Effects of High-density Stocking in a Recirculating Aquaculture System on Gill Morphology of Hybrid Striped Bass (*Morone saxatilis* x *M. chrysops*)

B.J. Smith, S.A. Smith, and C.J. Pfeiffer

Aquatic Medicine Laboratory
Virginia-Maryland Regional College of Veterinary Medicine
Department of Biomedical Sciences and Pathobiology
Duck Pond Drive, Blacksburg, VA 24061

ABSTRACT

The types and distribution of gill lesions observed in hybrid striped bass (*Morone saxatilis* x *M. chrysops*) reared in a commercial-scale recirculating aquaculture system are described. When placed in the system as fingerlings and reared there for eight months at typical stocking density, the gills of all examined fish presented a variety of extensive, non-specific lesions typically resulting from poor water quality. Lesions included epithelial cell hyperplasia, infiltration of the interfilamental region by mixed inflammatory cells, hyperplasia of mucous and lamellar epithelium, lamellar fusion and occasional filamental fusion. Up to 76% of the gill sample surface of individual fish was affected, with lesions being most severe in the distal filamental regions. Fish transferred to and maintained at low stocking densities in water of superior quality demonstrated that all lesions were fully reversible by five weeks post-transfer. This study demonstrates that culture of hybrid striped bass under intensive aquaculture management induced pathological changes in the gills, and suggests that maintenance of fish under improved water quality conditions will reduce gill lesions, which could potentially increase the fishes' performance.

INTRODUCTION

Recirculation aquaculture systems have become increasingly significant in the culture of numerous species of economically important fish. Benefits offered by recirculation systems include conservation of water and energy, decreased environmental pollution, and increased flexibility in the choice of aquaculture site location (Lucchetti and Gray 1988; Liao and Mayo 1974). Hybrid striped bass (*Morone saxatilis* x *M. chrysops*) are among an increasing number of species being successfully raised in intensive recirculation systems. In the interest of maximizing economic return, fish are commonly stocked at the highest population density possible without overtly affecting the fishes' health or growth. Unfortunately, high-density stocking practices exacerbate the inherent tendency of water in recirculation systems to carry high levels of contaminants such as particulates, metabolic wastes, parasites, and bacteria (Allen and Kinney 1981; Spotte 1979). The gills, being continuously and unavoidably in direct contact with these substances, respond with various structural and functional changes that can compromise respiratory, excretory, and osmoregulatory efficiency. (Roberts 1989; Ferguson 1989). By affecting gill function, even moderate changes in gill structure can affect the fishes' behavior, appetite, and homeostasis, and thus also have the potential to adversely affect growth and development.

The effects on gill morphology of rearing fish in recirculating systems under present management practices have not been closely scrutinized. However, conditions documented as significant stress factors, including poor water quality, overcrowding, and excessive handling, have long been known to occur in commercial recirculation systems (Amend 1970). Water quality parameters experienced in such systems may induce types and degrees of pathological change in the gills that have the potential to decrease fish performance and thereby decrease economic return. This study was undertaken to examine the hypothesis that high-density stocking of hybrid striped bass in recirculation systems as is commonly practiced in commercial aquaculture operations would induce significant gill pathology. Further, this study also aimed to characterize the nature of any gill lesions that developed, as well as to determine whether the resulting pathological changes were reversible.

METHODS

Fingerling hybrid striped bass (7-10 cm in length) were obtained from a commercial source where they had been reared in ponds. Six fish were arbitrarily selected as an incoming group to examine the microscopic anatomy of the gills at the outset of the study and determine whether significant underlying gill pathology was present at the outset of the study. These six fish were immediately anesthetized with tricane methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO, USA) and killed by cervical dislocation. Tissue samples consisting of the gill arch and associated holobranchs were obtained from the second gill arch of each fish and placed in fixative (5% glutaraldehyde, 4% formaldehyde, and 2.75% picric acid in 0.05% cacodylate buffer, pH = 7.4).

The remaining fish were divided into two groups and placed in recirculating systems where they were reared for eight months. One group (control; n = 21) was maintained at low population density (≤ 2.5 kg/m^3) in 1,893 liter (500 gallon) circular tanks with a sand filter for mechanical filtration and a trickle filter with Bio-Pac 150 (NSW Corp., Roanoke, VA, USA) as the biological filter. Water quality in this tank was main-tained within parameters defined as superior (i.e., they were better than those currently considered acceptable by the production industry: temperature 26 ± 1°C; pH 7.2-7.4; TAN < 0.1 mg/L; NO_2 < 0.01 mg/L; NO_3 < 50 mg/mL; DO 8-10 mg/L; alkalinity > 150 mg/L; hardness > 200 mg/L) throughout the eight-month period. The second group (experimental) (5000 fingerlings with fish graded and removed at appropriate intervals) was reared for the eight-month cycle in a 11,356 liter (3000 gallon) recirculation system with a sump discharge for mechanical filtration and a rotating biological contactor for biological filtration. Fish in both groups were fed a commercial diet (Floating Fish Nuggets, 40% protein, Zeigler Bros., Garners, PA, USA) twice a day at 5-6% body weight. At the end of the production cycle, fish density in the control group neared 2.5 kg/m^3, while that in the experimental group had reached 130 kg/m^3 of water. Water quality parameters in both systems were determined weekly. When water quality deteriorated beyond acceptable industry limits in the experimental group, appropriate water changes were performed.

At the end of the 8-month period, representatives from both the control and the experimental groups (six and nine fish, respectively) were

arbitrarily removed and gill samples obtained as previously described. The remaining 15 control fish were maintained in their original system, in which the stocking density remained low. From the experimental group, 15 fish were arbitrarily selected to investigate the reversibility of any lesions that had developed. These fish were placed in recirculation systems like that in which the control fish were held, and in which the population density was kept low (less than ≤ 2.5 kg/m^3) and the water was maintained at superior quality. Gill samples were obtained weekly as previously described from three arbitrarily-selected fish in each of the groups for a total of five weeks. Gill tissues from the experimental group were compared to those of control fish as well as to published normal anatomy for striped bass and their hybrids (Groman 1982; Pfeiffer et al. 2000).

Tissues were prepared for light microscopy (LM) by routine histological technique (Luna 1968; Hinton 1990), embedded in EM 400 embedding medium (Surgipath Medical Industries, Richmond, IL, USA), and sectioned at 6 µm. Separate slides from each sample were stained with hematoxylin/eosin (H&E) and the periodic acid-Schiff (PAS) reaction. For LM evaluation, samples were standardized to include a length of gill arch that included 10 to 20 gill filaments presenting

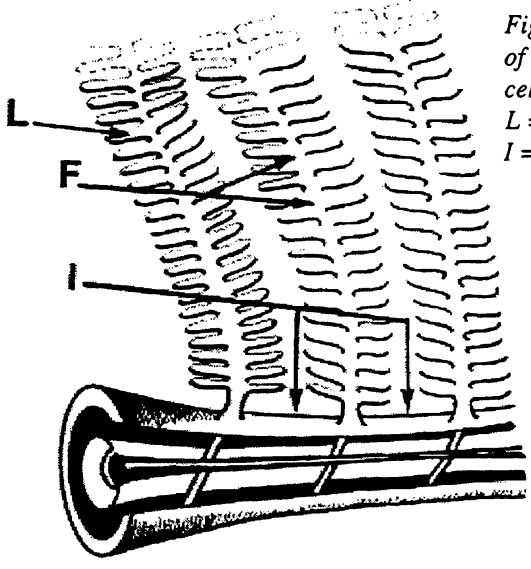


Figure 1. Schematic representation of sites on the gill at which mucous cells were enumerated. L = lamellar, F = filamental, and I = interfilamental sites.

lamellae along both sides of the length of the filament. For each sample, ten filaments were evaluated for morphological structure and pathologic change. Pathologic changes were evaluated using H&E-stained slides, characterized descriptively, and expressed as a percentage of filament length involved. To calculate the percentage of affected gill surface, the length of filament showing structural anomalies was divided by the total length of the filament. Slides stained with PAS were evaluated to detail alterations in number and location of mucous cells. Mucous cells were enumerated in the interfilamental regions at the base of 10 filaments, in the interlamellar spaces between the bases of the lamellae, and on the surfaces of the lamellae (Figure 1).

For transmission electron microscopy (TEM), tissue was fixed immediately at 4°C, washed in buffer, postfixed in 1% osmium tetroxide in 0.1 M cacodylate, washed in buffer again, dehydrated through a graded series (15% to 100%) of ethanol, transferred to propylene oxide, and embedded in Polybed 812 (Polysciences Inc., Warrington, PA, USA). Thin sections were doubly stained in lead citrate and uranyl acetate, and viewed in a JEOL/00 CX-11 transmission electron microscope (JEOL, Peabody, MA, USA) operating at 80 kv.

For statistical evaluation, the percentage abnormal gill surface and counts of the number of mucous cells in the interfilamental regions at the bases of the lamellae, in the interlamellar spaces, and on the surfaces of the lamellae were measured on 10 lamellae for each fish. For each of the four response variables, one measurement per fish was calculated by averaging the values from the 10 lamellae. Separate one-way ANOVA models were used to test whether sampling time had an effect on the percent abnormal gill surface and mucous cell counts from the three areas evaluated. ANOVA assumptions were evaluated by looking at normal probability plots of the residuals and plots of residuals against the predicted values. The normality of the residuals and equality of variances were substantially improved by a natural log transformation of the data. Sheffe's multiple comparisons procedure was used to make pairwise comparisons between sampling times. For all tests, a p-value of <0.05 was considered significant.

RESULTS

Incoming Fish

The histologic structure of the gills in the incoming group of fish was considered normal as compared with published reports of normal anatomy (Groman 1982; Pfeiffer et al. 2000).

Control Fish

Water Quality -- Water quality parameters remained within superior limits during the entire rearing period (data not shown).

Clinical Appearance of Control Fish -- Control fish appeared clinically normal on daily visual inspection throughout the study. The fish displayed normal swimming, feeding, social, and resting behavior, and grew at a rate typical of hybrid striped bass in commercial production.

Gill Histology -- The histologic structure of at least 94% of the gill surface in control samples was normal (Table 1, Figure 2) as was comparable to normal anatomy (Groman 1982; Pfeiffer et al. 2000). Filaments (Figure 3) were regularly arranged along the gill arch, with lamellae projecting freely and uniformly along the entire length of the filament. Interfilamental cells at the base of the filaments were present in appropriate types and numbers, consisting of a thin layer of mainly

Table 1. Percent Abnormal Gill Area in Control, High-Density, and Recovering Fish

	Percent Abnormal Gill Area									
	Number of Fish									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Overall Mean**
Control	5.9	5.1	5.4	5.1	5.7	5.6	--	--	--	5.5
High-density	61.5	71.5	64.0	67.5	76.1	73.7	72.8	69.9	71.7	69.9
Week 1 *Recovery*	45.9	48.9	47.0	--	--	--	--	--	--	47.3
Week 2 *Recovery*	25.9	27.4	25.6	--	--	--	--	--	--	26.3
Week 3 *Recovery*	23.3	23.8	23.3	--	--	--	--	--	--	23.5
Week 4 *Recovery*	11.0	10.9	10.2	--	--	--	--	--	--	10.5
Week 5 *Recovery*	5.7	4.8	5.3	--	--	--	--	--	--	5.3

For individual fish identified by number, each percentage reported is the mean for 10 filaments counted per fish. For the overall mean, the number reported is the arithmetic mean of all values within that group. For the control group, n = 6; for the high-density group, n = 9; for the recovering fish, n = 3. Dashes indicate a zero value in that category.

Table 2. Mucous Cell Numbers in Examined Regions of the Gill of Control, High-Density, and Recovering Fish

A. Interfilamental Space

	Number of Fish									
	1	2	3	4	5	6	7	8	9	Overall Mean
Control	3.9	3.7	2.8	3.0	3.3	3.2	–	–	–	3.3
High-density	9.0	11.5	8.3	11.0	9.3	10.1	8.2	9.6	10.5	9.7
Week 1 *Recovery*	5.4	5.7	4.9	–	–	–	–	–	–	5.3
Week 2 *Recovery*	4.5	4.8	5.0	–	–	–	–	–	–	4.8
Week 3 *Recovery*	3.5	3.7	3.1	–	–	–	–	–	–	3.4
Week 4 *Recovery*	3.2	3.1	3.2	–	–	–	–	–	–	3.2
Week 5 *Recovery*	2.8	2.9	3.2	–	–	–	–	–	–	2.9

For individual fish identified by number, the number reported is the mean of values for 10 interfilamental spaces in each fish. For the overall mean, the number reported is the arithmetic mean of all fish within that group. Dashes indicate a zero value in that category.

B. On Filament

	Number of Fish									
	1	2	3	4	5	6	7	8	9	Overall Mean
Control	26.1	28.8	28.6	27.4	28.5	28.2	–	–	–	27.9
High-density	220.6	203.2	227.2	220.6	226.6	240.8	214.5	218.8	219.1	221.3
Week 1 *Recovery*	163.6	162.5	159.0	–	–	–	–	–	–	161.7
Week 2 *Recovery*	106.6	103.7	104.0	–	–	–	–	–	–	104.8
Week 3 *Recovery*	52.4	46.8	55.8	–	–	–	–	–	–	51.7
Week 4 *Recovery*	31.8	29.1	28.8	–	–	–	–	–	–	29.6
Week 5 *Recovery*	27.4	29.0	27.9	–	–	–	–	–	–	28.1

For individual fish identified by number, the number reported is the mean of values for 10 filaments in each fish. For the overall mean, the number reported is the arithmetic mean of all fish within that group. Dashes indicate a zero value in that category.

C. On Lamellae

	Number of Fish									
	1	2	3	4	5	6	7	8	9	Overall Mean
Control	0.7	1.0	0.5	1.0	0.4	0.8	–	–	–	0.7
High-density	41.5	44.1	44.7	35.4	42.3	40.8	44.4	44.8	46.5	42.7
Week 1 *Recovery*	1.4	1.2	1.3	–	–	–	–	–	–	1.3
Week 2 *Recovery*	1.2	1.0	0.9	–	–	–	–	–	–	1.03
Week 3 *Recovery*	1.1	0.9	1.1	–	–	–	–	–	–	1.03
Week 4 *Recovery*	1.2	1.0	1.2	–	–	–	–	–	–	1.1
Week 5 *Recovery*	0.7	0.5	0.8	–	–	–	–	–	–	0.4

For individual fish identified by number, the number reported is the mean of values for 10 lamellae in each fish. For the overall mean, the number reported is the arithmetic mean of all fish within that group. Dashes indicate a zero value in that category.

undifferentiated cells, together with small numbers of chloride cells and isolated migrating leukocytes and eosinophilic granulocytes. Normal lamellar structure was typified by pillar cells separating capillary spaces, with the external lamellar surface covered by squamous epithelial cells. Occasional mucous cells were present in the interfilamental spaces. Greater numbers of mucous cells were found along the filaments, but were rare or absent along the lamellae (Table 2). Ultrastructurally, control gills (Figure 4) were typified by normal cellular structure of the principal cell types, including the surface epithelial cells of the secondary lamellae, pillar cells, and vascular endothelial cells.

Histological abnormalities were rarely observed in the control gills, and were restricted to small, localized sites of minor proliferation of interlamellar epithelial cells at the base of the lamellae, or, more rarely, in the interlamellar space.

Experimental (High-Density Stocked) Fish

Water Quality -- Experimental group water quality parameters generally remained within limits considered acceptable by production industry standards, though departures from acceptable limits occasionally occurred (Table 3). Near the end of the production cycle, despite the water quality parameters being generally acceptable, a higher concentration of suspended feed particles and organic debris often caused the water to become visibly turbid and brownish.

Clinical Appearance -- Throughout the study, the experimental fish appeared clinically normal on daily visual inspection. As with controls, the fish displayed normal swimming, feeding, social, and resting behavior, and appeared by visual inspection to grow at a rate typical of healthy hybrid striped bass.

Gill Histology of Experimental (High-Density Stocked) Fish -- Lesions of various types involving the majority of the gill surface (Table 1, Figure 2) were manifested in all market-aged fish at all levels along the length of the filament, though the full length of individual filaments was never involved. Twisting of filaments near lesions were generally most severe at the bases of and along the distal half of the filaments (Figure 5). The mid-regions of the filaments were affected to a lesser degree or not at all. Areas of severely affected tissue were often flanked by areas with less pathologic change.

Table 3. Water Quality Parameters from Tanks Holding Hybrid Striped Bass at High Density

Week	Temp. (22-28°C)	pH (7.2-8.6)	TAN (<2.0 mg/L)	NO_2 (<1.0 mg/L)	NO_3 (<150 mg/L)	DO (>5.0 mg/L)	ALK (>125 mg/L $CaCO_3$)	Hardness (>200 mg/L $CaCO_3$)
1	25.9	8.3	0.25	0.39	5	10.3	160	325
2	25.5	7.8	0.31	0.23	11	12.0	128	180
3	26.0	7.7	0.47	0.28	15	10.4	149	312
4	27.5	7.5	0.61	0.27	22	12.5	172	368
5	25.5	7.7	0.70	0.36	30	12.8	NA	NA
6	26.7	7.8	0.83	0.21	33	11.2	236	456
7	27.4	7.9	0.72	0.15	26	11.0	274	500
8	27.8	7.8	0.85	0.10	31	11.3	142	450
9	27.5	7.5	1.04	0.19	77	11.0	115	400
10	26.5	7.8	1.12	0.28	83	9.4	105	492
11	28.0	7.6	1.21	0.54	40	8.7	110	NA
12	28.2	7.6	1.28	0.19	83	11.5	115	NA
13	27.6	7.6	1.30	0.52	91	12.8	124	658
14	26.9	7.3	1.26	0.12	115	11.9	117	578
15	27.4	7.3	1.07	0.34	169	12.2	109	604
16	27.0	7.9	0.75	0.38	141	14.1	117	630
17	27.0	7.9	0.95	0.49	258	15.5	216	471
18	25.9	7.4	0.87	0.79	227	14.4	161	545
19	26.9	7.3	0.75	0.15	119	13.0	150	489
20	25.7	7.4	0.65	0.16	152	14.3	215	524
21	24.6	7.7	0.80	0.35	115	15.4	179	382
22	24.3	7.6	0.88	0.36	116	13.3	230	371
23	24.1	7.8	0.83	0.45	43	14.7	252	329
24	25.6	7.7	1.18	0.24	111	13.2	143	280
25	23.7	8.0	1.13	0.25	30	13.5	201	386
26	22.7	8.0	1.65	0.34	98	14.3	203	428
27	24.4	7.6	1.56	0.56	116	13.0	225	379
28	24.4	7.6	1.04	0.61	81	13.4	171	450
29	24.4	7.6	1.18	0.16	18	14.7	169	338
30	24.3	7.4	1.22	0.54	109	14.4	139	401
31	24.6	7.3	1.51	0.36	64	15.3	130	340
32	24.1	7.3	1.47	1.53	175	12.6	94	419
33	24.1	7.3	1.47	1.47	97	14.8	125	350

Numbers in parentheses under each parameter indicate desired limits for intensively-raised hybrid striped bass (modified for recirculation systems from Harrell et al. 1990). TAN indicates total ammonia nitrogen, *NA indicates* not available.

Lesions of the basal interfilamental region were typified by a thickening of the interfilamental cellular layers, caused by proliferation of epithelial cells and marked infiltration of the interfilamental region by mixed inflammatory cells. The thickening often extended to a level that engulfed some of the most proximal lamellae (Figure 6). The deepest layer of this thickened region contained primarily lymphocytes and macrophages, which extended from the basal region into and throughout the superficial layers as well. In the intermediate layers, these cells were joined by inflammatory cells possessing large eosinophilic granules. The superficial-most layer demonstrated increased numbers of mucous cells.

Lesions of the distal filaments were typified by proliferation of both epithelial and mucous cells on the lamellae (Figures 7-9). The number of mucous cells was significantly increased along both the filament and on the lamellae (Table 2; Figures 8,9). Mucous and other epithelial cellular proliferation and accumulation were sufficient to produce prominent, widespread lamellar fusion (Figures 8,9), which involved as much as a mean of 40% of the surface of affected filaments. In some areas, the fusion was so severe as to require careful examination of lamellar capillaries to distinguish adjacent lamellae (Figure 8).

Ultrastructurally, the gills of high-density stocked fish demonstrated a variety of pathologic changes. The lamellae demonstrated disruption of the pillar cells which resulted in the separation from their flanges supporting the central blood spaces (Figures 10,11). Further, marked enlargement of the subepithelial lymphatic spaces was evident, and the presence of granulocytes within these spaces was greatly increased (Figures 10,11). Membranous inclusions of obscure origin were common, both within the central blood spaces and the subepithelial lymphatic spaces. Small, electron-dense cytoplasmic granules became numerous within apices of the outer epithelial cells lining the secondary lamellae (Figure 10), though they were not prevalent in the control epithelia (Figure 4). Small cytoplasmic vacuoles were also noted in the outer epithelial cells covering the secondary lamellae.

Recovering Fish Transferred to Water of Superior Quality

Figure 2 and Table 1 show the percentage of gill surface with histopathological abnormalities in recovering fish. Evaluation of mucous cell numbers in these same fish is presented in Table 2.

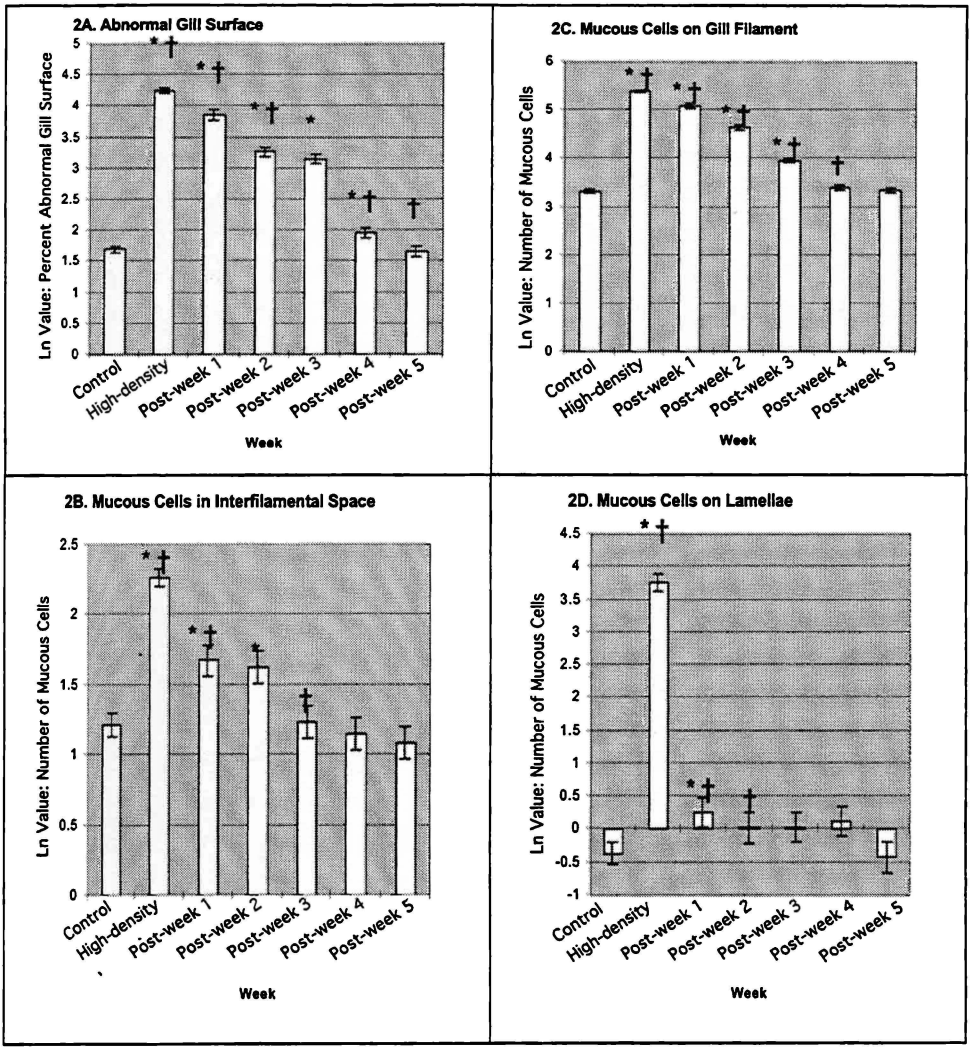


Figure 2. Percent abnormal gill surface (2A), and numbers of mucous cells in the interfilamental space (2B), on the filament (2C), and on the lamellae (2D) in control (n = 6), high-density (n = 9), and recovering (n = 3 in each group) fish.

Values shown are the group means of the natural log (Ln) transformed data. Error bars are 2x the SE of the mean.
** Indicates values significantly different from control,*
† Indicates significantly different from the immediately preceding measurement, $p < 0.05$.

One week post-transfer, gill tissue occasionally demonstrated resolving mild lesions of terminal vessel dilation (telangiectasis). Proliferative lesions and lamellar fusion in the distal region of the filaments were reduced to a mean of 45.9% of the gill surface (Table 1, Figure 2). The severity of these lesions was also greatly reduced as reflected in decreases in depth of proliferated cells in the interfilamental spaces and in the length of the filaments exhibiting lamellar fusion, as well as in absence of fusion of adjacent gill filaments. Mucous cells, though still present in elevated numbers (Table 3, Figure 2), appeared to be less active than in samples from high-density stocked fish, being smaller in size and presumably containing less mucus.

After two weeks of maintenance in superior quality water, areas of proliferation were further significantly decreased to a mean of approximately 25.9% of the gill surface (Table 1, Figure 2). The number of mucous cells on the lamellae were reduced to a level similar to controls, and remained so for the duration of the study (Table 3, Figure 2). Proliferation of mucous and epithelial cells as well as infiltration of inflammatory cells persisted in the regions between the basal interfilamental region, but were reduced from the one week post-transfer fish. The number of mucous cells in the interlamellar regions and on the filaments was decreased (Table 3, Figure 2), and the thickness of proliferated cells in the interlamellar spaces as well as along the lamellae was also reduced.

The types of lesions observed in the three week post-transfer fish were similar in distribution to the two week fish, but lesion severity continued to decrease. Numbers of mucous cells in the interfilamental regions and on the filaments approached control levels (Table 3, Figure 2). The thickness of proliferated cells in the interlamellar and interfilamental regions was further reduced, as was the proximal-to-distal length of involved lamellar regions. Lamellar fusion was no longer evident in any region of the gill.

By five weeks post-transfer, nearly all gill filament samples were similar in appearance to control tissues. Departures from normal histological structure were reduced to a mean of less than 10% of the gill surface (Table 1, Figure 2), and were characterized by only occasional areas of minor proliferation of epithelial cells in the interlamellar areas. Numbers of mucous cells were comparable to control levels in all locations.

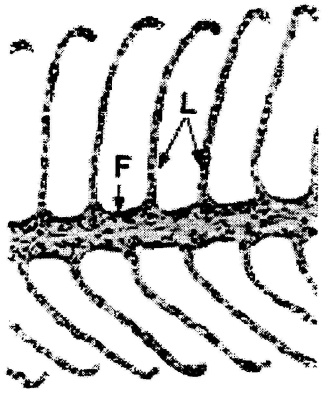


Fig.3

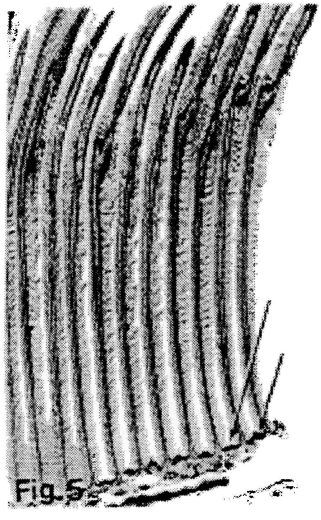


Fig.5

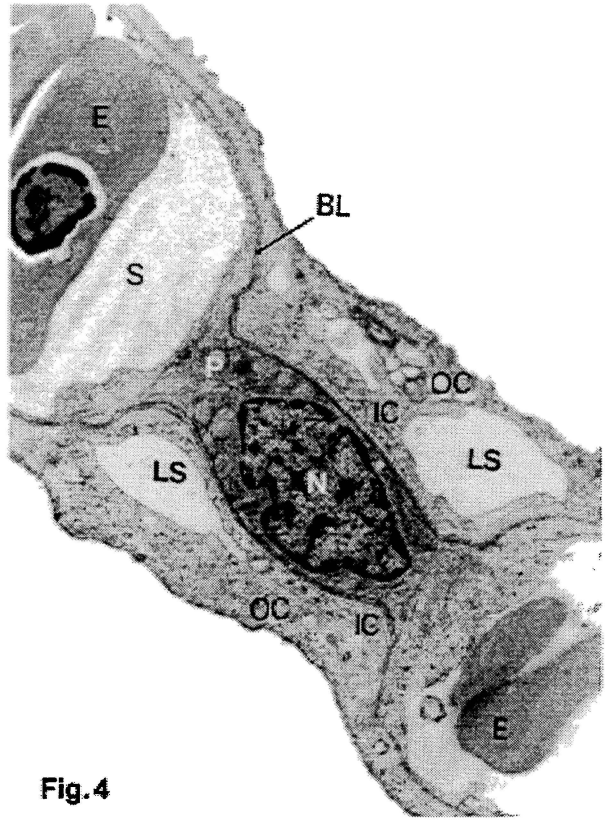


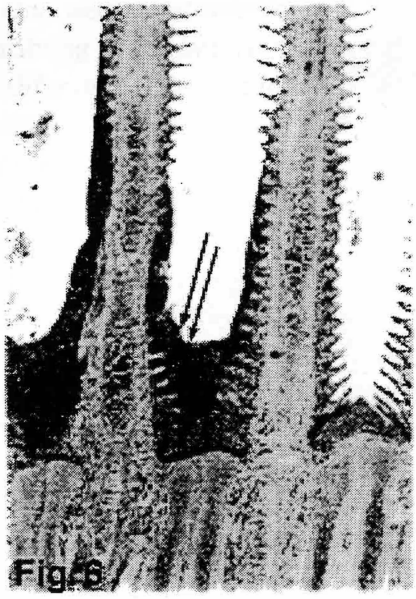
Fig.4

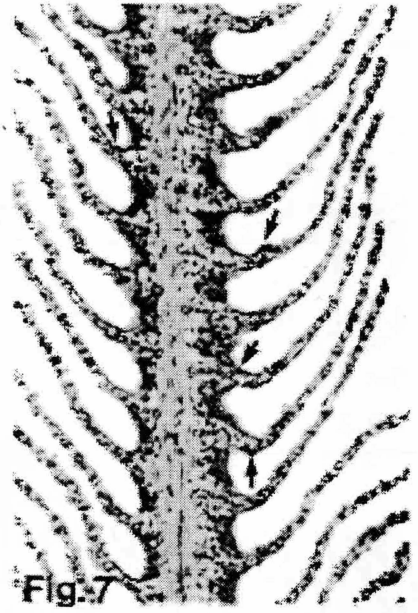
Figures 3-5

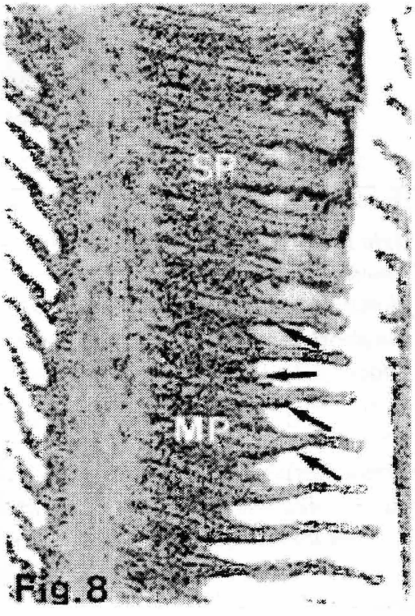
Figure 3. Light micrograph of normal gill filament. Lamellae (L) project freely from each side of the filament (F) with abundant water space between them. Squamous epithelial cells cover the surface of the lamellae, and the blood-water barrier is appropriately thin. Hematoxylin and eosin, x = 300.

Figure 4. Transmission electron micrograph of normal morphology of a secondary gill lamella showing pillar cell (P) with large, centrally-located nucleus (N) and a distinct basal lamina (BL) surrounding both the pillar cells and the central blood spaces (S). Portions of erythrocytes (E) are present within the respiratory channels. Outer (OC) and inner (IC) epithelial cells can be observed covering the lamellae, as well as subepithelial pymphatic spaces (LS). x = 19,440.

Figure 5. Light micrograph showing distribution of lesions along the length of gill filaments of high-density stocked fish. The basal interfilamental regions demonstrate some thickening of epithelial cell layers (arrows). Lamellar lesions are most prominent alone the distal region of the filaments, with lamellae along the more proximal part of the filament far less affected. Hematoxylin and eosin, x = 15.







Figures 6-8.

Figure 6. Light micrograph demonstrating thickening of epithelial cells in the basal interfilamental region in a high-density stocked fish. The layers of proliferated epithelial cells are infiltrated by mixed inflammatory cells, and mucous cells are numerous in the most superficial layer (arrows). Hematoxylin and eosin, x = 75.

Figure 7. Light micrograph demonstrating mild epithelial cell proliferation in high-density stocked fish. Proliferated layers of epithelial cells in the interlamellar spaces have extended only slightly distally along the length of the lamellae. Though the proliferation of epithelial cells is only mild, mucous cells (arrows) are greatly increased. Periodic acid-Schiff, x = 240.

Figure 8. Light micrograph of a segment of a filament from a high-density stocked fish showing moderate (MP) and severe (SP) epithelial cell proliferation. In the severe region, proliferation has proceeded to the point of lamellar fusion. Elevated numbers of mucous cells (arrows) are clearly demonstrated. Periodic acid-Schiff, x = 150.

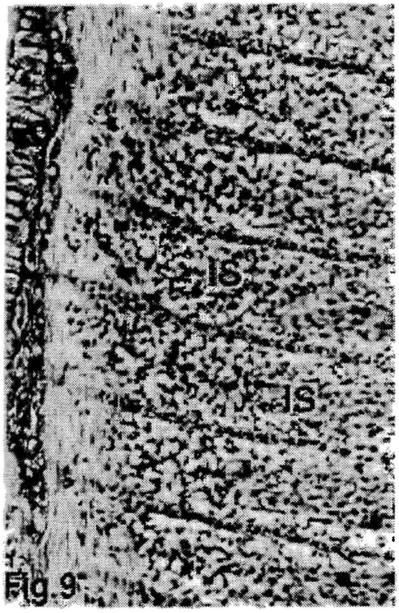


Figure 9. Light micrograph of a region of severe lamellar fusion. The entire interlamellar space (IS) is filled with proliferated and/or sloughed epithelial cells, effectively removing this area of the gill from respiratory function. Periodic acid-Schiff, x = 300.

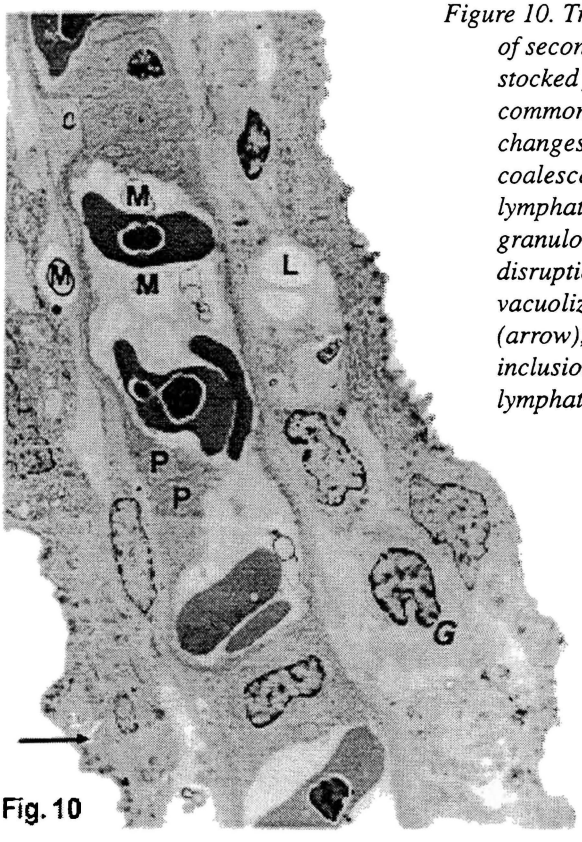


Figure 10. Transmission electron micrograph of secondary gill lamella of high-density stocked fish showing numerous, commonly-observed cytopathologic changes including enlargement and coalescence of the subepithelial lymphatic spaces (L), accumulation of granulocytes (G) within those spaces, disruption of pillar cells (P), vacuolization of epithelial cell cytoplasm (arrow), and presence of membranous inclusions (M) in the vascular and lymphatic spaces. x = 7540.

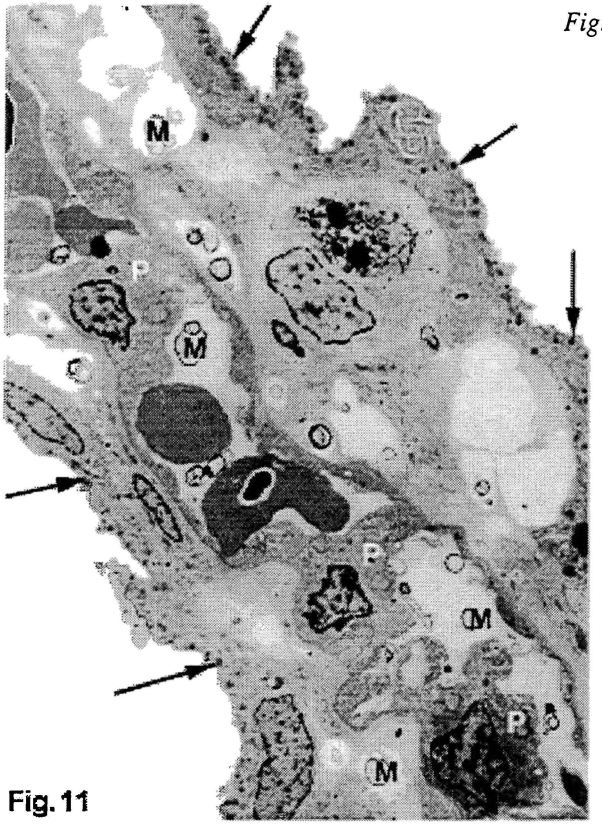


Fig. 11

Figure 11. Transmission electron micrograph of secondary gill lamella of high-density stocked fish showing alterations similar to those demonstrated in Figure 10, but at a more advanced stage of pathology. Greater thickening of gill lamella, more pronounced disruption of pillar cells (P), more numerous membranous inclusions (M) in both the vascular and lymphatic spaces, and enhanced accumulation of small, dense granules in the cytoplasm of the outer epithelial cells (arrow) are observed. x = 7540.

DISCUSSION

The minor areas of proliferative tissue present in the control fish were considered unremarkable. Such variation is typical of many species of wild and cultured fish inhabiting clean water (Ferguson 1989; Solangi and Overstreet 1982; Haensly et al. 1982; Ribelin and Migaki 1975), and hence is representative of the range of variation typically present in normal healthy fish. Though such foci do represent departures from normal, when small and few they are not considered significantly pathological to the gill as a functioning organ (Haensly et al. 1982). The similar foci observed in the five week post-transfer fish were also considered to be within normal limits.

The ultrastructural data clearly illustrate not only some specific cytopathological changes in the secondary gill lamellae resulting from over-crowding of the fish, but also the structural basis for impairment of respiratory gaseous transport as well as excretory and osmoregulatory

functions. In normal fish, the water-blood barrier consists of pillar cells flanges, a basal lamina, and thin layers of epithelial cell cytoplasm. In fish showing pathological changes, this transport barrier was significantly increased by either fusion of some of the secondary gill lamellae, or by a several-fold increase in the portion of the barrier consisting of the two-cell thick epithelial cell layer, as well as by mucus accumulation and enlargement/extension of the subepithelial lymphatic spaces. The origin of the membranous inclusions noted in the vascular and subepithelial lymphatic spaces remains obscure, but probably represent fragments of cellular membrane products (Hinton, personal communication). Nonetheless, their common occurrence in the crowded fish and absence in the uncrowded fish suggests that these bodies develop in response to some factor(s) experienced by the fish under high-density stocking. Such factors could involve stress on the fish, water quality, some other unidentified factor, or a combination of multiple factors. The ultrastructural changes observed in this study of hybrid striped bass resembled the acute inflammatory effects in gills reported in rainbow trout exposed to a specific water contaminant, zinc sulfate (Skidmore and Tovell 1972), which resulted in respiratory collapse and death.

Development of gill lesions was anticipated in fish reared at high population density in the intensive recirculating system. Though water quality parameters generally remained within limits considered acceptable, levels of potentially irritating substances such as suspended particulates or metabolic wastes were nonetheless elevated as compared to what would be encountered in open, unpolluted waters. Continuous exposure to these physical and chemical irritants likely contributed to the development of the changes observed.

The lesions that developed in these gills were non-specific in nature (Roberts 1989; Mallat 1985; Ribelin and Migaki 1975), and were similar to what has been described in studies involving exposure to diverse substances such as crude oil, ammonia, molluscicides, herbicides, and pesticides (Cruz et al. 1988; Soderberg 1985; Haensly et al. 1982; Solangi and Overstreet 1982; Eller 1969; Eller 1971). Further, similar effects have been reported in salmonids reared using re-used water or in recirculation systems (MacConnell 1989; Morrison and Piper 1988). The relatively few cell types present in the gill tissue together with the simple structural arrangement of those components result in only a limited range

of possible histopathological responses to any of a wide variety of insults (Roberts 1989). In particular, epithelial hyperplasia with or without fusion of adjacent lamellae, mucous cell hyperplasia, and inflammatory cell infiltration are characteristic of a chronic stress response (Roberts 1989; Ferguson 1989; Mallat 1985). This contrasts with certain conditions that induce specific changes, such as hyperplastic gill response in fish exposed to ammonia (Smart 1976; Larmoyeux and Piper 1973; Burrows 1964), increased chloride cells following exposure to acid water (Karlsson-Norrgren et al.1986; Leino and McCormick 1984) or nitrite (Gaino et al. 1984), and chloride cell degeneration and necrosis in nitrite and cadmium toxicity (Ferguson 1989). Proliferation of epithelial cells and particularly of mucous cells can contribute to the lamellar fusion that was prominent in the market-aged fish (Ferguson 1989; Roberts,1989).

Gill lesions from a number of etiologies may be distributed diffusely along the gill arch (Ferguson 1989; Mallat 1985), while other etiologies such as exposure to crude oil and other factors (Ferguson 1989; Solangi and Overstreet 1982) are characterized by a distal-filamental localization of lesions. Alterations observed in this study were more similar to the latter, being distributed mainly along the distal third to half of the arch. Severe histopathological alteration such as lamellar fusion effectively reduces the surface area of gills available for respiratory and other functions. Decreased oxygen intake caused by severe proliferative lesions and resulting impairment of respiratory exchange can be the primary cause of death in certain conditions such as zinc toxicity (Hughes 1972; Skidmore 1972,1970; Burton et al. 1972). However, when such lesions are only moderate and/or when not diffusely spread among the filaments, exchange across less-affected regions of the gills may adequately compensate for the damaged regions (Roberts 1989). Modifications in cardiovascular function have also been postulated to contribute to such compensation (Goldes et al. 1988). This is particularly true when fish are not achieving or sustaining high levels of physical activity (similar to the fish in this study and other intensive aquaculture systems), since in such instances the distal regions of the filament are not fully perfused (Ferguson 1989). Pantothenic acid deficiency (classical "nutritional gill disease") of rainbow trout presents a similar situation in which fish having severe lamellar fusion along the distal filamental region but little involvement of the more proximal filamental region

show little clinical evidence of distress when maintained under inactive conditions (Karges and Woodward 1984; Poston and Page 1982).

Dilation of small or terminal gill vessels (telangiectasis) has been described in association with chemical exposure, parasitic infestation, or metabolic wastes (Roberts 1989; Haensly et al. 1982; Redner and Stickney 1979; Smart 1976). This lesion also commonly develops in farmed fish following their physical handling associated with size grading and/or pond transfer (Roberts 1989). In the present work, resolving telangiectasis was observed only in the group sampled shortly after transference between tanks, rather than in any of the fish in groups maintained in the recirculation system for extended periods of time before sampling. This suggests that the telangiectasis developed related to the handling of the fish during transfer, rather than to water conditions in the recirculation system.

The alacrity with which mucous cell number on the gill lamellae is striking. The numbers of mucous cells decreased precipitously during the first week, decreasing from an overall mean of 42.7 cells in the high-density fish to 1.3 cells at 1 week post-transfer (Table 2, Figure 2). Lamellar mucous cell numbers then reached normal levels during the second week. In contrast, the number of mucous cells in the interfilamental spaces and on the gill filament did not reach normal levels until the third and fourth weeks, respectively. Further, the decrease in mucous cells in these other areas was more gradual, rather than being abrupt as on the lamellar surface. That the lamellar surface should clear of abnormally high numbers of mucous cells both rapidly and before other areas, may present an adaptive response in that the swift return of this area, which is the main respiratory surface, to normal structure would promote an expedient return to a normally-functioning state of this strategic portion of the gill.

The rapid resolution of the proliferative lesions was likely related to the extensive epithelial tissue component of the gills, the nature of the lesions, and the placement of the fish in clean water (i.e., removal from inciting conditions caused by less than superior water quality in the recirculation system). Epithelial tissues inherently possess rapid regenerative capacity, and organs with large amounts of epithelium are often capable of swift recovery from minor to moderate damage once the insult is removed. In keeping with such characteristics, gills are well-

characterized as able to recover rapidly from sub-lethal injury (Ferguson 1989; Goldes 1988; Fukuda 1983). Degenerative lesions resolve more quickly than necrotic lesions, since generation of new cells is not necessary. In this regard, the preservation of the basement membrane observed in this study likely facilitated rapid recovery. Placing the fish in superior quality water eliminated stimuli for continued excessive epithelial and mucous cell proliferation, as well as for excessive mucus production by proliferated cells. The flushing action of clean water is also documented as contributing to the removal of accumulated mucus and cells (Roberts 1989).

Though development of gill pathology in the experimental group was expected, the extent of the gill surface involvement was not. With significant structural alterations distributed over more than half of the gill surface, the absence of overt effects on the behavior and growth of the fish is noteworthy. Such apparent well-being of the fish could be misleading. Despite acceptable growth, the deteriorated condition of the gills could contribute to increased susceptibility of the fish to stressors like bacterial, viral, fungal, or parasitic infections or other forms of challenge. Further, resulting morbidity could be more severe than in fish where the gills were in better condition. For instance, increased water temperature and resulting decreased oxygen saturation could induce mortality in fish with damaged gills that otherwise might survive (Roberts 1989). Similarly, overcrowding of fish is known to be an important predisposing factor in numerous diseases (Roberts 1989; Ferguson 1989; Ribelin and Migaki 1975). Therefore, impairment of normal respiratory, excretory, and osmoregulatory function resulting from structural and functional compromise of the gill caused by such conditions may be a major contributing factor in the susceptibility of fish to other disease conditions, despite seemingly healthy condition and adequate growth.

Despite the fact that fish raised at high population densities in recirculation systems with less than superior quality water can survive and grow at an economically-profitable rate, the results of this study have important implications for the aquaculture industry. First, this study demonstrates that fish raised under present aquaculture practices in these systems develop extensive structural alterations along the majority of the exchange area of the gill surface, the character of which could potentially alter respiratory, excretory, and osmoregulatory functions. By affecting

such functions, growth performance and feed efficiency of the fish could be negatively impacted, as has been previously shown in channel catfish (Robinette, 1976; Soderberg et al. 1984). Therefore, production performance of fish as commonly reared in intensive recirculation systems, though considered acceptable, may not necessarily reach full potential. The reversibility of the lesions suggest that fish in production facilities could respond quickly to significant improvements in water quality. Practical and economic considerations of most production facilities preclude the maintenance of water conditions similar to those used in the recovery phase of this study. Nonetheless, moderate improvement of water conditions would likely decrease gill lesion severity. Such improvement could improve respiratory, osmoregulatory, and excretory functions of the gill, thereby potentially improving both the performance of the fish and ultimately increase economic return. Further, improving gill health prior to and after inducing significant stress on the fish by stressors such as handling procedures could assist in maintaining physical and physiological condition of the fish. Whether increased return generated by such practices will exceed the cost and logistics of implementation is yet to be determined, but invites investigation.

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