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Preventive Veterinary Medicine 83 (2008) 297–307

PREVENTIVE VETERINARY MEDICINE

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Factors associated with the incidence of bacterial gill disease in salmonid lots reared in Ontario, Canada government hatcheries

Christopher M. Good [a,*], Margaret A. Thorburn [b], Roselynn M.W. Stevenson [c]

[a] *The Conservation Fund Freshwater Institute, 1098 Turner Road, Shepherdstown, WV 25443, USA*

[b] *Department of Population Medicine, University of Guelph, Guelph, ON N1G 2W1, Canada*

[c] *Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON N1G 2W1, Canada*

Received 19 March 2007; received in revised form 23 August 2007; accepted 26 August 2007

Abstract

Bacterial gill disease (BGD) (causative agent: *Flavobacterium branchiophilum*) has been a persistent problem in early-rearing salmonids in the Ontario Ministry of Natural Resources (OMNR) fish hatchery system. Retrospective epidemiological investigations of BGD diagnoses and treatments in OMNR fish hatcheries during the period 1991–2001 were conducted using University of Guelph Fish Health Laboratory and OMNR central office data. All investigations were conducted at the lot-level, which is the major within-hatchery-level of population aggregation. Survivorship of BGD diagnosis in early-rearing lots within seven individual hatcheries ranged from 84.2 to 100%; within individual species groups, survivorship was lowest (84.6%) in brook trout (*Salvelinus fontinalis*) lots. Annual risk percentages (cumulative incidence) for BGD diagnosis within hatchery and species groups varied considerably among years. Multivariable proportional hazards survival analysis indicated that the species brook trout, and the Spring (March–May) season, were significantly associated with treatment for BGD. Combined, these results emphasize the importance of hatchery, species, and time on the development of BGD. Future observational research on this disease must consider these factors in their design and analysis.

Keywords: Aquaculture; *Flavobacterium branchiophilum*; Bacterial gill disease; Fish disease; Epidemiology; Salmonids

* Corresponding author. Tel.: +1 304 876 2815x279; fax: +1 304 870 2208.
E-mail address: c.good@freshwaterinstitute.org (C.M. Good).

doi:10.1016/j.prevetmed.2007.08.007

1. Introduction

Bacterial gill disease (BGD) is a major disease problem affecting freshwater aquaculture operations worldwide (Shotts and Starliper, 1999). The causative agent of BGD, *Flavobacterium branchiophilum*, is considered ubiquitous in the freshwater environment. When environmental conditions are favourable the pathogen is able to attach and proliferate on gill tissue, compromising gill function, and often leading to high mortality levels unless treatment is administered quickly and effectively. Environmental factors have long been recognized as being important in allowing *F. branchiophilum* to colonize gill tissue and cause clinical BGD (Bullock, 1972; Schachte, 1983; Wedemeyer, 1997). Despite the prevalence and impact of BGD, however, very little epidemiological research has been conducted to describe outbreaks of this disease under field conditions, and identify and quantify important risk factors that influence the occurrence of BGD on fish farms. Because diseases of farmed fish typically have a multifactorial etiology (Hedrick, 1998; Thorburn, 1999), epidemiological information gained from studying risk factors for diseases of cultured fish is considered essential for the prevention and control of such outbreaks (Georgiadis et al., 2001).

This study examined past occurrences of BGD in the Ontario Ministry of Natural Resources (OMNR) fish hatchery system, where BGD has been the most persistent disease problem (Penney, 2003). The OMNR system consists of ten hatcheries, located throughout the province, which raise native and non-native salmonid species for eventual stocking into the Ontario Great Lakes watershed. The OMNR routinely collects and compiles fish health data at its central offices (Peterborough), and at the Fish Health Laboratory (FHL – University of Guelph), which is used for diagnostic testing for clinical disease and annual screening for listed bacterial, viral, and parasitic pathogens. The method of diagnosis for BGD at the FHL is light microscopic examination of gill tissue from sampled affected moribund fish, and all records for gill examination and BGD diagnosis are kept on file at the FHL. The retrospective investigation described in this paper involved the examination of OMNR data collected as far back as 1991, and was undertaken between 2001 and 2003. The overall aim of this study was to gain a better understanding of the descriptive epidemiology of BGD in the OMNR hatchery system. Specific objectives were to quantify the rates of occurrence of BGD at the lot-level (see below) over several periods during the previous decade, and to describe the variability in occurrences among hatcheries, years and age groups. As well, the associations of some putative risk factors, including species and season, with BGD diagnosis and treatment were examined.

2. Materials and methods

Because younger fish are believed to experience relatively higher BGD morbidity and mortality rates (Shotts and Starliper, 1999), it was decided to limit this study to early-rearing fish (i.e. fish less than 9 months in age). Fish reared in the OMNR hatchery system are organized into ‘‘lots’’—fish of the same age and species that have always shared the same water supply and have originated from a discrete spawning population. Lots typically number in the hundreds of thousands of fish, and are usually distributed among multiple tanks within a facility. Because data from the central OMNR offices and the FHL are mostly collected at the lot-level (as opposed to the tank-level), the lowest level of valid epidemiological investigation of these data was the lot-level. Due to differences in record-keeping, data from the OMNR head offices and the FHL could not be combined into a single dataset, and therefore separate analyses were conducted for data from each source.

2.1. Fish health laboratory data

The FHL keeps yearly hatchery master lists, which were used to ascertain the total number of early-rearing lots at risk for BGD during each year of the study period, for each hatchery and for each species within the hatcheries. Fish health laboratory records summarizing all bacteriological and virological screening and diagnostic activities involving OMNR early-rearing hatchery fish from year classes 1994–2000 (covering the period from December 1994 to July 2001) were used to generate descriptive statistics for past BGD cases in OMNR hatcheries. In total, fish from 10 OMNR hatcheries, including ten different species, were submitted to the FHL during the study period. For each relevant study case, data gathered included: date of submission, hatchery of origin, lot identification code, fish species and age (in months), number of fish submitted, the condition of the fish, all tests carried out on the sampled fish, and test results.

The age (in months) that lots were first diagnosed with BGD was determined and used to create survivorship tables for specific hatcheries and specific species during the early-rearing period. The hatcheries included in the survivorship tables were those that, on more than one occasion, submitted fish with gill problems to the FHL during the study period. Seven of the 10 OMNR hatcheries met this criterion, and are referred to as Hatcheries A–G. In total, data from 293 early-rearing lots were available for the analyses. Cumulative percentages of surviving on a month-to-month basis (for each selected hatchery and species) through the early-rearing period were calculated by identifying the total number of early-rearing lots at risk of first-time BGD diagnosis within each selected hatchery and species category for the entire study period. These total values then served as the survivorship percentage denominators, and the cumulative numbers of lots surviving first-time BGD diagnosis within each consecutive age category served as the numerators. For the survivorship tables by hatchery, the percentages were calculated for all species combined within each hatchery. For the survivorship tables by species, the percentages were calculated by summing over all hatcheries that reared the specific species. The species selected to be included were those that (i) are major species reared in the OMNR hatchery system, and (ii) had repeated occurrences of gill problems over the study period. The five selected species were lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), splake (*S. namaycush* × *S. fontinalis*), rainbow trout (*Oncorhynchus mykiss*), and brown trout (*Salmo trutta*).

Annual risk (cumulative incidence) percentages were calculated for the selected hatchery and species categories described above. The risk percentages for first-time lot BGD diagnosis were calculated for each of the 7 years within the study period, for each hatchery and species category. All risk percentages for first-time BGD diagnosis were calculated for each hatchery or each species within each study year, in the manner described in the following example: for Hatchery A, the total number of early-rearing lots raised in 1998 (all species combined) was 5, so that with two instances of first-time lot gill examination at Hatchery A in 1998, the annual risk percentage was calculated as 2/5, or 40%. As well, risk percentages were calculated for repeated lot diagnoses of BGD within each hatchery and each species in each study year. For example, in 1995 Hatchery C had two lots diagnosed with BGD, with one of these lots being diagnosed with BGD a second time later on in the same year. Thus, the risk calculated for repeated lot BGD diagnoses at Hatchery C for 1995 was 1/2, or 50%.

To investigate the seasonal nature of BGD occurrences, the total number of first-time BGD diagnoses among early-rearing lots was calculated, and from this the average percentage of first-time BGD diagnoses for each month was calculated. These percentages were calculated as follows:

$$\left(\frac{\text{Number of first-time lot BGD diagnoses in month } x}{\text{Total first-time lot BGD diagnoses}}\right) \times 100$$

For example, out of 23 cases of first-time early-rearing lot BGD diagnoses occurring over the entire study period, three (13%) occurred during the month of March. Thus, a seasonal distribution of average monthly proportions of first-time early-rearing lot BGD diagnoses was created. In order to provide context to the above seasonal distributions, the total number of early-rearing lots existing in the hatcheries within each individual calendar month over a period of 2 years (July 1999–June 2001) was calculated; these values were summed to give a total lot-month value, and an annual distribution of early-rearing lots was created by calculating the proportion of early-rearing lots existing during individual calendar months out of the total lot-months existing during the 2 year period. The July 1999–June 2001 period used to create the monthly early-rearing lot distribution was selected because only the electronic Monthly Reports were reliable in noting additions and withdrawals of early-rearing lots for each month, and these electronic reports were only available from December 1998 onwards.

2.2. Centralized OMNR records

Data from monthly hatchery reports maintained at the OMNR head office (Peterborough) were organized and compiled for each OMNR hatchery, and included: number of fry in each lot existing at swim-up, all reported BGD treatments, all reported treatments for other reasons, lot age at treatment, and month and season of report. Season was categorized as follows: Spring (1) = March, April, or May; Summer (2) = June, July, or August; Fall (3) = September, October or November; and Winter (4) = December, January, or February. Due to inconsistencies in the Monthly Reports between years and hatcheries, a comprehensive data file summarizing all hatcheries between 1991 and 2001 could not be created, and so individual datasets were created and used for different analyses.

Records of actual BGD treatments in hatchery Monthly Reports were only reliable for certain hatcheries and only prior to the change to electronic record format. Also, certain binders of paper Monthly Reports were missing from the OMNR head office. Thus, in creating a dataset that focused on BGD treatment only the following Monthly records were available and reliable: Hatchery A (1991–1996), Hatchery B (1992–1994), Hatchery C (1992–1996), and Hatchery G (1991–1998). Once the dataset was completed, it was imported into Statistical Analysis System (SAS) software (SAS Institute, Cary, North Carolina, USA). First-time lot BGD treatment was selected as the dichotomous outcome variable, and design variables were created for hatchery (Hatcheries A–C, with Hatchery G serving as the reference level), season (Spring and Summer, with Fall and Winter combined as the reference level), and species (brook trout (*S. fontinalis*) and splake (*S. namaycush* × *S. fontinalis*), with lake trout (*S. namaycush*) serving as the reference level). Due to hatchery confounding, only these three species (which represent the majority of fish raised by OMNR, and all of which were raised at each of the four hatcheries during the intervals listed above) were included in the modeling described below. In total, 92 lots were available to be included in these analyses.

Bivariate logistic regression analyses (using PROC GENMOD in SAS) were run for all variables (hatchery, species, season, initial lot size, and prior treatment for other gill problems), to ascertain their associations ($p \leq 0.05$) with first-time lot BGD treatment. A multivariable proportional hazards survival analysis model was then built using PROC PHREG in SAS, with hatchery clustering controlled by specifying hatchery in the strata line of the program, and with age (in months) at treatment as the outcome (lots that were not treated prior to the end of the early-rearing period were considered censored (end of study) after their final month in early-rearing). A backward stepwise elimination model-building procedure was used, until only variables with $p \leq 0.05$ association with the outcome remained in the model. For the proportional hazards model, individual levels of design variables were removed and joined with the reference level if they were not significantly associated with the outcome.

Hatchery was included as a random component in the final multivariable analysis in order to control for the hatchery-level clustering suggested by the hatchery survivorship table. Also, certain species had to be excluded from the final analyses due to complete confounding with hatchery (e.g. one hatchery did not raise any indigenous species, and other hatcheries did not raise any, or only some, of the introduced species). Because the species distribution within hatcheries was not uniform across the hatchery system, only lots of the predominant indigenous species (lake trout, brook trout, and splake, which were raised at all four hatcheries in the dataset) were included in the analyses—otherwise, hatchery would have been a very strong confounder when examining species effects.

3. Results

The survivorship of within-hatchery early-rearing lots for first-time BGD diagnosis ranged from 84.2 to 100% up to the end of the early-rearing period (Table 1). Among the individual species examined for survivorship (Table 2), lake trout was the most likely to survive first-time BGD diagnosis during the early-rearing period, with 95.3% of lots not diagnosed with BGD by the end of 8 months. Brook trout were least likely to make it through early-rearing without being diagnosed with BGD; 15.4% of lots were diagnosed at least once with BGD by the end of 8 months.

Annual risk percentage for first-time lot BGD diagnosis, for individual selected hatcheries and species, are summarized in Tables 3 and 4. The maximum annual risk percentage calculated for individual hatcheries (Table 3) was 50.0% (Hatchery E, 1995 and 1996), and for individual species (Table 4) the maximum risk percentage was 50.0% (rainbow trout, 1995). Certain species

Table 1
Survivorship of first-time lot bacterial gill disease (BGD) diagnosis in Ontario Ministry of Natural Resources (OMNR) early-rearing fish lots, by hatchery[a], with all species combined (1995–2001)

Age (months)	Cumulative percentage of lots surviving without first-time BGD diagnosis to the specified age Hatchery						
	A	B	C	D	E	F	G
<2	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2 < 3	100.0	94.7	98.0	100.0	100.0	100.0	100.0
3 < 4	100.0	92.1	94.0	97.7	100.0	100.0	96.6
4 < 5	93.5	86.8	90.0	97.7	94.7	100.0	96.6
5 < 6	93.5	86.8	90.0	95.3	94.7	100.0	96.6
6 < 7	93.5	84.2	90.0	93.0	84.2	100.0	96.6
7 < 8	93.5	84.2	90.0	93.0	84.2	100.0	96.6
8 < 9	90.3	84.2	90.0	92.9	84.2	100.0	93.2

[a] Included are the seven OMNR hatcheries that reported gill problems more than once during the 1995–2001 period.

Table 2
Survivorship of first-time lot bacterial gill disease (BGD) diagnosis in early-rearing fish lots in seven Ontario Ministry of Natural Resources (OMNR) hatcheries[a], by major species reared (1995–2001)

Age (months)	Cumulative percentage of lots surviving without first-time BGD diagnosis to the specified age Species				
	Lake trout	Brook trout	Splake	Rainbow trout	Brown trout
<2	100	100	100	100	100
2 < 3	100.0	96.9	94.0	100	100
3 < 4	100.0	90.7	94.0	97.6	100
4 < 5	97.2	86.2	94.0	95.1	100
5 < 6	97.2	86.2	94.0	92.7	100
6 < 7	97.2	84.6	94.0	90.2	92.3
7 < 8	97.2	84.6	94.0	90.2	92.3
8 < 9	95.3	84.6	94.0	87.8	92.3

[a] Included are the seven OMNR hatcheries that reported gill problems more than once during the 1995–2001 period.

Table 3
Annual risk percentages for first-time bacterial gill disease diagnosis in Ontario Ministry of Natural Resources (OMNR) early-rearing lots, by hatchery[a], with all species combined (1995–2001)

Year	Hatchery						
	A	B	C	D	E	F	G
1995	0.0 (0/4)	0.0 (0/4)	28.6 (2/7)	0.0 (0/5)	50.0 (1/2)	0.0 (0/7)	25.0 (2/8)
1996	0.0 (0/4)	25.0 (1/4)	28.6 (2/7)	0.0 (0/5)	50.0 (1/2)	0.0 (0/7)	0.0 (0/9)
1997	12.5 (1/8)	0.0 (0/5)	0.0 (0/7)	0.0 (0/5)	0.0 (0/3)	0.0 (0/8)	0.0 (0/8)
1998	20.0 (1/5)	20.0 (1/5)	14.3 (1/7)	0.0 (0/7)	33.3 (1/3)	0.0 (0/9)	0.0 (0/8)
1999	0.0 (0/4)	16.7 (1/6)	0.0 (0/8)	33.3 (2/6)	0.0 (0/3)	0.0 (0/9)	0.0 (0/9)
2000	0.0 (0/3)	16.7 (1/6)	0.0 (0/7)	25.0 (2/8)	0.0 (0/3)	0.0 (0/7)	0.0 (0/9)
2001	33.3 (1/3)	25.0 (2/8)	0.0 (0/7)	0.0 (0/7)	0.0 (0/3)	0.0 (0/6)	0.0 (0/8)

[a] Included are the seven OMNR hatcheries that reported gill problems more than once during the 1995–2001 period.

and certain hatcheries experienced no (diagnosed) BGD during some years. In general, the risk percentages demonstrated wide fluctuation among study years. For example, while rainbow trout had the maximum risk percentage for first-time BGD diagnosis among all species, it also had four out of 7 years with a 0.0% risk percentage, compared to the more consistently affected brook

Table 4
Annual risk percentages for first-time bacterial gill disease diagnosis in early-rearing lots in seven Ontario Ministry of Natural Resources (OMNR) hatcheries[a], by major species reared (1995–2001)

Year	Species				
	Lake trout	Brook trout	Splake	Rainbow trout	Brown trout
1995	0.0 (0/16)	28.6 (2/7)	0.0 (0/3)	50.0 (3/6)	0.0 (0/3)
1996	0.0 (0/15)	37.5 (3/8)	0.0 (0/4)	0.0 (0/5)	33.3 (1/3)
1997	7.1 (1/14)	0.0 (0/10)	0.0 (0/5)	0.0 (0/6)	0.0 (0/4)
1998	6.3 (1/16)	16.7 (2/12)	0.0 (0/5)	20.0 (1/5)	0.0 (0/4)
1999	6.7 (1/15)	9.1 (1/11)	0.0 (0/5)	14.3 (1/7)	0.0 (0/4)
2000	0.0 (0/15)	10.0 (1/10)	20.0 (1/5)	0.0 (0/6)	25.0 (1/4)
2001	12.5 (2/16)	14.3 (1/7)	0.0 (0/5)	0.0 (0/6)	0.0 (0/4)

[a] Included are the seven OMNR hatcheries that reported gill problems more than once during the 1995–2001 period.

trout which had only 1 year without BGD. Risk percentages for repeated lot BGD diagnoses were calculated for only the 5 hatchery-years when repeated diagnoses occurred, and these ranged from 50.0% (Hatcheries C, D, and G) to 100.0% (Hatchery A) (results not shown). Likewise, risk percentages for repeated lot BGD diagnoses were calculated for the 5 species-years when repeated diagnoses occurred; these ranged from 6.67% (lake trout) to 20.0% (rainbow trout) (results not shown).

The results of investigating the seasonal nature of BGD diagnoses are presented in Figs. 1 and 2. No early-rearing lots received first-time BGD diagnosis in January or February, after which the percentage of all lots receiving first-time lot BGD diagnosis peaked in May, and declined in the Fall months (Fig. 1). For comparison, the annual distribution of early-rearing lots showed a slow increase between January and May, peaking in the month of June and dropping off significantly in the Fall months (Fig. 2).

Table 5 lists all initial variables considered for the multivariable survival analysis model, and summarizes their individual bivariate associations with the BGD treatment outcome. Two out of three hatchery design variables (Hatcheries B and C) were significantly associated with BGD treatment compared to the reference hatchery (Hatchery G). The brook trout design variable was also significantly associated with the outcome compared to the species referent (lake trout). As well, initial number of fry in a lot, and previous treatment for other gill infections, were positively associated with the outcome. Both Spring (March–May) and Summer (June–August) design variables were positively associated with the outcome compared to the Fall-Winter (September–February) reference level.

For the multivariable survival analysis model, only the brook trout design variable (compared to the combined splake and lake trout reference level) and the Spring season design variable (compared to all other seasons combined) were significantly associated with BGD treatment following the backward selection procedure (Table 6).

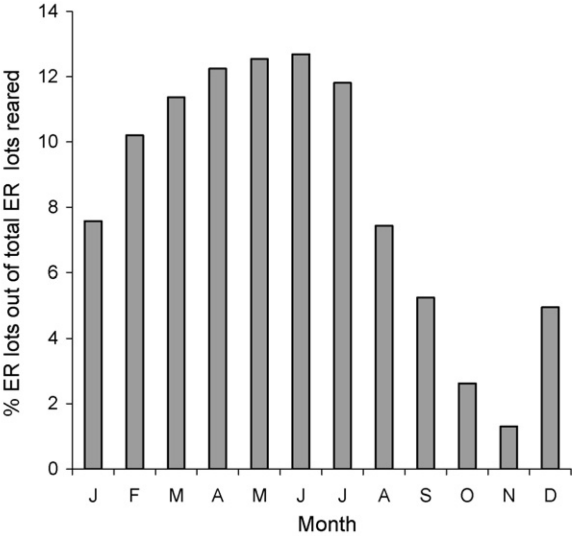

Fig. 1. Frequency of first-time early-rearing (ER) lot bacterial gill disease (BGD) diagnoses, expressed as average monthly percentages out of all early-rearing lots receiving first-time BGD diagnosis, from seven Ontario Ministry of Natural Resources fish hatcheries, 1995–2001.

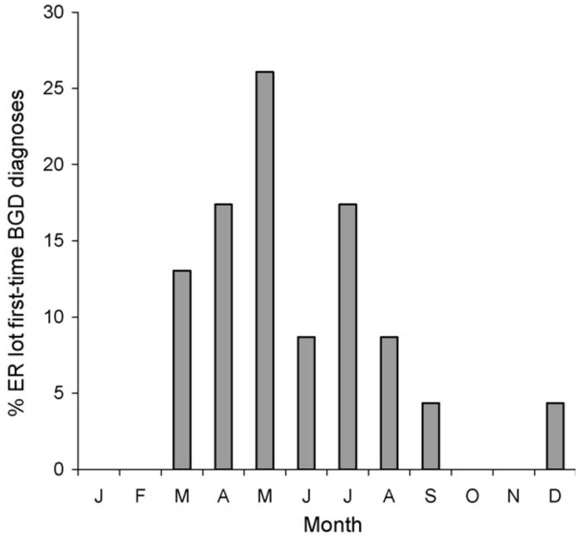

Fig. 2. Annual distribution of early-rearing (ER) lots, expressed as percentages of ER lots, by month, out of the total number of ER lots reared between July 1999 and June 2001, in seven Ontario Ministry of Natural Resources fish hatcheries.

Table 5
Bivariate logistic regression analyses of all initial variables examined for the multivariable survival analysis model for lot bacterial gill disease (BGD) treatment in Ontario Ministry of Natural Resources hatcheries reporting BGD treatment, 1991–1998

Variable	Bivariate[a] odds ratio (95% CI)	*p*-Value
Hatchery A[b]	1.469 (0.439, 4.916)	0.599
Hatchery B[b]	24.00 (2.57, 223.8)	0.0053
Hatchery C[b]	14.57 (2.566, 82.74)	0.0025
Brook trout[c]	5.250 (1.450, 19.01)	0.0115
Splake[c]	2.667 (0.590, 12.04)	0.2023
Initial number of fry in lot (log-transformed)	1.752 (1.146, 2.681)	0.0097
Previous treatment for (non-BGD) gill problems	4.078 (1.331, 12.50)	0.0139
Spring (March–May)[d]	5.877 (1.674, 20.64)	0.0057
Summer (June–August)[d]	7.428 (1.555, 35.48)	0.0119

[a] Bivariate analyses for design variables included all specified levels within the variable (e.g. Hatcheries A–C).
[b] Reference level selected for hatchery design variables was Hatchery G.
[c] Reference level selected for species design variables was lake trout.
[d] Reference level selected for season design variables was Fall-Winter (September–Febraury).

4. Discussion

This study found that the survivorship patterns among hatcheries and among species were highly variable during the early-rearing period and that annual BGD risk percentages among (and within) hatcheries and species were variable among years during the study period. These results underlined the influence of hatchery, species, and time on the occurrence of BGD. The final survival analysis model included brook trout and the Spring season, which further highlighted the importance of both species and season. The apparent seasonal pattern of BGD diagnoses – with

Table 6
Multivariable survival analysis model, controlling for hatchery clustering, with hazard ratios for risk of early-rearing lot treatment for bacterial gill disease (BGD) in Ontario Ministry of Natural Resources hatcheries reporting BGD treatment, 1991–1998

Variable	Hazard ratio (95% CI)	*p*-Value
Brook trout[a]	3.127 (1.378, 7.095)	0.0064
Spring (March–May)[b]	4.137 (1.191, 14.37)	0.0254

[a] Initial reference level selected for species design variables was lake trout; final reference level included lake trout and splake design variables.

[b] Initial reference level selected for season design variables was Fall-Winter (September–Febraury); final reference level included Fall-Winter and Summer (June–August) design variables.

diagnoses peaking in the Spring season – was also revealed graphically using submission records over a 6-year study period.

Speare and Ferguson (1989) suggested a seasonal pattern to BGD incidence, based on a rise in BGD submissions to their diagnostic laboratory during the Spring and Summer months. The authors cautioned, however, that these periods also coincide with fish being at younger ages (fry and fingerling stages) on many commercial fish farms, and thus the effect of season on BGD incidence might be confounded with higher risk developmental stages. This seasonality is supported by Teare (1997), who reported a seasonal incidence of chemotherapeutant usage (the majority of which was assumed to be for BGD) similar to this seasonal BGD submission distribution reported by Speare and Ferguson (1989). Fig. 2 supports the assertion that younger fish lots are at higher prevalence during the Spring and Summer months in OMNR hatcheries. Because fish are generally cultured beginning in December, peak numbers of early-rearing lots (between July 1999 and June 2001) occurred during the month of June, with the percentage of early-rearing lots reared dropping by November. Thus, if younger fish are at a higher risk for BGD infection, then this higher risk population in OMNR hatcheries coincides with the warmer months of the year. From these data it is difficult to separate the effects of season and fish age/size on BGD outbreaks. Although not age-controlled, bivariate regression analyses identified both Spring and Summer design variables as having significant association with BGD treatment. Likewise, the survival analysis model suggested a seasonal trend in BGD treatment, with Spring being significantly associated with BGD treatment. As well, Fig. 1 illustrates that early-rearing lot BGD diagnoses peaked during the month of May through the 1995–2001 period. Rising water temperature and higher precipitation levels occur during this time of year, both of which are considered to be risk factors for BGD outbreaks (Shotts and Starliper, 1999). Whether seasonal factors, such as these, or peaking populations of higher risk age groups, or both, are important risk factors for BGD outbreaks remains an area for future investigation.

The survivorship percentages summarized in Tables 1 and 2 suggest substantial differences among hatcheries and among species in the overall likelihood of first-time lot BGD occurrence, as well as the age at which BGD occurs. This agrees with the notion that particular species are naturally at a higher risk for developing BGD (e.g. Post, 1987), and that certain within-hatchery management activities, and/or site-specific environmental factors, might be important factors predisposing fish to the disease (Shotts and Starliper, 1999). It should be noted that, although datasets from different sources and different time periods were used, the results from the species survivorship table (Table 2) and the results of the multivariable survival analysis (Table 6) are in agreement, in that brook trout was identified as a higher risk species for both diagnosis of, and treatment for, BGD.

From the risk percentages summarized in Tables 3 and 4, it can be seen that the risk of first-time lot BGD diagnosis varies widely among study years for the different hatcheries and species examined. Since management protocols remain relatively constant from year to year in the OMNR hatchery system, the differences in annual risk percentages might be due to environmental factors, such as weather patterns, that change from year to year. A more long-term investigation of yearly or seasonal risk percentages involving more in-depth examination of management and environmental factors is required to further explain these findings.

The species brook trout was shown to be significantly associated with BGD treatment. This particular species has previously been shown to be at higher risk for bacterial pathogen detection than other salmonid species tested at the FHL (Good et al., 2001), and to have a substantially higher apparent prevalence for infectious pancreatic necrosis virus infection than other salmonid species raised in Ontario (Thorburn, 1996). The present results further indicate that brook trout is a higher risk species for infectious disease problems compared to other common salmonids raised in Ontario.

Bacterial gill disease sometimes persisted (or re-occurred) in lots in the study population after first-time lot BGD diagnosis (and subsequent treatment). Although not further investigated, this could suggest a number of things. First, treatment of BGD may have been less than 100% effective in clearing the organism from the gills and water of affected lots. Alternatively, predisposing environmental conditions might not have changed, so that with re-exposure of the lot to *F. branchiophilum* from the source water, a subsequent BGD outbreak might have been more likely to occur. Finally, lots that have had BGD in the past might have been physically weakened by the disease (and/or by the chemotherapeutic treatments administered) and hence more susceptible to further infections. It is worth noting that the bivariate analyses indicated that previous treatment for gill diseases other than BGD (i.e. protozoan infections) was associated with BGD treatment. It would not be surprising that fish whose health had already been compromised would be more susceptible to opportunistic infections, such as BGD. Interestingly, Bebak et al. (1997), in their retrospective study of hatchery-level risk factors for BGD, found that facilities with a history of BGD were more at risk for subsequent BGD outbreaks.

It is quite probable that many of the most important risk factors for BGD operate at levels other than the lot (e.g. at the hatchery- and tank-levels). However, much of the decision-making in the OMNR system occurs at the lot-level. Using lot-level data collected over several years, this study was able to identify some of the key sources of variability in BGD within the OMNR system. Specific hatcheries and specific species appear to be more consistently affected by BGD outbreaks. There also appears to be a fairly consistent seasonal distribution to the disease, but a great deal of annual variability in its incidence at the lot-level. Although further research is required to investigate hatchery- and tank-level risk factors for BGD, recommendations based on the findings of this study for lot-level prevention of BGD outbreaks include revised management protocols for brook trout lots (e.g. lower target stocking densities, higher water exchange rates), as well as increased attention to fish health (e.g. maintaining tank hygiene, reducing stressful activities, etc.) while managing lots during the Spring season.

5. Conclusion

There appear to be important hatchery-level factors that influence the occurrence of BGD in the OMNR fish hatchery system, and hence the design and analysis of future research should account for the hatchery effect. The species (and strains within species) reared by a given OMNR hatchery appear to strongly influence the level of BGD experienced at that site. Among all hatcheries observed, BGD risk percentages for individual species were inconsistent from year-to-year, and it is difficult to determine the contribution that hatchery-level factors made toward these calculated values. Detailed within-hatchery studies would avoid the potential problems of hatchery being confounded with species (and hatchery-level environmental or management factors). Brook trout were consistently associated with BGD compared to other species, and a seasonal trend in BGD diagnosis and treatment was noted, with peak occurrence in the Spring season. However, the variable size of the population at risk throughout the year needs to be taken into account when considering this apparent seasonal pattern.

Acknowledgements

The authors wish to thank the managers and personnel of the OMNR fish culture program and the FHL 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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