Rearing unit-level factors associated with bacterial gill disease treatment in two Ontario, Canada government salmonid hatcheries

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1. Introduction

Disease is a major constraint to aquaculture growth and production around the world (Bondad-Reantaso et al., 2005). In freshwater salmonid farms, bacterial gill disease (BGD) is often a serious problem, particularly in younger fish, and its overall impact is considered enormous (Shotts and Starliper, 1999). The ubiquitous causative agent of BGD, \textit{Flavobacterium branchiophilum}, can cause clinical disease and high mortality levels when environmental conditions are deleterious. A variety of environmental stressors are thought to be important in instigating or exacerbating BGD outbreaks (Bullock, 1972; Schachte, 1983; Wedemeyer, 1997), but very little epidemiological research has been carried out to identify and quantify BGD risk factors in fish farm settings. Disease outbreaks on fish farms often have multifactorial etiologies (Hedrick, 1998; Thorburn, 1999), and prevention and control of such outbreaks can be improved with information gained through epidemiological risk factor studies (Georgiadis et al., 2001a). Unfortunately, observational epidemiological research in aquaculture settings is relatively rare (Thorburn et al., 2001).
Historically, BGD has been an important production-limiting factor in Ontario fish farms (Daoust and Ferguson, 1993; Speare and Ferguson, 1989), and has consistently affected salmonid populations in Ontario Ministry of Natural Resources (OMNR) hatcheries for many years (Penney, 2003). Despite efforts by managers and personnel to reduce BGD in their hatchery system, the disease remains a persistent problem in OMNR early-rearing (i.e. <9 months in age) fish. The objective of this study was to identify, and quantify the effects of, important rearing unit-level factors associated with past BGD occurrences at individual OMNR hatcheries. The retrospective data available among the OMNR hatcheries were highly variable, which prevented system-wide analyses; however, within-hatchery analyses were possible for individual hatcheries. Although the data investigated were not recent (i.e. 1999), it is unlikely that factors associated with BGD occurrences have changed in any meaningful way since that time, and the need to understand these factors is still strong, particularly for those at the rearing unit-level. The factors investigated were those with putative association with BGD outbreaks, and which can feasibly be monitored or manipulated by operators, so that recommendations based on the findings of this study would be relevant and applicable to the everyday management of freshwater fish farms.

2. Materials and methods

Retrospective data were collected and compiled in 2003–2004 from individual hatchery records from two OMNR hatcheries (referred to as Hatcheries A and B). These hatcheries were relatively similar to one another in terms of water source (ground water), predominant species reared (brook trout (Salvelinus fontinalis) and lake trout (Salvelinus namaycush)), and geographic location (northern Ontario). Both hatcheries were selected from the available hatchery pool of ten OMNR facilities because: (i) their records for daily hatchery activities, including treatments, during the study period were relatively accurate and complete; (ii) they did not conduct the practices of prophylactic BGD treatment or “blanket” BGD treatment (i.e. treating the entire lot when only individual tanks were affected); and (iii) each had experienced serious problems with BGD. Specifically, BGD was particularly problematic at both selected hatcheries during the 1999 production year, and hence data collection was centered on 1999 year-class production units at these hatcheries. Due to differences in record-keeping practices, separate analyses were conducted for Hatcheries A and B. As well, because younger fish experience relatively higher BGD morbidity and mortality rates (Shotts and Starliper, 1999), this study’s focus was limited to early-rearing fish.

Given the amount of mixing and sorting of farmed fish as they age, it is often difficult or impossible to follow distinct groups of fish on farms over time (Thorburn, 1999). Thorburn et al. (2001) proposed a farm-tank-lot as a unit of analysis, to identify distinct populations within fish farms in order to deal with the problems of dynamic fish populations. In this study, because each hatchery had its own analysis, the tank-lot (i.e. the lot of fish existing in a given tank at any given time) was used as the unit of analysis. As fish from different lots are never mixed, all tank-lots were given unique identifiers in order to distinguish different fish lots within specific tanks at varying points during the study period.

Study data from Hatcheries A and B covered the periods January–November, 1999 (Hatchery A) and January–July, 1999 (Hatchery B). The study period was shorter for Hatchery B because fish at that facility were transferred from the early-rearing unit at an earlier age than fish at Hatchery A. Initially, charts were created to summarize spatial and temporal movements of fish lots among tanks at each hatchery, and served as a guide in creating datasets for weekly values of tank-lot variables. The final dataset for each hatchery summarized, by week, the following tank-lot-level variables: fish species, BGD treatment, fish transfers, mortality, amount fed, number of fish, and weight of individual fish (when measured). As well, records at Hatchery A provided data for additional tank-lot variables: water flow rate, water exchange rate, and tank type (supertrough versus raceway). Unfortunately, there were no specific data available from either hatchery describing the decisions to treat BGD during the study time frame (e.g. the diagnostic methodologies performed, if any, and by whom; the fish sampling protocol; etc.), and hence only BGD treatment, as opposed to actual BGD diagnosis, could serve as the dependent variable in this study’s analyses. In general, decisions to treat for BGD are often based on clinical signs (inappetance, flared operculae, crowding at the inlet, etc.) and are sometimes supported by on-site microscopic examination of gill tissues from affected fish (OMNR hatchery managers, personal communication). Although OMNR hatchery staff are experienced in detecting and treating diseases, research has not been carried out to determine the accuracy and agreement of BGD diagnoses and diagnosticians within and between OMNR facilities.

Samples of fish were weighed from each tank approximately every 4 weeks. Weekly estimates of growth were needed for the analyses. Growth in young fish is believed to follow a logarithmic pattern (Jobling, 1983); therefore, to provide weekly estimates of fish weight, the empirical values for each tank-lot were log-transformed, and PROC REG models for each tank-lot were created in SAS (SAS Institute, Cary, NC, USA), with (ln) weight as the dependent variable and week-of-age as the independent variable. R-squared values for all tank-lot models were >0.96. The derived formulae for each tank-lot combination were used to create weekly predicted weight values.

Other variables, namely biomass, standardized feed, and weekly mortality percentage, were created in SAS. Biomass was the entire weight of fish, in kilograms, existing in a tank at the start of a week, and was calculated by:

\[
\text{biomass} = \frac{\text{no. of fish} \times \text{estimated weight (g) of individual fish}}{1000}
\]

Standardized feed (referred to as “feed percentage”) was the amount of feed fed to a tank-lot, per week, as a
percentage of fish biomass in the tank-lot, and was calculated by:

\[
\text{feed} \% = \left( \frac{\text{amount of feed given (kg)}}{\text{tank-lot biomass}} \right) \times 100
\]

Weekly tank-lot mortality percentage was calculated as:

\[
\text{mort} \% = \left( \frac{\text{no. of fish mortalities during the week}}{\text{no. of fish at beginning of week}} \right) \times 100.
\]

For the Hatchery A dataset, standardized variables for tank-lot water flow and water exchange rates (averaged over a given week) were created in SAS (flow rate (L/min)/biomass, and exchange rate (number of water exchanges per hour)/biomass, respectively). Tank type design variables were also created, with supertrough tank type serving as the coded variable and raceway tank type as the referent. A tank-lot fish density variable (weekly average) was created by deriving tank-lot water volume from the recorded exchange rates, and then dividing the tank-lot biomass by this calculated water volume. At Hatchery B, all tanks housing the studied early-rearing population were of uniform size and shape, and water volume within these tanks was assumed to be constant across all tanks for each week of the study. Therefore, tank-lot biomass was considered to be a crude substitute for tank-lot density at Hatchery B.

For Hatchery A, species design variables were created for brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta), with lake trout (S. namaycush) serving as the reference level. Species design variables for Hatchery B were brook trout (S. fontinalis) and splake (S. fontinalis × S. namaycush), with lake trout (S. namaycush) serving as the reference level.

Lag variables were created using averages for the two-week period prior to BGD outbreaks. For Hatchery A, these included previous feed percentage (i.e. amount of feed fed to a tank-lot, per week, as a percentage of fish weight in the tank-lot, averaged for the 2 weeks prior to BGD treatment), previous water flow rate, previous water exchange rate (standardized), and previous mortality percentage. Hatchery B lag variables were previous feed percentage, and BGD treatment during the previous week due to the prolonged nature of outbreaks (and associated repeated treatments) at this hatchery.

For both hatchery datasets, SAS Insight was used to generate distributions of continuous variables for tank-lot weeks when BGD treatment was administered and those when no treatment was given. After examining these distributions, logarithmic transformations of certain variables were performed to normalize their distributions; these transformed variables included density, previous mortality percentage, and previous water flow and exchange rates (Hatchery A), and estimated fish weight (Hatcheries A and B). The datasets were then sorted by week within tank-lot, and bivariate analyses for all individual variables were conducted using PROC GENMOD coded for a repeated measures approach (repeated on week and controlling for lot-level clustering). Multivariable repeated measures logistic regression models for each hatchery were then built using PROC GENMOD, with the dependent variable being treatment for BGD in a given week. Logit was specified as the link function, and the correlation structure was specified as autoregressive. Lot-level clustering was accounted for by including lot in the repeated subject line of the program, and species effects were controlled by forcing species design variables into each model. Final models were selected using a backward stepwise approach such that variables were removed manually from the model until only those variables with \( p \leq 0.10 \) (using the Chi-square test) remained in the model. The \( p \leq 0.10 \) level of significance was selected as the cutoff for these final models to compensate for low power due to the limited number of BGD positive tank-lot weeks, and to provide focus for further prospective BGD risk factor studies. PROC REG was used to assess collinearity of continuous variables in the final multivariable models; no significant collinearity was detected.

3. Results

3.1. Hatchery A

Over the 41-week study period, there were 25 unique tank-lot combinations representing 466 individual tank-lot weeks. Among all tank-lot weeks, 24 (5.2%) were identified as BGD-positive. Table 1 summarizes continuous variables within tank-lot weeks with BGD treatment and those without treatment. Bivariable analyses, summarized in Table 2, indicated that supertrough tank type and biomass were significantly \( (p \leq 0.05) \) associated with BGD treatment. The final multivariable logistic regression model (Table 3), controlling for lot and species, indicated that previous feed percentage, \((\ln)\) previous mortality percentage, and \((\ln)\) exchange rate were significantly \( (p \leq 0.10) \) associated with BGD treatment.

3.2. Hatchery B

Over the 28-week study period, there were 26 unique tank-lot combinations representing 497 individual tank-lot weeks. Of the 497 tank-lot weeks, 71 (14.3%) had treatment for BGD. Continuous variables within tank-lot weeks with BGD treatment and those without treatment are summarized in Table 1. For the bivariable analyses (Table 2), all variables were significant at the \( p \leq 0.05 \) level, except for feed percentage and previous feed percentage. For the final multivariable model (Table 3), both splake and brook trout species design variables, and \((\ln)\) estimated weight and previous treatment were identified as significantly \( (p \leq 0.10) \) associated with BGD treatment.

4. Discussion

Feeding practices have been suggested, through experimental research, to be influential in the development of BGD (e.g., MacPhee et al., 1995). An important finding in the Hatchery A analyses was the significant association between treatment for BGD and higher previous feeding rates. This represents the first epidemiological verification, based on data collected from the field, of excessive feeding as a risk factor for BGD treatment. Various explanations
have been postulated regarding the importance of feeding in the development of BGD outbreaks. The accumulation of excess feed in tanks reduces water quality (e.g. increased suspended solids) (Daoust and Ferguson, 1993; Shotts and Starliper, 1999), which in turn is considered a predisposing factor for BGD outbreaks. Experimental studies, however, have reproduced BGD in water considered to be of good quality (Ferguson et al., 1991; Bullock et al., 1994); therefore, poor water quality as a result of excessive feeding might only be involved in amplifying the effects of outbreaks. MacPhee et al. (1995) reported that feeding was an important factor in experimental reproduction of BGD, but the authors were uncertain whether water quality parameters (e.g. high ammonia concentrations, high particulate matter levels), or actual physiological changes in fish during and after feeding, were responsible for the influence of feeding on the development of BGD. Because it was observed in their study that experimental fish fitted with stomach tubes still required feeding for BGD to be produced, MacPhee et al. (1995) suggested that feeding physiology might be more important in BGD development than feed-associated water quality parameters. No further experimental research, however, has been conducted to investigate the role of feeding in BGD development.

The possibility that feed percentage was confounded with fish size (which is closely correlated with fish age)
was considered. In other words, if smaller (younger), more at-risk fish receive more feed per gram of weight than larger (older), more resistant fish, then a distorted association between feed percentage and BGD treatment might have been observed in the absence of controlling for fish weight. While it was observed graphically that feeding rates do decline slightly as fish get larger over time, supplementary modeling indicated that previous feeding rate remained a significant risk factor for BGD treatment even when fish size (i.e. weight) was controlled (results not shown). Fish size itself was shown to be significantly associated with BGD treatment at Hatchery B through the negative association between individual fish weight and treatment, and this is in agreement with common observations that smaller fish are more frequently affected by BGD. This association, however, was not found at Hatchery A, and the reasons why older early-rearing fish at that facility were more likely to be treated for BGD remain unclear.

Low water exchange rate (in number of exchanges per hour) was also found to be significantly associated with BGD treatment at Hatchery A. The reduced dissolved oxygen and increased total ammonia levels seen with low water exchange rates are believed to be related to BGD outbreaks (Shotts and Starliiper, 1999). Bullock (1990) considered that maintaining good water quality was helpful in preventing BGD outbreaks, although outbreaks were still considered possible with proper husbandry practices. In a survey of Ontario fish farmers, Thorburn (1995) found that Ontario farmers who produced more fish with less water available tended to rely more heavily on preventive treatments (the majority of which were assumed to be for BGD) to maintain the health of their stocks. However, this finding was limited to larger fish (i.e. greater than 10 cm in length). The findings of the Hatchery A analyses, therefore, extend those of Thorburn (1995), in that lower exchange rates were associated with BGD treatment in younger, early-rearing fish in an Ontario hatchery.

The species brook trout was significantly associated with BGD treatment at both hatcheries (relative to lake trout) in the final repeated measures regression models. Although there has been no previous research aimed at identifying the relative susceptibility of various salmonid species to BGD, brook trout has been shown to be more at risk for being test-positive for two important bacterial pathogens (Aeromonas salmonicida and Yersinia ruckeri) than other salmonids raised in the OMNR hatchery system (Good et al., 2001). As well, Thorburn (1996) found that brook trout samples sent to Canadian diagnostic laboratories had a substantially higher apparent prevalence for infectious pancreatic necrosis virus than other salmonids (namely Atlantic salmon (S. salar), Arctic char (Salvelinus alpinus), and rainbow trout (Oncorhynchus mykiss)) sent to the same laboratories. The association between this species and BGD treatment found in this study provides further evidence of the relative susceptibility of brook trout to a variety of health problems affecting cultured salmonids. It should also be noted that the species splake, a hybrid cross of brook trout and lake trout (S. namaycush), was also shown to be significantly associated with BGD treatment at Hatchery B, which further suggests a genetic susceptibility related to the species brook trout. Finally, the species brown trout (S. trutta) was associated with BGD treatment at Hatchery A, and this represents the first finding suggesting the susceptibility (relative to lake trout) of this species to BGD.

Treatment for BGD during the previous week was also significantly associated with BGD treatment at Hatchery B. While this is certainly not considered a “risk factor” for BGD (i.e. treatment for BGD during the previous week more than likely represents efforts to control the same outbreak that is being treated in the current week), it illustrates: (i) the prolonged nature of past BGD outbreaks at the hatcheries involved in this study, and (ii) the ineffectiveness of either the individual chemotherapeutants used, or the treatment protocols employed, in quickly ending a BGD outbreak once it has occurred. This latter point was raised by Thorburn and Mocci (1993), who speculated that the common occurrence of treatment failure in Ontario (the majority of these treatments were assumed to be for BGD) was due to inappropriate treatment protocols. Similarly, while previous mortality at Hatchery A should not be considered a risk factor for BGD (due to the likelihood that BGD was, in fact, the cause of the observed mortality), the presence of this variable in the final model suggests that the disease was not being treated in a timely manner at that facility, or that other infections or environmental conditions predisposing fish to BGD were not being effectively remediated.

### Table 3

Final repeated measures logistic regression models, with odds ratios for risk of bacterial gill disease (BGD) treatment, in Ontario Ministry of Natural Resources early-rearing units at two hatcheries, January–July 1999.

<table>
<thead>
<tr>
<th>Site</th>
<th>Variable</th>
<th>Odds ratio (90% C.I.)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchery A</td>
<td>Species* Brook trout</td>
<td>3.829 (1.602, 9.158)</td>
<td>0.0110</td>
</tr>
<tr>
<td></td>
<td>Species Brook trout</td>
<td>3.965 (1.233, 12.74)</td>
<td>0.0517</td>
</tr>
<tr>
<td></td>
<td>(ln) Water exchange rate (turnovers/hr)</td>
<td>0.485 (0.378, 0.640)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Previous* feed percentage</td>
<td>1.073 (1.005, 1.146)</td>
<td>0.0073</td>
</tr>
<tr>
<td></td>
<td>(ln) Previous* mortality percentage</td>
<td>2.138 (1.420, 3.220)</td>
<td>0.0022</td>
</tr>
<tr>
<td>Hatchery B</td>
<td>Species* Brook trout</td>
<td>64.85 (12.12, 347.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Species Splake</td>
<td>6.189 (1.767, 21.67)</td>
<td>0.0164</td>
</tr>
<tr>
<td></td>
<td>(ln) Estimated wgt. (g)</td>
<td>0.064 (0.025, 0.170)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Previous* treatment</td>
<td>6.368 (3.133, 12.94)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Species design variables were entered together, lake trout (S. namaycush) as reference level.

b During the previous 2 weeks.
Although no statistical comparisons could be made between the two hatchery datasets, a comparison of the within-hatchery analyses suggests that hatchery-level factors are influential in the development of BGD. Accounting for clustering (herds, flocks, pens, etc.) when designing studies or analyzing data is very important in veterinary epidemiology (McDermott et al., 1994), because livestock populations invariably exist in groups, and incorrect inferences can be made between risk factor and outcome if clustering is not somehow accounted for (McDermott and Schukken, 1994). The influence of the hatchery as a cluster may be attributed to, among other things, differences in: (i) management practices (e.g. feeds used and amount given); (ii) the predominant species or strains of species cultured; (iii) hatchery infrastructure (e.g. tank types used, age of water pipes, etc.); (iv) water quality parameters; and (v) experience of personnel, particularly pertaining to the observation of their fish populations and the effectiveness of their efforts in preventing or minimizing the effects of disease.

The use of derived growth rate curves from empirical data of (ln) estimated average weight was considered a reasonable approach to generating weekly estimated weight values, due to the high r-squared values (all >0.96) calculated for each individual growth curve. Having weekly estimated average weight values was extremely important in these analyses, since this parameter (along with estimates of tank-lot fish numbers) was the basis for calculating values for weekly standardized variables, namely feed percentage, flow rates, exchange rates, and the lag variables created for each of these parameters. While the derived weekly weight estimates were considered relatively accurate, there may have been error in these estimates, particularly in relation to specific life stages of tank-lot fish. For example, estimated weight values might have been overestimated for fish during the first month of age, and then overestimated again during their final months in early-rearing. While examining the raw data and comparing empirical weight data with derived estimates illustrated no major discrepancies between the two sets of values, estimation error must still be considered when using this approach. However, because the same approach was used for all tank-lots being compared, it is highly unlikely that any error in estimating weight biased the results of the analyses in any one direction. Misclassification, if it indeed occurred, most likely affected both treated and non-treated tank-lots equally within specific age groups. Such non-differential misclassification bias could have decreased the likelihood of identifying significant risk factors in the analyses, but would most likely not have caused any spurious significant associations to be formed (Kleinbaum et al., 1982).

5. Conclusion

Certain tank-lot-level factors appeared to be associated with the BGD treatment in the OMNR fish hatcheries examined, including higher feeding rates, particular higher-risk species, lower water exchange rates, and higher mortality percentages. Each hatchery investigated in this study presented a unique scenario of how BGD manifests itself, and this underlines the importance of controlling for hatchery-level factors if tank-lots from multiple hatcheries are examined.

Acknowledgements

The authors wish to thank the managers and personnel of the two OMNR fish culture facilities for lending assistance with this project and for providing access to their data archives. Appreciation is also extended to the Ontario Ministry of Agriculture and Rural Affairs for providing funding for our BGD research.

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References


