

A COMPARISON OF THE EXTERNAL MICROBIAL ASSEMBLAGES BETWEEN  
NATIVE SOUTHERN STRAIN AND WILD NORTHERN BROOK TROUT,  
*SALVELINUS FONTINALIS*, OF HATCHERY ANCESTRY

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Western Carolina University in partial fulfillment of the  
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## ABSTRACT

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Hatchery reared, northern strain brook trout have been stocked in streams within the home range of southern strain brook trout in an effort to restore or enhance native trout populations since the late 1800s. But, brook trout native to the southern Appalachians are genetically distinct; raising ecological and ethical concerns regarding the impact of the past stockings. In this study, the external microbial assemblages on native southern and wild fish of hatchery ancestry were compared by characterizing colony morphologies and estimating densities of colony forming units. The hatchery-ancestry fish had significantly higher densities, and assemblages were more similar to that of the surrounding water than those of the southern strain fish. These results suggest that the native southern strain fish exhibit a greater ability to inhibit microbial growth in their epidermal mucus than do the fish with hatchery ancestry.

## INTRODUCTION

The brook trout (*Salvelinus fontinalis*) is the only native salmonid of the southern Appalachian Mountains (MacCrimmon and Campbell 1969). Brook trout have been stocked to restore or enhance depleted native trout populations (Lennon 1967, Jones 1975, Wilson 2011). However, the brook trout reared in these early hatcheries were not derived from the southern strain, but were of northern ancestry (Lennon 1967) and have distinct genetic differences (McCracken *et al.* 1993, Kreigler *et al.* 1995, Hayes *et al.* 1996, Galbreath *et al.* 2001). Northern strain brook trout are generally considered to be those found north of the New River drainage in Virginia, while the southern strains include the New River drainage and all waters south (Hayes *et al.* 1996). Using allozyme analysis, McCracken *et al.* (1993) found genetic differences between hatchery strain brook trout of northern descent and native southern strain populations. Wild northern strain fish of hatchery origin (NBKT) were found to have less genetic diversity than native southern strain brook trout (SBKT; Hayes *et al.* 1996). NBKT had only 4 mtDNA haplotypes, while SBKT populations had 12 haplotypes and exhibited almost as much intra-strain variation as they did with NBKT (Hayes *et al.* 1996).

Hayes *et al.* (1996) argued that the low diversity seen in the hatchery fish could be explained by the bottlenecking effect of the hatchery. However, northern strains also exhibited low genetic diversity in their native streams; it was hypothesized that this is due to the contraction and subsequent re-expansion of the northern brook trout's range after the mass glaciations of the Pleistocene (Hayes *et al.* 1996). In contrast, the genetic diversity of SBKT was shown to be much greater than that of the far northern populations and those of hatchery ancestry (Hayes *et al.* 1996). The difference between the two

strains may be explained by two hypotheses: 1) unlike the NBKT, who were forced into refugia during the Pleistocene glaciation, SBKT flourished in the South and when the glaciations ended they subsequently did not have a bottlenecking effect of reestablishment; and 2) after the Pleistocene glaciation, as the climate began warming and the lower reaches of major rivers became too warm, they had to seek refuge in the cooler headwater streams. Thus they became isolated into distinct populations increasing the chances of diversification across their southern range (Hayes *et al.* 1996).

Many concerns have been raised about introducing nonnative trout into native populations (Allendorf and Phelps 1980, Ferguson 1990, Krueger and May 1991). In particular, the loss of native genetic diversity through introgression of NBKT alleles is a possibility (Krueger and May 1991, Hayes *et al.* 1996). The process of hatchery rearing may have selected for traits or behaviors that are maladapted for natural streams (Hindar *et al.* 1991). Though the genetic differentiation between the strains has been known for some time, only recently have the ecological differences been quantified. Wesner *et al.* (2011) found differences between NBKT and SBKT in growth, behavior, and survival under experimental conditions. Hybridization with hatchery stock can swamp the genetic makeup of native trout (Hindar *et al.* 1991, Hansen and Loeschcke 1994). A loss of genetic integrity could disrupt unique naturally selected ecological and physiological responses, putting the genetic diversity and fitness of native fish populations at risk (Allendorf and Phelps 1980, Ferguson 1990).

Another potential ecological difference between the strains is how fish react to the microbial communities in their habitat. Fish have slow reacting specific immune responses that are affected by temperature (Ellis 1982, Bly and Clem 1991) and must



therefore rely heavily on their innate immune response (Subramanian *et al.* 2008). The epidermal mucosal layer of fish (slime) serves as an integral part of innate immunity (Ellis 1974, Ingram 1980) and is considered the fish's first line of defense against microorganisms (Hjelmeland *et al.* 1983, Austin and McIntosh 1988, Grinde *et al.* 1988, Fouz *et al.* 1990, Nagashima *et al.* 2001, Sarmaşik 2002). The process in which slime protects fish from harmful pathogens works in three layers: first the slime acts as a physical barrier between the fish and the environment; second, the slime is continually replenished and sloughed off, removing microbes that have attached (Pickering 1974, Alexander and Ingram 1992, Rombout *et al.* 1993, Aranishi and Nakane 1997, Ellis 2001); and third, the presence of broad-spectrum, defensive agents within the slime prevent or destroy growth of foreign invaders (Austin and McIntosh 1988, Ellis 2001, Hellio *et al.* 2002, Subramanian *et al.* 2008; reviewed in: Bols *et al.* 2001, Ellis 2001).

Subramanian *et al.* (2008) attempted to identify and describe the defensive agents within the slime using aqueous, organic, and acidic extracts of concentrated mucus from different fish species, including brook trout. The slime of brook trout was found to exhibit among the strongest antimicrobial properties of the tested fish. The agent which seemed to have the greatest antimicrobial properties was the small peptide molecules found in the acidic extracts. Fish become highly susceptible to infection (bacterial and fungal) after slime removal (Wedemeyer 1996, Madetoja *et al.* 2000). When Madetoja *et al.* (2000) challenged rainbow trout (*Oncorhynchus mykiss*) with intact mucal layers to immersion in baths containing known fish pathogens, no mortalities occurred. Fish in which the mucus had been removed resulted in mortalities of 27% of the sample, while fish with removed mucus and skin abrasions resulted in an average of 95% mortalities.

The antimicrobial activity of mucus differs among fish species as do the cells producing the mucus, which could lead to differences in mucus composition and thus variation in antimicrobial effectiveness (Shephard 1993, Subramanian *et al.* 2008). Mucus composition has also been shown to vary due to ecological and physiological conditions such as water quality and induced stress (Agarwal *et al.* 1979, Zuchelkowski *et al.* 1981, Blackstock and Pickering 1982, Pottinger *et al.* 1984, Lebedeva 1999). Isolation and differing selective pressures between these two strains of brook trout could have provided different trajectories of innate immune responses.

Though some antimicrobial agents have been identified and described from fish slime, much is still unknown (Subramanian *et al.* 2008). One fairly unexplored possibility is that of associative microbes living within the slime (Subramanian *et al.* 2008). Microbial species that have been identified in the mucus of fish have been shown to exhibit their own antimicrobial components (Ebran *et al.* 1999, Parret *et al.* 2005). Microorganisms associated with the exterior of host organisms can be beneficial in protecting the host from deleterious pathogens, forming a mutualistic relationship (Wingender *et al.* 1999). Studies of the external microbial assemblages of amphibians have shown that different species held different assemblages of microorganisms on their skin (Culp *et al.* 2007). Many of these microorganisms have been found to be unique to the host and were not constituents of the aquatic environment, suggesting a symbiotic relationship (Gilbert 1944, Culp *et al.* 2007). Specific analyses have shown that mutualistic relationships do exist between some salamanders and their skin flora (Lauer *et al.* 2007, Lauer *et al.* 2008). Thus the possibility of beneficial skin or mucus flora on brook trout acting as an antimicrobial agent should not be discounted.

Since much of the antimicrobial action of fish slime is believed to be broad-spectrum, reduced overall growth and especially that of environmental microorganisms within the slime could indicate a greater antimicrobial action and thus a greater ability to fight off potential pathogens (Ellis 2001, Hellio *et al.* 2002, Magnadóttir 2004, Balasubramanian *et al.* 2011). Results of differing antimicrobial activity would then potentially define selective differences of innate immunity in hatchery reared fish. Being that these differences in innate immune responses are genetically inherited (Secombes and Olivier 1997), the changes endured prior to the hatchery or selected for by hatchery pressures could persist through generations of stocked fish, raising concerns that potential hybridization between native and nonnative strains which may result in a loss of fitness by contaminating the genetic makeup of native fish (i.e. replacing their naturally selected immune responses for a maladaptive artificially selected response) (Allendorf and Phelps 1980, Currens *et al.* 1997, Lynch and O'Hely 2001, Davis 2006).

I extracted slime from NBKT and SBKT and cultured it in the lab to assess quantity and composition of the external microbial assemblages of these. There were three possible outcomes. The first was more microbial growth in the slime and/or an increased presence of environmental microbes in the microbial slime assemblage of NBKT. This could be indicative of either a lack of adaptation to the streams they have colonized. An alternative explanation is reduced mucal activity due to selection or lack thereof in the hatchery, perhaps because of the use of antibiotics and antimicrobials to control disease (Kirkan *et al.* 2003). The second possibility was that SBKT would exhibit a higher microbial count and a less endemic assemblage. This would result if either NBKT's historical ancestry in northern environments selected for fish with more

advanced immune systems based on environmental factors or if harsh conditions common to hatchery rearing (Tomasso *et al.* 1981, Piper *et al.* 1989, Winfree *et al.* 1998, Ellis *et al.* 2002) selected for more effective innate immunity. The final possibility was that negligible variation in mucosal immune responses exists in these sub-populations, suggesting that isolation (either hatchery or historic geographic) has not resulted in selection of differences in mucal activity. Based on the endemicity of the native strain and potentially mal-selective pressures of fish hatcheries, I hypothesized that SBKT would exhibit reduced density and diversity of colony forming units.

## METHODS

The study sites were two tributaries of each of three separate major rivers in western North Carolina (French Broad, Pigeon, and Tuckasegee). Study sites were chosen on the basis of being distinct, isolated watersheds so as to control for any effect of the water itself, and to look at the microbial diversity of isolated populations. Each pair of streams consisted of one stream previously identified by the NC Wildlife Resource Commission to be populated with SBKT, and one occupied by NBKT (Table 1). The streams chosen for the French Broad were Sawmill Creek and Shoal Creek, containing SBKT and NBKT respectively. From the Pigeon I sampled Scapecat Creek – hybridized population originally believed to be SBKT - and Flat Laurel Creek -NBKT. From the Tuckasegee I sampled Mull Creek –SBKT - and Beechflat Creek - NBKT. The samples from Scapecat Creek were later removed from the SBKT category when I learned that fish of mixed native and hatchery ancestry (hybrid brook trout - HBKT) had been identified from this stream (Galbreath *et al.* 2001). Both the mucus and water samples were used in the statistical analyses; however, they were placed into their own category of HBKT and HBKT stream.

Two rounds of sampling were undertaken. The first sampling was conducted in 2011 between October 8<sup>th</sup> and November 19<sup>th</sup> and the second during July 2012 (17<sup>th</sup>-19<sup>th</sup>). Shoal Creek was not sampled during 2012 due to the difficulty of sampling and low capture rate, and thus its paired stream Sawmill Creek was also omitted that year.

Upon arrival at a sample site, a grab-sample of stream water for microbial analysis was taken by immersing a sterilized 50 ml conical tube in non-turbulent but swift flowing water. Temperature, pH, % DO, and conductivity of the stream were then

measured using a dissolved oxygen meter (model YSI 650, YSI Inc., Yellow Springs, OH, USA). Before fish collection all submersible capture gear was sterilized in 15% house-hold bleach. Resealable polyethylene bags were sterilized overnight using 70% isopropyl alcohol then rinsed and filled with 500 ml of 0.85% saline solution and placed on ice.

I collected brook trout were via electro-fishing or hook and line from each stream. Hook and line was used when shocking was not effective either due to extremely low conductivity or unmanageable terrain making capture after shocking difficult or dangerous). Because the amount of slime on the fish is relative to the surface area (size) of the fish, the collection was not based on numbers of fish but by a cumulative total fish length of 50-100 cm per sample site. Collected fish were placed in a sterilized resealable plastic bag containing 500 ml of saline solution and lightly shaken for 15 seconds for slime extraction. Fish were removed from the bag by hand using a fresh nitrile glove after each catch. The slime was pooled for each stream reach, reusing the same bag of saline solution for each new capture. After slime extraction, the bag was placed on ice. The total length of the fish was measured and then they were immediately released.

Negative controls were used to test for the occurrence of outside contamination. Using the same sterile saline solution and plastic-bag-setup procedure (but without fish), the process of the slime extraction was mimicked. All samples of water, extracted slime, and negative controls were stored on ice while transported back to Western Carolina University and refrigerated at 4°C to minimize growth and preserve spec imines until culture.

The microbiota assemblage of the slime, water, and controls were examined by plating samples (either the slime from the saline solution or the water) on an R2A nutrient agar medium. A dilution series using a 0.85% saline solution was conducted using the undiluted sample, a 1:10 dilution, and a 1:100 dilution. 100  $\mu$ l of each concentration from each sample were plated on three different T100x15 mm Petri dishes. Dishes were plated and evenly spread under a sterile hood using a blower with laminar flow. After plating, the dishes were stored upside down in the dark at ambient temperature (approximately 20°C), undisturbed for one week. After one week the dishes were observed and plates with few to no colonies were marked and were then placed in refrigeration at 4°C to minimize further colony growth. This would indicate if further growth was occurring after refrigeration. Colony forming units (CFUs) were counted on each plate. Individual CFUs were observed and described using a six-characteristic microbe check list similar to the protocol described by Breakwell *et al.* (2007). While CFUs were counted from samples taken during both sample periods, only samples from the first sample season were used for the colony morphology descriptions. Thus analyses that used the morphology data only reflect a total of three samples from each grouped origin.

The plates used to determine the counts were the three plates from the dilution series for each sample that fell within the 20-200 CFU range. If no plate's count for a sample fell within the range, the nearest appropriate plates were used. If the CFU count for all plates was less than 20, then the plates with the highest values were used, and if all CFU counts were greater than 200, then the plates with the lowest values were used. If there were more than three plates within the optimal range the three closest to the middle

of the 20-200 range (i.e. nearest 110) were used. The median CFU density (number per mL of sample) from the three subsamples was used as the CFU density for the sample.

Each sample was analyzed as independent even though some samples were from the same locations but at different times. Independence was assumed based on the belief that the microbial assemblages would represent new populations due to the continuous renewal of the mucal layer and the rapid frequency in which microbes reproduce. The data were transformed ( $\log(x)$ ) to meet the assumptions of normality and homoscedasticity. To measure differences in the microbial abundance among the samples data were analyzed by ANOVA. Analysis of variance was followed by pairwise comparisons of means using Tukey's correction to maintain an experiment-wise error rate of 0.05. For abundance analysis water and mucus samples were analyzed separately due to the differences in the initial dilution of samples.

To measure the uniqueness of CFU composition, the abundance of morphological colony types uniquely represented in a particular sample and shared by no other samples (referred to in this paper as "private CFUs", based on my terminology and not related to other scientific literature) was totaled for each sample and a proportion was calculated using the number of private colonies divided by the total number of colonies for the sample "percent private CFU abundance" (Appendix Tables 1 and 2). This is interpreted as a test for potential endemicity of microbes in different populations and as a screen for antimicrobial activity, based on the premise that absence of environmental microbes suggests inhibition. An ANOVA followed by Tukey's pairwise comparisons was performed using arcsine transformed data. The raw count/classification data (Appendix Table 1) was summarized into a table of occurrence, based on the presence or absence of



specific CFU types within the different sample locations (Appendix Table 3). A similar private CFU analysis based on the richness of private CFU types was performed “percent private CFU richness”. Richness in this context refers to the number of distinct CFU types for each sample. The ratios of private to total CFU types were calculated and analyzed in the same manner as the previous private CFU analysis. This test of private CFUs was conducted to remove the bias from abundant CFU types. For example, a sample that exhibits high abundance of a particular private CFU type and low private CFU richness would result in the previous analysis “percent private CFU abundance” showing a high percent of private CFUs, whereas the “percent private CFU richness” analysis would show a low percent of private CFUs.

Table 1. Field sites showing locations and characteristics of streams containing northern strain brook trout derived from hatchery ancestry (N), native, southern strain brook trout (S), and fish of mixed genetic origin (H).

Stream	Drainage	Strain	Latitude	Longitude	Elevation (m)
Sawmill Creek - above falls	French Broad	S	35.191101	-82.82089	824
Shoal Creek	French Broad	N	35.260136	-82.849281	925
Scapecat Creek	Pigeon	H	35.380413	-82.892151	1065
Flat Laurel Creek	Pigeon	N	35.327363	-82.901912	1521
Mull Creek	Tuckasegee	S	35.365794	-83.020409	1055
Beechflat Creek - above falls	Tuckasegee	N	35.352388	-83.015501	1082

## RESULTS

Water conditions were similar among the streams (Table 2). Temperature differed between the two seasons with the July 2012 sampling season running warmer (15.0-16.9°C) than the October through November 2011 season (7.9-12.0°C). Conductivities were similar and typical of high elevation mountain streams (5-18  $\mu$ S). Dissolved oxygen had little variation (84.3-91.4 sat%) as did pH (typical range 6.14-7.30 pH), except for a single outlying data point, the second sampling of Flat Laurel Creek exhibited a much higher level of acidity than the other streams (4.98 pH) and also than itself the previous season (7.30 pH).

All fish capture totals (Table 3) were within the desired range of 50-100 cm except for Shoal Creek from which I was only able to sample two fish (summed length of 42 cm). Fish capture was markedly easier and quicker in the streams inhabited by SBKT, and in general it took much longer to acquire fish from northern strain streams. Though the total length of trout collected differed among streams, I kept the paired streams (NBKT and SBKT of the same river system) within similar total fish length ranges.

The control plates yielded no CFUs, with the exception of one replicate. A small growth was seen in along the edge in a single 1:100 dilution. The frequency of microbes per plate was proportionate to the dilution except for one Mull Creek fish sample. One plate from the 1:100 dilution (2 CFUs) yielded more CFUs than the 1:10 dilutions (0 CFUs); however, in this case the CFU count was extremely low throughout all the plates and during later scrutiny it was observed that there were four 1:100 plates labeled and only two 1:10 plates labeled. This error went unnoticed because most of the plates from both dilutions yielded no CFUs. In addition, the total occurrence of CFUs

from Mull Creek fish was so low that it could be realistically considered as no growth. However, I took a broad approach and the plate was evaluated despite having fewer than 20 CFUs. Most samples' dilution series produced at least three plates in the 20-200 CFU range. Most of the locations that yielded no plates reaching the optimal range belonged to SBKT samples as a result of their low yields. One stream (Shoal Creek) produced very high CFU counts (Table 4, Appendix Table 2), but samples appeared uncontaminated due to the fact that the dilutions still produced the correct proportions and microbes described occurred in most of the plates from that stream and even appeared in plates from other sample locations.

The undiluted HBKT samples from Scapecat Creek were different from the SBKT samples in appearance, due to the presence of spreading CFUs (irregular colonies that had no uniformity and established themselves over a large area of the plate). Though "spreaders" were not uncommon within the water and NBKT samples they were relatively small or absent from the SBKT samples. All "spreaders" were counted as a single colony, unless completely divided by edged space. The filmy, layered appearance within plates with heavy "spreaders" made it difficult to characterize some CFU forms and discern whether they were individual colonies or of the same colony. CFUs that were formed within "spreaders" were counted for abundance data but were not characterized to avoid possible misidentification. Selected pictures (replicate "A" from each dilution of each sample) are provided in the Appendix (Figure 1).

SBKT had significantly lower median CFU density than NBKT ( $p = 0.016$ ) (Table 5, Figure 1). The water samples exhibited similar abundance values with no significant differences (Table 6, Figure 1). HBKT exhibited CFU counts between SBKT

and NBKT and thus was not significantly different from either of the two strains. The water samples, regardless of occupying strain, and NBKT samples all had very similar CFU densities.

The presence of potentially fungal-like colonies was also noted by features of dullness, opaqueness, and fibrous or rhizoid projections. These fungal-like colonies were not infrequent within the water, NBKT, and HBKT samples, but were virtually absent from the SBKT samples (Appendix table 1).

Based on abundance, SBKT possessed a significantly higher percent of total private CFUs in comparison to NBKT ( $p = 0.023$ ), SBKT streams ( $p = 0.030$ ), and NBKT streams ( $p = 0.015$ ; Tables 7 and 8, Figure 2). The majority of the microbial composition of the native trout was CFUs that were unique to each sample of SBKT (Appendix table 3). The other samples exhibited much higher abundances of shared colonies. Based on the richness analysis, when compared to NBKT and all of the stream samples, SBKT exhibited a significantly higher percent of private CFU types than NBKT ( $p = <0.001$ ) and the water samples (SBKT.W  $p = 0.002$ , NBKT.W  $p = <0.001$ , HBKT.W  $p = 0.006$ ), which exhibited similarly insignificant differences among each other (Tables 9 and 10, Figure 3). However, HBKT exhibited a significantly greater percent of private CFU forms in comparison to NBKT streams ( $p = 0.046$ ) and a higher mean percent than NBKT ( $p = 0.057$ ) but not significant at the 95% confidence level. Only a single CUF type cultured from samples of SBKT was shared by one other sample (Table 11) But CFUs cultured from the water in which the brook trout were living often shared many types with other stream samples and with NBKT samples. HBKT exhibited shared CFUs with the environment and with NBKT but do a lesser degree than NBKT.

Table 2. Water parameters taken at each stream reach prior to sampling.

Abbreviations: temperature (Temp), specific conductivity (SP Cond), dissolved oxygen saturation (DO sat%).

Location	Strain	Date	Time	Temp (°C)	SP Cond ( $\mu$ S)	DO (sat%)	pH
Beechflat Creek	N	10/8/2011	12:37	12.0	18	86.7	6.14
Mull Creek	S	10/8/2011	16:06	12.0	15	87.2	6.81
Scapecat Creek	H	10/23/2011	12:46	9.1	11	88.2	7.14
Flat Laurel Creek	N	10/25/2011	16:22	7.9	5	86.3	7.30
Sawmill Creek	S	11/3/2011	10:35	9.6	9	91.4	7.13
Shoal Creek	N	11/3/2011	12:45	9.1	11	85.3	6.92
Beechflat Creek	N	7/17/2012	10:00	15.0	10	89.6	6.39
Mull Creek	S	7/18/2012	8:41	15.2	15	89.9	6.97
Scapecat Creek	H	7/19/2012	8:34	16.7	14	87.5	7.07
Flat Laurel Creek	N	7/19/2012	15:10	16.9	6	84.3	4.98

Table 3. Individual and total capture lengths (in cm) of brook trout sampled at each field site. The number “2” refers to the sample taken during the second season.

SBKT			NBKT					HBKT	
Mull	Sawmill	Mull 2	Beechflat	Flat Laurel	Shoal	Beechflat 2	Flat Laurel 2	Scapecat	Scapecat 2
11	15	10	15	15	19	20	20	9	14
8	15	13	17	14	23	25	15	7	12
6	14	15	15	18		19	18	10	15
16	9	13	9	19			20	10	13
9	9	12		17				7	6
13								15	
								22	
63	62	63	56	83	42	64	73	80	60

Table 4. Median CFU abundance of fish and water samples at each field site. CFU totals are an average of the three plates closest to the 20-200 range.

Origin	Strain	Median abundance (CFUs per ml)
<b>Fish Samples</b>		
Mull	S	2.00E+01
Sawmill	S	1.10E+02
Mull 2	S	5.00E+01
Beechflat	N	8.40E+02
Flat Laurel	N	2.20E+02
Shoal	N	1.15E+05
Beechflat 2	N	5.90E+03
Flat Laurel 2	N	1.07E+04
Scapecat	H	1.60E+02
Scapecat 2	H	4.20E+02
<b>Water Samples</b>		
Mull	S	1.21E+03
Sawmill	S	1.16E+03
Mull 2	S	1.10E+04
Beechflat	N	6.00E+02
Flat Laurel	N	4.30E+02
Shoal	N	7.20E+03
Beechflat 2	N	6.20E+03
Flat Laurel 2	N	2.30E+03
Scapecat	H	8.40E+02
Scapecat 2	H	1.10E+03



Table 5. Statistical summaries of ANOVA and Tukey's pairwise comparisons among median estimates of CFU abundance (per ml) in fish samples. Abbreviations: degrees of freedom (df), sum of squares (SS), mean squared (MS), f value (F), p value (P), standard error (SE), t value (t), fish (F), water (W).

ANOVA					
Source	df	SS	MS	F	P
Sample Origin	2	39.58	19.792	5.508	0.0366
Error	7	25.15	3.594		
Tukey Pair-wise Comparisons:					
Comparison	Estimate	SE	t	P	
NBKT.F-HBKT.F=0	2.79	1.586	1.759	0.2488	
SBKT.F-HBKT.F=0	-1.688	1.73	-0.976	0.6119	
SBKT.F-NBKT.F=0	-4.479	1.384	-3.235	0.0334	

Table 6. Statistical summaries of ANOVA and Tukey's pairwise comparisons among median estimates of CFU abundance (per ml) in water samples. Abbreviations: degrees of freedom (df), sum of squares (SS), mean squared (MS), f value (F), p value (P), standard error (SE), t value (t), fish (F), water (W).

ANOVA					
Source	df	SS	MS	F	P
Sample Origin	2	1.125	0.5627	0.39	0.691
Error	7	10.093	1.4419		
Tukey Pair-wise Comparisons:					
Comparison	Estimate	SE	t	P	
NBKT.W-HBKT.W=0	0.6949	1.0047	0.692	0.774	
SBKT.W-HBKT.W=0	0.9518	1.0962	0.868	0.674	
SBKT.W-NBKT.W=0	0.257	0.8769	0.293	0.954	

Table 7. Percent of private CFUs per sample based on total abundance. From October/November samples only.

Sample Location	Strain	Total CFUs	Total Private CFUs	%Private CFUs
<b>Fish Samples</b>				
Mull	S	4.00E+00	4.00E+00	100.00
Sawmill	S	3.90E+01	3.10E+01	79.48
Beechflat	N	1.87E+02	3.70E+01	19.78
Flat Laurel	N	6.70E+01	2.10E+01	31.34
Shoal	N	3.33E+04	2.00E+00	0.0059
Scapecat	H	6.00E+01	4.30E+01	71.66
<b>Water Samples</b>				
Mull	S	4.44E+02	3.40E+01	7.65
Sawmill	S	3.29E+02	4.20E+01	12.76
Beechflat	N	3.45E+02	2.60E+01	7.53
Flat Laurel	N	4.23E+02	1.60E+01	3.78
Shoal	N	6.62E+02	7.00E+01	10.57
Scapecat	H	1.82E+02	4.60E+01	25.27

Table 8. Statistical summaries of ANOVA and Tukey's pairwise comparisons for percent private CFUs based on total abundance. Abbreviations: degrees-of-freedom (df), sum-of-squares (SS), mean-squared (MS), F-value (F), p-value (P), standard error (SE), t-value (t).

ANOVA					
Source	df	SS	MS	F	P
Sample Origin	5	1.9014	0.3803	7.303	0.0156
Error	6	0.3124	0.0521		
Tukey Pair-wise Comparisons:					
Comparison	Estimate	SE	t	P	
HBKT.W-HBKT.F=0	-0.48273	0.32271	-1.496	0.6734	
NBKT.F-HBKT.F=0	-0.65519	0.26349	-2.487	0.2552	
NBKT.W-HBKT.F=0	-0.74115	0.26349	-2.813	0.1774	
SBKT.F-HBKT.F=0	0.32629	0.27948	1.168	0.8336	
SBKT.W-HBKT.F=0	-0.68662	0.27948	-2.457	0.2636	
NBKT.F-HBKT.W=0	-0.17246	0.26349	-0.655	0.9805	
NBKT.W-HBKT.W=0	-0.25842	0.26349	-0.981	0.9061	
SBKT.F-HBKT.W=0	0.80902	0.27948	2.895	0.1617	
SBKT.W-HBKT.W=0	-0.20389	0.27948	-0.73	0.9694	
NBKT.W-NBKT.F=0	-0.08596	0.18632	-0.461	0.9958	
SBKT.F-NBKT.F=0	0.98148	0.20831	4.712	0.0232	
SBKT.W-NBKT.F=0	-0.03143	0.20831	-0.151	1.0000	
SBKT.F-NBKT.W=0	1.06744	0.20831	5.124	0.0155	
SBKT.W-NBKT.W=0	0.05454	0.20831	0.262	0.9997	
SBKT.W-SBKT.F=0	-1.01291	0.22819	-4.439	0.0303	

Table 9. Percent of private CFU richness per sample based on occurrence of unique CFU types. From October/November samples only.

Sample Location	Strain	Total CFUs	Total Private CFUs	% Private CFUs
<b>Fish Samples</b>				
Mull	S	4.00E+00	4.00E+00	100.00
Sawmill	S	1.40E+01	1.30E+01	92.85
Beechflat	N	8.50E+01	3.30E+01	38.82
Flat Laurel	N	5.00E+01	1.30E+01	26.00
Shoal	N	1.50E+01	3.00E+00	20.00
Scapecat	H	3.00E+01	2.20E+01	73.33
<b>Water Samples</b>				
Mull	S	8.80E+01	3.00E+01	34.09
Sawmill	S	7.00E+01	2.30E+01	32.85
Beechflat	N	8.10E+01	1.90E+01	23.45
Flat Laurel	N	6.40E+01	1.40E+01	21.87
Shoal	N	1.13E+02	3.70E+01	32.74
Scapecat	H	5.80E+01	2.00E+01	34.48

Table 10. Statistical summaries of ANOVA and Tukey's pairwise comparisons for percent private CFU richness based on occurrence. Abbreviations: degrees of freedom (df), sum of squares (SS), mean squared (MS), f value (F), p value (P), standard error (SE), t value (t).

ANOVA					
Source	df	SS	MS	F	P
Sample Origin	5	1.319	0.26386	23.3	0.000729
Error	6	0.068	0.01133		
Tukey Pair-wise Comparisons:					
Comparison	Estimate	SE	t	P	
HBKT.W-HBKT.F=0	-0.40054	0.15051	-2.661	0.21019	
NBKT.F-HBKT.F=0	-0.47102	0.12289	-3.833	0.05719	
NBKT.W-HBKT.F=0	-0.49433	0.12289	-4.022	0.04682	
SBKT.F-HBKT.F=0	0.40736	0.13035	3.125	0.12444	
SBKT.W-HBKT.F=0	-0.4112	0.13035	-3.155	0.12049	
NBKT.F-HBKT.W=0	-0.07049	0.12289	-0.574	0.98895	
NBKT.W-HBKT.W=0	-0.09379	0.12289	-0.763	0.96336	
SBKT.F-HBKT.W=0	0.8079	0.13035	6.198	0.00610	
SBKT.W-HBKT.W=0	-0.01066	0.13035	-0.082	1.00000	
NBKT.W-NBKT.F=0	-0.0233	0.0869	-0.268	0.99968	
SBKT.F-NBKT.F=0	0.87839	0.09716	9.041	<0.001	
SBKT.W-NBKT.F=0	0.05982	0.09716	0.616	0.98497	
SBKT.F-NBKT.W=0	0.90169	0.09716	9.281	<0.001	
SBKT.W-NBKT.W=0	0.08313	0.09716	0.856	0.94285	
SBKT.W-SBKT.F=0	-0.81857	0.10643	-7.691	0.00197	

Table 11. Frequencies of shared CFU types among the different samples at each field site. The diagonal indicates the total discrete CFU type occurring in a sample and the off-diagonal contains the number of shared CFU type between the two samples indicated by column and row. Abbreviations: Mull Creek (ML), Beechflat Creek (BF), Scapecat Creek (SC), Flat Laurel Creek (FL), Sawmill Creek (SM), Shoal Creek (SH), suffix designation for fish sample (F), water sample (W).

Origin	Strain	ML.F	ML.W	BF.F	BF.W	SC.F	SC.W	FL.F	FL.W	SM.F	SM.W	SH.F	SH.W
ML.F	S	4											
ML.W	S	0	88										
BF.F	N	0	31	85									
BF.W	N	0	38	32	81								
SC.F	H	0	2	2	2	30							
SC.W	H	0	14	14	14	7	58						
FL.F	N	0	18	13	14	3	15	50					
FL.W	N	0	19	15	18	3	13	22	64				
SM.F	S	0	0	0	0	0	0	0	1	14			
SM.W	S	0	17	15	17	3	11	9	19	0	70		
SH.F	N	0	3	3	3	0	1	1	2	0	6	15	
SH.W	N	0	29	27	36	3	15	16	24	0	32	10	113

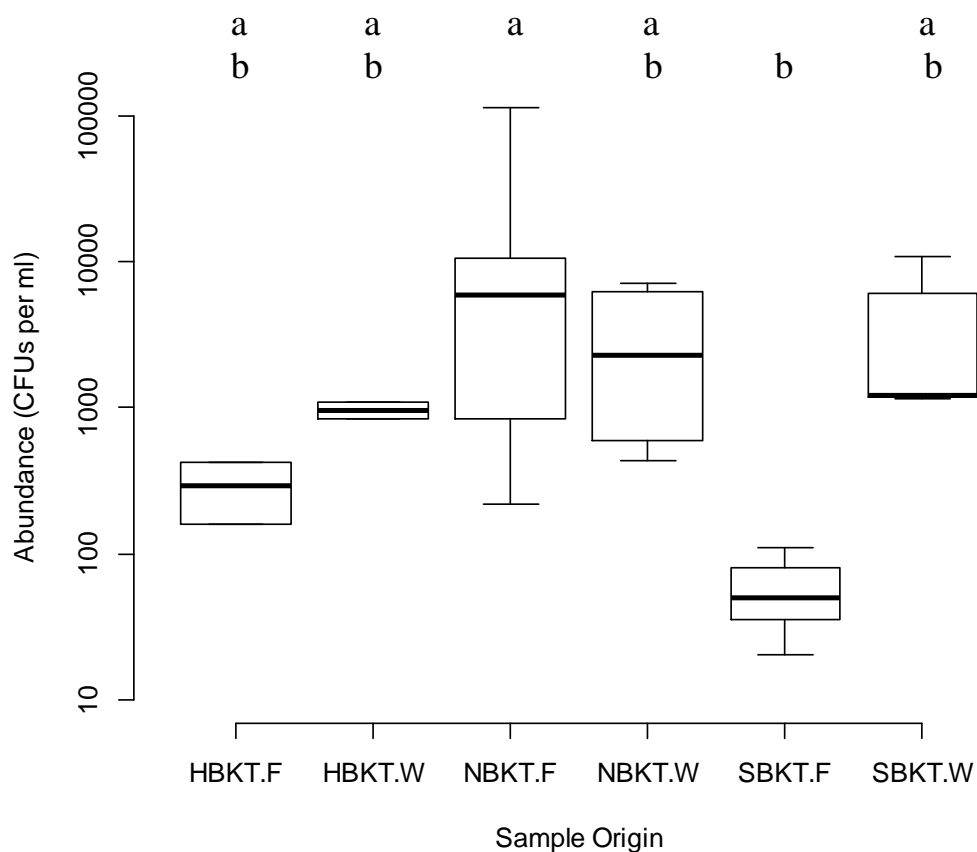


Figure 1: Box plot showing ranges of median CFU abundance for each grouped sample. Letters above plots represent the results of pair-wise comparisons. Origins with the same letter were not found to be significantly different. “F” refers to fish mucus samples while “W” refers to water samples. Water and fish samples were collected in different dilutions and should not be compared for relatedness or differences in abundance.



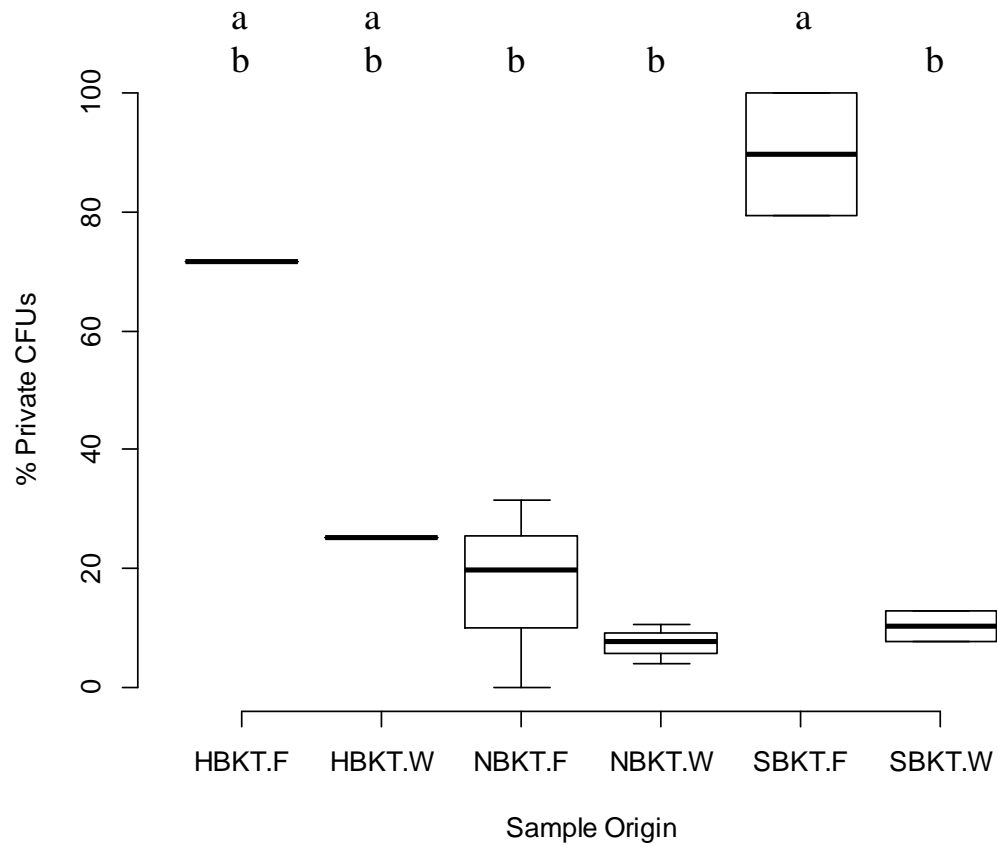


Figure 2. Box plot showing ranges of percent private CFUs based on total abundance for each grouped sample. Letters above plots represent the results of pair-wise comparisons. Origins with the same letter were not found to be significantly different. SBKT show significantly higher percentage of private colonies; however, this describes each individual sample and not the grouped location.

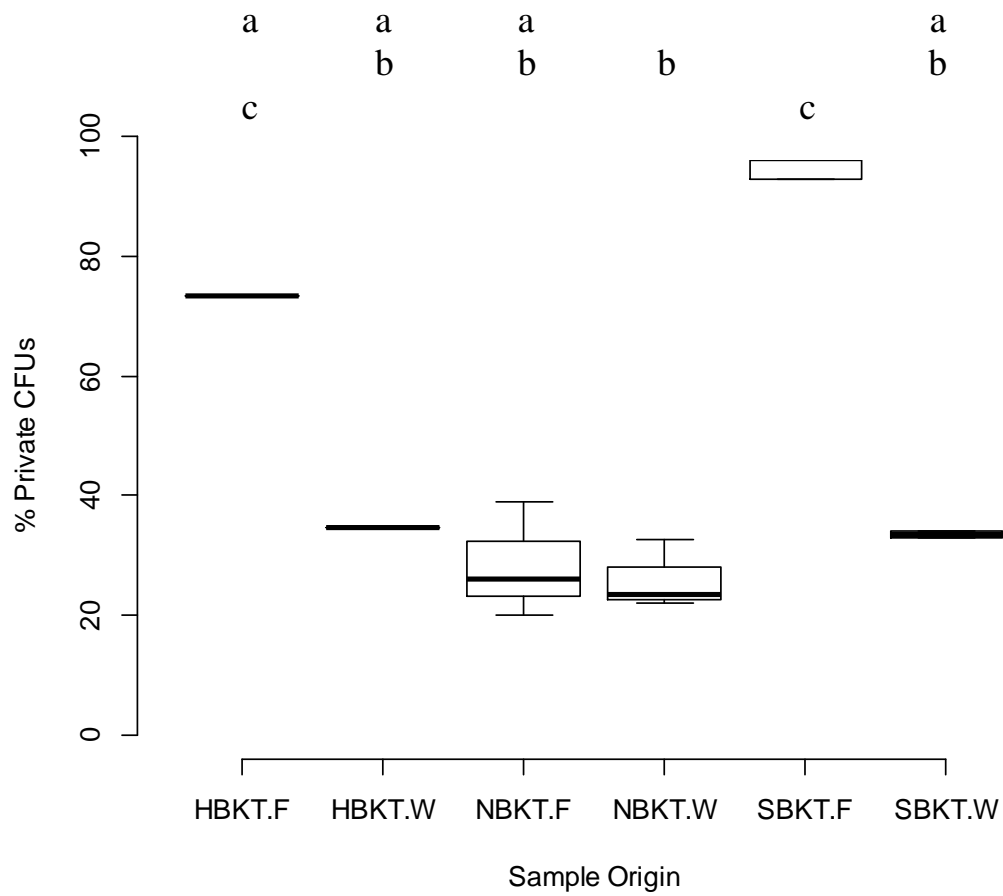


Figure 3. Box plot showing ranges of percent private CFUs based on richness of individual CFU types for each grouped sample. Origins with the same letter were not found to be significantly different.

## DISCUSSION

Differences in microbial growth (both in quantity and composition) were observed between native southern and hatchery derived northern strain brook trout (Tables 5, 8, and 10; Figures 1, 2, and 3). The differences seen between the two strains were most likely due to their physiological differences rather than due to differences in their environments. Environmental parameters were mostly consistent among streams (Table 2). The high acidity of the second sample of Flat Laurel Creek may have been due to a rain event the night before in addition to the frequent rain experienced that week. No significant differences were observed in the CFU quantity or composition of water samples from the streams based on the strain of the resident brook trout (Tables 6, 8, and 10; Figures 1, 2, and 3).

SBKT had significantly lower CFU density than did NBKT (Table 5, Figure 1). The native southern brook trout could either have increased broad-spectrum antimicrobial activity, in either intensity or effectiveness. An alternative hypothesis, not mutually exclusive from the previous, is that the innate antimicrobial action is specialized and more effective against the local microbial community (Ellis 1999, Magnadóttir 2004). Not only did the SBKT exhibit reduced growth in terms of reduced CFU density, but they also seemed to exclude the number of CFU types found in the water as evidenced by their high percentage of private CFUs (Tables 8 and 10, Figures 2 and 3). The extent of private CFUs within SBKT appears to extend to the population level, with samples showing uniqueness among the different sample sites. This could be an indication of endemic microbes specifically associated with the SBKT, but due to their differentiation among the populations and their low abundance transiently associated CFUs should not

be ruled out. NBKT showed a much lower percent of private CFUs based on both total abundance and richness and contained several repeated representatives from the water samples. This indicates that not only is there higher occurrence of environmental microbes within NBKT but that a broader spectrum of microbial species is uninhibited. Even though the test did not specifically screen for pathogens, non-pathogenic opportunistic microbes may pose a significant and more omnipresent threat to fishes' health (Ellis 2001, Magnadóttir 2004). Common water microbes such as *Saprolegnia* readily infect fish after injury, mucal removal, or other stressors (Pickering and Willoughby 1982).

Due to the inhibitive properties of fish slime, the majority of the cultured microbes may have only been transiently associated with the outermost layer and therefore not part of a stable population established within the mucosal layers (Cahill 1990, Ellis 2001). The data could be representative of a short window of what was present on the exterior of the fish at the moment of extraction (Cahill 1990). This effect of ephemeral microbial association may be responsible for the high percent of private CFUs observed in the SBKT samples (Tables 8 and 10, Figures 2 and 3). In essence, some of the observed growth may not be representative of a stable or established population of microbes that could grow in the mucus uninhibited. However, nonpathogenic microbes are more readily inhibited than pathogenic species which have developed strategies to allow for their penetration of the slime (Jung *et al.* 2000, Ellis 1999, 2001, Magnadóttir 2004). Thus, the CFUs cultured from slime samples represents a sample of the river water microbes the slime was exposed to and/or a sample of associated species of organisms that penetrated the mucus and are established or both.

Regardless of how the microbes came to be and whether they represent pathogenic microbes or not, a clear trend in microbial quantity and composition was observed within the samples. Considering the broad-spectrum activity of the slime (Hellio *et al.* 2002, Subramanian *et al.* 2008), the data represent a significant difference in the number of CFUs present in two strains of fish from the same species. The analysis of possible reasons and pressures attributing to the differences in presumed antimicrobial effectiveness and microbial composition could help us understand how hatchery environments and/or historic isolating factors can affect the reaction of fishes' mucosal-based innate immune systems to the microbial environment.

Overall, it appears that the SBKT in their natural habitat show far greater antimicrobial abilities than do the NBKT in nonnative waters. This is most likely due to either artificial selection from a hatchery environment or an artifact of historic isolation in their native habitat. The latter option would suggest a difference based on evolution to combat endemic microbes (Ellis 1999, Magnadóttir 2004) or different historic environmental factors altering the innate immune response (Blackstock and Pickering 1982, Lebedeva 1999), and thus an increased susceptibility to microbial establishment may be seen when they are subjected to alien microbes in new environments.

The SBKT have been genetically isolated, not only from NBKT, but from other populations of SBKT since the Pleistocene glaciations (Hayes *et al.* 1996). Their isolation and island population distribution could lead to selection for specialized immune responses limiting establishment and possible infection from local microbes (Agarwal *et al.* 1979, Zuchelkowski *et al.* 1981, Blackstock and Pickering 1982, Pottinger *et al.* 1984, Ellis 1999, Lebedeva 1999, Magnadóttir 2004). Thus the NBKT

may not be as well adapted to the habitats of southern Appalachia (Lennon 1967).

Though the brook trout strains being sampled historically evolved in allopatry (Hayes *et al.* 1996) the physiological requirements of brook trout are fairly similar between the strains. There are differences in their traditional environmental habitats, but it is likely that they would seek similar physiological optimums (e.g. temperature, pH, dissolved oxygen, etc.) regardless of geographic location (MacCrimmon and Campbell 1969, Rashleigh *et al.* 2005, Ficke *et al.* 2009). Thus similar environmental pressures, including microbial load, would be expected.

Due to the broad-spectrum antimicrobial action of the slime identified in brook trout (Subramanian *et al.* 2008) and the relative similarities between natural northern and southern strain environments (Rashleigh *et al.* 2005, Hudy and Thieling 2008), I argue that it is more likely that the majority of differences in antimicrobial activity found in this study arose due to hatchery rearing. A hatchery environment in which natural stream microbes have been removed may result in a loss of resistance in to common stream microorganisms over time (Davis 2006). Hatchery origin rainbow trout, historically unexposed to the myxosporean parasite *Ceratomyxa shasta*, exhibited significantly higher mortality rates after exposure than native rainbow trout from streams in which the parasite resides (Currens *et al.* 1997). Shrimpton *et al.* (1994) found that coho salmon (*Oncorhynchus kisutch*) raised in hatcheries for the first part of their lives showed increased susceptibility to pathogens when placed in a natural setting in comparison to wild fish. This may be a result of anthropogenic conditioning occurring in hatcheries (Maynard *et al.* 1994, Reisenbichler and Rubin 1999, Hansen 2002, Álvarez and Nicieza 2003, Davis 2006). Genetic, behavioral, and physiological differences selected by

domestication in hatcheries are well documented (Hindar *et al.* 1991, Maynard *et al.* 1994, Reisenbichler and Rubin 1999, Ellis *et al.* 2002, Hansen 2002, Álvarez and Nicieza 2003) and aspects of domestication have been shown to reduce survival of fish when stocked in natural systems (Shrimpton *et al.* 1994, Currens *et al.* 1997, Reisenbichler and Rubin 1999, Hansen 2002, Davis 2006). A degree of the reduced survival may be attributed to the low genetic variation commonly seen in hatchery fish (Hindar *et al.* 1991, Kreigler *et al.* 1995, Hayes *et al.* 1996, Hansen *et al.* 2001) which has been shown to have a negative effect on the fish's immune system (Allendorf and Phelps 1980).

The hatcheries could be providing an environment that allows for reduced antimicrobial activity. However, some researchers have concluded that hatchery rearing may produce fish that are more resistant to pathogens because of the intense selection due to generally poorer environmental conditions (Ruzzante 1994, Davis 2006). Water in hatcheries is generally of poorer quality than would be found in a natural environment (Tomasso *et al.* 1981) due to waste accumulation by fish in unnaturally high densities (Piper *et al.* 1989) as well as decomposing material from uneaten food (Cho *et al.* 1994, Conte 2004). High densities can also lead to greater rates of infection due to unnaturally close proximity and frequently compromised mucosal layer due to physical abrasion from aggressive fish interactions (Pickering 1989, Winfree *et al.* 1998, Ellis *et al.* 2002). In addition to the direct health impacts, chronic stress, which can result from the previous conditions (reviewed in: Conte 2004, Davis 2006), can have a negative impact on general health as well (Pickering and Duston 1983, Dhabhar and McEwen 1996, 1997, Barton 2002, Ellis *et al.* 2002, Magnadóttir 2004, Davis 2006). These conditions would lead one

to suspect a selective pressure for a more responsive and stronger immune system in hatchery reared fish. However, other aspects of hatchery rearing could be reducing selection for broad-spectrum innate resistance.

Though no longer an accepted practice in modern aquaculture, the heavy and unregulated use of antibiotics was once common (Watts *et al.* 2001, Benbrook 2002, Thurman *et al.* 2002, Anand *et al.* 2011), due to the increased potential for fish infection from the typically poorer water conditions and the overcrowding of fish (Klinger and Floyd 1998, Ellis *et al.* 2002). Broad-spectrum antibiotics are known to compromise innate mucosal defense in humans (Brandl *et al.* 2008). The mucosal innate immune system is fairly analogous among vertebrates, but out of necessity fish have higher concentrations of mucus forming cells and a larger arsenal of defensive agents (Alexander and Ingram 1992, Ellis 2001). This is because fish have a more primitive acquired immune system and are intimately in touch with the microbial environment, (Ellis 2001, Magnadóttir 2004). A history of persistent antibiotic use could lead to immunosuppression (Anand *et al.* 2011), as has been shown to occur in common carp (*Cyprinus carpio* L.; Rijkers *et al.* 1980). Heavy antibiotic use leading to immunosuppression may remove the pressure for individuals to have adequate immune responses. However, there is also the potential that antibiotics may select for immunosuppressed individuals. Because sperm cells are non-self, inflammation and infection can cause them to be targeted by defensive agents. Immunosuppressed fish therefore have more viable sperm which would suggest greater reproductive success (Måsvær *et al.* 2004). In a natural environment this is a positive selective pressure. Fish that are resistant to foreign-invaders which could cause inflammation would experience



greater reproductive success, while those that were immunosuppressed but susceptible would suffer reduced fitness from infection (Måsvær *et al.* 2004). However, in a hatchery with antibiotics causing the immunosuppressive tendencies, the selection towards the more immunocompromised individual would not be based on increased pathogen resistance, which could lead to the selection of fish with the greatest immunosuppression, without the benefit of resistance.

In conjunction with the use of antibiotics, other factors of hatchery life could lead to a reduction of inherited immune responses (Bosakowski and Wagner 1994, Carballo *et al.* 1995). The application of antibiotics in the presence of continuously stressful environments may lead to a selective pressure against natural stress responses for indication of infection. In a hatchery setting where stress is omnipresent, chronic stress responses tend to be detrimental to fish health (Dhabhar and McEwen 1996, 1997, Barton 2002, Conte 2004, Davis 2006). Chronic stressors may result in energetically taxing responses (Davis *et al.* 1985, Pickering 1990). Prolonged stress is generally seen to compromise the immune system (Maule *et al.* 1989, Pickering 1989, Dhabhar and McEwen 1996, 1997, Ortuño *et al.* 2001, Davis 2006), including reduced levels of mucal secretion (Barton 1987) and inhibition of defensive agents within the mucus (Hjelmeland *et al.* 1983). However, stress is not entirely detrimental (Barton 2002). Stress responses are evolutionary adaptations to signal potential threats and to initiate the appropriate reaction (Barton 2002, Volpato *et al.* 2007). Responding to stress in the appropriate way is beneficial and exposure to acute stress enhances the immune system (Pickering and Pottinger 1989, Dhabhar and McEwen 1996, 1997, Chrousos 1998, Davis 2006) and initially leads to an increase in mucal production (Barton 1987).

Due to these persistent stressors, hatchery fish might benefit from a reduced stress response and higher tolerances, and thus the selection of fish with higher stress tolerances has likely occurred in hatcheries (Woodward and Strange 1987, Davis 2006). This could promote higher survival and success in hatchery systems due to the removal of the deleterious effects of chronic stressors. Under hatchery conditions negative reactions to chronic stress may supersede the benefits of responding to acute stressors, thus the beneficial adaptation for hatchery conditions may result in the general suppression of stress and loss of an adaptation for natural habitats (Davis 2006). This may result in hatchery fish responding inappropriately to potential stressors in natural settings (Reisenbichler and McIntyre 1977, Chilcote *et al.* 1986, Pickering and Pottinger 1989, Hindar *et al.* 1991, Barton 2002, Davis 2006,). Hatchery derived traits have been shown to be maladaptive and to reduce the success of fish in the wild, in addition these traits may share genetic inheritance and lead to generations of poorly adaptive fish in natural environments (Reisenbichler and Rubin 1999, Hansen 2002).

The water quality of hatcheries can impact mucosal effectiveness (Lang *et al.* 1987, Grinde 1989, Mock and Peters 1990), due to waste accumulation from high fish densities (Piper *et al.* 1989). Hatcheries have higher ammonia levels than would occur in natural environments (Piper *et al.* 1989) and ammonia is known to interfere with mucus renewal (Lang *et al.* 1987) and reduce the presence of defensive agents in the slime of rainbow trout (Grinde 1989, Mock and Peters 1990). Other toxicants also interfere with mucal production and suppress the innate immune system (reviewed in: Carballo *et al.* 1995, Bols *et al.* 2001). Hatchery substrate has a significant effect on the disease susceptibility and welfare of trout (Bosakowski and Wagner 1994) and other fish based

on abrasiveness (Mahoney *et al.* 1973) (i.e. steel or concrete enclosures lead to greater instances of fin, scale, and mucus removal). Other common hatchery practices attributing to epidermal abrasions include handling, transport, and overcrowding (Abbott and Dill 1985). In conjunction with the treatment of antibiotics (Benbrook 2002, Thurman *et al.* 2002), the reduction of mucal effectiveness through abrasions and toxicants may result in the repeated replacement of slime as a pointless and expensive process. This could create a pressure within hatchery settings selecting for less slime production and less investment of defensive agents within the slime.

Since the environmental microbes were largely inhibited in SBKT, the possibility of endemic microbes specific to the fish may explain the presence of the private CFUs (Tables 8 and 10, Figures 2 and 3; Austin 1982, 1983). Several microbes have been identified within the mucus of fish that exhibit their own antimicrobial properties (Ebran *et al.* 1999, Parret *et al.* 2005). In addition, if uninhibited, the integument of fish could present itself as a beneficial environment for the microbe. Living in a microbially hostile environment would present little competition for the microbe and relatively homeostatic conditions as well as a stable carbon source (Bordas *et al.* 1996). Evidence of microbial mutualisms associated with the integument of red-backed salamanders (*Plethodon cinereus*) has been shown (Lauer *et al.* 2007) and similar relationships may exist within the mucus of fish (Subramanian *et al.* 2008).

Because the sampling of the hybridized stream Scapecat Creek was accidental, the sample of HBKT was from only a single stream. However, it is interesting to note that CFU abundance (Figure 1) and the percent of private CFUs were intermittent between the two pure strains (Figures 2 and 3). Aspects of innate immune response are known to be

heritable (Secombes and Olivier 1997); and though the NBKT studied in this experiment were of hatchery descent, these fish represent wild populations separated by their hatchery predecessors by many generations. If the selective pressures of ecological isolation or hatchery conditioning led to these differences in innate immune activity, then these characteristics have persisted through several generations and have strong basis for genetic inheritance. This creates a problematic situation in terms of introgression between the strains. It is well documented that in many occasions hatchery-reared brook trout and other salmonids experience lower survival than wild fish after being stocked in natural settings (Greene 1952, Miller 1952, Salo and Bayliff 1958, Reimers 1963, Reisenbichler and McIntyre 1977, Fraser 1981, Webster and Flick 1981,). Though there have been several identified causes for this trend, the possibility of a more effective innate immune system in native fish may be an additional factor attributing to the reduced survivability commonly seen in transplanted hatchery fish. A concern over the loss of genetic integrity in native fish through the introgression of introduced hatchery stock has been a well discussed topic of concern (Allendorf and Phelps 1980, Ferguson 1990, Krueger and May 1991). Galbreath *et al.* (2001) demonstrated that introgression has frequently occurred between SBKT and NBKT. Though the degree to which wild trout hybridize with cultivated fish is only partially known and understood (Galbreath *et al.* 2001, Hansen 2002) empirical evidence shows that at least in some situations it does occur in spite of selective forces acting against hatchery raised fish (reviewed in: Hansen 2002). Hansen (2002) showed that introgression between native and hatchery brown trout (*Salmo trutta*) does not occur as frequently as expected if both strains were equally contributing to new progenies of fish. It is hypothesized that the lack of introgression of

the hatchery stock into the gene pool of native trout may be due to the poor survival and maladaptedness of the introduced strain (Hansen 2002). Though maladaptive traits will be selected against in a natural environment, introgression still occurs and may have detrimental effects on native populations (Lynch and O'Hely 2001, Hansen 2002). Negative effects on fitness may also occur long after stocking has ceased (Lynch and O'Hely 2001). However, introgression may also explain some trends favoring heterosis in wild X hatchery hybrids. Webster and Flick (1981) showed reduced survival of hatchery ancestry brook trout reared in a wild setting when compared to wild fish but found the survival of hybrids to be as great as or greater than the wild fish. However, the presence of poor alleles is still potentially detrimental. Though a degree of initial heterosis may be exhibited in early generations, the continuous introgression of fish with impaired innate immune systems could swamp the adaptive genetics of native fish and lead to an overall population of fish with maladapted innate immune responses (Allendorf and Phelps 1980, Hindar *et al.* 1991, Hansen and Loeschcke 1994). Though not conclusive due to small sample size, it appears likely that the introgression of hatchery brook trout into native populations may reduce the activity of the innate immune system, potentially threatening the fitness of wild populations.

## CONCLUSIONS

The inherited immunities or microbes associated with an organism's ability to protect itself from potentially harmful invaders may be shaped by the historic environment of the organism. Propagation of many generations of fish in unnatural hatchery settings may also apply evolutionary pressures, making them less fit in natural settings. The fish in the streams sampled represent artifacts of historical hatcheries and stocking practices and do not necessarily reflect the artificial pressures of hatcheries today. Antibiotics, immunization, and chronic anthropogenic stress may play major roles in the suppression of the immune system in hatchery fish. These changes could become problematic if these fish hybridize with native fish. These results suggest further reasons for caution when stocking fertile fish in areas where they could interbreed with native fish species. While in North Carolina, hatchery fish are no longer stocked into streams with naturally reproducing populations, and in general, hatcheries are moving toward production of non-fertile fish for recreational stocking (Davis 2006, Wilson 2011), selective pressures of modern hatcheries may still have a negative effect on the innate immune response. Thus, hatcheries rearing fish intended for supplementation, should consider rearing fish in more natural settings with lower densities promoting better water quality, reducing the need of antimicrobial additives. Additionally hatchery fish may benefit from exposure to acute stressors while minimizing chronic stressors, promoting a more natural and robust stress response. The mimicking of natural settings and reduction of anthropogenic influences should more appropriately create natural conditions for hatchery fish intended for supplementation, and may reduce the potential for genetic or physiological changes due to hatchery selection.

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Table 1. Continued.

Sample	ID	Cnt Size in mm				Consistency					Colony Appearance					Edge					Elevation					Notes
		#	<1	1-2	2-7	OPQ	TRL	SHY	DUL	CRC	IRR	FIL	RHZ	ENT	UND	LOB	ERO	FIL	CRL	FLT	RAI	CVX	UMB			
M.W 1:X A	WWW'	2		+																						
M.W 1:X A	CCC'	1			+																					
M.W 1:X A	47'	1			+																					
M.W 1:X A	DDD'	1																								
M.W 1:X A	25'	1			+																					
M.W 1:X A	22B'	1			+																					
M.W 1:X A	88'	1			+																					
M.W 1:X A	40'	1			+																					
M.W 1:X A	34'	1			+																					
M.W 1:X A	7'	7																								
M.W 1:X A	DD'	6																								
M.W 1:X A	20'	9																								
M.W 1:X B	I'	1			+																					
M.W 1:X B	57'	1			+																					
M.W 1:X B	ZZ'	1																								
M.W 1:X B	O'	2			+																					
M.W 1:X B	35B'	2			+																					
M.W 1:X B	KK'	1			+																					
M.W 1:X B	40'	1			+																					
M.W 1:X B	FF <sup>80</sup> '	3			+																					
M.W 1:X B	1 <sup>80</sup> '	1			+																					
M.W 1:X B	X'	1			+																					
M.W 1:X B	87'	1			+																					
M.W 1:X B	HH'	1			+																					
M.W 1:X B	27'	1			+																					
M.W 1:X B	RR3'	1			+																					
M.W 1:X B	NNN'	1																								
M.W 1:X B	EEE'	1			+																					
M.W 1:X C	88'	1			+																					
M.W 1:X C	34'	1			+																					
M.W 1:X C	GGG'	1			+																					
M.W 1:X C	35B'	1			+																					
M.W 1:X C	25'	1			+																					
M.W 1:X C	22'	1			+																					
M.W 1:X C	62'	1			+																					
M.W 1:X C	RR3'	1			+																					
M.W 1:X C	YYY'	1			+																					
M.W 1:X C	13'	1			+																					
M.W 1:X C	10'	1			+																					
M.W 1:X C	CCC'	1			+																					
M.W 1:X C	UU'	1			+																					
BF.F 1:100A	E	1			+	Wht/Bg			+										+							
BF.F 1:100B	F	1			+	Yl Cn, Clear Eg			+	+									+					1 Overly defined pointed lobe		
BF.F 1:100C	L	1			+	Pl Bg, Li Eg			+										+							
BF.F 1:10A	M	1			+	Pl Yl/Og, Dk Eg			+	+									+			Li	Li	Scattered CVX/UMB areas within		
BF.F 1:10A	N	1			+	Li Yl/Gn, Li Eg			+	+														Within M		
BF.F 1:10A	O	1			+	Wht Cn, Cl Eg		Cn	Eg	+												+		Within M		
BF.F 1:10A	O'	2			+																					
BF.F 1:10A	P	1			+	Cl Yl, Dk at Eg		Blo	Mo	+									Li	+				Mostly Cl and FLT w/ Dk OPQ CVX YL Blotches of CVX areas		
BF.F 1:10A	Q	1			+	Pure Wht Cn, Li Eg		Cn	Eg	+													Hv			
BF.F 1:10A	Q'	1			+																					
BF.F 1:10A	K'	1			+	Yl Cn, Pl Og Eg		Cn	Eg	+													+	Sim to B but smaller and Dif		
BF.F 1:10B	G	1			+	Pl Yl/Bg Nearly Cl			+															Slight Cn Depression, mild Tex		
BF.F 1:10B	H	1			+	Yl/Bg			+																	







Table 1. Continued.

Sample	ID	Cnt Size in mm		Color	Consistency	Colony Appearance					Edge					Elevation					Notes			
		#	<1			1-2	2-7	OPQ	TRL	SHY	DUL	CRC	IRR	FIL	RHZ	ENT	UND	LOB	ERO	FIL		CRL	FLT	RAI
BF.W 1:10A	13	1			+	Wht Cn, Mo Cl Eg, Wht outer		+	+		Cn					Hv							+	Wht Cn, Mo Cl Eg w/ Wht outer fRge
BF.W 1:10A	14	1			+	Li Og/Yl, Cl Eg		+	+		+				+				+	+				More entire color, not Egg-Like
BF.W 1:10A	15	1				Li Yl Cn, Cl Eg		+	+		Cn	Cn				+				+				Lg Eg, Sim to NNN
BF.W 1:10A	16	1				Br Yl Cn, Cl/Wht Eg		+	+		Cn	Cn					Hv						Li	PR
BF.W 1:10A	17	1				Cl/Yl Eg, Li Og Cn		+	+		+									+				Dimpled Like golfball, sSmooth Cn
BF.W 1:10A	20'	1			+	Mo Cl, Li Og/Wht Cn		+	+		+				+								+	Might be Sim to HH
BF.W 1:10A	20'	1			+																			
BF.W1:10B	AAA*	1																						
BF.W1:10B	BBB'	1																						Dividing
BF.W1:10B	UUU'	1																						
BF.W1:10B	7'	1																						
BF.W1:10B	7"	1																						
BF.W1:10B	7"	1			+																			
BF.W1:10B	DD'	1				3cm																		Eg Li UND due to overlap
BF.W1:10B	FF'	1																						
BF.W1:10B	17'	1																						
BF.W1:10B	14'	1			+																			
BF.W1:10B	18	1				Cl Lg Eg, Li Og Cn		+	+		Mo				Li					+				Sim to 17 but not dimpled
BF.W1:10B	19	1			+	Og/Ph		+	+		+				+						+			Uniform UND, Crater-Like
BF.W1:10B	CCC'	1																						Dk flecks within, due to overlap, probably a Dif colony
BF.W1:10B	21	1				Cl Lg Eg, Grad to Yl Cn		+	+		Cn	+			Mo	Li					+			PR
BF.W1:10B	22	1			+	Pl Yl, Sm Clearer Eg		+	+		+				+					+				PR
BF.W1:10B	20'	3			+																			
BF.W1:10B	HH'	1			+																			
BF.W1:10B	39'	1			+																			
BF.W1:10B	23	1			+	Yl Cl Eg		+	+		+				+					+				
BF.W 1:10C	AAA'	1																						
BF.W 1:10C	CCC'	1			+																			
BF.W 1:10C	7'	1																						
BF.W 1:10C	7"	2																						
BF.W 1:10C	7"	2			+																			
BF.W 1:10C	W'	2			+																			
BF.W 1:10C	22'	1			+																			
BF.W 1:10C	24	1				Cl w/ very Sm Bg Cn		+	+		Li			Mo	Li					+				Mo straight ENT Eg, w/ Light breaks, Mo IRR but round shape
BF.W 1:10C	14'	1			+	Lg Very Li Og/Yl Cn, Cl Eg		+	+		+				+				Li			Li		
BF.W 1:10C	27	1			+	Pl Wht/Og		+	+		+				+				Li			+		
BF.W 1:10C	29	1			+	Li Og Cn, Cl Eg		+	+		+				+				+				Li	
BF.W 1:10C	24'	1																						
BF.W 1:10C	25	1				Cl Eg, Yl/Og Eg		+	+		Cn	Eg			+								Cn	
BF.W 1:10C	26	1			+	Sherbert Og		+	+		+				+								+	
BF.W 1:10C	92	1			+	Pl Yl, Dkr Cn		+	+		+				+								Li	
BF.W 1:10C	1*	1			+	Cl Eg, Yl Cn		+	+		+				+									+
BF.W 1:X A	62	1			+	Cl		+	+		+			Mo	Li				Cn					+
BF.W 1:X A	NNN'	1			+																			+
BF.W 1:X A	7'	10			+																			
BF.W 1:X A	RR'	1																						
BF.W 1:X A	WWW'	3			+																			
BF.W 1:X A	ZZ'	1			+																			
BF.W 1:X A	AAA*	2																						
BF.W 1:X A	FF*	1			+	Cl		+	+		+			Li							+			Raised and dips in sLightly
BF.W 1:X A	FF'	1			+																			
BF.W 1:X A	30	1			+	Pr, Dkr Eg & Cn		+	+		+								+				Li	Cn and outer Eg Dkr
BF.W 1:X A	X'	2			+																			
BF.W 1:X A	W'	1			+	Lg Og Cn, Cl/Wht Eg		+	+		+			Li					+			+		Sim to W



















Table 1. Continued.

Sample	ID	Cnt Size in mm Color			Consistency	Colony Appearance					Edge					Elevation			Notes					
		#	<1	1-2		2-7	OPQ	TRL	SHY	DUL	CRC	IRR	FIL	RHZ	ENT	UND	LOB	ERO		FIL	CRL	FLT	RAI	CVX
SM.W 1:X B	35B'	1			+																			
SM.W 1:X B	WW'	1																						
SM.W 1:X B	FF2'	1		+																				
SM.W 1:X B	J3'	1																						
SM.W 1:X B	MM2'	1			+																			
SM.W 1:X B	BB2'	1			+																			
SM.W 1:X B	42A'	3			+																			
SM.W 1:X B	22B'	1		+																				
SM.W 1:X B	GG3'	1																						
SM.W 1:X B	PP2'	1			+																			
SM.W 1:X B	86'	1			+																			
SM.W 1:X B	44B'	1			+																			
SM.W 1:X B	W'	1		+																				
SM.W 1:X B	62'	2			+																			
SM.W 1:X B	10A'	1		+																				
SM.W 1:X B	VV'	1			+																			
SM.W 1:X B	34'	1			+																			
SM.W 1:X B	19'	5	+	+																				
SM.W 1:X B	J'	1			+																			
SM.W 1:X B	16A'	1			+																			
SM.W 1:X C	WW2'	2	+			Mo		+		+				+									+	
SM.W 1:X C	FF2'	1			+																			
SM.W 1:X C	41B'	1																						
SM.W 1:X C	86'	1			+																			
SM.W 1:X C	11'	1			+																			
SM.W 1:X C	GG3'	1																						
SM.W 1:X C	34'	1			+																			
SM.W 1:X C	12A'	1		+																				
SM.W 1:X C	GGG'	1			+																			
SM.W 1:X C	35'	1			+																			
SM.W 1:X C	J'	1			+																			
SM.W 1:X C	EE'	1			+																			
SM.W 1:X C	35B'	1		+																				
SM.W 1:X C	64'	1	+																					
SM.W 1:X C	J3'	1																						
SM.W 1:X C	44B'	1			+																			
SM.W 1:X C	TT2'	1			+																			
SM.W 1:X C	16B'	1																						
SM.W 1:X C	42A'	1		+																				
SH.F 1:100A	PP2	62		+	Bg Cn, Mlk Wht Eg			+		+												+	Sim to D2 but Dif, not as OPQ, no Rg, under 1 cm, More CRC	
SH.F 1:100A	QQ2	24		+	Bg Cn, Mlk Wht Eg	LiCn	Mo	+														Li	Larger Cn, CRC, less pointed UMB, Dk Cn	
SH.F 1:100A	RR2	5		+	Lg Bg Cn, Sm Li Bg Eg	Cn	Eg	+														+		
SH.F 1:100A	SS2	9		+	Dk Bg Cn, Mlk Wht Eg	LiCn	+	+		Li	+											Li	Larger Cn, More IRR but smaller Cn than RR2	
SH.F 1:100A	TT2	15		+	Off Wht	Mo	Li	+								+				Mo				
SH.F 1:100A	PP2'	49		+																				
SH.F 1:100A	QQ2'	30																						PR 2, one w/ More Y1 Cn, other w/ Wht and less OPQ
SH.F 1:100A	RR2'	2																						
SH.F 1:100B	SS2'	8																						
SH.F 1:100B	DD'	2																						
SH.F 1:100B	PP2'	90			+																			
SH.F 1:100C	QQ2'	36			+																			
SH.F 1:100C	RR2'	2		+																				
SH.F 1:100C	SS2'	10																						
SH.F 1:100C	DD'	5																						PR

Table 1. Continued.

Sample	ID	Cnt Size in mm		Color	Consistency	Colony Appearance					Edge					Elevation				Notes	
		#	<1 1-2 2-7			OPQ	TRL	SHY	DUL	CRC	IRR	FIL	RHZ	ENT	UND	LOB	ERO	FIL	CRL		FLT
SH.F 1:10A	PP2'	SP	+	+																	
SH.F 1:10A	QQ2'	SP	+	+																	
SH.F 1:10A	SS2'	SP	+	+																	
SH.F 1:10A	CCC'	1			+																
SH.F 1:10A	HH'	1			+																
SH.F 1:10A	DD'	1	+	+																	
SH.F 1:10A	UU2'	1		+	Y1	+		+		+									+		Shadowed Eg
SH.F 1:10B	PP2'	SP																			
SH.F 1:10B	QQ2'	SP																			
SH.F 1:10B	SS2'	SP																			
SH.F 1:10B	RR2'	SP																			
SH.F 1:10B	CCC'	1																			
SH.F 1:10B	DD'	1																			
SH.F 1:10C	PP2'	SP																			
SH.F 1:10C	QQ2'	SP																			
SH.F 1:10C	SS2'	SP																			
SH.F 1:10C	RR2'	SP																			
SH.F 1:10C	CCC'	1																			
SH.F 1:10C	DD'	1																			
SH.F 1:10C	VV2'	1	+		Y1/Og, Sm Li Eg	+		+		+			Mo		Li				+		Dkr blotches inside
SH.F 1:X A	PP2'	SP																			
SH.F 1:X A	QQ2'	SP																			
SH.F 1:X A	SS2'	SP																			
SH.F 1:X A	RR2'	SP																			
SH.F 1:X A	WW2'	2	Mo		Gry/Wht	+		+		+									+		Grainy Tex Several that appear Sim
SH.F 1:X A	DD2'	1		+																	
SH.F 1:X A	UU2'	2		+																	
SH.F 1:X B	PP2'	SP																			
SH.F 1:X B	QQ2'	SP																			
SH.F 1:X B	SS2'	SP																			
SH.F 1:X B	RR2'	SP																			
SH.F 1:X B	UU2'	2		+																	
SH.F 1:X B	XX2'	1			Pl Og/Ph			+		+				+					+		RAI in connected lumps
SH.F 1:X B	YY2'	1			Cl, few soLid Pts.	Pts		+	+		+		Hv				Mo				IRR Cn
SH.F 1:X C	PP2'	SP																			
SH.F 1:X C	QQ2'	SP																			
SH.F 1:X C	SS2'	SP																			
SH.F 1:X C	RR2'	SP																			
SH.F 1:X C	UU2'	3		+																	
SH.F 1:X C	YY2'	1																			
SH.F 1:X C	ZZ2'	1		+	Y1/Bg, Dkr Y1 Cn	Mo		+		Li			Hv	Li						Li	Lumpy but Li CVX shape
SH.W 1:100A	O2'	1		+																	
SH.W 1:100A	1B	1			PK/PH			+	+	+			+	VyLi			+	Mo	Li		Ent but not complete CRC, Li RAI on CRL
SH.W 1:100A	2B	1		+	Tng	+	LiEg	+		Mo			Li	Li					Hv		SMooth but not quite CRC
SH.W 1:100A	3B	1		+	Bwn/Og	+		+		+			Li						Hv		
SH.W 1:100B	YY'	1																			
SH.W 1:100B	86'	1		+																	
SH.W 1:100B	40'	1		+																	
SH.W 1:100B	19'	1		+																	
SH.W 1:100B	4B	1			Pl Y1/Og, Sm Cl Eg			+	+	+			Mo	Li			+		CRL		PR, RAI at CRL, CL FLT Eg
SH.W 1:100B	J'	1																			
SH.W 1:100B	5B	1			Pl Y1/Og			+	+	+			Hv				+		CRL	Li	Fg at Eg outside of CRL, RAI at CRL
SH.W 1:100B	6B	1		+	Tng			+	+	+			+	Li						+	PR
SH.W 1:100B	7B	1			Pl Og Cn, Grad to Cl Eg			+	+	+			Li	Li			Li	Mo			Li RAI on Cn & CRL, Pts in pattern toward Eg



Table 1. Continued.

Sample	ID	Cnt Size in mm				Color	Consistency				Colony Appearance				Edge				Elevation				Notes	
		#	<1	1-2	2-7		OPQ	TRL	SHY	DUL	CRC	IRR	FIL	RHZ	ENT	UND	LOB	ERO	FIL	CRL	FLT	RAI		CVX
SH.W 1:10B	YY'	1																						
SH.W 1:10B	DD'	5	+	+	+																			
SH.W 1:10B	20'	6																						
SH.W 1:10B	24A'	1		+																				
SH.W 1:10B	FF8'	6			+																			
SH.W 1:10B	25B'	1			+																			
SH.W 1:10B	27B	1		+		Pl Yl/Og			+	+		+						Li		Li		Li	PR	
SH.W 1:10B	26B'	1			+																			
SH.W 1:10B	28B	1				Lg Cloudy Bg Cn, Mlk Wht Eg	LiCn	Mo		+				+								+	Hv frills on Sm Eg	
SH.W 1:10B	28B'	1																						
SH.W 1:10B	29B	1				Sm Bg Cn, CL Eg		+	+		+			+			+					+	Lg Frills	
SH.W 1:10B	30B	1			+	Bwn/Og Cn, Wht Eg	+		+	+				+	+							+		
SH.W 1:10B	30B'	2			+																			
SH.W 1:10B	31B	1		+		Bwn/Og Cn, Cl Eg	Cn	Eg	+		+			+									+	
SH.W 1:10B	32B	1			+	Yl/Bwn Cn, Cl/Wht Eg	Li	+		+				+					+					
SH.W 1:10B	32B'	2			+																			
SH.W 1:10B	33B	1			+	Bg/Mlk Wht, Dkr Cn	Li			+				+									Li	PR frilled Eg
SH.W 1:10B	34B	1		+		Cl	+	+		+				+		+						+	PR !!!!	
SH.W 1:10B	35B	1		+		Og/Bwn Cn, Cl Eg	+	+		+				+			Li					+	not quite CRC, Dkr Pts	
SH.W 1:10B	36B	1			+	Yl/Og	+	+		Mo			Mo	Li							Li		Hv frills on Eg, Sim to QQ2	
SH.W 1:10B	37B	1		+		Bwn/Og Cn, Cl Eg	+	+		+				+								Li		
SH.W 1:10C	39'	1			+																			
SH.W 1:10C	19'	4		+	+																			
SH.W 1:10C	Y'	1			+																			
SH.W 1:10C	18'	1			+																			
SH.W 1:10C	QQ2'	1			+																			
SH.W 1:10C	OO'	1			+																			
SH.W 1:10C	11'	1			+																			
SH.W 1:10C	RR2'	6			+																			
SH.W 1:10C	86'	2			+																			
SH.W 1:10C	SS2'	1			+																			
SH.W 1:10C	35'	1			+																			
SH.W 1:10C	VV'	3		+																				
SH.W 1:10C	U2'	1			+																			
SH.W 1:10C	DD'	3	+		+																			
SH.W 1:10C	37B'	2			+																			
SH.W 1:10C	32B'	2			+																			
SH.W 1:10C	26B'	3			+																			
SH.W 1:10C	31B'	1			+																			
SH.W 1:10C	FF8'	1			+																			
SH.W 1:10C	W'	2			+																			
SH.W 1:10C	38B	1			+	Dull Yl, Dkr Cn	+	+		+				+		+							+	
SH.W 1:10C	39B	1			+	Pl Yl, Dkr Pts	+	+		Li				+							Li		Sim to 36 but More UND and More Pts	
SH.W 1:10C	39B'	1			+																			
SH.W 1:10C	40B	1		+		Bg/Og, Dkr Cloudy Cn	Li	+		+			+									Li	PR	
SH.W 1:10C	41B	1			+	Cl	+	+		Li				+			Cn			+				
SH.W 1:10C	40'	1			+																			
SH.W 1:10C	42B	1			+	Cl	+	+		+				+	+	Li				+				
SH.W 1:10C	43B	1		+		Mo Cl, Li Yl Cn	+	+		+				+					+		Li		Li	Frills on Eg
SH.W 1:10C	HH'	1		+																				
SH.W 1:10C	TT'	1		+																				
SH.W 1:10C	44B	1		+		Wht/Gry Cn	+	+		Oval			+									Li		
SH.W 1:10C	O'	1		+																				
SH.W 1:10C	19'	1		+																				







Table 2. Total CFU counts in each plate in the different dilution series for each sample.

Selected median values in “**bold**”.

Sample	Count	Sample	Count	Sample	Count
M F 1:100 A	2	SC F 1:100 A	0	SM F 1:100 A	0
M F 1:100 B	0	SC F 1:100 B	0	SM F 1:100 B	0
M F 1:100 C	0	SC F 1:100 C	0	SM F 1:100 C	0
M F 1:10 A	0	SC F 1:10 A	3	SM F 1:10 A	0
M F 1:10 B	0	SC F 1:10 B	7	SM F 1:10 B	1
M F 1:10 C	0	SC F 1:10 C	4	SM F 1:10 C	2
M F 1:X A	<b>2</b>	SC F 1:X A	17	SM F 1:X A	13
M F 1:X B	0	SC F 1:X B	13	SM F 1:X B	<b>11</b>
M F 1:X C	0	SC F 1:X C	16	SM F 1:X C	12
M W 1:100 A	6	SC W 1:100 A	3	SM W 1:100 A	1
M W 1:100 B	3	SC W 1:100 B	3	SM W 1:100 B	6
M W 1:100 C	7	SC W 1:100 C	5	SM W 1:100 C	6
M W 1:10 A	26	SC W 1:10 A	12	SM W 1:10 A	12
M W 1:10 B	22	SC W 1:10 B	15	SM W 1:10 B	25
M W 1:10 C	24	SC W 1:10 C	33	SM W 1:10 C	16
M W 1:X A	130	SC W 1:X A	80	SM W 1:X A	104
M W 1:X B	<b>121</b>	SC W 1:X B	94	SM W 1:X B	137
M W 1:X C	105	SC W 1:X C	84	SM W 1:X C	<b>116</b>
BF F 1:100 A	2	FL F 1:100 A	4	SH F 1:100 A	<b>115</b>
BF F 1:100 B	6	FL F 1:100 B	0	SH F 1:100 B	91
BF F 1:100 C	1	FL F 1:100 C	0	SH F 1:100 C	143
BF F 1:10 A	27	FL F 1:10 A	0	SH F 1:10 A	1000+
BF F 1:10 B	21	FL F 1:10 B	5	SH F 1:10 B	1000+
BF F 1:10 C	14	FL F 1:10 C	1	SH F 1:10 C	1000+
BF F 1:X A	106	FL F 1:X A	12	SH F 1:X A	10000+
BF F 1:X B	74	FL F 1:X B	<b>22</b>	SH F 1:X B	10000+
BF F 1:X C	<b>84</b>	FL F 1:X C	24	SH F 1:X C	10000+
BF W 1:100 A	2	FL W 1:100 A	4	SH W 1:100 A	4
BF W 1:100 B	6	FL W 1:100 B	2	SH W 1:100 B	15
BF W 1:100 C	1	FL W 1:100 C	0	SH W 1:100 C	16
BF W 1:10 A	22	FL W 1:10 A	12	SH W 1:10 A	37
BF W 1:10 B	21	FL W 1:10 B	17	SH W 1:10 B	66
BF W 1:10 C	19	FL W 1:10 C	9	SH W 1:10 C	<b>72</b>
BF W 1:X A	74	FL W 1:X A	35	SH W 1:X A	142
BF W 1:X B	51	FL W 1:X B	60	SH W 1:X B	186
BF W 1:X C	<b>60</b>	FL W 1:X C	<b>43</b>	SH W 1:X C	224

Table 2. Continued.

Sample	Count	Sample	Count
M2 F 1:100 A	0	SC2 F 1:100 A	0
M2 F 1:100 B	0	SC2 F 1:100 B	1
M2 F 1:100 C	1	SC2 F 1:100 C	1
M2 F 1:10 A	1	SC2 F 1:10 A	9
M2 F 1:10 B	1	SC2 F 1:10 B	9
M2 F 1:10 C	1	SC2 F 1:10 C	8
M2 F 1:X A	4	SC2 F 1:X A	46
M2 F 1:X B	6	SC2 F 1:X B	55
M2 F 1:X C	<u>5</u>	SC2 F 1:X C	<u>42</u>
M2 W 1:100 A	12	SC2 W 1:100 A	5
M2 W 1:100 B	13	SC2 W 1:100 B	3
M2 W 1:100 C	24	SC2 W 1:100 C	0
M2 W 1:10 A	121	SC2 W 1:10 A	30
M2 W 1:10 B	<u>110</u>	SC2 W 1:10 B	17
M2 W 1:10 C	83	SC2 W 1:10 C	17
M2 W 1:X A	326	SC2 W 1:X A	114
M2 W 1:X B	306	SC2 W 1:X B	98
M2 W 1:X C	288	SC2 W 1:X C	<u>110</u>
BF2 F 1:100 A	13*	FL2 F 1:100 A	19
BF2 F 1:100 B	9	FL2 F 1:100 B	23
BF2 F 1:100 C	8*	FL2 F 1:100 C	10
BF2 F 1:10 A	<u>59</u>	FL2 F 1:10 A	123
BF2 F 1:10 B	71	FL2 F 1:10 B	<u>107</u>
BF2 F 1:10 C	58	FL2 F 1:10 C	90
BF2 F 1:X A	328	FL2 F 1:X A	448
BF2 F 1:X B	287	FL2 F 1:X B	522
BF2 F 1:X C	353	FL2 F 1:X C	337
BF2 W 1:100 A	15	FL2 W 1:100 A	5
BF2 W 1:100 B	21	FL2 W 1:100 B	4
BF2 W 1:100 C	21	FL2 W 1:100 C	5
BF2 W 1:10 A	56	FL2 W 1:10 A	27
BF2 W 1:10 B	68	FL2 W 1:10 B	21
BF2 W 1:10 C	<u>62</u>	FL2 W 1:10 C	<u>23</u>
BF2 W 1:X A	281	FL2 W 1:X A	333
BF2 W 1:X B	232	FL2 W 1:X B	312
BF2 W 1:X C	286	FL2 W 1:X C	299

\* 13 and 8 colonies within “normal” recordable size ranges occurring with pin-point colonies that were too numerous to count.

Table 3. Presence or absence of unique CFU types within each sample. Abbreviations: presence indicated by (1), absence by (0), CFU species type (Sp#), lower CUF type corresponds to the designation in (Appendix Table 1).

CFU Type	Sp 1	SP 2	Sp 3	Sp 4	Sp 5	Sp 6	Sp 7	Sp 8	Sp 9	Sp 10
Sample Origin	A	B	C	D	B*	50	51	52	K	54
MF	1	1	1	1	0	0	0	0	0	0
MW	0	0	0	0	1	1	1	1	1	1
BF F	0	0	0	0	0	0	0	0	1	0
BF W	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	1	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	1	0

Table 3. Continued.

	Sp 11	Sp 12	Sp 13	Sp 14	Sp 15	Sp 16	Sp 17	Sp 18	Sp 19	Sp 20	Sp 21
Sample Origin	55	56	57	58	59	60	TTT*	65	67	68	69
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	1	1	1	1	1	1	1	1	1	1
BF F	0	0	1	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	1	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 22	Sp 23	Sp 24	Sp 25	Sp 26	Sp 27	Sp 28	Sp 29	Sp 30	Sp 31	Sp 32
Sample Origin	70	71	72	73	74	75	76	77	78	79	81
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	1	1	1	1	1	1	1	1	1	1
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	1	0	0	1	0	0	0	0	0

Table 3. Continued.

	Sp 33	Sp 34	Sp 35	Sp 36	Sp 37	Sp 38	Sp 39	Sp 40	Sp 41	Sp 42	Sp 43
Sample Origin	82	83	84	85	86	87	88	89	90	91	E
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	1	1	1	1	1	1	1	1	1	0
BF F	0	0	0	0	0	0	0	0	0	0	1
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	1	0	1	0	0	0	0
FL F	0	0	0	0	0	0	1	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	1	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	1	0	1	0	0	0	0

Table 3. Continued.

	Sp 44	Sp 45	Sp 46	Sp 47	Sp 48	Sp 49	Sp 50	Sp 51	Sp 52	Sp 53	Sp 54
Sample Origin	F	L	M	N	O	P	Q	G	H	I	J
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	1	0	0	1	0	1	0
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	1	0	1	0	0	1	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	1	0	1	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	1	0
FL W	0	0	0	0	0	0	0	0	0	1	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	1	0	1	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	1	0	0	1	0	1	1



Table 3. Continued.

	Sp 55	Sp 56	Sp 57	Sp 58	Sp 59	Sp 60	Sp 61	Sp 62	Sp 63	Sp 64	Sp 65
Sample Origin	K	II	JJ	KK	LL	MM	NN	OO	PP	QQ	S
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	1	0	0	0	0	0	0	0
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	0	0	1	0	0	1	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	1	0	0	0	0	0	0
FL F	0	0	0	1	1	0	0	0	0	0	0
FL W	0	0	0	1	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	1	0	0	1	0	0	1

Table 3. Continued.

	Sp 66	Sp 67	Sp 68	Sp 69	Sp 70	Sp 71	Sp 72	Sp 73	Sp 74	Sp 75	Sp 76
Sample Origin	T	U	V	W	X	Y	Z	AA	BB	CC	DD
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	1	1	0	0	0	0	0	1
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	0	1	1	0	0	0	0	0	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	1	1	0	0	0	0	0	0	0
FL F	0	0	0	1	1	0	0	0	0	0	1
FL W	0	0	0	0	1	0	0	0	0	0	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	1	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	1
SH W	0	0	1	1	1	1	0	0	0	0	1

Table 3. Continued.

	Sp 77	Sp 78	Sp 79	Sp 80	Sp 81	Sp 82	Sp 83	Sp 84	Sp 85	Sp 86	Sp 87
Sample Origin	EE	FF	GG	HH	SS	UUU	VVV	WWW	XXX	20	40
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	1	0	1	0	0	0	1	0	1	1
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	1	1	0	1	1	1	0	1	1	1	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	1	1	1
FL F	0	1	0	0	0	0	0	0	0	1	0
FL W	1	1	0	1	0	0	0	0	0	1	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	1	1	0	0	0	1	0	1
SH F	0	0	0	1	0	0	0	0	0	0	0
SH W	1	1	0	1	1	0	0	0	1	1	1

Table 3. Continued.

	Sp 88	Sp 89	Sp 90	Sp 91	Sp 92	Sp 93	Sp 94	Sp 95	Sp 96	Sp 97	Sp 98
Sample Origin	ZZZ	57	63	64	AAA	BBB	CCC	DDD	EEE	FFF	GGG
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	0	1	1	1	1	1	1	1	0	1
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	0	0	1	1	1	1	0	0	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	1	0	0	0	0	0	1	0	0
FL F	0	0	1	1	0	0	0	0	0	0	0
FL W	0	0	1	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	1	0	0	0	0	0	0	1
SH F	0	0	0	0	0	0	1	0	0	0	0
SH W	1	0	0	0	0	0	0	0	0	0	1

Table 3. Continued.

	Sp 99	Sp 100	Sp 101	Sp 102	Sp 103	Sp 104	Sp 105	Sp 106	Sp 107	Sp 108	Sp 109
Sample Origin	HHH	III	JJJ	KKK	LLL	MMM	NNN	OOO	PPP	QQQ	RRR
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	1	0	0	0	0
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	0	0	0	0	1	0	0	0	0
SC F	0	0	0	0	1	0	0	0	0	0	0
SC W	0	0	0	0	1	0	0	0	0	0	0
FL F	0	0	0	0	1	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	1

Table 3. Continued.

	Sp 110	Sp 111	Sp 112	Sp 113	Sp 114	Sp 115	Sp 116	Sp 117	Sp 118	Sp 119	Sp 120
Sample Origin	SSS	TTT	RR	TT	UU	VV	WW	XX	YY	ZZ	7
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	1	0	0	0	0	1	1
BF F	1	1	1	1	1	1	1	1	1	1	1
BF W	0	0	1	1	0	1	0	1	0	1	1
SC F	0	0	0	0	0	0	0	0	0	1	0
SC W	0	0	0	0	0	0	0	0	0	1	1
FL F	0	0	0	1	0	0	0	0	0	1	0
FL W	0	0	1	0	0	0	1	0	1	1	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	1	0	1	1	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	1	1	0	1	0	0	1	0	0

Table 3. Continued.

	Sp 121	Sp 122	Sp 123	Sp 124	Sp 125	Sp 126	Sp 127	Sp 128	Sp 129	Sp 130	Sp 131
Sample Origin	YYY	46	61	1	2	3	4	5	6	AAA*	8
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	0	1	0	0	0	0	0	0	0	0
BF F	1	1	1	0	0	0	0	0	0	0	0
BF W	1	1	0	1	1	1	1	1	1	1	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	1	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	1	0	1	0	0	0	1

Table 3. Continued.

	Sp 132	Sp 133	Sp 134	Sp 135	Sp 136	Sp 137	Sp 138	Sp 139	Sp 140	Sp 141	Sp 142
Sample Origin	9	10	11	12	13	14	15	16	17	18	19
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	1	1	0	1	1	1	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	1	1	1	1	1	1	1	1	1	1	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	1	0	0	0	0	0	0
FL F	0	0	1	0	1	0	0	0	0	0	0
FL W	0	0	1	0	1	0	0	0	0	0	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	1	0	0	0	0	0	0	0	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	1	0	0	1	0	0	0	1	1



Table 3. Continued.

	Sp 143	Sp 144	Sp 145	Sp 146	Sp 147	Sp 148	Sp 149	Sp 150	Sp 151	Sp 152	Sp 153
Sample Origin	21	22	23	24	27	29	25	26	92	1*	62
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	1	0	0	1	1	1	0	0	1	1
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	1	1	1	1	1	1	1	1	1	1	1
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	1	0	0	0	0
FL F	0	0	0	0	0	0	0	0	1	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	1	0	0	1	0	0	0	1	1

Table 3. Continued.

	Sp 154	Sp 155	Sp 156	Sp 157	Sp 158	Sp 159	Sp 160	Sp 161	Sp 162	Sp 163	Sp 164
Sample Origin	FF*	30	32	33	34	35	36	37	39	41	41*
MF	0	0	0	0	0	0	0	0	0	0	0
MW	1	1	1	0	1	0	0	0	1	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	1	1	1	1	1	1	1	1	1	1	1
SC F	1	0	0	0	0	0	0	0	0	0	0
SC W	1	0	0	0	0	1	0	0	0	0	1
FL F	1	0	0	0	0	1	0	0	1	0	0
FL W	1	1	0	0	1	1	0	1	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	1	1	0	0	1	1	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	1	0	0	0	1	0	0	1	0	0

Table 3. Continued.

	Sp 165	Sp 166	Sp 167	Sp 168	Sp 169	Sp 170	Sp 171	Sp 172	Sp 173	Sp 174	Sp 175
Sample Origin	42	43	44	45	48	7*	47	92	49	A3	B3
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	1	1	0	0	0	0
BF F	0	0	0	0	0	1	0	0	0	0	0
BF W	1	1	1	1	1	1	1	1	1	0	0
SC F	0	0	0	0	0	0	0	0	0	1	1
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	1	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	1	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	0	1	1	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 176	Sp 177	Sp 178	Sp 179	Sp 180	Sp 181	Sp 182	Sp 183	Sp 184	Sp 185	Sp 186
Sample Origin	C3	D3	E3	F3	G3	H3	I3	J3	K3	L3	M3
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	1	1	1	1	1	1	1	1	1	1	1
SC W	0	0	0	0	0	0	0	1	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	1	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 187	Sp 188	Sp 189	Sp 190	Sp 191	Sp 192	Sp 193	Sp 194	Sp 195	Sp 196	Sp 197
Sample Origin	N3	53	O3	P3	Q3	J3*	R3	T3	86*	S3	25A
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	1	1	1	1	1	1	1	1	1	1	0
SC W	0	0	0	0	0	0	0	0	0	0	1
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 198	Sp 199	Sp 200	Sp 201	Sp 202	Sp 203	Sp 204	Sp 205	Sp 206	Sp 207	Sp 208
Sample Origin	26A	27A	28A	29A	30A	31A	32A	33A	34A	35A	36A
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	1	0	0	0	1	0	0	0	0	0
SC W	1	1	1	1	1	1	1	1	1	1	1
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 209	Sp 210	Sp 211	Sp 212	Sp 213	Sp 214	Sp 215	Sp 216	Sp 217	Sp 218	Sp 219
Sample Origin	37A	38A	39A	40A	41A	42A	43A	44A	45A	46A	47A
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	1	0	0	0	0	0
SC W	1	1	1	1	1	1	1	1	1	1	1
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	1	0	0	0	1	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	1	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	1	0	1	0	0	0

Table 3. Continued.

	Sp 220	Sp 221	Sp 222	Sp 223	Sp 224	Sp 225	Sp 226	Sp 227	Sp 228	Sp 229	Sp 230
Sample Origin	48A	49A	51A	U3	V3	W3	X3	Y3	Z3	AA3	BB3
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	1	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	1	1	1	0	0	0	0	0	0	0	0
FL F	0	0	0	1	1	1	1	1	1	1	1
FL W	1	0	0	1	0	0	0	0	1	1	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	1	0	0	0



Table 3. Continued.

	Sp 231	Sp 232	Sp 233	Sp 234	Sp 235	Sp 236	Sp 237	Sp 238	Sp 239	Sp 240	Sp 241
Sample Origin	CC3	DD3	EE3	FF3	GG3	HH3	II3	JJ3	KK3	LL3	MM3
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	1	0	0	1	0	0	1	0	0
FL F	1	1	1	1	1	1	1	1	1	1	1
FL W	0	0	1	0	1	0	0	0	0	1	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	1	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 242	Sp 243	Sp 244	Sp 245	Sp 246	Sp 247	Sp 248	Sp 249	Sp 250	Sp 251	Sp 252
Sample Origin	NN3	OO3	PP3	QQ3	RR3	SS3	TT3	UU3	VV3	WW3	2A
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	1	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	1	0	0	0	0	0	0	1	0	0	0
FL F	1	1	1	1	1	1	1	1	1	1	0
FL W	0	0	0	1	1	1	0	0	0	0	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	1	0	0	1	0	0	0	0	0	0

Table 3. Continued.

	Sp 253	Sp 254	Sp 255	Sp 256	Sp 257	Sp 258	Sp 259	Sp 260	Sp 261	Sp 262	Sp 263
Sample Origin	3A	9A	10A	11A	12A	13A	14A	15A	16A	17A	18A
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	1	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	1	1	1	1	1	1	1	1	1	1	1
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	1	0	1	0	0	0	1	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	1	0	0	0

Table 3. Continued.

	Sp 264	Sp 265	Sp 266	Sp 267	Sp 268	Sp 269	Sp 270	Sp 271	Sp 272	Sp 273	Sp 274
Sample Origin	20A	21A	22A	23A	24A	3A*	4A	5A	6A	8A	A2
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	1	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	1	1	1	1	1	1	1	1	1	1	0
SM F	0	0	0	0	0	0	0	0	0	0	1
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	1	0	0	0	0	1	0

Table 3. Continued.

	Sp 275	Sp 276	Sp 277	Sp 278	Sp 279	Sp 280	Sp 281	Sp 282	Sp 283	Sp 284	Sp 285
Sample Origin	B2	C2	D2	E2	F2	G2	H2	I2	J2	H2	K2
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	1	0	0	0	0	0	0	0
SM F	1	1	1	1	1	1	1	1	1	1	1
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 286	Sp 287	Sp 288	Sp 289	Sp 290	Sp 291	Sp 292	Sp 293	Sp 294	Sp 295	Sp 296
Sample Origin	L2	M2	N2	O2	P2	Q2	R2	S2	<u>Z2</u>	T2	U2
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	1
SM F	1	1	0	0	0	0	0	0	0	0	0
SM W	0	0	1	1	1	1	1	1	1	1	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	1	0	0	0	0	0	0	1

Table 3. Continued.

	Sp 297	Sp 298	Sp 299	Sp 300	Sp 301	Sp 302	Sp 303	Sp 304	Sp 305	Sp 306	Sp 307
Sample Origin	V2	W2	X2	Y2	AA2	BB2	CC2	DD2	EE2	FF2	GG2
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	1	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	1	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	1	1	1	1	1	1	1	1	1	1	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	0	0	0	0	0	0	0	0	0	0	0

Table 3. Continued.

	Sp 308	Sp 309	Sp 310	Sp 311	Sp 312	Sp 313	Sp 314	Sp 315	Sp 316	Sp 317	Sp 318
Sample Origin	HH2	II2	JJ2	KK2	LL2	MM2	NN2	OO2	PP2	QQ2	RR2
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	1	0	0	0	0	1	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	1	1	1	1	1	1	1	1	1	1	1
SH F	0	0	0	0	0	0	0	0	1	1	1
SH W	0	0	0	0	0	0	0	0	1	1	1



Table 3. Continued.

	Sp 319	Sp 320	Sp 321	Sp 322	Sp 323	Sp 324	Sp 325	Sp 326	Sp 327	Sp 328	Sp 329
Sample Origin	SS2	TT2	UU2	VV2	WW2	DD2	XX2	YY2	ZZ2	1B	2B
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	1	0	0	1	0	0	0	0	0	0
SH F	1	1	1	1	1	1	1	1	1	0	0
SH W	1	0	1	1	1	0	0	1	0	1	1

Table 3. Continued.

	Sp 330	Sp 331	Sp 332	Sp 333	Sp 334	Sp 335	Sp 336	Sp 337	Sp 338	Sp 339	Sp 340
Sample Origin	3B	4B	5B	6B	7B	8B	9B	10B	11B	12B	13B
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	1	1	1	1	1	1	1	1	1	1

Table 3. Continued.

	Sp 341	Sp 342	Sp 343	Sp 344	Sp 345	Sp 346	Sp 347	Sp 348	Sp 349	Sp 350	Sp 351
Sample Origin	14B	15B	16B	17B	18B	19B	20B	21B	22B	23B	24B
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	1	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	1	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	1	0	1	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	1	0	0	0	0	0	1	0	0
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	1	1	1	1	1	1	1	1	1	1

Table 3. Continued.

	Sp 352	Sp 353	Sp 354	Sp 355	Sp 356	Sp 357	Sp 358	Sp 359	Sp 360	Sp 361	Sp 362
Sample Origin	25B	26B	27B	28B	29B	30B	31B	32B	33B	34B	35B
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	1
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	0	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	0	0	0	0	0	0	0	0	0	0	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	1	1	1	1	1	1	1	1	1	1

Table 3. Continued.

	Sp 363	Sp 364	Sp 365	Sp 366	Sp 367	Sp 368	Sp 369	Sp 370	Sp 371	Sp 372	Sp 373
Sample Origin	36B	37B	38B	39B	40B	41B	42B	43B	44B	46B	45B
MF	0	0	0	0	0	0	0	0	0	0	0
MW	0	0	0	0	0	0	0	0	0	0	0
BF F	0	0	0	0	0	0	0	0	0	0	0
BF W	0	0	0	0	0	0	0	0	0	0	0
SC F	0	0	0	0	0	0	0	0	0	0	0
SC W	0	0	0	0	0	0	0	0	1	0	0
FL F	0	0	0	0	0	0	0	0	0	0	0
FL W	0	0	0	0	0	0	0	0	0	0	0
SM F	0	0	0	0	0	0	0	0	0	0	0
SM W	1	0	0	0	0	1	0	0	1	0	1
SH F	0	0	0	0	0	0	0	0	0	0	0
SH W	1	1	1	1	1	1	1	1	1	1	1

Table 3. Continued.

Sample Origin	Total CFU Richness	Total shared CFUs
M F	4	0
MW	88	58
BF F	85	52
BF W	81	62
SC F	30	7
SC W	58	38
FL F	50	37
FL W	64	50
SM F	14	1
SM W	70	47
SH F	15	12
SH W	113	76

Figure 1. Photos of selected plate cultures to visually demonstrate growth trend within samples. Only the first replicate (A) of each dilution is depicted.

Mull Creek fish from first sample.



Beechflat Creek fish from first sample.

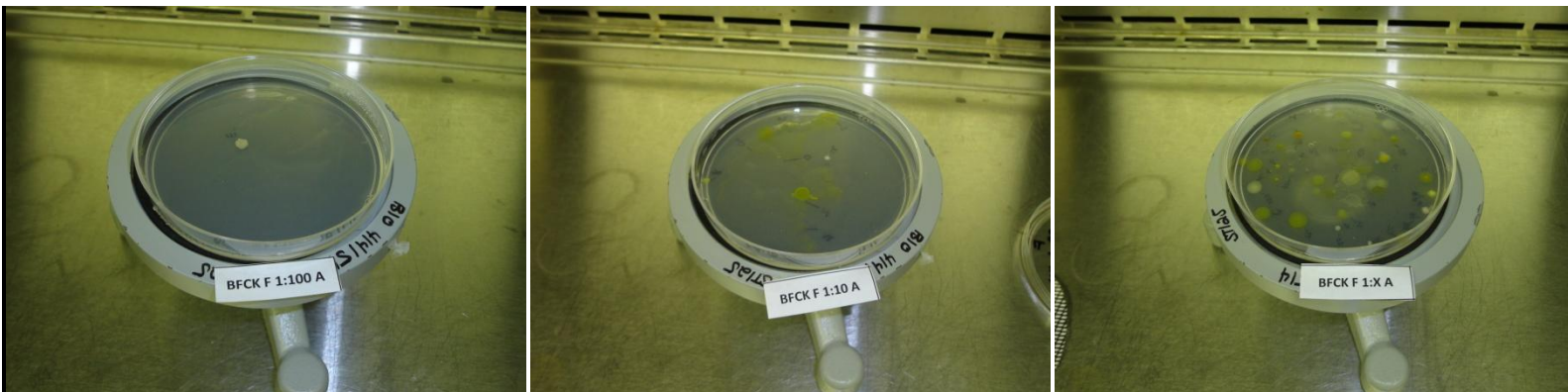
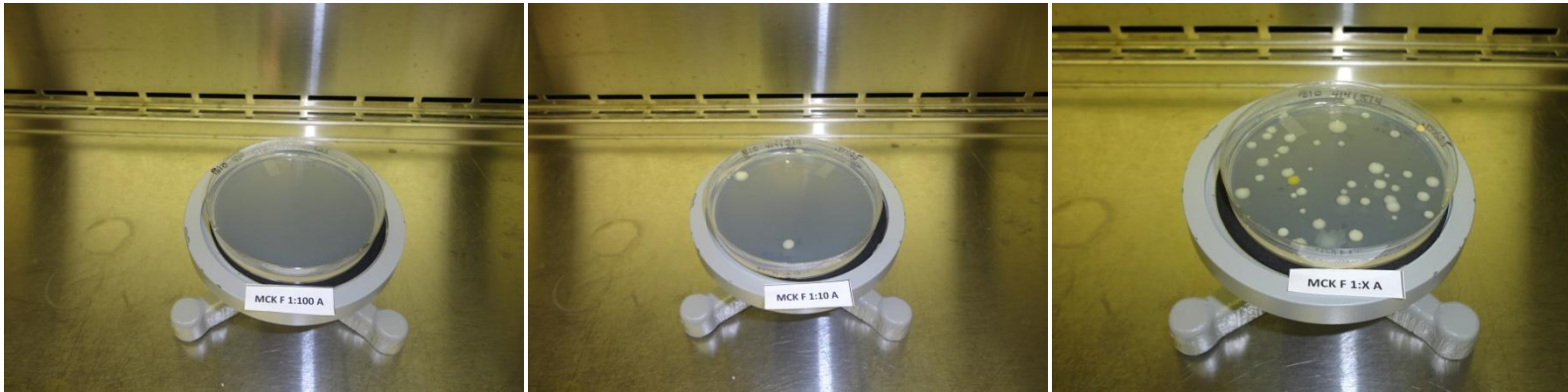


Figure 1. Continued.

Mull Creek fish from second sample.



Beechflat Creek fish from second sample.

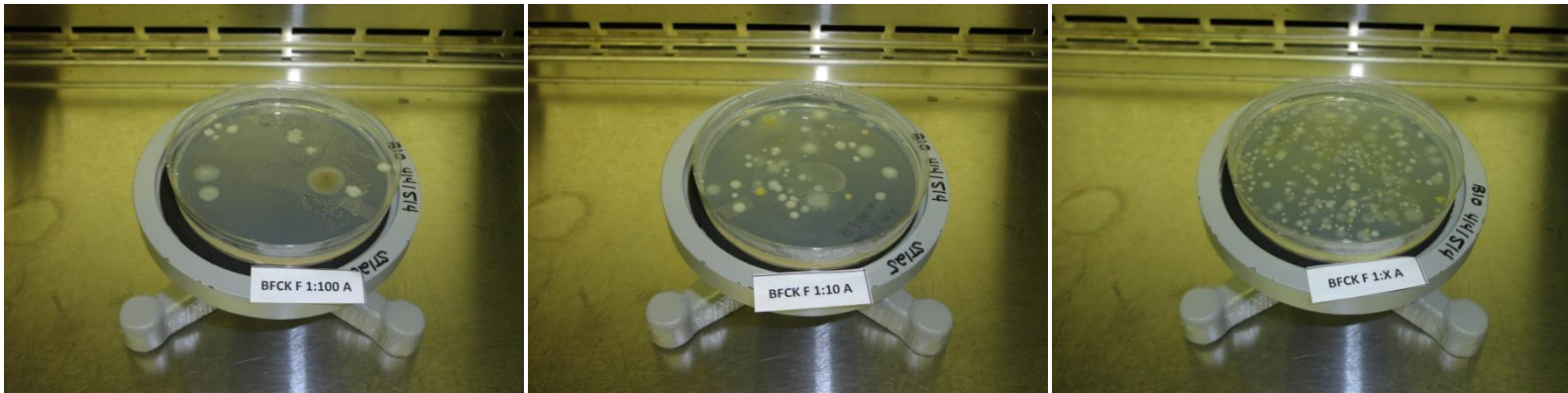
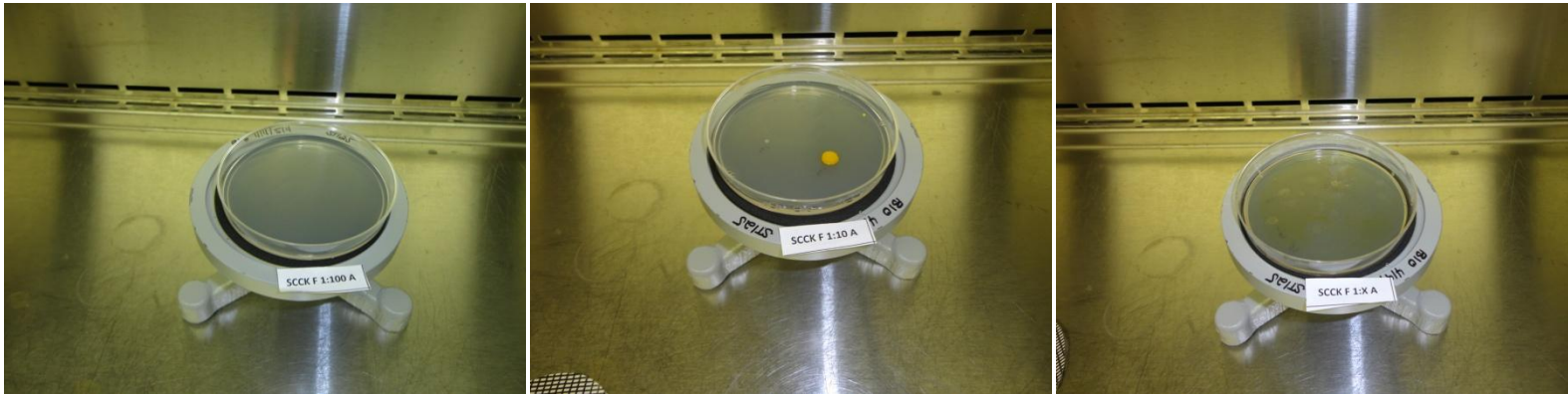




Figure 1. Continued.

Scapecat Creek fish from first sample.



Sawmill Creek fish from first sample.



Figure 1. Continued.

Mull Creek water from first sample.



Beechflat Creek water from first sample.

