possible that the overall population may have shifted to individuals with heightened resistance to the pathogen (Purcell et al., 2008). Earlier studies on coho salmon have shown that resistance to BKD is linked to the transferrin gene (Suzumoto et al., 1977; Winter et al., 1980). The authors reported that coho salmon with the transferrin genotype of 'AA' are three times more likely to die from a *R. salmoninarum* infection than coho salmon with the transferrin genotype of 'CC.' It is possible that more fish which are less susceptible to the pathogen will survive, and produce offspring that are also less likely to become infected with *R. salmoninarum*, thereby reducing the prevalence of the disease over time.

An additional factor that may explain the minimal presence of *R. salmoninarum* in state fish hatcheries is the improved screening process for signs of diseases that was initiated at MDNR egg-collection weirs in the 1990s but expanded in the early 2000s. Once gametes were removed from adult *Oncorhynchus* spp., the fish were examined externally (presence of ulcers, furuncles, lesions, etc.) and internally (pale or swollen organs, granulomas, hemorrhages, etc.) by trained MDNR staff and experienced fish health professionals for signs of disease. If fish were suspected to be harboring *R. salmoninarum*, the gametes were not used for production. By using this method, fish affected by the acute form of BKD were removed from the broodstock population, in addition to those that developed the chronic form of BKD (characterized by the formation of renal granulomas); thereby reducing the potential amount of *R. salmoninarum* that would be passed on to the progeny. Elliott et al. (1995) found significant differences in clinical BKD signs in Chinook salmon progeny from parent broodstock with a low prevalence of *R. salmoninarum* infection (low-BKD), which would mimic a chronic infection, when compared to a broodstock with a high prevalence of infection (high-BKD), which would be similar to an acute infection. Compared to the fish in the low-BKD group, a higher proportion of fish in high-BKD group had evidence of organ and tissue pathology, such as exophthalmia, corneal opacity, pale and/or frayed gills, fin erosion, swollen or mottled kidneys, enlarged spleens, and abnormal livers. While it is possible that the fish with low intensities of infection will not be detected by a screening method such as visual observation of the disease when compared to highly infected fish, fish with the low intensity of infection pose less of a risk of passing *R. salmoninarum* to their offspring. Similar to Elliott et al. (1995), Pascho et al. (1991) found that within the progeny that had positive ELISA results, most of the low-BKD group had low intensity infections, while the majority of the fish in the high-BKD group had high intensity infections.

In this context, a long-term study (1993–2005) of *R. salmoninarum* infections in Chinook salmon in Idaho hatcheries has demonstrated the effectiveness of establishing a program such as screening the broodstock by an ELISA method and then culling based on the results, and continuing it on a yearly basis (Munson et al., 2010). It was found that the ELISA-based (as outlined in Munson et al., 2010) broodstock screening program reduced the prevalence, the intensity of infection, and mortality rates due to *R. salmoninarum* in Chinook salmon juveniles and broodstock. Our findings in Michigan corroborate with those of Munson et al. (2010) in Idaho.

Combining broodstock culling with the more stringent biosecurity measures implemented at the MDNR hatcheries as of 2002, have minimized, not only the vertical transmission from broodstocks, but also limited potential for horizontal transmission of *R. salmoninarum*. As recommended by Danner and Merrill (2006), each of the six state fish hatcheries utilizes separate nets, brushes, and buckets for each of the raceways that are cleaned and disinfected on a regular basis. Additionally, disinfecting footbaths and mats are placed at the entrance to all facilities to reduce the possible cross-contamination between facilities.

Another method of disease prevention that the MDNR implemented in 2002 is the use of the antibiotic erythromycin as a therapeutic treatment for hatchery salmon and trout. It is considered to be extra-label use to use erythromycin as a therapeutic; therefore the MDNR used it under the supervision of a veterinarian. *Renibacterium salmoninarum* is known to be susceptible to exposure to erythromycin (Stoffregen et al., 1996). While MDNR uses erythromycin baths, Evelyn et al. (1986a) and Lee and Evelyn (1994) demonstrated that the intramuscular injection of erythromycin into broodstock fish can minimize the vertical transmission of *R. salmoninarum* into the ova. Additionally, Evelyn et al. (1986a) showed the antibiotic persisted within the eggs after attempts were made to leach it out; suggesting that it reduces the initial vertical transmission from parent to offspring; and potentially lowers the risk of horizontal transmission. It is likely that the erythromycin therapeutic baths that MDNR implemented on all egg lots acted in a similar fashion and further contributed to minimizing the potentials of vertical transmission. Unfortunately, as the MDNR could not risk the loss of valuable propagated fish, it was not possible to include a negative control (i.e., a hatchery with no newly implemented enhanced biosecurity measures) for the sake of comparison for this study.

Our finding of differences in *Renibacterium salmoninarum* prevalence among the three *Oncorhynchus* species matches the results of Starliper et al. (1997) and Beacham and Evelyn (1992). Starliper et al. (1997) demonstrated that strains of *R. salmoninarum* from coho salmon have the potential to be more harmful than strains of the bacteria from Chinook salmon. In six out of eight different salmonid hosts, a strain of *R. salmoninarum* from coho salmon from Manistee, Michigan was found to be more virulent than a strain from Chinook salmon from Manistee, Michigan (Starliper et al., 1997). Also, Starliper et al. (1997) found that coho and Chinook salmon were more susceptible to *R. salmoninarum* than rainbow (steelhead) trout. Furthermore, in a study by Beacham and Evelyn (1992), juvenile coho and Chinook salmon were infected with *R. salmoninarum* to better understand how the bacterium affected mortality rates, the mean time to death, and growth rates of the three species. It was concluded that although they had a longer time to death, coho salmon had a higher percent

of mortality than Chinook salmon (Beacham and Evelyn, 1992). A possible contributory factor that may help explain the greater prevalence of *R. salmoninarum* in coho salmon in this study is that broodstock of this species were only collected from streams in the Lake Michigan watershed, which overall had a greater prevalence of *R. salmoninarum* compared to fish collected from Lake Huron tributary streams.

The generally greater prevalence rate of *R. salmoninarum* in HB coho salmon versus MI coho salmon suggests that the strains may differ in their susceptibility to BKD. The studies of Withler and Evelyn (1990) documented that such variations in disease susceptibility can exist between strains of coho salmon. These investigators exposed two strains of coho salmon from British Columbia to *R. salmoninarum* to determine the likelihood of resistance to *R. salmoninarum*, as determined by survivability and time to death. The Kitimat River strain of coho salmon had greater survival and a longer time to death when compared to the Robertson Creek strain of coho salmon.

In addition to our study, several other studies have documented low occurrences of *R. salmoninarum* in *O. mykiss*, at least when compared to occurrence in brook trout, brown trout, Chinook salmon, and coho salmon (Bullock et al., 1971; Mitchum and Sherman, 1981; Mitchum et al., 1979; Hsu et al., 1991; Sakai et al., 1991; Jansson et al., 1996; Starliper et al., 1997). Additionally, Mitchum and Sherman (1981) found that *O. mykiss* had the lowest mortalities and the least severe clinical signs of BKD in a study investigating the horizontal transmission of *R. salmoninarum* from infected wild brook trout to newly stocked hatchery-raised brook trout, brown trout, and rainbow trout. Hsu et al. (1991) also found that steelhead in Lake Ontario had lower prevalences *of R. salmoninarum* and also lower detectable antigen levels than coho or Chinook salmon among fish returning to the Salmon River Fish Hatchery (Altmar, New York) based on monoclonal-antibody-based ELISA.

The prevalence of *R. salmoninarum* shedding in this study was low, with the exception of the HB coho salmon, which may be attributable to the finding that HB coho salmon had the highest overall prevalence of *R. salmoninarum* in the kidney and spleen of the parental broodstock. The prevalence of shedding *R. salmoninarum* in *Oncorhynchus* spp. broodstocks in this study corresponded to the prevalence of the pathogen in the progeny. For example, in 2004, 72.5% of HB coho salmon were positive for *R. salmoninarum* and 52.5% of them were capable of shedding the bacteria. As a result, 43.3% of their progeny were positive for the pathogen. Interestingly, in 2005, the prevalence of *R. salmoninarum* in broodstock decreased to 18.3% with 11.7% of them shedding the bacteria. Consequently, none of the progeny were positive for *R. salmoninarum*. While vertical transmission clearly plays a role in the infection of the progeny, there are several other factors that can affect the prevalence of the disease as well, such as density of the fish in the raceway, environmental conditions (water temperature, dissolved oxygen levels, etc.), and biosecurity measures at the facility.

It is clear that female salmonids contribute to vertical transmission by having *R. salmoninarum*-infected ovarian fluid, but the role of male salmonids in vertical transmission is less understood. The shedding in this study occurred fairly equally between male and female fish; however, in the case of SRW-CHS and LMRW-STT, the males had a somewhat higher shedding prevalence than the females. Evelyn et al. (1986b) concluded that male coho salmon and steelhead did not play a significant role in vertical transmission as a result of their studies examining infection rates in eggs fertilized with infected or non-infected milt. Based on our findings, it is at least plausible that male SRW-CHS and LMRW-STT could be contributing to the vertical transmission of *R. salmoninarum* in LGL, although to what extent this is occurring is not known.

The overall decline of *R. salmoninarum* from 2001 to 2010 in the three feral broodstock and propagated fish stocks has shown that in addition to a possible heightened genetic resistance, preventative measures such as an improved screening process, broodstock culling, and enhanced biosecurity measures can be successful in reducing the prevalence of a pathogen in hatcheries and perhaps in returning broodstock from the Great Lakes. The broad decline in the prevalence of *R. salmoninarum* in the various fish species and stocks in this study is most likely due to a combination of improved visual inspections and culling conducted at the weirs, implementation of increased biosecurity measures at the hatcheries, reduced rearing stress, and iodophor and erythromycin disinfection of eggs. While the decline of *R. salmoninarum* in these three salmonid species is promising, BKD continues to be a potential problem in the LGL basin. Lake whitefish (*Coregonus clupeaformis*), which are in a closely related subfamily to salmonids (i.e., Corgeninae), have been shown to heavily infected with *R. salmoninarum*. Recently, Faisal et al. (2010) documented the presence of *R. salmoninarum* in approximately 66% of the lake whitefish populations sampled in northern Lake Huron by the Q-ELISA method described above, with predominant clinical signs of infection. This high infection prevalence in another susceptible fish species attests for the continuous strong presence of *R. salmoninarum* in the LGL ecosystem. It further strengthens the finding of this study that the decline of the bacterial presence in *Oncorhynchus* species is the result of disease management measures undertaken in Great Lakes salmonid gamete collection weirs and state fish hatcheries.

Conflict of interest statement

The authors declare that there was no conflict of interest throughout the duration of this study.

Acknowledgments

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Appendix A.

Table A.1
Listing and description of models fit to the *R. salmoninarum* prevalence and shedding data. Analysis column indicates models fit only to propagated pre-stocking fingerling prevalence or broodstock gamete shedding data.

Model	Description	Analysis
Intercept	Common intercept; no change over time	All
Intercept + time	Common intercept; common change over time	All
Intercept + species × time	Common intercept; species specific change over time	All
Intercept + SS × time	Common intercept; species/stock/strain specific change over time	All
Intercept + SSH × time	Common intercept; species/stock/strain/ hatchery specific change over time	Propagated pre-stocking fingerlings
Intercept + sex × time	Common intercept; sex specific change over time	Broodstock shedding
Intercept + SX	Common intercept; species/sex specific change over time	Broodstock shedding
Intercept + SSX × time	Common intercept; species/stock/strain/sex specific change over time	Broodstock shedding
Species	Species specific intercept; no change over time	All
Species + time	Species specific intercept; common change over time	All
Species + species × time	Species specific intercept; species specific change over time	All
Species + SS × time	Species specific intercept; species/stock/strain specific change over time	All
Species + SSH × time	Species specific intercept; species/stock/strain/ hatchery specific changes over time	Propagated pre-stocking fingerlings
Species + sex × time	Species specific intercept; sex specific change over time	Broodstock shedding
Species + SX × time	Species specific intercept; species/sex specific change over time	Broodstock shedding
Species + SSX × time sex specific change over time	Species specific intercept; species/stock/strain/ Broodstock shedding	
SS	Species/stock/strain specific intercept; no change over time	All
SS + time	Species/stock/strain specific intercept; common change over time	All
SS + species × time	Species/stock/strain specific intercept; species specific change over time	All
SS + SS × time	Species/stock/strain specific intercept; Species/stock/strain specific change over time	All
SS + SSH × time	Species/stock/strain specific intercept; species/ stock/strain/hatchery specific change over time	Propagated pre-stocking fingerlings
SS + sex × time	Species/stock/strain specific intercept; sex specific change over time	Broodstock shedding
SS + SX × time	Species/stock/strain intercept; species/sex specific change over time	Broodstock shedding
SS + SSX × time	Species/stock/strain specific intercept; species/stock/strain/sex specific change over time	Broodstock shedding
SSH	Species/stock/strain/hatchery specific intercept; no change over time	Propagated pre-stocking fingerlings
SSH + time	Species/stock/strain/hatchery specific intercept; common change over time	Propagated pre-stocking fingerlings
SSH + species × time	Species/stock/strain/hatchery specific intercept; species specific change over time	Propagated pre-stocking fingerlings
SSH + SS × time	Species/stock/strain/hatchery specific intercept; species/stock/strain specific change over time	Propagated pre-stocking fingerlings
SSH + SSH × time	Species/stock/strain/hatchery specific intercept; species/stock/strain/hatchery specific change over time	Propagated pre-stocking fingerlings
Sex	Sex specific intercept; no change over time	Broodstock shedding
Sex + time	Sex specific intercept; common change over time	Broodstock shedding
Sex + species × time	Sex specific intercept; species specific change over time	Broodstock shedding
Sex + SS × time	Sex specific intercept; species/stock/strain specific change over time	Broodstock shedding
Sex + sex × time	Sex specific intercept; sex specific change over time	Broodstock shedding
Sex + SX × time	Sex specific intercept; species/sex specific change over time	Broodstock shedding
Sex + SSX × time	Sex specific intercept; species/stock/strain/sex specific change over time	Broodstock shedding
SS + SS × time	Species/stock/strain specific intercept; Species/stock/strain specific change over time	All
SX	Species/sex specific intercept; no change over time	Broodstock shedding
SX + time	Species/sex specific intercept; common change over time	Broodstock shedding
SX + species × time	Species/sex specific intercept; species specific change over time	Broodstock shedding
SX + SS × time	Species/sex specific intercept; species/stock/strain specific change over time	Broodstock shedding
SX + sex × time	Species/sex specific intercept; sex specific change over time	Broodstock shedding
SX + SX × time	Species/sex specific intercept; species/specific change over time	Broodstock shedding
SX + SSX × time	Species/sex specific intercept; species/stock/strain/sex specific change over time	Broodstock shedding
SSX	Species/stock/strain/sex specific intercept; no change over time	Broodstock shedding
SSX + time	Species/stock/strain/sex specific intercept; common change over time	Broodstock shedding
SSX + species × time	Species/stock/strain/sex specific intercept; species specific change over time	Broodstock shedding
SSX + SS × time	Species/stock/strain/sex specific intercept; species/stock/strain specific change over time	Broodstock shedding
SSX + sex × time	Species/stock/strain/sex specific intercept; sex specific change over time	Broodstock shedding
SSX + SX × time	Species/stock/strain/sex specific intercept; species/sex specific change over time	Broodstock shedding

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2012.06.003.

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