Clinical and pathological features of common gill diseases of cultured salmonids in Ontario

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Abstract

We reviewed the clinical presentations and histopathology of 118 diagnostic submissions of trout with infectious gill diseases from commercial trout farms within Ontario. Bacterial gill disease (BGD) (56%) and nodular gill disease (NGD) (26.2%) together accounted for 82.2% of these submissions. Submissions of fish with BGD occurred in every month of the year, but were proportionally more frequent in late winter and spring than in late summer and fall. Chemotherapy of groups of fish with gill diseases was common prior to submission, but the success rate of such treatment was low (29% for BGD; 21% for NGD). Specific therapeutic protocols implemented following etiological diagnosis of BGD were successful in 80% of the previously unresponsive cases and in 88.8% of previously untreated cases. The gills of trout collected within 48–96 h of treatment and apparent clinical recovery lacked bacteria and necrotic epithelial cells, but features such as lamellar fusion and epithelial hyperplasia were similar between recovered and affected fish.

Résumé

Aspects cliniques et pathologiques des principales maladies des branchies de salmonidés de culture en Ontario

Une revue de la présentation clinique et de l'histopathologie fut effectuée sur 118 spécimens provenant de truites atteintes de branchite infectieuse. Les échantillons provenaient de fermes d'élevage commercial de truites en Ontario. La maladie bactérienne des branchies (MBB) (56%) et la maladie nodulaire des branchies (MNB) (26.2%) totalisaient 82.2% de toutes les soumissions de spécimens. La présence de MBB se retrouvait à tous les mois de l'année mais semblait proportionnellement plus élevée à la fin de l'hiver et au printemps qu'à la fin de l'été et durant l'automne. La chimiothérapie de certains groupes de poissons avec une atteinte des branchies était commune avant la soumission des spécimens bien que le taux de succès de la thérapie fut bas (29% pour MBB et 21% pour MNB). Cependant, l'implantation de protocoles thérapeutiques spécifiques basés sur l'établissement d'un diagnostic étiologique furent efficaces à 80% dans les cas de MBB qui n'avaient pas au préalable répandu et à 88.8% dans les cas qui n'avaient pas reçu de traitement. Les branchies recueillies 48–96 heures après le début du traitement chez des truites apparemment guéries ne démontraient une absence de bactéries det de cellules épithéliales nécrotiques. Cependant, une fusion des lamelles et une hyperplasie épithéliale se retrouvaient à la fois chez des poissons guéries et ceux qui étaient encore affectés.

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Introduction

Bacterial gill disease (BGD) is a major cause of disease and mortality in commercially reared salmonids (1–4). The incidence is sufficiently high in Ontario, Canada to be a major factor in limiting production of rainbow trout (*Salmo gairdneri*) (5). The population at risk for BGD is usually limited to fry and small fingerling size salmonids reared under intensive conditions (3). Morbidity and mortality are usually explosive (6). Morbid fish have labored respiration and flared opercula; they are lethargic, anorexic, ride high in the water column, and crowd water inlets (6–8). Based on clinical signs, differential diagnoses include oxygen deficiency, nitrite excess, gill damage from other toxicants (9), protozoal gill diseases (6), and branchial columnaris disease (3).

This disease can be diagnosed using either whole mount or histological gill preparations (2,5,6,10) and is characterized by large numbers of noninvasive filamentous gram-negative bacteria between the gill lamellae (4,5). In contrast with BGD, the filamentous bacteria of columnaris disease are locally invasive, and create large regions of epithelial and connective tissue necrosis (2). Necrosis is not a reported feature of BGD (3,4). Gills from trout with BGD, have large numbers of *Flavobacterium* spp. (3,10,11). *Flavobacterium* differs from the *Flexibacter* of columnaris disease by being nonswarming and nongliding (10,12).

Attempts to experimentally reproduce BGD have met with variable success. Early reports described failure to reproduce this disease when normal trout were exposed to large numbers of bacteria isolated from cases of BGD (13,14). Bullock (14) reproduced BGD by exposing normal fish to fish with BGD. More recently there have been several reports of successful colonization of the gills of normal fish after exposure to *Flavobacterium* isolates (10,11,12,15). Clinical and pathological features of the infected fish were milder than those seen in natural infections.

Outbreaks of BGD are frequently associated with deteriorating water conditions (4,5,6,16), and some authors consider BGD to represent a secondary infection of gill tissues previously altered by aberrations in water quality (14,17). Others feel that such aberrations can promote subclinical cases of BGD into clinical cases (3).

A range of morphological changes is reported in gill tissue from trout with BGD, including heavy bacterial colonization, lamellar fusion, epithelial hyperplasia, and variable (usually minimal) inflammation (5,7,13,18). These changes have been ascribed to the effects of metabolic products released from the bacteria, including proteases and "hyperplasia inducing factors" (18,19). Lamellar fusion and epithelial hyperplasia cause a reduction of gill surface area by occlusion of the interlamellar spaces (13,16,18,20). The thickness of the exchange membrane is increased by the combined effects of epithelial proliferation, and the occluding mat of mucus and bacteria (1,3,14,16). In combination, these changes are believed to physiologically hinder gas, ionic, and osmotic homeostasis (5,20).

Nodular gill disease (NGD) in commercial trout is another important disease in Ontario (5,21); it is distinct from BGD and is characterized by low level, chronic mortality. This disease is typified by several unique pathological alterations of the gills, including multifocal areas of massive filamental epithelial hyperplasia, and the presence of large numbers of angular "A-cells". These A-cells resemble amebae, and are in close association with the surfaces of hyperplastic tissue.

Our purpose in this study was to reexamine clinical case acquisitions of fish with infectious gill diseases submitted to the Fish Pathology Laboratory, Ontario Veterinary College, from trout farms in Ontario, Canada.

Materials and methods

Diagnostic submissions, which involved infectious causes of gill disease from commercial trout farms in Ontario from 1982–1988, were reviewed. The histopathology from each case was reexamined in a blind fashion to confirm or change the original diagnosis. Species involved were rainbow trout and brook trout (*Salvelinus fontinalis*). The proportion of outbreaks attributed to BGD and to NGD was determined; resubmissions from the same cases were included in this study only to assess recovery.

History from data forms accompanying case reports usually included the date of submission, age or weight range of the affected fish, the type of rearing facility, and the duration and severity of the clinical disease. Records included whether or not the fish had been treated prior to submission, along with the chemotherapeutic agent(s) used, and the clinical response. The proportion of diagnosed BGD case submissions which occurred in the different months of the year was determined for the years 1986, 1987 and 1988.

For a subset of BGD cases, in which follow-up histopathology was available, the effectiveness of the following therapeutic regimes was assessed. Subsequent to laboratory diagnosis of BGD, therapeutic recommendations included the use of *either* formalin *or* chloramine-T (sodium para-toluenesulphonchloramide) based on the experience of the farm manager in using these chemotherapeutics, or their local availability. Treatment doses were often tailored to the specifics of the housing system and the degree of gill damage which the fish were suffering.

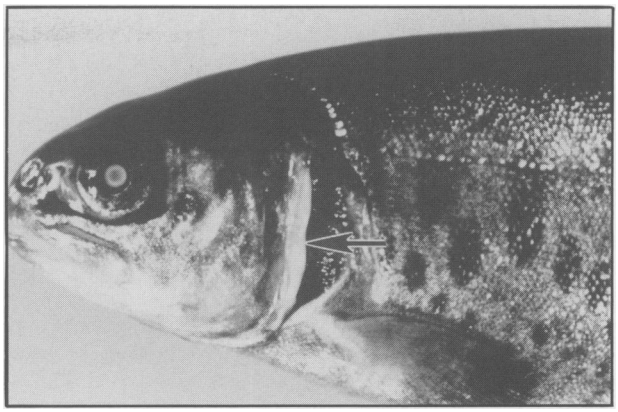

Figure 1. Rainbow trout fingerling with bacterial gill disease. The trailing edge of the operculum is eroded (arrow), and the tips of the gill filaments are exposed.

In most cases, the dose of formalin recommended was 167 mg/L of water for a 1 h bath repeated daily for three days. Alternatively, when chloramine-T was recommended, it was used at 8–10 mg/L of water, again for 1 h baths repeated daily for three days. Along with other recommendations, the owner was strongly encouraged to withhold food for 6 h before and after each treatment, to increase water flow and aeration rates to the units, and to reduce stocking densities if at all possible. Therapy was judged to be successful if there was a marked reduction in morbidity and mortality, combined with histological evidence of a sharp reduction in the bacterial density on the gills of fish from the affected unit, by 48–96 h after initiation of therapy.

Fish collected for histology were killed by spinal severance after anesthesia with MS-222 (tricaine methanesulfonate), or by overdose with MS-222 alone. Tissues were fixed in either 10% phosphate-buffered formalin, or Bouin's fixative, for 24–48 h, routinely processed for histology, and 6 μm hematoxylin and eosin stained sections of gills and systemic organs were examined.

Results

From 1982–1988, (excluding 1985 when data were not in a form suitable for this survey) there were 118 submissions which involved infectious gill diseases. These included 66 outbreaks of BGD (56% of the cases), and 31 cases of NGD (26.2% of the cases).

The majority of BGD outbreaks studied involved fish up to fingerling size (77% of cases); the remainder involved larger (200–450 g) fish in grow-out units (large circular units, raceways, or ponds).

Clinical signs and gross pathology of BGD and NGD

A range of common clinical signs was reported for those BGD cases which involved fry and fingerling trout; these included initial sudden anorexia and listlessness. Affected fish distributed themselves toward the edges of the housing unit or near the water surface. Opercula of these fish were bilaterally flared, with an irregularly eroded margin (Figure 1). Respiratory rates were markedly increased, and some fish exhibited labored and accentuated use of the buccal and opercular pumps.

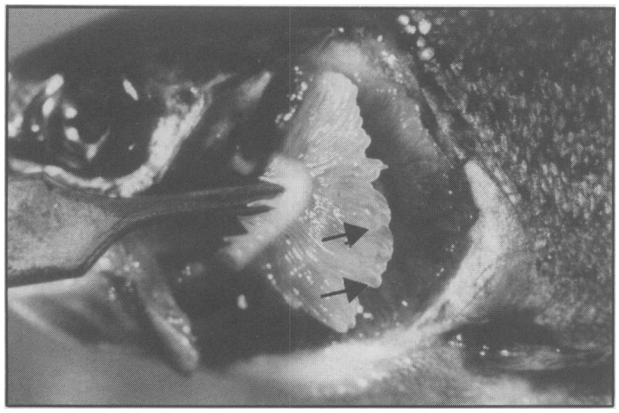

Figure 2. Rainbow trout fingerling with nodular gill disease. Proliferation of gill epithelium has transformed the filament tips into variably sized nodules (arrows).

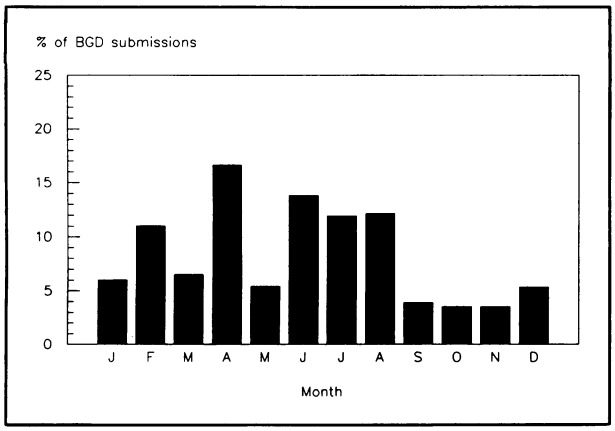

Figure 3. Frequency of submission of cases of BGD expressed as the average monthly proportions of the annual totals of bacterial gill disease case submissions from commercial trout farms in Ontario, 1986–1988.

Grossly, the gills appeared congested and were covered with mucus and debris. Catarrhal exudate also covered the epithelial lining of the branchial cavity. Terminal parts of filaments were markedly displaced from those of neighboring hemibranchs of adjacent arches. This imparted a ragged or flared appearance to the gill.

In untreated cases of BGD, morbidity increased rapidly within 24–96 h of the initial onset of clinical disease within the population. Frequently, in excess of 80% of the fish would become affected such that the entire population seemed markedly listless. These fish faced the direction of water flow and gathered at the water inflow sites (a common behavioral sign of respiratory distress) despite the water in the housing unit carrying abundant oxygen (sometimes in excess of that in incoming water) due to artificial aeration. Fish *in extremis* clogged outflow pipes or outflow screens. With the onset of high (>50%) morbidity, many fish began to die, with up to 10% of the population dying per day. The opercula of dead fish remained flared. By 48 h after the initiation of appropriate therapy, morbidity and mortality were greatly reduced.

In BGD outbreaks which affected larger fish (200–450 g), the progression of the disease was slower.

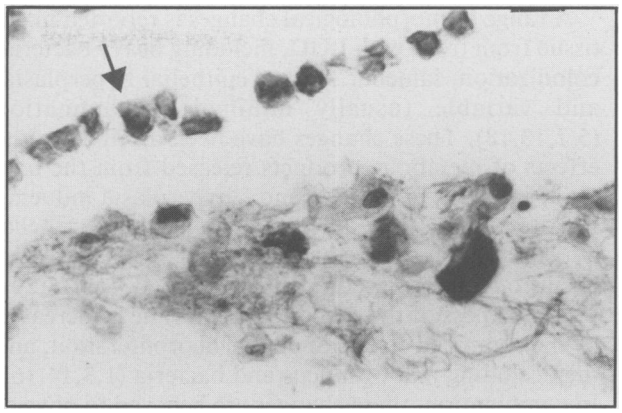

Figure 4. A group of nodular gill disease "A-cells" (arrow) accompanying filamentous bacteria in a mixed bacterial gill disease-nodular gill disease infection of a rainbow trout. Bar = 10 μm.

Widespread morbidity and heavy mortality did not occur until 5–10 days after the onset of morbidity within the population. Fish *in extremis* had a range of hyperkinetic agonal activities. These included gasping at the water surface, described by the farmer as "piping" because the fish hung vertically while gasping. Other terminology such as "sharking" is in reference to a common combination of clinical signs, wherein the fish darkened, and swam rapidly and aimlessly. In ponds, such affected fish sometimes dashed into the shallows and beached themselves.

In contrast to BGD, cases of NGD affecting fry, fingerling, or larger (200–450 g) fish had a chronic clinical history with a slowly progressive increase in morbidity over a period of up to three weeks. Clinical signs in affected fish were similar to those described for BGD. Sudden increases in morbidity and mortality occurred when the affected population was graded, moved, or subjected to adverse water conditions, Grossly the gills of fish with NGD had large numbers of white, partially translucent nodules of proliferated epithelium. These were especially prominent at the tips of filaments (Figure 2).

Seasonal patterns of BGD case submission rates

Although differences existed between years in the monthly pattern of BGD submissions, differences between seasonal patterns were minimal. When the proportions for each month were averaged over the three-year period, it appeared that submissions increased during a broad period from late winter until late summer (Figure 3).

Mixed infections involving BGD

Thirty percent (20 of 66) of the BGD cases examined had pathogens other than the filamentous bacteria present in tissue section. A-cells, typical of NGD were found in 45% of these 20 mixed infections (Figure 4). Dual infection with fungi, whose hyphae resembled *Saprolegnia* sp., accompanied BGD in five of the mixed cases (Figure 5). Protozoa (*Ichthyobodo necator, Ichthyophthirius multifiliis, Trichodina* sp., and *Chilodonella* sp.) accompanied BGD in the remaining six cases.

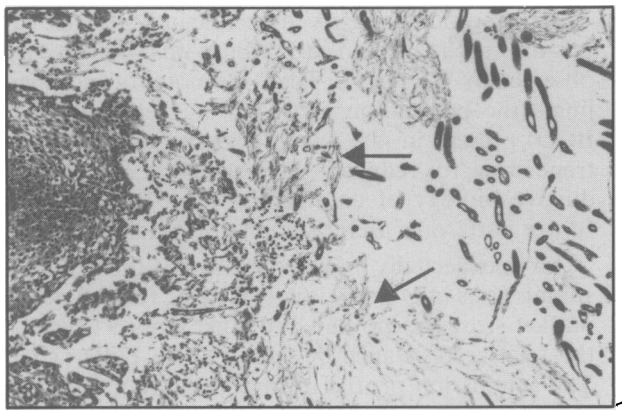

Figure 5. Saprolegniasis and focal gill necrosis from a rainbow trout with chronic bacterial gill disease. The irregular masses of fungal mycelia (arrows) overlie necrotic areas. Hyphae are nonseptate, and branch irregularly. Bar = 60 μm.

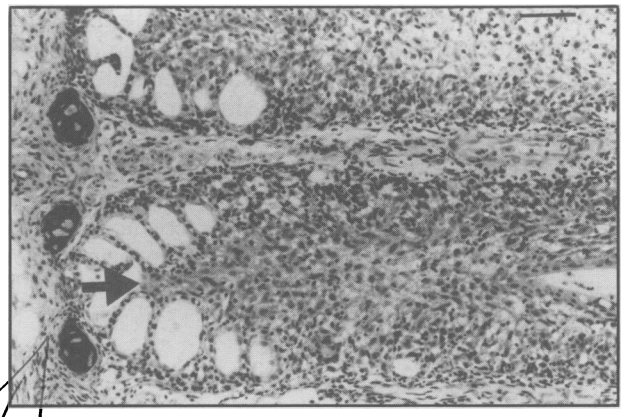

Figure 6. Gill from markedly moribund rainbow trout with bacterial gill disease. Mats of bacteria and exfoliated necrotic lamellar epithelial cells (arrows) are present between lamellae. Only a minor degree of lamellar fusion and epithelial hyperplasia is present. Bar = 40 μm.

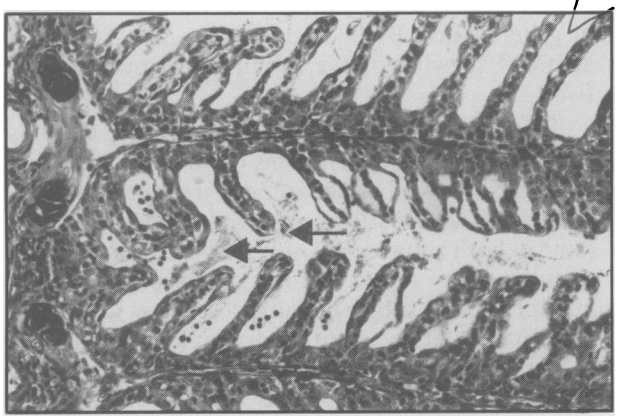

Figure 7. Gill from clinically recovered rainbow trout five days after treatment for bacterial gill disease with chloramine-T. Compared to Figure 6 there is a marked reduction in the numbers of bacteria and exfoliated necrotic cells; however there is dramatic lamellar fusion and epithelial hyperplasia (arrow). Bar = 40 μm.

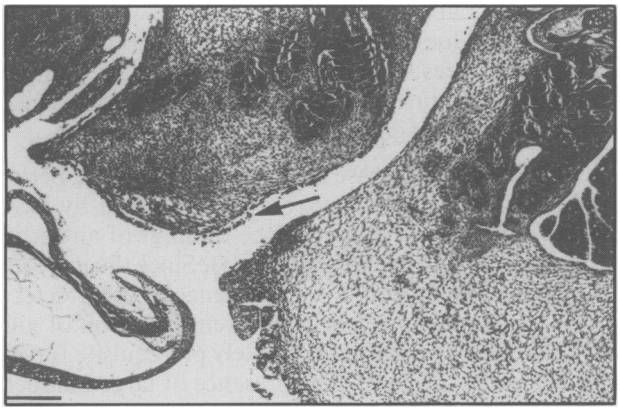

Figure 8. Massive numbers of "A-cells" (arrow), closely apposed to the surface of a hyperplastic nodular mass of spongiotic epithelium. From a case of nodular gill disease in a rainbow trout. Bar = 200 μm.

Apparent efficacy of therapy for BGD

Of the BGD cases submitted, 32% (21 of 66) had been treated for gill disease by the farm manager within 48 h prior to collection of fish samples for diagnostic assistance. Of these 21 cases, 10 had been treated with formalin, seven with chloramine-T, and four with salt treatment regimes. Failure of treatment was recorded in 15 of the 21 cases. Failure was judged to have occurred when morbidity and/or mortality had diminished only slightly, remained the same, or had increased. Histologically, fish from failed treatments still had moderate to large numbers of filamentous bacteria on their gills along with morphological changes consistent with BGD. Formalin had failed in 7 of 10 cases, chloramine-T in four of seven cases, and salt in all four cases where it had been used by the farmer to treat gill diseases prior to diagnosis. Implementation of suggested treatments following diagnosis apparently resolved both the morbidity and infection in 12 out of these 15 cases within 48–96 h. This was based on reevaluation of clinical signs and histological examination of fish collected after treatment. Untreated control groups were unavailable because of the ethical problems associated with persuading farmers to leave untreated groups of diseased fish (populations generally exceeded 10,000 fish per group) when high daily mortality rates are so typical of BGD.

Fish had not been treated prior to submission in 68% (45 to 66) of BGD cases. Implementation of therapeutic recommendations was apparently effective in resolving both the morbidity and infection in 16 of the 18 cases (88.8%) monitored after treatment. For the ethical reasons stated above, untreated control groups of diseased fish were not available. The two failures were recorded from farms using pond culture techniques where severe unresolved water chemistry aberrations had occurred.

Except for the absence of bacteria, and a marked reduction in the numbers of necrotic lamellar epithelial cells, the gills from clinically recovered fish examined within the first week after treatment, resembled those of the previously submitted sick fish. In 12 of 33 cases examined, the extent of epithelial hyperplasia and mucus cell metaplasia, was even more dramatic in the recovered fish than in the sick ones (Figures 6 and 7).

Effectiveness of therapy for NGD

Of NGD cases submitted, 74% (23 of 31) had received treatment prior to submission for diagnosis. In 14 of

these 23, repeated treatments with combinations of chemicals had been administered over the course of several weeks. Despite treatment, moderate to large numbers of A-cells were still present on the gills in 18 of 23 of the cases (Figure 8). In seven of eight of those cases not previously treated, filamentous bacteria in low to moderate numbers accompanied A-cells on the gill. By contrast, filamentous bacteria accompanied A-cells in only 2 of 23 treated cases. Insufficient information was available to assess the chemotherapeutic regimes suggested for NGD.

Discussion

The trout farming industry in Ontario has undergone a recent phase of dramatic expansion in the number of commercial operations. Data are presently unavailable within the industry to assess total and proportional mortality rates. The scope of this study therefore has been based on diagnostic submissions to the Fish Pathology Laboratory. Although the resulting submission profile does not necessarily reflect population proportional rates, it does nevertheless represent the proportional rates among clinical situations where farmers seek diagnostic assistance.

Infectious gill disease submissions during the period under study were almost totally dominated by BGD and NGD. This is similar to the findings of an earlier survey by Daoust and Ferguson (5). Since the majority of BGD cases occurred in the absence of other detectable pathogens, prior or concurrent infection of gills with other pathogens is an unlikely prerequisite for the pathogenesis of BGD. The presence of large numbers of bacteria (*Flavobacterium* sp.) may however, hamper survival or mask the presence of other pathogens. In addition, the detection of smaller infective agents, such as viruses and mycoplasmas, would not be possible histologically.

Of the mixed infections involving BGD, *Saprolegnia* sp. fungus was common. This fungus is cited as a secondary opportunistic agent which complicates a range of fish diseases (22), and is described as such when it complicates BGD (6,16). In this survey, A-cells of NGD were the most common pathogen involved in mixed infections predominated by BGD. Amebae commonly feed on bacteria (23), and gills infected with bacteria may therefore become a favorable habitat to support colonies of amebae (24). Almost all of the untreated cases considered to be dominated by NGD, had low to moderate numbers of filamentous bacteria. This is in contrast to the remarkable absence of bacteria in most of the treated cases of NGD. The absence of bacteria in this latter group may reflect the reduction in bacterial numbers brought about by the repeated use of chemotherapeutics prior to submission of samples for diagnostic assistance. Although a greater range of untreated cases of NGD would need to be examined, preliminary evidence suggests a close pathobiological association between the A-cells of NGD, and the bacteria of BGD as suggested by Daoust and Ferguson (21). The distinct possibility is that NGD may represent a secondary infection of gills subclinically infected with BGD.

The empirically observed decline in BGD case submissions in the fall and early winter was similar to that observed by Warren (6). There are more fry and fingerlings (the population generally at greatest risk for BGD) present in late winter and spring (6). Present trends towards reducing the seasonality of the reproductive biology of commercial trout may alter the future pattern of BGD submission rates.

An unexpectedly high proportion of the BGD case submissions in Ontario involved large fingerlings and market-size fish, compared to other reports which cite the relative resistance of these fish to BGD (2,3,6). The submission profile in this study may reflect the recent growth in the trout farming industry, and the trend towards specializing in either hatchery or grow-out operations. The production of fry is by experienced operators who generally diagnose and properly treat BGD as it occurs. Many new farmers are involved primarily in purchasing fingerlings and growing them to market size. Their inexperience with fish husbandry in general, and BGD in particular, may impose an artificial skewing of the submissions towards larger fish.

Treatment protocols carried out after the diagnosis of BGD performed well. This contrasts sharply with the poor success of owner-initiated treatment, although the chemotherapeutic agents used were generally the same in both situations. Unsuccessful owner-initiated treatment may reflect the use of the proper chemotherapeutics at subtherapeutic levels, or insufficient amelioration of those husbandry problems which may have promoted the outbreak. In addition, on-going mortality would reduce the stocking density and improve the odds of survival and recovery of the remaining fish at subsequent retreatment after diagnosis. A major factor to consider is that an unknown and probably large number of BGD outbreaks are successfully diagnosed and treated by experienced fish farmers, without the need for diagnostic assistance. Many of these farmers would only seek diagnostic assistance for those gill disease cases which were not responding. This, in combination with submissions from farmers inexperienced with proper treatment procedures, would give the appearance, through the nature of the present survey, that owner initiated therapy had poor success.

Histological comparison of the gills of affected fish to clinically recovered fish, highlights the difficulty in drawing pathophysiological inferences from histological assessment of gill damage. Clinically recovered fish examined shortly after successful therapy often had the same or an even greater degree of lamellar fusion, epithelial hyperplasia and increased lamellar thickness, than did clinically affected fish prior to therapy. Histologically, the only empirical difference was a marked reduction in the numbers of bacteria and exfoliating necrotic cells on the gills of recovered fish. Similar to the results of this study, other workers have observed that fish with BGD recovered rapidly within days after treatment (2,3,25). They also noted that therapy led to effective removal of bacteria from the gill surface. In contrast, it takes several weeks for the regression of lamellar fusion and hyperplasia (25).

Such observations may indicate that the presence of the bacteria themselves, and their on-going active effects on the gill (such as necrosis) are important for the clinical expression of BGD. Lesions such as lamellar fusion and hyperplasia may represent a healing response which, despite imparting surface area changes, may have a pathophysiological effect which is too subtle for clinical detection. CVJ

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