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Abstract

In the first of a three-part study of Brewery Creek, an arm of the Ottawa River, water samples were assayed for total bacterial counts, numbers of lipolytic and proteolytic bacteria, and pollution indicator species. For all parameters studied during the entire sampling period, counts were significantly higher at our downstream station on the creek than at our upstream station.

Part way down the creek, in an area where sewage and other effluent pipes were common, all counts increased sharply, and they remained high until Brewery Creek rejoined the Ottawa River. Counts also increased considerably during a period when the flow was reduced to permit construction, but did not vary much from one season to another. At both upper and lower ends of the creek, the ratio of direct total count to total viable count was low, suggesting that the bacteria are largely saprophytic. Ratios of fecal coliforms to fecal streptococci indicated a mixture of animal and human wastes entering the water. The overall results showed that Brewery Creek is grossly polluted with a constant input of organic wastes from various sources and that considerable numbers of lipolytic and proteolytic degradative bacteria are introduced into the creek waters via these wastes.

During the 1971-72 winter and part of the 1972 spring period, water samples were assayed for numbers of lipolytic

and cytochrome oxidase positive bacteria at different temperatures of incubation. This second study showed that numbers of lipolytic and oxidase positive bacteria increased about 100 fold from the upstream to the downstream station at all temperatures of incubation. During this period, the lipolytic population at the upstream site appeared to be composed entirely of psychrophilic species, approximately 2/3's of which were oxidase positive. Paradoxically, at our downstream station, oxidase positive bacteria made up over 1/2 of the lipolytic population even though two samples, taken from a major sanitary sewer situated mid-way on the creek, contained primarily cytochrome oxidase negative lipolytic species. To explain our downstream results, we have suggested that the meat-packing plant effluent may represent the major source of cytochrome oxidase positive species, which would counterbalance the predominantly oxidase negative input from sewage. Another possibility is that oxidase negative species may die off more rapidly in the creek waters.

Analysis of the cytochrome oxidase positive bacteria at both sites showed that a greater proportion of these exhibited lipolysis at lower temperatures (4, 10, and 20C) than at higher temperatures (30, 37, and 42C) of incubation. This suggests that oxidase positive psychrophilic species are more important in metabolizing fatty wastes than are their mesophilic homologues, and, for this reason,

are a major contributor to the self-purification of polluted waterways.

In order to know which types of lipolytic psychrophiles were present in Brewery Creek, a total of 383 isolates were classified, mostly to the generic level, during the same 1971-72 winter and 1972 spring period. At the upstream, less polluted station, pseudomonads were predominant. At the downstream site Acinetobacter and Aeromonas were also common. These three groups were also the major ones obtained from a sewage sample. At both sites smaller numbers of Alcaligenes, Moraxella, Chromobacterium and Enterobacteriaceae were also isolated. Most of these isolates, judged psychrophilic by their ability to grow at 4C, would also grow at 30C. Eighty of these would also grow at 37C, and 16 (all aeromonads) were strongly lipolytic at this temperature. However, for most, lipolysis was strongest at 20C or below; suggesting, again, that psychrophiles probably play an important degradative role in polluted aquatic systems. From the taxonomic view-point, the difficulties encountered in trying to classify the isolates, particularly beyond the generic level, suggest that insufficient attention has been paid to psychrophilic organisms in existing classification schemes.

Résumé

A partir d'échantillons d'eau prélevés dans le ruisseau de la Brasserie, un embranchement de la rivière Ottawa, des numérations bactériennes totales, lipolytiques, protéolytiques, ainsi que des numérations d'espèces bactériennes indicatrices de pollution, ont été déterminées. Etudiés sous trois aspects différents, ces premiers travaux ont montré que les eaux du cours inférieur contenaient un nombre de bactéries significativement plus élevé que les eaux du cours supérieur, et ce, pour tous les paramètres étudiés pendant notre période complète de prélèvements. Sur le parcours du ruisseau (environ à mi-chemin) plusieurs tuyaux déversent des eaux usées d'origine domestique et autres. Des analyses d'eau, déterminées à cet endroit, ont montré une très forte augmentation de toutes nos numérations bactériennes, et ce, jusqu'au point où les eaux retournent dans la rivière Ottawa. Pendant la période où le débit du ruisseau fut réduit afin de permettre des travaux de construction, on a noté également une hausse considérable de germes, sans grandes variations saisonnières. Dans les eaux du cours supérieur et inférieur, nous avons obtenu des valeurs peu élevées pour les rapports numérations totales sur numérations viables, ce qui suggère que la population bactérienne est essentiellement composée d'espèces saprophytes. Les rapports colibacilles fécaux sur streptocoques fécaux indiquent la présence de déchets animaux et humains dans les eaux du ruisseau.

Tous ces résultats nous ont permis de conclure que le ruisseau de la Brasserie est fortement pollué par une arrivée constante de déchets organiques provenant de sources variées, et qu'un nombre considérable de germes lipolytiques et protéolytiques est déversé dans les eaux du ruisseau grâce à ces déchets.

Pendant l'hiver 1971-72 et une partie du printemps 1972, nous avons déterminé, à partir d'échantillons d'eau, des numérations bactériennes lipolytiques et cytochrome oxydase-positives à différentes températures d'incubation. Cette deuxième étude a montré que les numérations de bactéries lipolytiques et oxydase-positives dans les eaux du cours inférieur étaient 100 fois plus élevées que celles des eaux du cours supérieur, et ce, à chaque température d'incubation. Pendant cette période, la population lipolytique du cours supérieur semblait être composée entièrement d'espèces psychrophiles, les 2/3 de celle-ci étant des bactéries oxydase-positives. Paradoxalement, plus de la moitié de la population lipolytique des eaux du cours inférieur était composée d'espèces oxydase-positives, bien que les eaux d'un important égout domestique, déversées à mi-chemin sur le cours du ruisseau, contenaient principalement des espèces lipolytiques cytochrome oxydase-négatives (résultats de l'analyse de deux échantillons d'eaux d'égout).

Afin d'interpréter les résultats des eaux du cours supérieur, nous avons suggéré que l'égout de la manutention

des viandes représentait peut-être la source principale d'espèces oxydase-positives; ce qui contrebalancerait obligatoirement l'addition principalement oxydase-négative des eaux d'égouts. Il est également possible que les espèces oxydase-négatives subissent un plus grand taux de mortalité dans les eaux du ruisseau.

L'analyse de bactéries oxydase-positives dans les eaux des cours supérieur et inférieur du ruisseau ont montré qu'une plus forte proportion de celles-ci étaient lipolytiques à des températures basses (4, 10, et 20C) plutôt qu'à des températures d'incubation plus élevées (30, 37, et 42C). Ceci porte à croire que les espèces psychrophiles oxydase-positives jouent un rôle plus important que leurs homologues mésophiles en ce qui a trait à la dégradation de déchets lipidiques, et que ces psychrophiles contribuent largement à l'auto-purification d'eaux polluées.

Afin de connaître les types de psychrophiles lipolytiques présents dans le ruisseau de la Brasserie, nous avons identifié (au niveau du genre, autant que possible) 383 souches durant l'hiver 1971-72 et le printemps 1972. Dans les eaux moins polluées du cours supérieur, le genre Pseudomonas dominait, tandis que dans le cours inférieur les genres Acinetobacter et Aeromonas étaient également communs. Ces trois groupes représentaient aussi les genres principaux trouvés dans l'analyse d'un échantillon d'eaux

d'égout domestique. Dans les deux cours d'eau, on a isolé également un nombre moindre des genres Alcaligenes, Moraxella, Chromobacterium, et de la famille des Enterobacteriaceae. La majorité des souches isolées, reconnues comme étant des psychrophiles par leur croissance à 4C, poussaient également à 30C. Un total de 80 de ces souches poussaient aussi à 37C, parmi lesquelles 16 (tous des Aeromonas) déployaient une forte lipolyse à cette température. Néanmoins, pour la plupart des souches, la plus forte lipolyse était observée à 20C ou moins. Par conséquence, ceci laisse à supposer que les psychrophiles jouent un rôle dégradatif important dans les systèmes aquatiques pollués. En ce qui a trait à la taxonomie, des difficultés se sont présentées dans l'identification des souches isolées, particulièrement au delà du niveau générique. Il apparait donc qu'un manque d'importance a été attribué aux psychrophiles dans la systématique actuelle.

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

Introduction

I) Water Pollution by organic matter

General considerations

Because of their convenience, waterways are widely used for dumping wastes. As a result of the increasing world population and continued expansion of industry, aquatic routes are subjected to a constant input of organic pollutants. The wastes from dairy operations, food-processing and meat-packing plants, slaughterhouses, as well as sewage wastes from large municipalities are common causes of this type of water pollution. The dumping of such wastes is rightfully an area of concern today, when we consider that only 3% of the world's water supply is fresh water, 89% of which is not readily accessible for human consumption (Wollman, 1962). In the future, localized water shortages may occur, especially in densely populated areas where industry requires enormous quantities of water for its operation. This problem is compounded because the very areas requiring the most water are also the greatest sources of pollution. As the demand for water increases, reutilization of waste waters will have to be considered if needs are to be met (Tallon, 1969). Since organic wastes represent one of the major forms of pollution today, knowledge of the microbiological forces at work in degrading such compounds will yield information as to the extent to which autopurification in aquatic systems may proceed.

Sewage composition: Its general effects on aquatic systems.

Organic pollutants consist principally of animal excreta and plant and animal refuse. Carbohydrates, fats, and proteins are the major classes of organic wastes constituting sewage (Painter and Viney, 1959), although specific wastes tend to vary in composition depending upon their origin (Dugan, 1972; Albritton, 1952). Nucleic acids, vitamins, hormones, electrolytes, minerals, and other miscellaneous organic compounds excreted by man also enter sewage systems (Albritton, 1952).

Hynes (1966) lists five major effects of sewage effluents:

1) Addition of poisonous substances

Metals and toxic organic compounds, present in concentrations which will kill saprophytic bacteria involved in biological oxidation of sewage, impede the re-purification of the river. In time, however, dilution of such toxins may allow normal biological oxidation to proceed.

2) Addition of suspended solids

The rate of settling of suspended solids depends on the density, size of the particles, and turbulence of the water. Since a majority of the bacteria in rivers seem to be attached to suspended matter (Wuhrmann, 1963), organic degradation will be enhanced by a rapid flow since oxidation can proceed in an aerobic environment. Stagnant conditions will increase the rate of settling and anaerobic conditions

created in the river bed will diminish aerobic degradation of pollutants.

3) De-oxygenation

Biological oxidation of organic pollutants results in de-oxygenation of an aquatic system. Re-oxygenation depends on factors such as flow rate, turbulence, depth of the water, and photosynthesis. Oils and detergents present at the water surface also reduce the rate of re-oxygenation and slow down the re-purification process.

4) Addition of non-toxic salts

NaCl, universally present in sewage, may have much the same effect as toxic poisons but is usually reduced in concentration by dilution. On the other hand, nutrient salts (phosphates, nitrates, etc) which can be products of organic degradation, may increase plant growth and have important biological consequences on the river flora.

5) Heating of the water

Sewage water is usually warmer than that of the receiving waterbody. As a result of inflowing sewage, water temperatures of aquatic systems may remain elevated for many miles downstream. This will enhance the effects of de-oxygenation.

Hynes (1966) concludes that river purification can still proceed if the action of these 5 sewage effects is moderate, and the river is long and large enough to let dilution, time and degradative processes normalize conditions once more. However, the influence of such diverse factors is, in most cases, beyond calculation.

Role of heterotrophic microorganisms in relation to organic pollution.

Self-purification must necessarily start with the degradation of organic pollutants by the oxidative processes of aerobic heterotrophic microorganisms. As Alexander (1971) states, this mineralization process, or the conversion of an organic form of an element to the inorganic state, is a means whereby microorganisms decrease the biochemical complexity of the ecosystem. It is also a process on which other forms of life, namely, autotrophs, depend for their own life processes. This latter process, called immobilization (the conversion of an inorganic nutrient element to an organic complex) completes this cycle.

Henrici (1938) and Stark and McCoy (1938) were the first to show, by means of plate counts, that an increased concentration in organic matter in water caused a corresponding increase in heterotrophic bacterial counts. Later, Silvey and Roach (1964) studied the annual microbiotic cycles of blue-green algae and gram-negative heterotrophic bacteria in Southwestern Reservoir lakes (USA). Their results showed that, as algal blooms began to disintegrate, the gram-negative heterotrophic bacterial population immediately began to increase and that the mineralization process was taking place. Such cycles, if studied in polluted systems, could cause considerable confusion in the interpretation of experimental results.

Silvey and Roach, as well as showing the mineralization role of heterotrophic bacteria at the expense of the blue-green algae, showed the continuity of such a cycle, as by-products formed by the metabolizing heterotrophs were in turn used by other forms of life. A chemo-organotrophic Caulobacter sp. recently isolated from a fresh-water pond by De Bont, Staley, and Pankratz (1970) exhibited this nutrient cycling principle. Its appearance in enrichment cultures was always delayed, indicating to these investigators that it probably utilized the excreted by-products of other heterotrophic microorganisms which grew in the enrichment culture broth.

Recently, Kuznetsov (1968) studied the role of micro-organisms in nutrient cycling in lakes and reservoirs (USSR). He found that in reservoirs supplied with allochthonous organic matter from bogs and water-sheds, bacterial biomass was significantly higher than phytoplankton biomass. In fact, the destruction of organic matter in most lakes exceeded its synthesis. Kuznetsov also showed a direct relationship between destruction of organic matter and total number of bacteria. As organic matter increased, so did total number of bacteria, and vice-versa. In sewage plants providing primary and secondary treatment 85% or more of the organic load may be removed due to the action of aerobic heterotrophic bacteria, with microorganisms in the receiving water-body continuing the purification process (Tallon, 1969).

Biological degradation of organic matter, and hence of organic pollutants, is an essential function of heterotrophic microorganisms. Such microbes, however, are not the cure-all for water purification, as intermediate metabolic products and others such as lignins, tannins, pesticides, and humic acid* substances may be resistant to further breakdown and may require special treatment processes (Tallon, 1969). Nevertheless, the bulk of organic breakdown is performed by these heterotrophs and, for this role, they are indispensable.

The following sections summarize certain quantitative and qualitative aspects of this area of knowledge in respect to degradative aerobic heterotrophs.

II) Aerobic heterotrophic bacteria

Populations in unpolluted water

Up to the year 1940 most of the work in microbial ecology had been done on freshwater lakes in Europe and North America. These early investigations (reviewed by Collins, 1963) were essentially quantitative plate count studies of the vertical and horizontal distribution of bacteria in lakes, as influenced by seasonal and other physical factors. Similar studies were undertaken in the 1930's when direct total count techniques were developed (Collins, 1963). The investigations,

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* Humic acid substances are large-molecule organic acids that are partially broken down. They exist in a colloidal state and readily dissolve in water (Ruttner, 1970).

mentioned earlier, by Henrici (1938) and Stark and McCoy (1938) showed that the concentration of organic matter was an important factor accounting for greater numbers of bacteria.

The first serious attempt to classify freshwater bacteria was undertaken by Taylor (1942). In his study, 800 cultures of bacteria were isolated from samples taken from lakes and some of their inflowing waters in the Lake District of north-west England. The Gram reaction, glucose oxidation, gelatin liquefaction, nitrate reduction and litmus milk reactions were recorded for all cultures. From this work, Taylor noted the following: 1) Almost all the isolates (95%) were gram-negative rods; 2) Chromogenesis was observed for most colonies on sodium caseinate agar; 3) The physiological activity of most cultures was weak, though gelatin liquefaction was fairly common. No striking physiological differences were noted between cultures isolated from the unpolluted Thirlmere lake with those from the other lakes and inflows which were subject to both animal and human pollution.

Of the genera widely distributed in lake water, Collins (1963) reported that the following formed the greatest proportion of the population: Pseudomonas, Achromobacter, Alcaligenes, Chromobacterium, Flavobacterium and Micrococcus. Rodina and Kuz'mitskaya (1964), recognizing the part played by heterotrophic bacteria in the conversion of organic matter, identified 248 bacterial isolates from Lake Lagoda (a large Russian oligotrophic lake). The results of their clas-

sification showed the following genera to be present among their isolates: 1) Non sporing rods; Pseudomonas, Chromobacterium, Mycobacterium, Bacterium*, and Pseudobacterium*; 2) Sporing rods; Bacillus; 3) Cocci; Micrococcus, Sarcina.

Silvey and Roach (1964) studied the cycles of blue-green algae and gram-negative heterotrophic bacteria in pollution-free Southwestern Reservoir lakes (U.S.A.). They found the maximum numbers of bacteria during the summer months of the order of 7×10^4/ml, characterized largely by the following genera: Alcaligenes, Aerobacter and Pseudomonas.

Hoadley and McCoy (1966) used selective media for the isolation of pseudomonads from unpolluted and polluted stations in a 6-mile creek flowing into Lake Mendota (Wisconsin). They found three different types of pseudomonads at their unpolluted station; the mean bacterial count was 6,960 per ml obtained from total plate counts. The characteristics of the three types isolated are reproduced in the table below:

Types of pseudomonads isolated by Hoadley and McCoy (1966) from 6-mile creek

Type	Gram reaction	Glucose oxidation	Oxidase	Pigmentation		Growth at	
				Pyocyanin	Fluorescein	37C	42C
A	-	+	-	-	+	-	-
B	-	+	-	purple pigment	+	-	-
F	-	+	+	-	+	-	-

The status of these three types in the polluted section of the 6-mile creek is discussed in the next section.

* These genera are not recognized by Bergey's Manual: Rodina and Kuz'mitskaya (1964) identified them from Krasil'nikov's

Baumann (1968) has reported the ubiquitous occurrence of Acinetobacter in ponds, lakes, rivers and creeks of central California. Members of this genus comprised at least 0.001% of the total heterotrophic population in water and in some cases were the predominant organisms. In view of their nutritional versatility (previously shown by Baumann, Doudoroff and Stanier, 1968b), this author inferred that acinetobacters probably play important roles in the aerobic mineralization of organic matter.

Recently, Bell, Hoskins and Hodgkiss (1971) studied the bacterial flora associated with the surface of stream-incubating salmon eggs of the Qualicum River (B.C.). They isolated 18 heterotrophic species, which were representatives of the 7 following genera: Pseudomonas, Aerobacter, Chromobacterium, Actinomyces, Acinetobacter, Aeromonas and Cytophaga. In this relatively unpolluted river, total bacteria, as determined from plate counts, never exceeded 10^3 per ml.

Heterotrophic bacteria in polluted waters

The bulk of the qualitative and quantitative work on aerobic heterotrophs in polluted systems has been done on those populations endemic to processes of sewage treatment plants, such as activated sludge. The particulate matter and clumped or individual bacterial cells composing activated sludge (Pipes, 1967) are constantly utilized as an active inoculum of microorganisms in the biological treatment of sewage wastes (Dugan, 1972).

Some of the more recent quantitative studies performed in sewage treatment plants have shown that the number of aerobic heterotrophs in activated sludge is between 10^6 to 10^9 bacteria per ml (Prakasam and Dondero, 1967; Gayford and Richards,1970; Benedict and Carlson, 1971). In raw sewage the number is in the order of 10^6 - 10^7 bacteria per ml (Prakasam and Dondero, 1967). Qualitative studies of activated sludge are common but further work is necessary because of differences in the systems studied and techniques employed (Prakasam and Dondero, 1967). One of the more comprehensive studies on activated sludge was performed by Dias and Bhat, 1964. The dominant bacteria were representatives of the following genera: Achromobacter, Aerobacter, Alcaligenes, Bacillus, Brevibacterium, Corynebacterium, Comamonas, Flavobacterium, Micrococcus, Pseudomonas, Spirillum and Zooglea. Of their more than 300 isolates, an average of 88.1% were found to be gram-negative. Other workers have also found these genera in activated sludge (reviewed by Benedict and Carlson, 1971). Benedict and Carlson (1971) found representatives of the genera Acinetobacter, Caulobacter, Cytophaga, Hyphomicrobium, Micrococcus, bacterium, and Sphaerotilus as well. Out of 111 isolates identified by these investigators, 63% were gram-negative.

Aside from the studies on the heterotrophic microorganisms involved in sewage treatment plant processes, few investigations have been conducted on polluted aquatic systems as such. Several investigators have indirectly noted activities

of aerobic heterotrophic bacteria by comparing direct total counts (a) to plate counts (b) (Beling and Jannasch 1955; Jannasch, 1958; Collins, 1963). An a/b ratio in the order of tens of thousands was characteristic of water low in organic matter; for more polluted water the a/b value was often less than a hundred and sometimes less than ten. Ruttner (1970) has summarized the findings of several investigators and states that the higher a/b ratios obtained in non-polluted aquatic systems indicate that true water bacteria (autotrophs) do not thrive on a peptone substrate (present in plate count media), whereas degrading heterotrophs dominant in polluted waters do. In Beling and Jannasch's (1955) study of polluted sections of the Fulda river (Germany) a/b ratios ranged from 26.7 to 58.8. Similar calculations, based on Razumov and Korsh's (1961) data from various water reservoirs (USSR), indicate that as organic pollution increases, heterotrophic bacteria increase and the a/b ratio decreases. Examination of Collin's (1963) data from English lakes yields ratios ranging from 6 to 11,278 depending on the degree of organic pollution. However, Jannasch (1958) found that in exceptionally clean waters, such as continually flowing brooks and springs the plate count exceeded the direct total count. This was accounted for by clumping effects and the occurrence of very small bacterial forms not visible on the membrane filter surfaces. In all other water types studied, the total direct count was always much higher. Morphological studies of colonies from culture plates and of bac-

terial forms present on membranes used for direct counts showed a much higher % of rods (gram-negative) on the culture plates than on the membranes.

This led Jannasch to conclude that the gram-negative heterotrophs were the major groups involved in organic degradation. Korsh (1961) confirmed this when morphological studies of membrane filters showed that in clean reservoirs coccoid forms of bacteria predominated (60-65% of the total number), while in polluted reservoirs rod-shaped forms were dominant.

Qualitative investigations of bacteria in polluted aquatic systems are not common. Dias and Bhat (1964) isolated 150 sewage bacteria which they compared to their activated sludge isolates, described previously. These sewage isolates were not generically identified, but they reported that 25% were coliforms on the basis of gas production from glucose fermentation. On this basis and in view of the fact that 123 strains produced acid from glucose breakdown, Dias and Bhat concluded that they differed vastly from their sludge isolates.

Hoadley and McCoy (1966) isolated 3 types of pseudomonads from unpolluted and polluted sections of a 6-mile creek flowing into Lake Mendota (Wisconsin). These authors found an increase in all three types immediately after a major unchlorinated sewage treatment plant effluent. At the next station downstream from the sewage effluent, they found a sharp decrease in types A and B, but not in type F. Hoadley and McCoy

suggested that type F pseudomonads were, in fact, multiplying in the creek waters on organic waste substrates. They concluded that the type F pseudomonads might be potentially useful as a means of detecting sewage contamination of waterways. Druce and Thomas (1970) classified 232 psychrophilic strains as well as 1,096 mesophilic strains of bacteria isolated from untreated dairy farm water supplies in Wales. Representatives of the genera Micrococcus, Bacillus, Pseudomonas, Achromobacter, Alcaligenes, Acinetobacter, Flavobacterium, Chromobacterium, Serratia, Enterobacteriaceae and unclassified gram-positive nonsporeforming rods were found among their isolates. In all, the gram-negative rods constituted 93% of these isolates. Recently, Warskow and Juni (1972) isolated 56 Acinetobacter strains from various rivers, ponds and swamps, as well as 26 Acinetobacter strains from sewage. These authors showed that the ubiquity of this genus in aquatic systems, coupled with its nutritional versatility, makes it an important organism in the cycling of organic matter in nature.

III) Degradative heterotrophic bacteria

Lipolytic and proteolytic heterotrophs

Studies of self-purification of natural aquatic habitats are practically nonexistent, though some information has been obtained in model experiments with artificial water channels. Wuhrmann (1972) described such an experiment in which a preset biocenosis* was developed in an artificial flowing water

* biocenosis: "a community of organisms whose composition

channel to which known quantities of bio-degradable substrates (various carbohydrates) were added. In the experiment, Sphaerotilus (a heterotrophic bacteria), Fusarium (a fungus), Diatoma hiemale (a diatom), and Cladophora (a green algae) were "seeded" in the artificial water channel and represented the biocenosis. The results showed that all of the added substrates were metabolized almost entirely by Sphaerotilus. Degradation by the other organisms was found to be insignificant.

Although the application of such model experiments to natural conditions is not yet possible, Wuhrmann advocates that a thorough knowledge of the type and quantity of biomass involved in metabolizing pollutants is a first step in the right direction. Since organic analyses of solids in raw sewage have shown that fats (34.4% of the organic composition of sewage) and proteinaceous substances (27.1%) of the organic composition of sewage) are the major constituents (Dugan, 1972), it is likely that lipolytic and proteolytic microorganisms will play a major role in their degradation. Fats and proteins are degraded by hydrolases (lipases and proteinases) of many species (see Table 1) and the lower molecular weight compounds produced can then serve as energy-yielding organic nutrients for the growth and development of most heterotrophic organisms (Dugan, 1972). Of the 23 bacterial genera listed in Table 1, 14 are known to contain lipolytic species, while 17 contain proteolytic species. Eleven genera also contain species known to display both pro-

perties. The fact that the majority of these genera contain lipolytic and proteolytic types is undoubtedly indicative of the importance of fat and protein breakdown in aquatic habitats.

Wuhrmann (1963) reported that more than 99% of the total bacteria in a moderately turbid river were attached to the suspended matter present in its waters. Since microbial hydrolases are predominantly extracellular (cell-surface) enzymes (Dugan, 1972) it is natural to expect bacteria to be concentrated around usable substrates.

Psychrophilic heterotrophic bacteria

Psychrophilic (cold-loving) bacteria have been designated by a variety of names (psychro-cartericus; cold-conquering: psychrotolerant; cold-tolerant: psychrotrophic; cold-thriving) since their discovery at the turn of this century (reviewed by Stokes, 1963). All definitions were based on minimum, optimum or maximum temperatures for growth. Stokes (1963) proposed the retention of the term "psychrophile" because of its long and wide usage, and defined psychrophiles as microorganisms which grow rapidly enough at 0C to become macroscopically visible in about one week regardless of temperature optima or maxima. Another recent definition is that psychrophiles are microorganisms capable of growth at temperatures below 5C (Keogh, 1968).

Table 1

References referring to proteolytic and lipolytic properties of aerobic heterotrophic bacteria found in unpolluted and polluted waters (from Section II)

Bacterial types and genera	Lipolysis	Reference source	Proteolysis	Reference source
Gram-negative rods				
Acinetobacter	+	Baumann et al. (1968b)	-	Baumann et al. (1968b)
Aerobacter (enterobacteriaceae)	+	Davis and Ewing (1964) Lentsner, Toom and Tammaru (1967)	+	Skerman (1967)
Aeromonas	+	Chakrabarty, Adhya and Pramanik (1970)	+	Kazanas (1968)
Alcaligenes	+	Fryer, Lawrence and Reiter (1967)	+	Kazanas (1968)
Caulobacter	*		-	Bergey (1957)
Chromobacterium	+	Breuil and Gounot (1972)	+	Skerman (1967)
Comamonas			+	Skerman (1967)
Cytophaga	+	Skerman (1967) Bell et al. (1972) McCurdy (1969)	+	Mitchell, Hendrie and Shewan (1969) Skerman (1967) McCurdy (1969)
Flavobacterium	+	Breuil and Gounot (1972) Dempster (1968)	+	Kazanas (1968)

Pseudomonas	+	Stanier, Palleroni and Doudoroff (1966) Breuil and Gounot (1972)	+	Kazanas (1968) Martley, Jayashankar, and Lawrence (1970)
Serratia (enterobacteriaceae)	+	Davis and Ewing (1964) Lentsner et al. (1967)	+	Skerman (1967)
Sphaerotilus			+	Bergey (1957)
Spirillum			+	Bergey (1957)
Zooglea				
Gram-positive rods				
Actinomyces	+	Breuil and Gounot (1972)	-	Skerman (1967)
Bacillus	+	Sierra (1957)	+	Kazanas (1968)
Brevibacterium			+	Skerman (1967) Kazanas (1968)
Corynebacterium	+	El Sadek and Richards (1957) Dempster (1968)	+	Skerman (1967) Kazanas (1968)
Microbacterium	-	Skerman (1967)	+	Kazanas (1968)
Mycobacterium	+	Lubert, Smith and Thornton (1949) Sierra (1957)	-	Skerman (1967)
Gram-negative cocci				
Hyphomicrobium			-	Bergey (1957) Skerman (1967)

Gram-positive cocci				
Micrococcus	+	Sierra (1957) Fryer et al. (1967) Dempster (1968)	+	Kazanas (1968) Martley et al. (1970)
Sarcina			+	Kazanas (1968)

* The absence of a + or - sign indicates that reference to lipolysis or proteolytis for a particular genus has not been found in our search of the literature.

Psychrophilic bacteria have been extensively studied in the food and dairy industries because of their spoilage role (Ayres, 1955; Witter, 1961; Dempster, 1968; Panes and Thomas, 1968; Eller, 1969). All such studies have demonstrated that psychrophiles were predominantly gram-negative rods. Table 2 shows the various genera containing psychrophiles which have been reported in the literature. Although members of several gram-positive genera have been shown to be psychrophilic, they have always been found in relatively small numbers. Moreover, several investigations (reported by Witter, 1961) have shown that members of the genus Pseudomonas are the dominant spoilage organisms in dairy products, and in frozen meat, fish, poultry and egg products.

In contrast to the extensive studies of psychrophiles in the food and dairy industries, data on their incidence in aquatic habitats are limited. Stokes and Redmond (1966) showed the ubiquity and numerical importance of psychrophilic bacteria in aquatic systems. They found that psychrophiles constituted a major fraction of the bacterial population: 16 to 47% in stream and river water; 41 to 76% in lake water. On this basis, they concluded that psychrophiles may be important in the cycling of matter in water. Druce and Thomas (1970), in a study of untreated dairy farm water supplies in Wales, found that nearly 50% of the total bacterial population was composed of psychrophiles. Their qualitative findings are discussed further on. A study by Larkin (1970) on the incidence of psychrophiles in lakes, rivers, and bayous of a

Table 2

Psychrophilic genera and references to some investigators who have reported their presence in: 1) food and dairy products and 2) in aquatic habitats.

Genus	Isolated from food and dairy products	Isolated from aquatic habitats
Gram-negative rods		
Acinetobacter	Witter (1961) Thornley (1967) Dempster (1968) Eller (1969)	Baumann et al. (1968b) Druce and Thomas (1970) Bell et al. (1971)*
Aeromonas	Eddy and Kitchell (1959) Witter (1961) Dempster (1968)	Bell et al. (1971)*
Alcaligenes	Witter (1961) Stokes (1963) Panes and Thomas (1968)	Druce and Thomas (1970)
Arthrobacter	Witter (1961)	Breuil and Gounot (1972)
Caulobacter		DeBont et al. (1970)
Chromobacterium	Ayres (1955) Witter (1961)	Druce and Thomas (1970) Bell et al. (1971)* Breuil and Gounot (1972)
Cytophaga		Bell et al. (1971)*
Enterobacteriaceae	Witter (1961) Stokes (1963) Dempster (1968) Panes and Thomas (1968)	Druce and Thomas (1970) Bell et al. (1971)*
Flavobacterium	Witter (1961) Stokes (1963) Dempster (1968)	Druce and Thomas (1970) Breuil and Gounot (1972)
Moraxella		Bell et al. (1971)*

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Arthrobacter	Witter (1961)	Breuil and Gounot (1972)
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Cytophaga		Bell et al. (1971)*
Enterobacteriaceae	Witter (1961) Stokes (1963) Dempster (1968) Panes and Thomas (1968)	Druce and Thomas (1970) Bell et al. (1971)*
Flavobacterium	Witter (1961) Stokes (1963) Dempster (1968)	Druce and Thomas (1970) Breuil and Gounot (1972)
Moraxella		Bell et al. (1971)*

Pseudomonas	Witter (1961) Dempster (1968) Panes and Thomas (1968) Eller (1969)	Castel and Garrard (1941) Druce and Thomas (1970) Bell et al. (1971)* Breuil and Gounot (1972)
Vibrio	Witter (1961)	
Gram-positive rods		
Actinomyces	Ayres (1955) Witter (1961)	Bell et al. (1971)* Breuil and Gounot (1972)
Bacillus	Ayres (1955) Witter (1961)	Druce and Thomas (1970)
Brevibacterium	Witter (1961)	
Corynebacterium	Witter (1961) Stokes (1963) Dempster (1968)	
Lactobacillus	Ayres (1955) Witter (1961)	
Microbacterium	Witter (1961)	
Gram-positive cocci		
Diplococcus	Ayres (1955)	
Micrococcus	Witter (1961) Stokes (1963) Dempster (1968)	
Sarcina	Ayres (1955) Witter (1961)	Bell et al. (1971)*
Streptococcus	Ayres (1955) Witter (1961)	

* Bell et al (1971) studied the bacterial flora associated with the surface of stream-incubating pacific salmon eggs in the unpolluted Qualicum river, B.C. Although these authors isolated these strains from 25C-incubated plates, they may, in fact, be psychrophiles, since ambient water temperatures at time of sampling never exceeded 6C.

more temperate climate (Louisiana), showed the complete absence of psychrophilic bacteria during the summer months in this region. During the winter period, however, psychrophiles formed up to 31% of the total bacterial population. Breuil and Gounot (1972), in a survey of various soils and waters, whose temperature at time of sampling did not exceed 8C, found average psychrophilic bacterial counts of 1.4×10^6/ml in relatively polluted sections of the Loire river (France). From 2.7×10^4 (2%) to 8.6×10^5/ml (6%) of these psychrophiles displayed lipolytic properties, indicating their probable role in the degradation of fats in this river.

Qualitative studies of psychrophiles in aquatic habitats have been relatively rare, although there have been several useful investigations. Castell and Garrard (1941), during an extensive bacteriological survey of water supplies across Canada, found that 85% of the well waters from rural areas which were free of intestinal pollution contained proteolytic and lipolytic bacteria able to grow at 4-5C. The great majority of these were reported as belonging to the genus Pseudomonas. Mitchell et al. (1969) have reported the incidence of psychrophilic strains of Cytophaga from waters associated with salmonid fish diseases. DeBont et al. (1970) have recently described a psychrophilic Caulobacter species isolated from a freshwater pond having a growth range from 1C to 30C. Druce and Thomas (1970), mentioned earlier, identified the following psychrophilic genera in their study of untreated farm water supplies: Pseudomonas, Acinetobacter, Alcaligenes,

Flavobacterium, Chromobacterium, several types of Entero-bacteriaceae, Bacillus and Micrococcus. Of their 232 isolates, the first four genera were the most numerous, making up 95.8% of their total isolates.

Bell et al. (1971), in their study of the unpolluted Qualicum river, B.C., isolated 67 bacterial strains, 64 of which were gram-negative. Twenty-eight of these were pseudomonads. Acinetobacter, Aeromonas, Chromobacterium, Enterobacteriaceae, Moraxella, Actinomyces, Cytophaga and Sarcina species were also identified. Possession of lipase was reported for all Pseudomonas, Acinetobacter, Cytophaga and Moraxella isolates, as well as for 1 of 3 Aeromonas species. Even though their strains were isolated from plates incubated at 25C, they were likely to be psychrophiles, since water temperatures at time of sampling never exceeded 6C. Breuil and Gounot (1972), in their water and soil studies, found a predominance of gram-negative rods comprising the lipolytic psychrophilic population, with Pseudomonas species being the most numerous lipolytic group. Other genera were made up of Flavobacterium, Chromobacterium, Arthrobacter and Actinomyces species.

Thus far, quantitative studies have pointed to the ubiquity and numerical importance of psychrophilic bacteria in aquatic habitats, while qualitative studies have consistently shown, that the majority of psychrophiles are gram-negative rods. For example, gram stains performed by Larkin (1970), in his study of water psychrophiles in the Louisiana region,

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showed that this population was exclusively made up of gram-negative rods. Also, the genera commonly isolated generally display proteolytic and lipolytic properties (Table 1) and Pseudomonas species often predominate.

Whereas most psychrophilic bacteria are generally capable of growth to 30C (Werkman and Wilson, 1951; Stokes, 1963), their mesophilic counterparts usually show a minimal growth temperature of about 10C (Witter, 1961; Stokes, 1963). Since waters, especially in Canada, are below 10C during much of the year, the bulk of biodegradation of pollutants in aquatic systems must necessarily be performed by psychrophiles. Although data on enzymatic activities of psychrophiles are scarce, a few studies have indicated the important role the latter may play in the cycling of matter in water. Nashif and Nelson (1953a) demonstrated that the lipase of Pseudomonas fragi was preferentially produced at low temperatures and none was produced when this organism was grown at 30C or higher. The same authors (1953b) showed that optimum temperature for lipase production by several species of the genus Pseudomonas was 21C or below. Breuil and Gounot (1972) studied the lipolytic activity on different lipid substrates of 161 lipolytic psychrophiles isolated from various soils and waters. They found no significant differences in activity at 4C and 20C. Peterson and Gunderson (1960) studied the proteolytic enzymes from Pseudomonas fluorescens. They reported that the liberation of the extracellular proteolytic enzymes was greatest at 0C and decreased with increasing

Wuhrmann (1972) has stated that an increased metabolic rate due to increasing temperature does not necessarily mean that self-purification will be greater in summer. As he points cut, water flow is at a minimum during the cold season with the result that the biomass actively engaged in metabolizing pollutants has the advantage of a longer contact time with the polluting material. Oxygen levels are also highest at cold temperatures thus favoring biodegradation by aerobic heterotrophs. Further investigations of the characteristics, production and activity of enzyme systems from psychrophilic bacteria will undoubtedly contribute to the better understanding of the roles which psychrophiles play in nature.

IV) Assessment of bacterial pathogens in water

An important aspect of water pollution is the potential health hazard conditions created by the presence of microbial pathogens. Wastes from municipal, meat-packing, dairy plant, and storm water run-off discharges form the major part of domestic sewage in many cities. Dugan (1972) states that microorganisms account for nearly 90% of the mass of fecal discharges and for this reason represent a major component of domestic sewage. As a result of these concentrations of fecal wastes, bacterial pathogens derived from humans, farm animals (livestock, poultry, animal pets) and wild animals are found in raw sewage (Geldreich, 1972). Some of the most common bacterial pathogens present in domestic

wastes include representatives of the following genera: Salmonella, Shigella, Leptospira, enteropathogenic Escherichia coli, Pasteurella, Vibrio and Mycobacterium, all of which cause serious, if not fatal, illnesses (Geldreich, 1972).

Unfortunately, the presence of such pathogens in contaminated waters is seldom determined directly. Their small numbers, the time-consuming procedures involved in identifying them, and their usually rapid die-off make the direct search for these organisms impractical for routine purposes (Grabow, 1970). A further impediment lies in the fact that methods of identification are too long and tedious to be performed on a routine basis (Geldreich, 1972). It is for these reasons that bacteriological water quality is assayed by indirect means through the use of so-called indicator bacteria. Total coliform, fecal coliform, and fecal streptococci counts are the most widely used indicators of fecal pollution today (Standard Methods, 1971). The finding of such bacteria in water is indicative of recent fecal pollution and of a potentially dangerous health hazard, since they may well indicate the presence of intestinal pathogens whose origins are identical to those of the indicator bacteria (Grabow, 1970).

While standards assigned for these indicator bacteria are uniform for drinking purposes (0 total coliforms, fecal coliforms, and fecal streptococci per 100 ml of water) (Grabow, 1972), those assigned for recreational purposes

(swimming, bathing, boating; water discharges into streams and surf zones; water industrial uses) show a great deal of variance (Gallagher and Spino, 1968; Foster, Hanes and Lord, 1970; Grabow, 1970) and have, for the most part, been assigned arbitrarily. Grabow (1970) states that the bacterial flora of water is determined by so many factors which vary from one source of supply to another that it is impossible to lay down specific standards for waters as a whole. Grabow suggests, to compensate for this, the establishment of a set standard for each individual water body, where departure from normalcy in indicator counts can be at once viewed with suspicion. Foster et al. (1970) reported the great variation in standards set in different states in the U.S.A. as well as the disagreement as to the technique to be used in obtaining such indicator counts.

One of the major problems associated with the setting of standards has been the lack of epidemiological proof for such criteria, a fact deplored by various authors (Gallagher and Spino, 1968; Foster et al. 1970; Geldreich, 1972). Gallagher and Spino (1968) tried to ascertain whether there was any level of total and fecal coliforms below which the probability of isolating Salmonella was negligible, by comparing coliform data with Salmonella data gathered in stream surveys throughout the United States. Their results showed little apparent correlation between levels of total or fecal coliforms and the isolation of Salmonella. For example, at one of their sampling stations located below a re-

creation area, the mean total coliform count was 800/100 ml, the fecal coliform count was 20/100 ml, and S. montevideo and S. muenchen were isolated. At another station (Richland Creek), the mean total coliform count was 16,000/100 ml, the mean fecal coliform count was 3,100/100 ml, yet no Salmonella species were isolated. Despite their obvious shortcomings, these indicator bacteria in water confirm the presence of some degree of fecal pollution depending on counts obtained, and serve as a warning that enteric pathogens may also be present. In recent years, the increased findings of enteropathogenic Escherichia coli serotypes warrants the continued utilisation of these indicator groups for water quality control (Grabow, 1970; Geldreich, 1972).

Recent studies by Geldreich and Kenner (1969) have shown that fecal coliform (FC) to fecal streptococci (FS) ratios could be applied to distinguish between animal and human fecal pollution. These authors conducted an intensive study of the occurrence of fecal streptococci and fecal coliform densities for humans and various types of warm-blooded animals. Analysis of their comparative data indicated that fecal streptococcus densities were significantly higher than fecal coliform densities in all warm-blooded animal feces examined except for that of humans. In human feces, the FC/FS ratio was 4.4 (median value) or higher, whereas ratios for fecal discharges from all other warm-blooded animals were 0.7 or less. All domestic wastewater samples showed ratios of 4.4 or greater. Samples taken from storm-waters, canneries

(manufacturing sauerkraut and green beans), meat-packing plants, dairy plants and private wells showed one of 0.7 or less. Sugar mills, prairie watersheds, irrigation waters, recreational waters as well as public water intakes showed ratios ranging from <0.7 to >4.4.

Besides the coliform indicator groups, Clostridium perfringens has also been considered as an indicator of fecal pollution. Its spore-forming capacity, which allows it to survive a long time in water, is of value in determining pollution that has occurred at one time in the past, if its presence in water is found without subsequent findings of coliform counts (Grabow, 1970). Another gram-positive anaerobic intestinal dweller, considered by some to have value in indicating fecal pollution, is Lactobacillus bifidus (Grabow, 1970). Finnish investigators (Gyllenberg, Niemella and Sormunen, 1960) had shown the specificity of this organism for the animal and human intestinal tract, where it outnumbered the coliform and streptococcus groups by 10 to 100 times. Unlike the coliforms and streptococci which showed initial multiplication after storage in water, the bifidus group die-off rates were constant, indicating that this group of bacteria would be useful in assessing recent fecal contamination of water. The lack of mention of these two anaerobic indicators in Standard Methods (1971) suggests that efforts to include them as routine indicators of fecal pollution have not met with any success in this continent.

Another waterborne bacteria of known pathogenicity, which has been considered as an adjunct to the coliform tests is Pseudomonas aeruginosa. Selenka, and Ruschke (1965) have reported finding 33,000 P. aeruginosa per ml in sewage from the city of Uberlingen (Population 11,000), Germany; this sewage contained hospital and slaughterhouse wastes, as well as domestic sewage.

The presence of P. aeruginosa in water and feces, coupled with the knowledge that it causes ear, urinary, and other types of infections has prompted many investigators to recommend its detection on a routine basis (Bonde, 1966; Hoadley and McCoy, 1966; Wolf, 1972). The inclusion of a section on tentative techniques for the enumeration of this bacteria in Standard Methods (1971) with regards to swimming pools and bathing-place waters is indicative of the growing concern shown towards this pathogen.

Surprisingly, counts of the genus Acinetobacter, a known pathogen, have not, as yet, been included in routine analyses for the evaluation of water quality. Most investigators have been conducting tests to clarify the taxonomic position of this genus (DeLey, Bain and Shewan, 1967; Baumann et al. 1968b; Thornley, 1968; Gilardi, 1969; Breuillard, Michel and Lemoine, 1970). Because of the wide divergence of opinions concerning the taxonomy of the genus Acinetobacter*, clinical bacteriologists are faced with classification problems in properly

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* The genus Acinetobacter in this thesis is defined according to the recommendation of Baumann et al. 1968b: Non-motile. Gram-negative rod. Aerobic. Catalase-positive. Cytochrome

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identifying pathogenic species of this group (Leduc et al. 1969). Acinetobacter strains have been isolated from the gastrointestinal, genital, and respiratory tracts of humans (Daly, Postic and Kass, 1962), from the human skin (Taplin, Rebell and Zaias, 1963), and from almost every fluid and organ system of the human body (Robinson, Garrison and Brown, 1964). These strains were associated with sepsis, meningitis, gastroenteritis, pneumonitis, post-operative empyema, bronchitis, cerebral abscesses, bacterial endocarditis, chronic synovitis, bacteremia, osteomyelitis, wound infections and skin lesions (Enjalbert, Brisou and Kambou, 1959; Daly et al. 1962; Taplin et al. 1963; Mandel, Wright and McKinnon, 1964). In fact, most taxonomic work on this genus was performed utilizing organisms isolated from clinical specimens (Baumann et al. 1968b; Gilardi, 1969; Breuillard et al. 1970; Pintér and Kántor, 1971; Bergogne et al. 1971). The increasing resistance to various drugs by this organism has also been noted by several investigators (Breuillard et al. 1970; Pintér and Kántor, 1971; Bergogne et al. 1971). The ubiquitous occurrence of Acinetobacter from a variety of waters (Baumann, 1968) and the recent isolation of Acinetobacter strains in sewage by Warskow and Juni (1972) demands that such organisms be accounted for in assessing water quality in view of the serious potential health hazards which they present.

The lack of epidemiological evidence for standards assigned to the coliform group of indicators (total coliforms,

fecal coliforms, and fecal streptococci) coupled with the various other types of bacteria associated with fecal pollution and/or pathogenicity for which no standards have as of yet been proposed, indicates some of the problems which remain to be solved in the field of water quality assessment.

STATEMENT OF PROBLEM

Water is widely used for the disposal of organic pollutants. Because adequate waste treatment is lacking in many areas, organic pollution is frequently caused by wastes from domestic sewage, food-processing plants, meat-packing plants, slaughterhouses, and hospitals. Since many of the bacteria introduced by these wastes may threaten health, or the aesthetic and recreational qualities of the environment, knowledge of their numbers, types, and activities in polluted systems is essential.

Brewery Creek, allegedly polluted with domestic sewage and meat-packing plant wastes, was chosen as the site for our investigations. To obtain an estimate of the degree of organic pollution, counts of pollution indicator bacterial groups were taken for a period of approximately 1½ years (Jan. 5, 1971 to May 1, 1972).

Since fats and proteinaceous substances are the major constituents of organic wastes in sewage, we also determined numbers of lipolytic and proteolytic bacteria present in Brewery Creek during this period. At present, little is known about the importance, types, and activities of these degradative populations in aquatic systems. Whether indigenous to the water, or introduced in wastes, these bacteria may play important roles in degrading the lipid and protein components of the wastes, and thus contribute to self-purification of the water.

Since water temperatures of aquatic systems, in this country especially, seldom exceed 20C at the warmest period of the year, psychrophilic species are likely to perform the bulk of this degradation. To obtain some knowledge of these important bacteria, we also conducted an intensive quantitative and qualitative study of psychrophilic bacteria exhibiting lipolysis. Isolates were classified, where possible to the generic level, and temperature optima determined for both growth and lipolysis.

Materials and Methods

Sampling

Weekly triplicate water samples were taken below the bridge at Wright Street (upstream) and below that at Fournier Blvd. (downstream), Fig. 1, from January 20th, 1971, to November 23rd, 1971. After this date, sampling was continued on a bi-monthly basis until May 1, 1972. At both stations, samples were taken at mid-depth (approximately 1 meter down at Wright; 1.5 meter down at Fournier) where flow of water was swiftest, using a Meyer sampling bottle, as described and illustrated in Ruttner (1970). Water temperature was immediately recorded with a centigrade thermometer after each sample was drawn from the water. During the period when the water froze over, Fig. 2, the ice was broken with an axe at the same locations and sampling proceeded in the usual manner. All samples were analyzed within 2 hours of collection. On July 12, 1971 and July 17, 1972, triplicate samples were taken at 7 different sites along Brewery Creek (Fig. 1) to attempt to find the site(s) on the creek where major waste dumpings occurred. In addition to the regular sampling sites described above, occasional samples were taken from Lemieux Island, l'Escale, and the Ottawa Rowing Club on the Ottawa River (Fig. 1) during the spring, summer, and fall of 1971.

Fig. 1 Brewery Creek, showing the location of the various sampling stations: 1) Wright St. (upstream station; 2) Montcalm St.; 3) Railway bridge; 4) Montclair St.; 5) after rapids; 6) Fournier Blvd. (downstream station); 7) mouth of Brewery Creek; 8) Lemieux Island station; 9) l'Escale station; 10) Ottawa Rowing Club station. MPP - meat packing plant. S-major sewage effluent pipe from which samples were obtained.

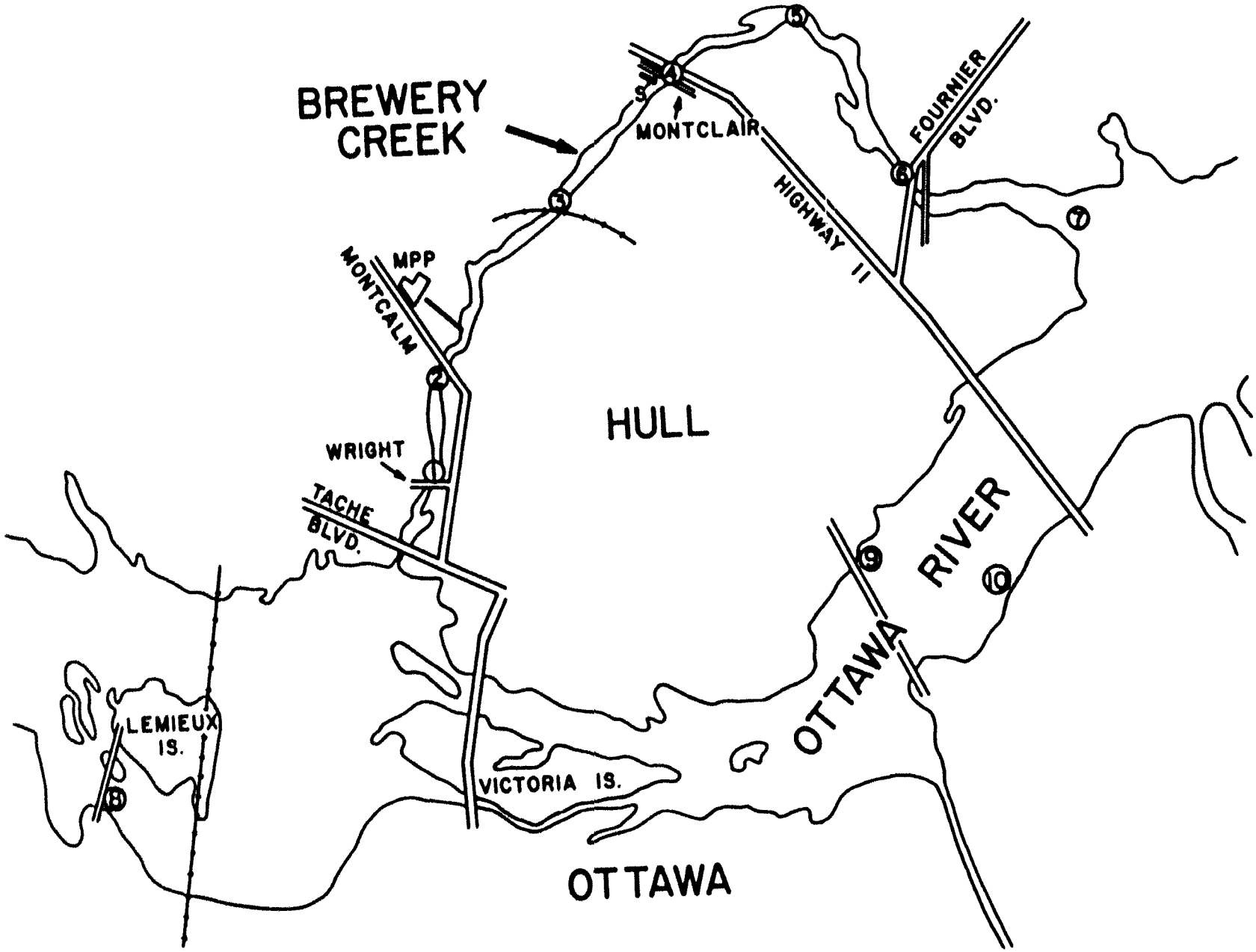
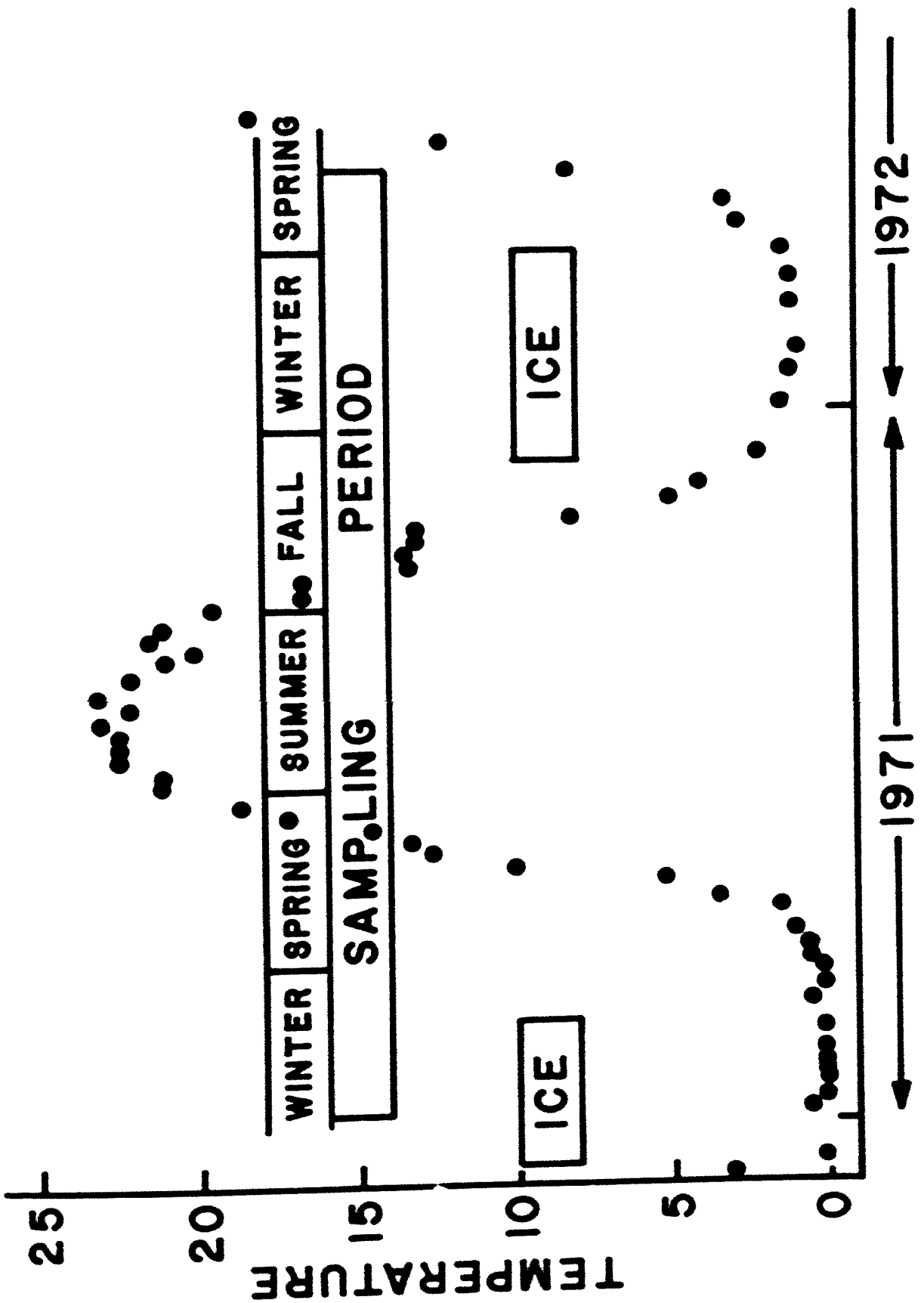

BREWERY CREEK
MONTCLAIR
FOURNIER BLVD.
HIGHWAY 11
MPP
MONTCALM
HULL
WRIGHT
TACHE BLVD.
OTTAWA RIVER
LEMIEUX IS.
VICTORIA IS.
OTTAWA

Fig. 2 Extent of ice coverage and mean water temperatures at the upstream (Wright St.) and downstream (Fournier Blvd.) sampling stations during the 1971-72 study period.


TEMPERATURE
25
20
15
10
5
0
1971
1972
WINTER
SPRING
SUMMER
FALL
WINTER
SPRING
SAMPLING PERIOD
ICE
ICE

I) Materials and methods pertaining to seasonal variation in major degradative and pollution indicator groups.

For all plating procedures, except total coliform, fecal coliform, and fecal streptococci analyses, counts were determined from 20C-incubated plates. The standard spread-plate technique was used to disperse appropriate dilutions of bacteria on all culture media employed.

Total counts (Extent of analysis: June 28, 1971 to May 1, 1972).

Direct total bacterial counts were obtained using a modification of Razumov's method, as described by Korsh (1961). A 10 ml aliquot of each sample was filtered through a 47 mm white gridded membrane filter of 0.45 μ pore size. (Millipore No. HAWG 047SO). The bacteria trapped in the membrane pores were fixed with a 0.4% aqueous solution of formaldehyde. The filters were then dried, and placed in a petri plate on filter paper saturated with carbolated erythrosin (1 g stain per 100 ml of a 5% phenol solution). After 24 hours of staining, the filters were decolorized by placing them on filter paper saturated with distilled water. Decolorization was repeated until the filters appeared pale pink in color. The filters were dried again, after which prepared sectors were placed on a slide and impregnated with immersion oil. Examination was made with an 100 X immersion objective and a 16 X ocular, with a square micrometer disc in one of the oculars. The number of bacteria (N) in each sample was calculated according to the formula:

$$N = \frac{Sn \times 10^6}{s\ V} \quad \text{bacteria/ml}$$

where S is equal to the area of the filtration area of the filter (mm^2), s is the area of the micrometer disc (measured in u^2 with a stage micrometer at the same magnification), n is the average number of bacteria per micrometer area, and V is the volume (in ml) of water filtered.

Total viable plate counts (Extent of analysis: Jan. 20, 1971 to Nov. 15, 1971)

Total viable bacterial plate counts were obtained on Foot and Taylor's medium (1949) in which the peptone concentration had been increased from 0.05% to 0.3% so as to yield larger colonies. Plates were incubated at 20C for 10 days.

Lipolytic counts (Extent of analysis: entire sampling period)

Aliquots of suitable dilutions were spread on Starr's Spirit Blue Agar for the detection of lipolytic colonies (Starr, 1941). This medium is commercially available as Bacto-Spirit Blue Agar (Difco) and Bacto-lipase reagent (a cotton-seed oil substrate). Plates were incubated at 20C for 5 days. Lipolytic colonies were readily distinguished by the zone of clearing around the colony.

Proteolytic Counts (Extent of analysis: Jan. 20, 1971 to Nov. 15, 1971)

Aliquots of suitable dilutions were spread on plates of skim milk agar. The medium was prepared by combining 8 g nutrient broth and 15 g agar in 950 ml distilled water with

5 g Bacto-skim milk powder dissolved in 50 ml distilled water. The two solutions were autoclaved separately. Clear zones surrounding the colonies gave evidence of casein hydrolysis. All plates were incubated at 20C for 5 days.

Total coliform, fecal coliform, and fecal streptococci analyses (Extent of analysis: Entire sampling period)

All counts of these three sewage pollution indicator bacteria were obtained using the membrane filtration media and techniques prescribed by the American Public Health Association (Standard Methods, 1971).

II) Materials and methods pertaining to the enumeration and classification of lipolytic psychrophiles. (Extent of analysis: Nov. 23rd, 1971 to May 1, 1972)

Lipolytic and cytochrome oxidase bacterial counts

Aliquots of suitable dilutions were spread on Spirit Blue Agar, as mentioned before, for the detection of lipolytic colonies. Plates were incubated as follows: 4C, 14 days; 10C, 10 days; 20C, 5 days; 30C, 2 days; 37C, 1 day; 42C, 1 day. The procedure of Gaby and Hadley (1957) was used to determine the numbers of cytochrome oxidase positive (COP) colonies growing on these plates. Equal volumes of 1% aqueous solution of dimethyl-p-phenylenediamine oxalate and 1% α-naphthol in ethanol were added to the plates. The solutions were mixed uniformily on each plate; cytochrome oxidase positive colonies turned blue within one minute. In this manner, counts of lipolytic, total COP, and COP lipolytic

colonies were obtained from countable (suitably diluted) plates at all temperatures of incubation. Prior to the cytochrome oxidase test, a number of isolated lipolytic colonies were picked from the 4C plates for more detailed characterization (this procedure is described below).

Classification of lipolytic psychrophiles and lipolytic sewage isolates

In conjunction with the quantitative study of lipolytic bacteria mentioned above, qualitative determinations of lipolytic colonies were performed. During the November 23, 1971 to May 1, 1972 period, 11 triplicate samples (taken approximately bi-monthly) were obtained from the downstream (Fournier Blvd.) station. During this time, the temperature was below 10C and the water was ice-covered from early December to late March (Fig. 2). Lipolytic bacteria were also classified from 2 samples taken from the upstream (Wright St.) station on March 20, and May 1, and from a July 17 (1972) sewage sample obtained directly from one of the effluent pipes entering Brewery Creek. Sampling locations are shown in Fig. 1.

Lipolytic psychrophiles were classified from the Fournier, Wright, and sewage, 4C-incubated plates. A number of lipolytic mesophiles were also identified from 20C-incubated plates of the sewage sample. The same dilutions consistently yielded an acceptable number of colonies per plate (30 to 300) and were used for the isolations. These were: Fournier, 500 fold; Wright, 10 fold; 4C sewage, 10^4; 20C, sewage 10^5). All

lipolytic colonies were picked which fell within a variable sized square in the centre of each plate. The size was chosen so that approximately 10 colonies were isolated from each plate. Thus, for each triplicate sample from a specific station, on the average 30 colonies were isolated and classified.

Isolates were re-streaked on Spirit Blue agar plates and incubated at 10C. Colony morphology was recorded, and a single colony then transferred to tryptone-yeast extract broth (1% Bacto-tryptone; 0.5% Bacto-yeast extract) and incubated at 10C. In order to determine thermal tolerance and the temperature range of lipolytic activity, the broth cultures were streaked onto Spirit Blue agar plates, which were then incubated at 4, 10, 20, 30, 37 and 42C. The strength of lipolysis was scored according to the extent of spreading of the clear zone around the colonies; less than 2 mm, weak; 2 to 6 mm, moderate; greater than 6 mm, strong.

All isolates were identified, where possible to the generic level, according to the scheme of Cowan and Steel (1965), which involves the tests listed below. (Unless otherwise stated, all tests requiring incubation were carried out at 10C). A total of 330 colonies were isolated from the downstream station (Fournier), 66 from the upstream station (Wright), and 27 each from the sewage samples incubated at 4C and 20C.

Acid and gas from glucose. The following medium was used: glucose, 7g; tryptone, 10g; yeast extract, 5g; bromcresol purple (BCP) stock solution, 2 ml; distilled water to 1000 ml. The BCP stock solution, containing 1.6% w/v of the indicator dissolved in 50% ethanol, was gravity filtered. Each tube, containing 5 ml of broth and an inverted Durham tube, was inoculated with a loopful of the tryptone-yeast extract broth culture, then examined for acid and gas production daily up to two weeks.

Catalase. A 3% solution of H_2O_2 was used to flood isolated colonies grown on Spirit Blue agar for evidence of O_2 evolution.

Cytochrome oxidase. The method of Gaby and Hadley (1957), as described earlier, was used.

Gram stain. The Hucker modification (Pelczar, 1957) was used. Cultures were stained after 24 hours growth in tryptone-yeast extract broth.

Motility. Motility was determined by phase-contrast microscopic examination of each culture after 1 to 2 days incubation in tryptone-yeast extract broth.

Oxidation / Fermentation of glucose. The method of Hugh and Leifson (1953) was used. Peptone was replaced by tryptone in the medium since our isolates had originally been picked from tryptone-containing Spirit Blue agar.

Other secondary tests performed were as follows:

Ammonia from arginine. The Streptococcus isolate was tested for the liberation of ammonia from arginine according to the procedure of Niven, Smiley and Sherman (1942).

Arginine di-hydrolase. The method of Thornley (1960) was used as a means of differentiating between Aeromonas and Vibrio, according to the dichotomous key of Bain and Shewan (1968).

Citrate utilization. Simmon's citrate agar (Difco) was used to show citrate utilization by Acinetobacter and Moraxella.

EMB Agar. All Moraxella and Acinetobacter isolates were streaked on EMB agar (Difco). Eosin Y and Methylene blue, the dyes in EMB agar, are incorporated by these organisms (Nelson and Shelton, 1965) to produce blue colonies. All Enterobacteriaceae isolated were also streaked on this medium for evidence of sheen.

Endo Agar (Difco). This medium was used to show green sheen production by Enterobacteriaceae (Standard Methods, 1971).

Hemolysis. The Streptococcus isolate was tested for hemolysis on plates prepared from Bacto-Blood Agar base (Difco) to which 5% (v/v) defibrinated sheep's blood had been added. Prepared plates were obtained from the Qualicum Company (Ottawa, Ontario).

Multitest Micromethod System for identification of Enterobacteriaceae. Enterobacteriaceae isolates were classified with the aid of the Analytab systems of 20 biochemical tests (Analytab Products Inc., New York). (Incubation was at 37C, except for 1 isolate, which did not grow at this temperature).

Penicillin sensitivity. All Moraxella species were tested, according to the method of Baumann, Doudoroff and Stanier (1968a), by spreading aliquots of individual broth cultures

on HIA (Heart Infusion Agar; Difco) plates, made with 1% Ionagar, to which 1 unit of penicillin G per ml was added after the plates had cooled to 40C.

Pigmentation. Fluorescein production by pseudomonads was observed under U.V. light on King's B Agar (King, Ward and Raney, 1954).

poly-B-OH-butyrate reserves. Moraxella species were examined for poly-β-OH-butyrate (PHB) granules with Hartman's Sudan Black B stain (1940). Cultures were grown on the medium described by Stanier, Palleroni and Doudoroff (1966), with Na acetate as the carbon source.

Proteolysis. All isolates were examined on gelatin agar for gelatin hydrolysis, and on skim milk agar for casein hydrolysis. Gelatin agar contained, per liter of distilled water, 8 g nutrient broth, 15 g agar, and 4 g gelatin. Skim milk agar was prepared as described earlier. Zones of gelatin hydrolysis were detected by precipitating unhydrolyzed protein with a solution of 15 g $HgCl_2$ in 20 ml concentrated HCl and 100 ml distilled water.

Sensitivity to Vibriostatic agent 0/129. (2,4-diamino-6,7-di-isopropyl pteridine). The filter paper disc method of Schubert, as modified by Bain and Shewan (1968) was used to differentiate Aeromonas from Vibrio.

III) Analysis of data

Seasonal variation in major degradative and pollution indicator groups

In order to statistically treat the data, counts

obtained from each triplicate sample were pooled to give an average value for each calendar season of the year separated into Spring, Summer, Fall, and Winter for all parameters studied. The 95% confidence interval limits were calculated to demonstrate significant differences.

Median values and the corresponding seasonal range of FC/FS (fecal coliform to fecal streptococci) ratios were reported, as suggested by Geldreich and Kenner (1969).

Total (membrane filter) bacterial counts (a) to total viable (Foot and Taylor Agar) bacterial plate counts (b) were calculated by dividing the mean seasonal a value by the corresponding mean seasonal b value.

Quantitative study of lipolytic bacteria

During this sampling period (Nov. 23rd, 1971 to May 1, 1972), where lipolytic, total COP, and COP lipolytic counts were gathered from plates incubated from 4C to 42C, no statistically significant differences in numbers could be demonstrated from sample to sample. To show meaningful differences between the two sites and between the results at different incubation temperatures, results were pooled to give an average "winter" value* for each parameter studied. The 95% confidence interval limits were calculated to demonstrate significant differences.

All of the separate percentage values (at each temperature of incubation) were calculated for that part of the lipolytic population which was COP, and for that part of the

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* This actually included the 1971-72 winter as well as part of the 1972 spring.

total COP population which was lipolytic. Each value was then transformed into a corresponding arc sin value (arc sine transformation test; Schefler, 1969). From here, a mean arc sin value was calculated as well as corresponding 95% arc sin confidence intervals for each of the 2 percentage parameters. These arc sin mean and 95% confidence interval values were then re-transformed into the corresponding percentages and graphed (Figures 8 and 9).

RESULTS

I) Seasonal variation in major degradative and pollution indicator groups

Our original intent was to obtain samples for approximately one year to see if there was seasonal variation in numbers of degradative or pollution indicator groups. However, in early fall 1971, the regional government of the Outaouais district began construction of a sewage collector pipe along Brewery Creek. The intake of water at Taché Blvd., approximately 100 yards upstream from our upstream (Wright St.) sampling station (Fig. 1), was reduced considerably to allow materials and equipment on some parts of the creek bed. The reduced flow has continued to the present. One advantage of this reduction was that it was then possible to determine the location of various effluent pipes which had previously been under water. In October 1971 we located 7 storm sewer pipes, 14 sewage effluent pipes, and one major waste effluent pipe from a meat packing plant (Fig. 1).

Because of the altered flow characteristics, we extended our sampling period to the end of May, 1972. Figure 2 shows the average water temperature during the sampling period, and the periods of ice cover. The reduced flow resulted in a marked concentration of sewage and other wastes, which is reflected in the increased bacterial counts, described below. The concentration of wastes probably also accounted for the slightly higher water temperature during the second winter.

Total bacterial counts: Direct membrane filtration and viable count methods

Membrane filtration total bacterial counts, taken from the 1971 summer to the 1972 spring, are shown in Fig. 3. Significant differences between upstream and downstream stations are evident, as well as the increase in total counts at the downstream site caused by the flow reduction. Total viable plate counts, taken from the winter 1970-71 to fall 1971 (Fig. 3) also show a significant difference between the two sites, and show the concentration effect at the downstream site.

Several investigators have shown that the ratio of direct count to viable count is highest in non-polluted waters, indicating that true water bacteria do not thrive on a medium containing peptone, which is often used to obtain viable counts, whereas saprophytic bacteria dominant in polluted systems do (Beling and Jannasch, 1955; Korsh, 1961; Kuznetsov and Karzinkin, 1930; Ruttner, 1970). For example, Kuznetsov and Karzinkin (1930) showed an unpolluted lake to have a direct count/viable count ratio of 18,200 while a canal effluent had a ratio of 93.

We have calculated this ratio for the two seasons in which our viable count and direct count values overlap (Table 3). The small ratios obtained indicate that a large percentage of the bacterial flora at both sites are saprophytic. The particularly low ratio (1.9), obtained at the downstream site (Fournier) in the fall of 1971, is indicative

Fig. 3 Total counts obtained either by staining bacteria fixed on membrane filters (o) or plating on Foot and Taylor agar (Δ). 95% confidence limits are indicated by the vertical lines.

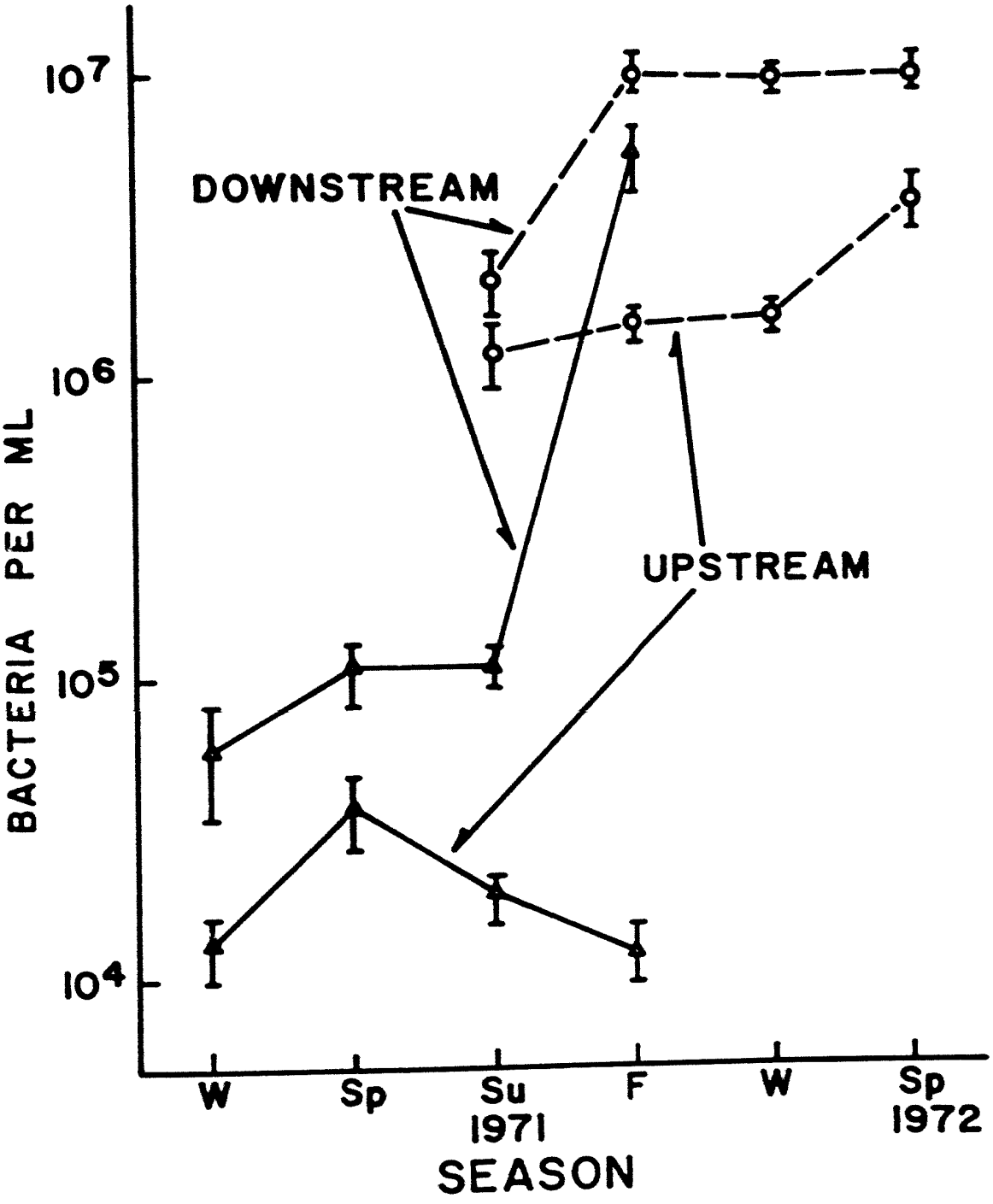

10^7
10^6
10^5
10^4
BACTERIA PER ML
DOWNSTREAM
UPSTREAM
W
Sp
Su
F
W
Sp
1971
1972
SEASON

TABLE 3

Total (a) to viable (b) count ratios obtained at the upstream (Wright Street) and downstream (Fournier Boulevard) stations, Brewery Creek, during the sampling period.

Season	Upstream (Wright St.)			Downstream (Fournier Blvd.)		
	a	b	a/b	a	b	a/b
Summer 1971	1.2×10^6	1.9×10^4	64.2	2.1×10^6	1.1×10^5	18.5
Fall 1971	1.5×10^6	1.2×10^4	122.1	1.0×10^7	5.4×10^6	1.9

Note: Total counts (a) were determined by the membrane filtration technique; viable counts (b) were determined by Foot and Taylor agar plate counts.

of the concentration of saprophytic bacteria caused by the flow reduction.

Pollution indicator groups

Total and fecal coliforms and fecal streptococci are commonly used as indicators of sewage pollution. Counts of all three obtained at the downstream station were significantly higher than those obtained upstream (Fig. 4). The reduction of flow in the fall of 1971 caused a significant increase for all counts at the downstream site, and some increase at the upstream site as well (the latter being 100 yards downstream from the damming location (Fig. 1). At the downstream site there was a decrease, particularly in total coliforms, during the winter and spring of 1972, perhaps due to a more rapid die-off of these organisms in the cold. However, counts were still considerably higher than in the corresponding seasons of the preceeding year.

Median values for the ratio of fecal coliforms to fecal streptococci are shown in Table 4. According to Geldreich and Kenner (1969), ratios less than 0.7 indicate fecal material of animal origin whereas ratios higher than 4.4 indicate human wastes. At the upstream site, all values indicate a mixture of animal and human wastes, except in the summer of 1971, when the ratio was significantly lower. The wastes from farms upstream on the Ottawa river, and from birds, insects and rodents, whose fecal material contains predominantly fecal streptococci (Geldreich and Kenner, 1969),

Fig. 4 Counts of total (o) and fecal (⊙) coliforms and fecal streptococci (Δ) obtained at the two major sampling stations. 95% confidence limits are indicated.

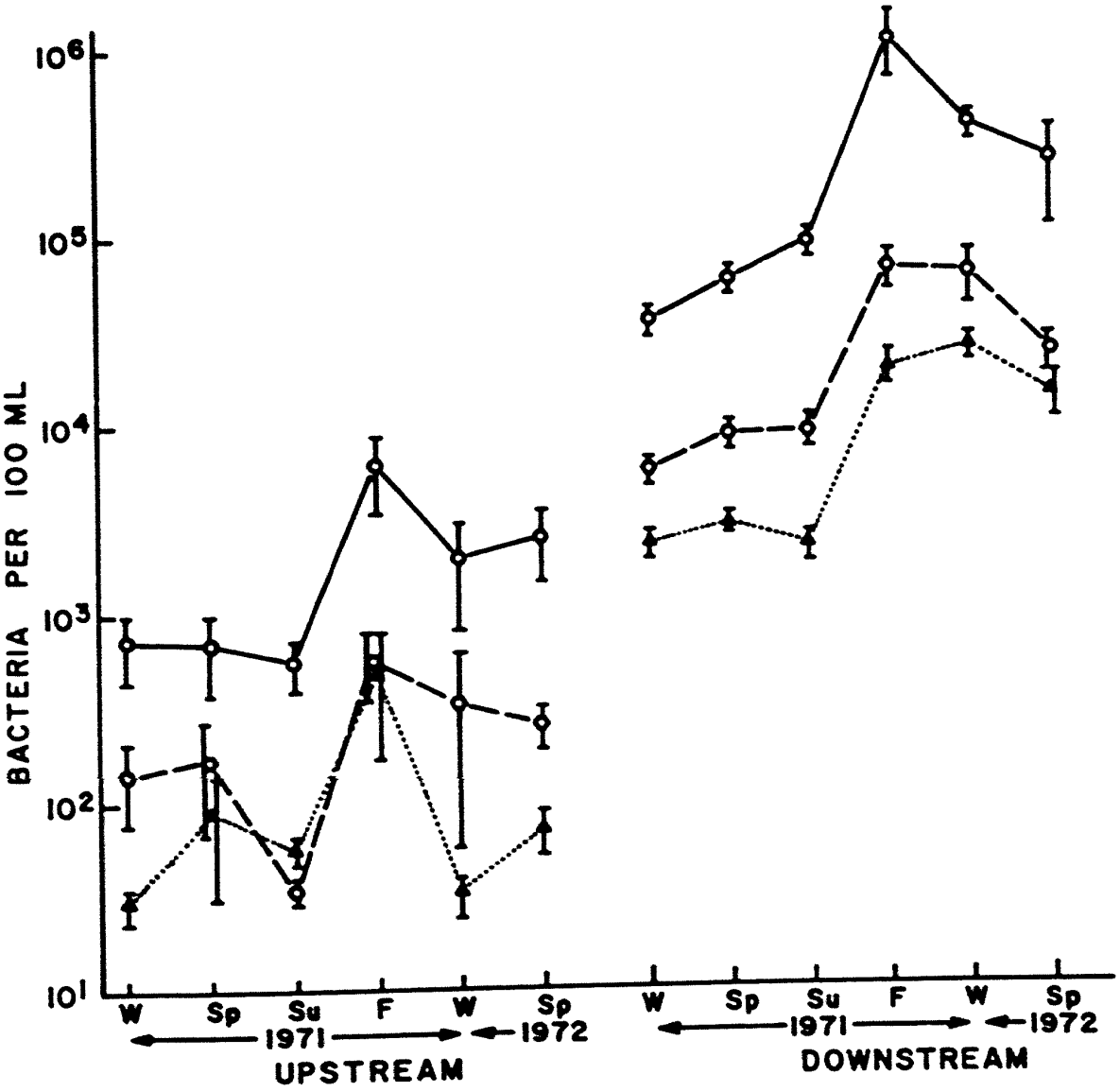

BACTERIA PER 100 ML
10⁶
10⁵
10⁴
10³
10²
10¹
W
Sp
Su
F
W
Sp
1971
1972
UPSTREAM
DOWNSTREAM

TABLE 4

Fecal coliform to fecal streptococci (FC/FS) ratios obtained at the upstream (Wright Street) and downstream (Fournier Boulevard) stations, Brewery Creek, during the sampling period.

Season	Upstream(Wright St.)		Downstream(Fournier Blvd.)	
	Median value	Seasonal range	Median value	Seasonal range
Winter 1970-71	3.8	1.17 to 16.88	3.10	0.51 to 16.43
Spring 1971	4.05	0.33 to 121.4	3.07	1.19 to 6.83
Summer 1971	0.66	0.32 to 1.22	4.32	0.55 to 12.0
Fall 1971	1.63	0.08 to 20.67	4.07	0.73 to 19.75
Winter 1971-72	3.21	1.59 to 50.0	1.91	0.39 to 4.83
Spring 1972	4.11	1.5 to 5.48	1.64	0.97 to 3.33

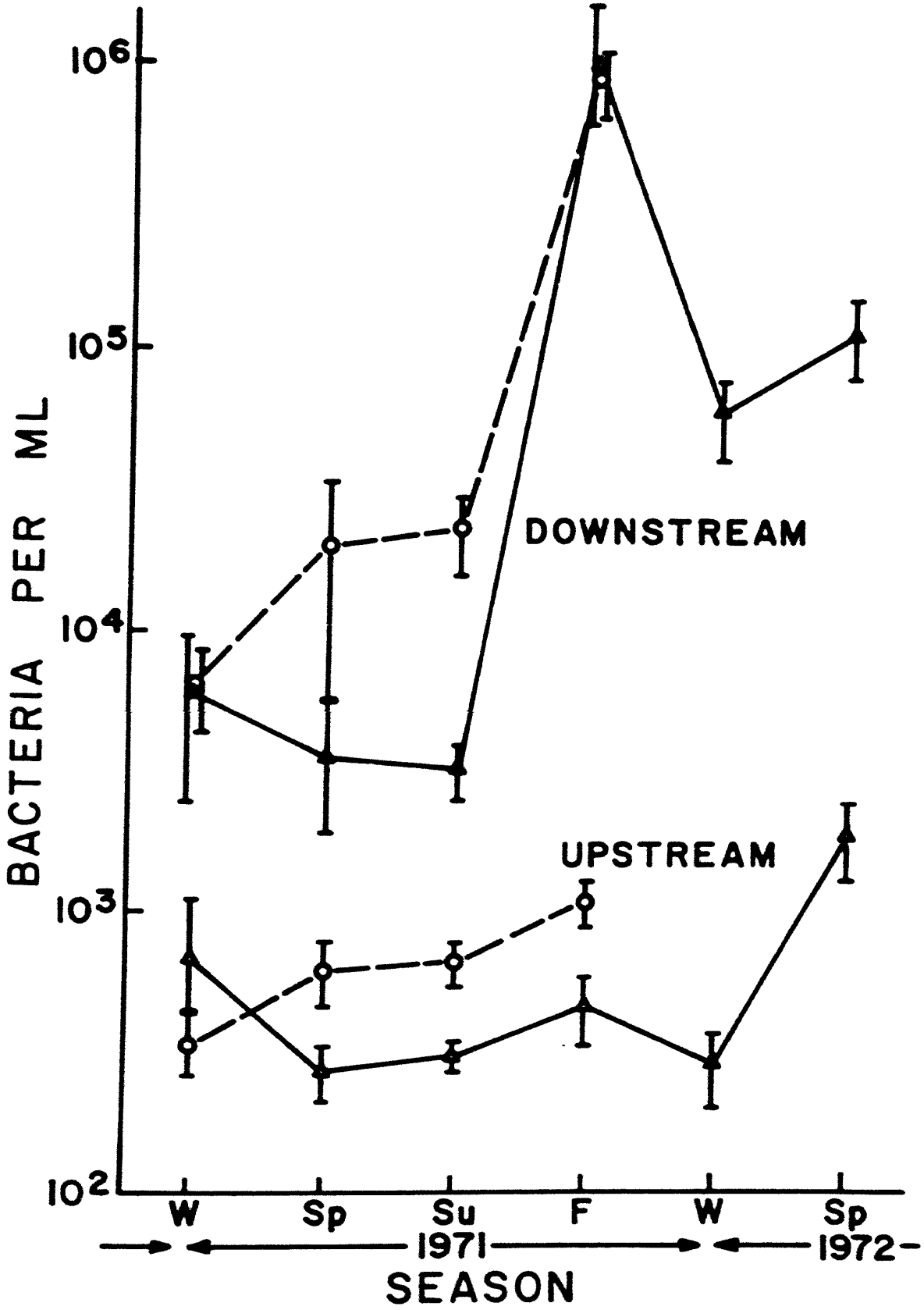
probably accounted for this low ratio. The range in this ratio varied greatly except during the summer of 1971.

At the downstream station, the ratios indicate a greater proportion of human fecal material, although the range again shows considerable variation. Though storm sewer effluents as well as the meat-packing plant effluent (Fig. 1), would largely contribute fecal streptococci, fecal coliforms would predominate in domestic sewage effluents (Geldreich and Kenner, 1969).

Degradative Populations

Both proteolytic and lipolytic bacterial counts were significantly higher at the downstream station (Fig. 5), and the reduction in flow rate increased this difference. Quantitatively the two populations did not differ to a great extent. However, there was a significant increase in the proteolytic counts between the winter of 1970-71 and the following summer, while in the same period the lipolytic counts decreased (though not significantly). This increase also occurred for total viable plate counts and total coliform counts, and could be explained by decreased flow, increased dumping of wastes, or increased survival of mesophiles which would die off more rapidly in the winter months. Three facts suggest that the third possibility is the most likely. (a) The flow generally increases from winter to spring. (b) There is no evidence that the dumping of sewage or wastes from the packing plant follow a seasonal pattern. (c) Most lipolytic bacteria appear to be psychrophilic, or

Fig. 5 Proteolytic (o) and lipolytic (Δ) bacterial counts obtained at the two major sampling stations. 95% confidence limits are indicated.

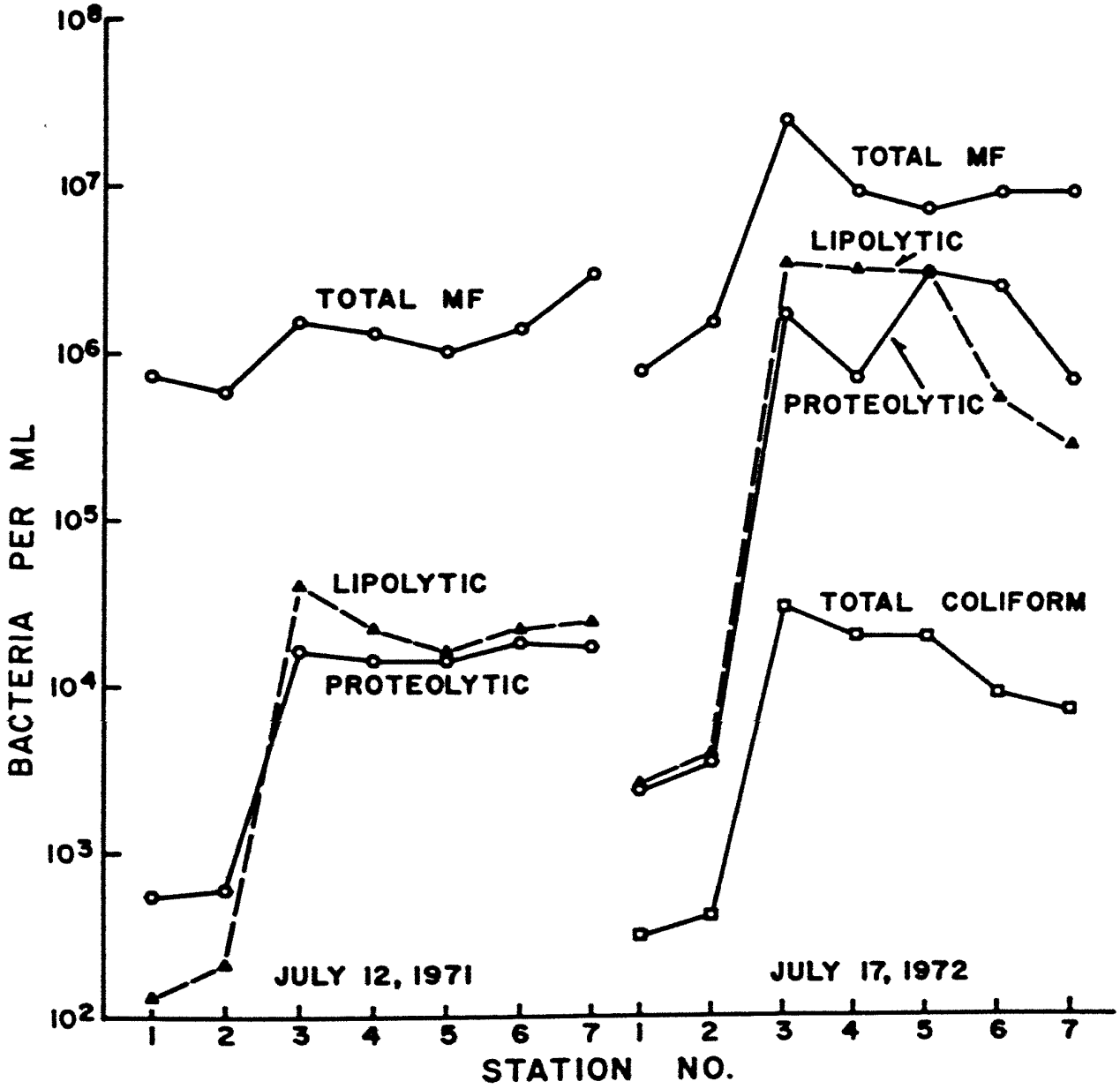

BACTERIA PER ML
10⁶
10⁵
10⁴
10³
10²
DOWNSTREAM
UPSTREAM
W
Sp
Su
F
W
Sp
1971
1972
SEASON

at least psychro-tolerant (Witter, 1961). At the 20C incubation chosen for this study, most psychrophilic bacteria will grow, as will the mesophiles, yet the lipolytic count does not increase from winter to summer.

7-site sampling in Brewery Creek

In an attempt to pin-point the locations on the creek where major bacterial input occurred, samples were taken at 7 different sites in Brewery Creek (Fig. 1). On July 12, 1971, total, lipolytic, and proteolytic counts were obtained. The experiment was repeated one year later (July 17, 1972), but total coliform counts were also included in this second sampling series. In both cases, counts of all parameters studied rose sharply between stations 2 and 3 and generally stayed at the same level up to station 7 where Brewery Creek flows back into the Ottawa River (Fig. 6). A total of 5 effluent pipes are located between sites 2 and 3; 4 of these are sewage effluent pipes, while the other is the major effluent of a meat-packing plant. Nine sewage effluent pipes are located after station 3. Since the numbers remain fairly constant, the additional input may be balanced by die-off of bacteria in the water. Counts obtained on July 12, 1971 were significantly lower than those obtained on July 17, 1972 under conditions of reduced water flow begun in the 1971 fall season.

Fig. 6 Total "MF" counts (obtained by staining bacteria fixed on membrane filters), lipolytic, and proteolytic counts obtained at all 7 sampling stations in Brewery Creek on two occasions. Total coliforms were also determined in the second occasion.


BACTERIA PER ML
10^8
10^7
10^6
10^5
10^4
10^3
10^2
TOTAL MF
LIPOLYTIC
PROTEOLYTIC
TOTAL MF
LIPOLYTIC
PROTEOLYTIC
TOTAL COLIFORM
JULY 12, 1971
JULY 17, 1972
1 2 3 4 5 6 7 1 2 3 4 5 6 7
STATION NO.

Ottawa River sampling stations

In addition to the work done in Brewery Creek, occasional water samples were taken at Lemieux Island, l'Escale and Ottawa Rowing Club stations on the Ottawa River (Fig. 1). At the Lemieux Island station, 7 spring samples, 3 summer samples, and 1 fall sample, were taken. Four summer samples and 1 fall sample were taken at l'Escale station, while 3 summer samples were taken at the Ottawa Rowing Club station. The results are shown in Table 5 and Table 6.

a) Lemieux Island Station

This station (Fig. 1), located near the Lemieux Island water purification plant, was sampled mainly for comparison with our upstream (Wright Street) station in Brewery Creek. Statistical tests were not applied to compare the parameters analyzed at these two stations because of the scarcity of samples taken at Lemieux Island. However, the counts obtained at the Lemieux Island station (Table 5) were very similar to those obtained at the Wright Street station, except for total and fecal coliforms, and fecal streptococci in the fall of 1971 (during this season, Brewery Creek was partially dammed as previously explained). FC/FS and a/b ratios were calculated (Table 6), and were also fairly close to those obtained at Wright St. (Table 4). It seems very likely, therefore, that bacterial counts at these two stations were very similar throughout at least the first part of our sampling period.

TABLE 5

Average bacterial counts[a] of parameters studied at Lemieux Island (L.I.), l'Escale (l'E.), and Ottawa Rowing Club (ORC) sampling stations on the Ottawa River during spring, summer, and fall 1971.

	L.I.			l'E.		ORC
Season	Spring	Summer	Fall	Summer	Fall	Summer
Mean water temperature	11C	22.5C	13.5C	22C	13C	21C
Total bacteria[b]		1.1×10^6	1.5×10^6	3.3×10^6	1.6×10^6	2.1×10^6
Viable bacteria[c]	2.7×10^4	2.9×10^4	1.1×10^4	6.3×10^5	4.6×10^4	1.1×10^5
Lipolytic bacteria	200	340	260	1.4×10^4	1×10^3	1.8×10^3
Proteolytic bacteria	600	430	630	4.3×10^4	4.3×10^3	1.8×10^4
Total coliforms	140	550	350	2×10^4	1.8×10^3	4.8×10^3
Fecal coliforms	25	50	70	960	210	160
Fecal streptococci	15	60	170	170	110	250

[a] Counts are expressed per ml except those for total and fecal coliforms and fecal streptococci (per 100 ml).

[b] Determined by the membrane filtration technique.

[c] Determined by Foot & Taylor agar plate counts.

TABLE 6

Fecal coliform (FC) to fecal streptococcus (FS) ratio and direct total bacterial count (a) to total viable bacterial count (b) ratio at Lemieux Island, l'Escale, and Ottawa Rowing Club sampling stations during spring, summer, and fall of 1971.

Sampling station	Season	FC/FS ratio		a/b ratio[a]
		Median value	Range	
Lemieux Island	spring	2.17	0.23 to 8.0	
	summer	0.87	0.55 to 1.14	37.93
	fall	0.37	0.37 to 0.52	136.36
l'Escale	summer	3.53	1.24 to 13.13	5.24
	fall	2.10	1.67 to 2.10	34.78
Ottawa Rowing Club	summer	0.74	0.32 to 1.06	21.0

[a] Direct total bacterial counts (a) were determined by the membrane filtration counting technique; total viable bacterial counts (b) were determined by Foot & Taylor agar plate counts.

b) L'Escale and Ottawa Rowing Club stations

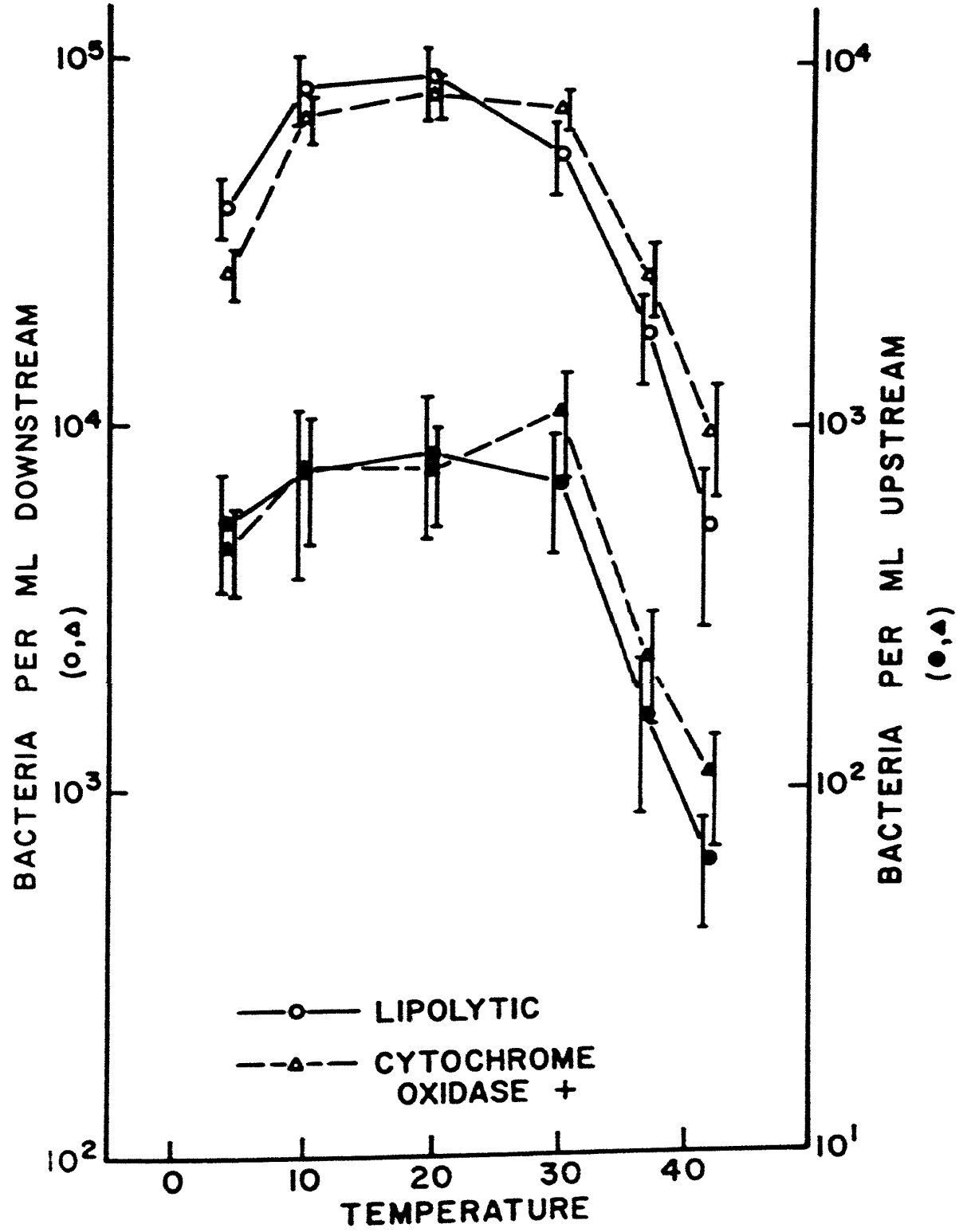
These two sites were sampled so that we could compare the degree of pollution in the main channel of the Ottawa River with that occurring in Brewery Creek. Bacterial counts (Table 5) and ratios (Table 6) indicate a higher degree of pollution in these areas of the Ottawa River than at Lemieux Island. Waste inputs between this latter station and the l'Escale and Ottawa Rowing Club station probably contribute to this increase in counts. However, the counts are all considerably lower than those obtained at the downstream station (Fournier Blvd.) on Brewery Creek.

II) Enumeration of lipolytic psychrophiles

Aside from showing the degree of contamination of Brewery Creek, the first part of this research showed high counts of lipolytic and proteolytic degradative bacteria present in the water. The results reported in this section summarize a detailed quantitative study of Brewery Creek lipolytic bacteria. This investigation was carried out from Nov. 23, 1971, to May 1, 1972. During much of this period, the water was ice-covered and the temperatures near 0C (Fig. 2). The goals of this survey were to obtain information on numbers, general types, activities, and probable sources of these bacteria.

The numbers of lipolytic and cytochrome oxidase positive bacteria which grew on Spirit Blue agar depended on the temperature of incubation (Fig. 7), but were always about 100 fold higher at the downstream station. Since the samples

Fig. 7 Average counts of lipolytic and cytochrome oxidase positive bacteria obtained at different temperatures of incubation during the 1971-72 winter and part of the 1972 spring sampling periods. ●, ▲ upstream (Wright St.) station; O, Δ downstream (Fournier Blvd.) station. 95% confidence limits are indicated.


BACTERIA PER ML DOWNSTREAM (o,Δ)
BACTERIA PER ML UPSTREAM (●,Δ)
10⁵
10⁴
10³
10²
10⁴
10³
10²
10¹
0
10
20
30
40
TEMPERATURE
LIPOLYTIC
CYTOCHROME OXIDASE +

were taken during a period when the water temperature was below 10C, one might expect to find only psychrophiles in the water. If this were the case, then the counts would be approximately equal at 4, 10, and 20C. A total of 383 lipolytic psychrophiles (i.e. 4C isolates) from Wright and Fournier were tested for growth at temperatures up to 42C. All grew at 20C, 97.7% at 30C, 20.1% at 37C, and only 3.7% at 42C. Other workers have also reported that most psychrophiles grow equally well at 4C and 20C (Stokes and Redmond, 1966; Werkman and Wilson, 1951). At the upstream site, the numbers of bacteria which grew at 4C and at 20C, when plated directly from the water samples, were not significantly different (Fig. 7). At 37C and 42C significantly fewer grew; this decrease closely paralleled the decrease in the numbers of psychrophilic isolates capable of growth at 37C and 42C. The results suggest that the population at this site is almost entirely psychrophilic.

In contrast, at the downstream site the number capable of growth at 20C was significantly higher than at 4C, indicating a population of both mesophiles and of psychrophiles. The mesophilic population at Fournier can be attributed to incoming wastes. Untreated sewage enters the creek at various locations, and effluent also enters from a meat packing plant situated mid-way along the creek. However, since the mesophiles do not grow at 4C, they are not likely to contribute significantly to lipid degradation during the winter months.

At the upstream site approximately 2/3 of the lipolytic population were cytochrome oxidase positive (Fig. 8), at all temperatures. At the downstream site, a significantly lower percentage (52%) of the psychrophilic (4C) population were cytochrome oxidase positive. The lower percentage suggests that oxidase negative psychrophiles are entering the water with sewage or other effluents. To test this possibility, we analyzed samples taken on two different occasions from a major sanitary sewer entering midway along the creek (Fig. 1). The results (Table 7) show that potential psychrophiles make up only 1/3 to 1/10 of the lipolytic population in sewage, but the oxidase negative species predominate at both 4C and 20C. Thus, the inflow of sewage could account for the lower percentage of oxidase positive lipolytic psychrophiles at the downstream site.

At the 20C incubation temperature and above, approximately 2/3 of the lipolytic bacteria were cytochrome oxidase positive at both sites (Fig. 8), but the total numbers were considerably higher downstream, as shown above. Growth alone cannot account for the increase between the two sites, since the population at the downstream site is roughly 1/2 mesophilic, while that at the upstream site appears to be entirely psychrophilic. Since the sewage mesophiles are predominantly oxidase negative, there must be a major source of oxidase positive lipolytic mesophiles entering the creek. This source could be the meat packing plant, but we were unable to obtain a sample directly from its effluent.

Fig. 8 Percentages of lipolytic bacteria at the two major sampling stations which were cytochrome oxidase positive at the various temperatures of incubation. The narrow bars indicate the 95% confidence interval.

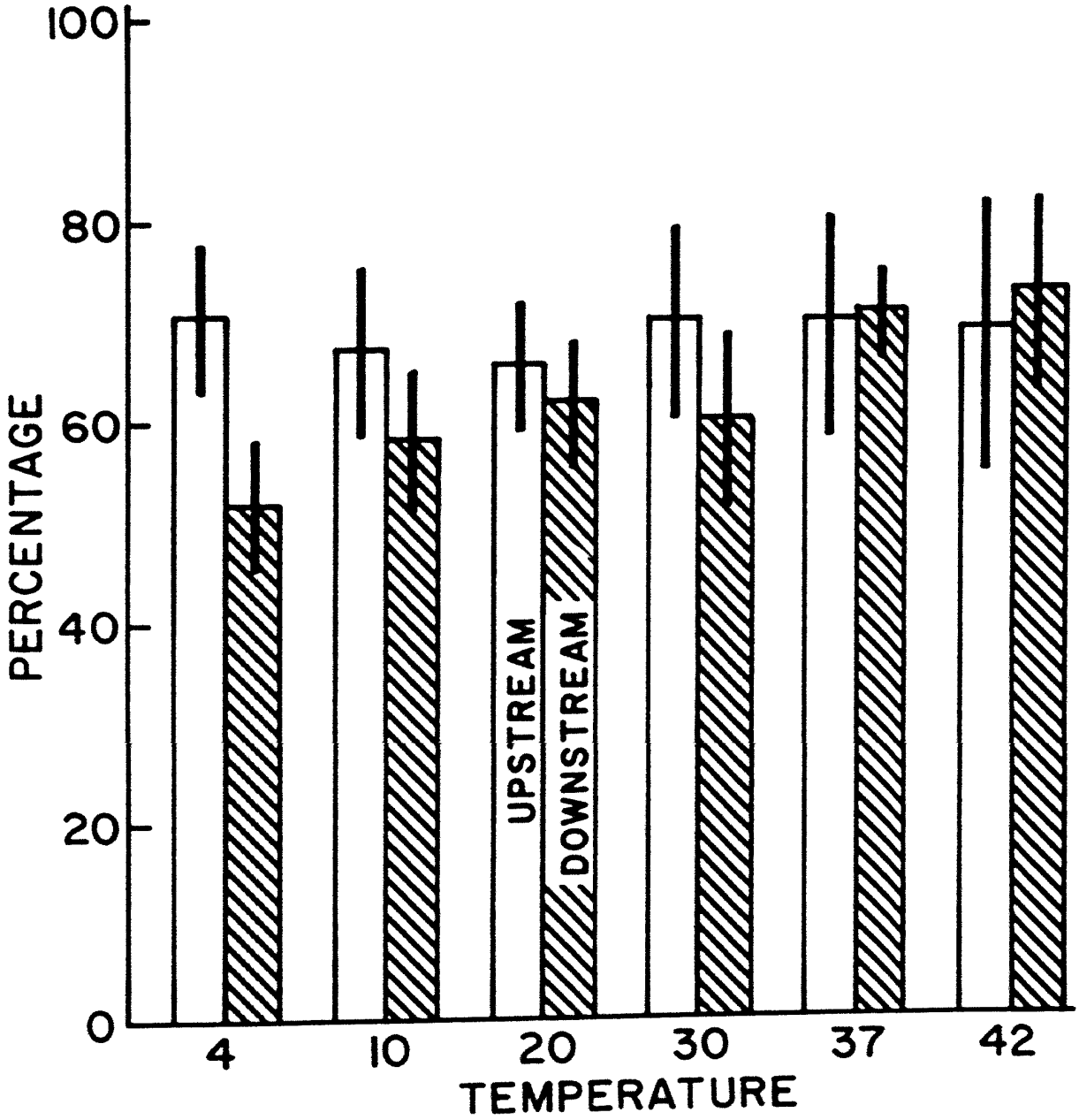

100
80
60
40
20
0
PERCENTAGE
UPSTREAM
DOWNSTREAM
4
10
20
30
37
42
TEMPERATURE

TABLE 7

Analysis of sewage samples

Date	Total Count	Total Coliforms	Lipolytic	Cytochrome oxidase +	% of lipolytic which are c.o. +
17/7/72	1.8×10^7	4.1×10^5	2.6×10^5	1.3×10^5	33.4 (4 C)
			3.3×10^6	1.8×10^6	18.8 (20 C)
17/11/72	2.5×10^7	1.3×10^5	2.5×10^5	4.7×10^4	8.0 (4 C)
			6.3×10^5	1.5×10^5	19.0 (20 C)

Note: All counts expressed per ml.

The percentages of cytochrome oxidase positive bacteria from both sites which were lipolytic at the various temperatures of incubation are shown in Fig. 9. At both locations, a higher percentage were lipolytic at the lower temperatures. Sjöström (1959) also found that the great majority of fat-splitting bacteria were psychrophilic. This may indicate that lipolytic enzymes, in the case of these bacteria, are dependent on lower temperatures for their activity, and that they become non-functional at the higher temperatures (Nashif and Nelson, 1953a, 1953b; Witter, 1961). On the other hand, the majority of oxidase positive bacteria capable of growth at higher temperatures may simply not possess lipolytic enzymes.

III) Classification of lipolytic psychrophiles

A total of 450 lipolytic psychrophiles were isolated and characterized, as far as possible, to the generic level. The overall results are summarized in Table 8. The major characteristics of the different groups are summarized below.

Pseudomonads

All the lipolytic pseudomonads isolated possessed the following characteristics: motile, gram-negative rods, catalase positive, cytochrome oxidase positive, aerobic, glucose oxidative. The majority were fluorescent on King's B medium, and would hydrolyze both casein and gelatin. These isolates fit the description of *P. fluorescens* (Stanier *et al.*, 1966), while the less common, non-proteolytic fluorescent isolates fit the description of *P. putida* (Stanier *et al.*, 1966). There

Fig. 9 Percentages of the total cytochrome oxidase positive bacteria at the two major sampling stations which were lipolytic at the various temperatures of incubation. The narrow bars indicate the 95% confidence interval.

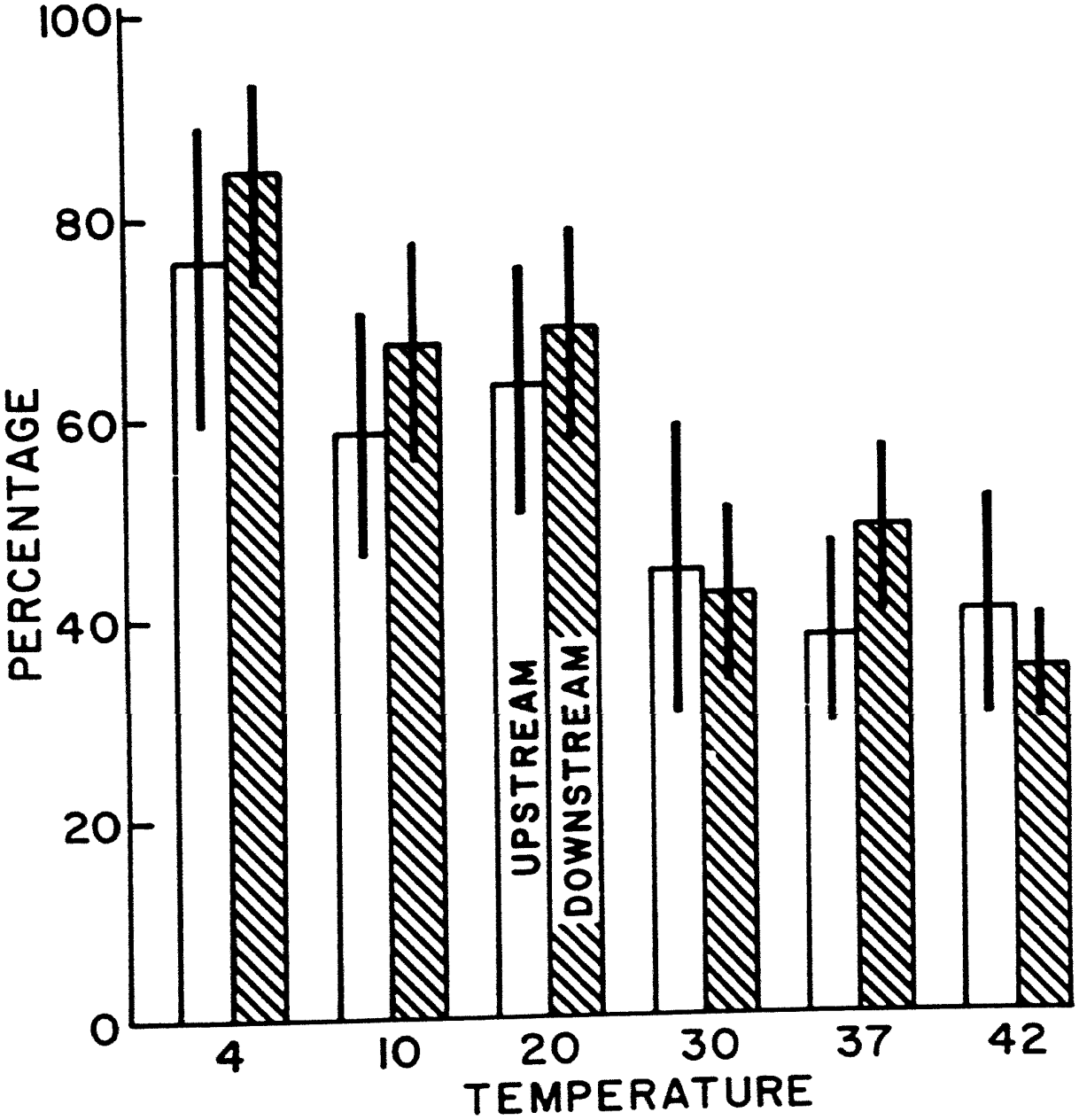

100
80
60
40
20
0
PERCENTAGE
UPSTREAM
DOWNSTREAM
4
10
20
30
37
42
TEMPERATURE

TABLE 8

Classification of 450 lipolytic psychrophiles isolated from Brewery Creek

Group	Downstream (Fournier Blvd.)	Upstream (Wright St.)	Sewage		Total
			20C incubation	4C incubation	
Pseudomonas	153	44	0	5	202
Acinetobacter	108	4	19	20	151
Aeromonas	30	0	5	0	35
Alcaligenes	9	7	0	1	17
Moraxella	10	3	0	0	13
Chromobacterium	5	3	0	0	8
Enterobacteriaceae	2	3	1	0	6
Vibrio	1	0	0	0	1
Streptococcus	1	0	0	0	1
Lost[1]	11	2	2	1	16

[1] Failed to grow after sub-culturing

were also a large number of non-fluorescent isolates which may be other P. fluorescens and P. putida isolates incapable of pigment formation on the media provided. However, there were also differences in thermal tolerance.

All the pseudomonads grew over a temperature range of 4C to 30C, and none at 42C. Of the 127 presumptive P. fluorescens isolates, 28 grew at 37C, as did 5 of the 17 presumptive P. putida isolates. However, only 2 of the 59 non-fluorescent types grew at 37C. The numbers in each group found at each site, and their thermal tolerances, are summarized in Table 9.

Visual inspection of the zone of lipolysis on Spirit Blue agar indicated that all isolates produced an equally large zone at 4, 10 and 20C by the end of incubation period, though not all produced a strong zone of lipolysis (6 mm or more in diameter). Fewer, particularly among the P. fluorescens group, were strongly lipolytic at 30C. The results for all groups are summarized in Table 10.

Acinetobacter

All the lipolytic Acinetobacter isolates had the following characteristics: non-motile, gram-negative rods exhibiting pleomorphism, aerobic, catalase positive, cytochrome oxidase negative, not proteolytic.

The incorporation of eosin and methylene blue by Acinetobacter on EMB agar has been noted by several investigators (Eller, 1969; Hugh and Reese, 1967; Nelson and Shelton, 1965; Taplin et al.,1963). All of our isolates, except for one from Fournier, produced blue colonies on EMB agar plates in-

TABLE 9

Classification of Pseudomonas isolates

Site	Group	Number of isolates	Number growing at 37C
Fournier (downstream)	P. fluorescens	91	18
	P. putida	15	4
	non-fluorescent	47 (34)*	2 (1)*
Wright (upstream)	P. fluorescens	31	8
	P. putida	2	1
	non-fluorescent	11 (10)*	0
Sewage	P. fluorescens	4	2
	non-fluorescent	1	0

* ( ) - number which were proteolytic.

TABLE 10

Lipolytic activity of isolates

Group	No. of isolates	Strong* lipolysis at: 4	10	20	30	37
P. fluorescens	126	112	112	98	42	5
P. putida	17	16	16	12	12	2
non-fluorescent pseudomonads	59	54	49	45	39	0
psychrophilic acinetobacters	132	101	99	99	70	0
20C sewage acinetobacters	19	-	16	10	9	7
psychrophilic aeromonads	30	28	28	28	20	16#
20C sewage aeromonads	5	-	5	5	5	5
Alcaligenes	17	17	17	17	6	1
Moraxella	13	13	10	10	6	0
Chromobacterium	8	8	8	8	0	0
Enterobacteriaceae	6	3	1	1	1	0
Streptococcus	1	0	0	0	0	0
Totals	434	329	344	338	193	28

* Zone of clearing greater than 6 mm diameter.

6 were also lipolytic at 42C.

cubated at 20C. This blue-coloured growth has also been noted for all our Moraxella isolates. Randomly chosen isolates from our other lipolytic genera failed to give similar coloration when grown on EMB.

Citrate utilization and glucose oxidation appear to be two useful criteria for subdividing the acinetobacters (Baumann et al., 1968b; Thornley, 1967). The number of isolates from each site which could utilize citrate is given in Table 11. Only 2 isolates oxidized glucose. One was from the downstream site, the other from sewage at 20C.

Thermal tolerances of the Acinetobacter isolates are also shown in Table 11. All but two of the psychrophilic isolates grew to at least 30C. Out of the 19 isolates from 20C-incubated plates (from sewage), all except 1 grew at 37C, while 14 failed to grow at 4C. As in the case of the Pseudomonas isolates, most of the acinetobacters were strongly lipolytic at 20C or below (Table 10). At 37C only 7 of the 20C isolates were lipolytic.

Moraxella

According to Baumann et al. (1968a, 1968b), the "moraxellas" should be separated into Moraxella and Acinetobacter primarily on the basis of the cytochrome oxidase test. The latter are oxidative negative. Our isolates were divided on this basis, but are otherwise quite similar. All lipolytic Moraxella isolates possessed the following characteristics: non-motile gram negative rods, aerobic, catalase positive, not proteolytic, cytochrome oxidase positive. As in the case of Acinetobacter, all 13 isolates gave blue-coloured growth

TABLE 11

Properties of *Acinetobacter* isolates

Site	Number of isolates	Citrate utilization	Numbers growing at 30C	37C	42C
Fournier (downstream)	108	6	106	11	0
Wright (upstream)	4	1	4	2	0
Sewage-4C	20	0	20	4	0
Sewage-20C	19	12	19	18	3

on EMB at 20C. Baumann et al. (1968a) also found that Moraxella could be differentiated from Acinetobacter on the basis of the latter's resistance to 1 unit per ml penicillin G. However, three of our oxidase positive isolates were resistant to this concentration (Table 12).

Baumann et al. (1968a) have subdivided the moraxellas on the basis of nutritional and physiological properties. Their group I is characterized by PHB accumulation, no hydrolysis of gelatin, no oxidation of glucose and failure to utilize citrate as a carbon source. All of our isolates accumulated PHB, and, as noted above, were not proteolytic. However, 2 oxidized glucose and 7 were capable of citrate utilization (Table 12). The possible taxonomic position of our isolates is discussed below.

Thermal tolerances were similar to those of the Acinetobacter isolates. Only one grew above 30C, and 3 failed to grow at 30C (Table 12). The latter 3 showed strong lipolysis only at 4C and 10C, while all the others showed strong lipolysis at 20C as well (Table 10).

Alcaligenes

The 17 lipolytic Alcaligenes isolates possessed the following properties: motile gram-negative rods, aerobic, catalase positive, oxidase positive, do not attack glucose. Three of the 9 strains isolated from the downstream site were proteolytic.

Thermal tolerances were similar to those of Acinetobacter and Moraxella. Only one isolate grew above 30C, and 3 failed

TABLE 12

Properties of *Moraxella* isolates

Site	Number of isolates	Penicillin resistance	Citrate utilization	Glucose oxidation	Numbers growing at 30C	37C	42C
Fournier (downstream)	10	2	5	2	8	0	0
Wright (upstream)	3	1	2	0	2	1	1

Note: No *Moraxella* isolates were obtained from sewage at 4C or 20C.

to grow at 30C (Table 13). All strains, however, showed strong lipolysis from 4C to 20C, and several were lipolytic at 30C (Table 10).

None of the isolates fit precisely the description of the 6 species in Bergey's Manual (1957).

Aeromonads

All the lipolytic aeromonads isolated had the following characteristics: motile gram-negative rods, catalase-positive, cytochrome oxidase positive, facultatively anaerobic, glucose attacked fermentatively (with or without gas production). These were further differentiated from Vibrio on the basis of resistance to the vibriostatic agent 0/129 (Bain and Shewan, 1968).

Bain and Shewan (1968) have subdivided the aeromonads on the basis of gas production from glucose and growth at 37C. They also believe that the aeromonads are invariably arginine dihydrolase positive, except for a specialized group of animal pathogens which are also sensitive to the vibriostatic agent. However, Cowan and Steel (1965) assign a variable result to the arginine dihydrolase reaction because of conflicting reports and taxonomy in the literature. Twelve of our isolates were negative, and thus do not fit Bain and Shewan's scheme. Otherwise they would be classified as A. formicans, since none produce gas from glucose (Table 14). The breakdown of the other isolates, according to Bain and Shewan's scheme, is shown in Table 14.

TABLE 13

Thermal tolerance of *Chromobacterium* and *Alcaligenes* isolates

Genus	Site	Number of isolates	Numbers growing at 30C	Numbers growing at 37C
Chromobacterium	Fournier (downstream)	5	5	0
	Wright (upstream)	3	3	0
Alcaligenes	Fournier (downstream)	9	9	0
	Wright (upstream)	7	4	1
	Sewage-4C	1	1	0

Note: No *Chromobacterium* isolates were obtained from sewage; no *Alcaligenes* isolates were obtained from sewage at 20C.

TABLE 14

Properties of *Aeromonas* isolates

Site	Arginine dihydrolase	Gas from glucose	Growth at 37C	Number of isolates	Numbers growing at 42C
Fournier (downstream)	+	+	+	10	6
	+	+	-	1	0
	+	-	+	7	3
	-	-	+	7	2 (note a)
	-	-	-	5	0 (note b)
Sewage - 20C	+	+	+	4	0 (note c)
	+	-	+	1	0

[a] One was not proteolytic

[b] One failed to grow at 30C

[c] Two failed to grow at 4C

All Aeromonas isolates, except one from Fournier, were proteolytic.

The thermal tolerance of the 4C isolates was markedly higher than that of the groups described above. Twenty-four of the 30 isolates from Fournier grew at 37C, and 11 at 42C (Table 14). Only one failed to grow at 30C. All 5 sewage isolates from 20C grew to 37C, but none grew at 42C. Two also failed to grow at 4C. The majority of the isolates were also strongly lipolytic over their entire growth range (Table 10).

Vibrio

The one Vibrio (F57-B8) isolated came from the downstream (Fournier) site. Its basic characteristics were the same as those of the aeromonads, but it was differentiated from them on the basis of its sensitivity to the vibriostatic agent 0/129. The isolate was proteolytic, arginine dihydrolase negative, and produced no gas from glucose. It grew to 42C, but was strongly lipolytic only at 10C and 20C (Table 10).

Chromobacterium

All the lipolytic Chromobacterium isolates possessed the following characteristics: motile gram-negative rods, aerobic, catalase positive, cytochrome oxidase negative, produce purple pigment, attack glucose oxidatively, proteolytic. None were chromogenic on King's B agar plates incubated at 10C, and the cytochrome oxidase test was therefore performed under these conditions.

The isolates were quite similar in their properties. All grew to 30C, but none at 37C (Table 13). All were strongly

lipolytic from 4C to 20C (Table 10). According to Cowan and Steel (1965) their psychrophilic nature defines them as C. lividum.

Enterobacteriaceae

The individual properties of 6 Enterobacteriaceae isolated are shown in Table 15. All had the following similar characteristics: gram-negative rods; facultatively anaerobic; catalase-positive; cytochrome oxidase negative; attack glucose fermentatively with gas production.

One (S20-C1), isolated from sewage at 20C, was classified as Klebsiella pneumoniae on the basis of the API scheme. Cold tolerant species of Klebsiella have previously been isolated from meats (Eddy and Kitchell, 1959), milk (Dempster, 1968; Panes and Thomas, 1968; Witter, 1961) and other food products (Panes and Thomas, 1968), and lipolytic isolates have also been described (Dempster, 1968). The remaining 5 isolates were all atypical.

Both Fournier isolates exhibited strong lipolysis at 4C. The Wright St. isolates W59-B4 and W59-C3 were weakly lipolytic from 4C to 30C, while W62-B4 exhibited equally strong zones of clearing from 4C to 30C. The sewage isolate identified as K. pneumoniae displayed weak lipolysis from 4C to 37C.

Streptococcus

Our only gram-positive isolate (F52-B2), classified as a Streptococcus, had the following properties: non-motile coccus; facultatively anaerobic; catalase-negative; cytochrome

TABLE 15

Properties of Enterobacteriaceae isolates

Isolate[1] (ref. no.)	Proteolysis	Sheen on EMB	Sheen on Endo	Gas from glucose	Motility	Growth at 30C	Growth at 37C	Growth at 42C	API identification
F56A1	-	+	+	+	+	+	+	+	atypical
F59A6	+	-	+	+	+	+	+	-	atypical
W59B4	+	-	-	+	+	+	+	-	atypical
W59C3	+	+	-	+	-	+	-	-	atypical
W62B4	-	+	-	+	+	+	+	-	atypical
S-C-1(20C)	-	+	not done	+	-	+	+	-	Klebsiella pneumoniae

[1] F; Fournier (downstream): W; Wright (upstream): S; sewage.

oxidase negative; ferments glucose. It formed punctiform cream-colored colonies on Spirit Blue Agar and microscopic examination revealed long chain formation. This organism fits the description (Bergey, 1957) of S. cremoris on the basis of growth range (4C to 30C), no ammonia from arginine, no haemolysis on sheep blood agar, no proteolysis. Lipolysis was weak at 4C and 10C and absent at 20C and 30C.

DISCUSSION

I) Seasonal variation in major degradative and pollution indicator groups.

Brewery Creek branches off from the Ottawa River at a point above the major sources of industrial pollution in the Ottawa area, and in fact not far from the Lemieux Island water purification plant. However, indicator bacterial counts (total coliforms, fecal coliforms and fecal streptococci) obtained at our upstream (Wright St.) station indicate that the water entering Brewery Creek is already slightly polluted. The ratios of fecal coliforms to fecal streptococci obtained at this site suggest a mixture of human and animal wastes in the water, except in the summer of 1971 when animal wastes prevailed.

Human and animal wastes are added to Brewery Creek via 7 storm sewers, 15 sewage effluent pipes, and one major meat-packing plant effluent discharge. As a result, significantly higher numbers of coliforms and fecal streptococci, and of proteolytic and lipolytic bacteria are found at the downstream sites. The high counts obtained throughout our sampling period indicate a constant input of untreated wastes in this system.

The low ratio of total direct count to total viable count obtained in the summer and fall of 1971 also indicate the presence of a large number of saprophytic bacteria.

Even at the relatively unpolluted upstream station, the ratios were low in comparison to those obtained by other investigators for unpolluted aquatic systems (Beling and Jannash, 1955; Korsh, 1961; Kuznetsov and Karzinkin, 1930; Ruttner, 1970).

Though effluent pipes are distributed along the entire length of Brewery Creek, the major increase in bacterial counts occurred between sites 2 and 3. In this stretch effluent enters from the meat packing plant and from at least four sanitary sewers. The nearly uniform counts obtained from station 3 onward may well reflect a balance between additional input and die-off of organisms already in the water.

It is clear from the report that Brewery Creek is heavily polluted by sewage and fatty wastes, but these results, unfortunately, do not show the specific contribution made by each. The degree of contamination of the water, however, is such that it is unfit for virtually any use by the time it flows back into the Ottawa River. It undoubtedly contributes to pollution of the Ottawa River, and, from the health aspect, may present potentially hazardous conditions for humans, animals, and river communities alike.

After construction of sewage collector pipes is completed, a reexamination of water quality in Brewery Creek would seem justified to see what improvement has actually taken place.

II) Enumeration of lipolytic psychrophiles.

The predominantly psychrophilic population of lipolytic bacteria present upstream is either indigenous to the water, added to the water via waste input further up in the Ottawa River, or present because of a combination of these 2 possibilities. On the other hand, the lipolytic population present downstream, significantly higher in numbers, is made up of a mixture of psychrophiles and mesophiles. Both types enter with sewage, as results have shown. Although we were unable to obtain direct samples of the meat-packing plant effluent and to evaluate its contribution as a source of lipolytic bacteria, several studies have shown that vast numbers of lipolytic bacteria are present in meat-packing plant operations (Ayres, 1955; Empey and Scott, 1939; Haines, 1933). The meat-packing plant effluent may therefore represent an important source of these bacteria.

Whereas lipolytic mesophiles probably die-off rapidly during the winter, their psychrophilic counterparts may actually multiply by metabolizing fatty wastes. Moreover, the significant increase in lipolytic psychrophiles downstream may be partly due to bacterial division of the numbers already present upstream.

The cytochrome oxidase test as a taxonomic aid

In 1961, Steel (1961) performed the oxidase test on 1,660 strains of various bacterial genera. Only a minority of these were cytochrome oxidase positive. Many

of the cytochrome oxidase positive types found by Steel have extremely fastidious nutritional requirements (for example, Neisseria), and would not likely grow on a medium such as Spirit Blue agar. Those that should grow on Spirit Blue agar include the following genera, all of which have been shown to include lipolytic species: Aeromonas (Chakrabarty et al., 1970; Fryer et al., 1967), Alcaligenes (Fryer et al., 1967), Bacillus (Sierra, 1957), Moraxella (Baumann et al., 1968a), Pseudomonas (Chakrabarty et al., 1970; Fryer et al., 1967; Stanier et al., 1966; Tvsset et al., 1969) and Vibrio (Chakrabarty et al., 1970; Sierra, 1957; Tysset et al., 1969). The results of our own taxonomic studies on lipolytic isolates from Brewery Creek (Table 8) confirm this prediction; all, except Bacillus species, have been isolated from 4C Spirit Blue plates during the winter sampling period. The great majority, however, were Pseudomonas sp. The absence of Bacillus species is probably due to their generally higher growth temperature requirements, and to the fact that they are more commonly found in soil than in water. The cytochrome oxidase test, therefore, appears to be of potential value in specifying the types of bacteria present in water samples.

The results of analyses of 4C-incubated plates downstream showed that only 52% of the lipolytic psychrophiles were cytochrome oxidase positive (Fig. 8). We have suggested that this may be due to the input of predominantly

cytochrome oxidase negative types via sewage wastes, as analyses of the 2 sewage samples have shown. At all other temperatures of incubation downstream, however, approximately 2/3's of the lipolytic population were cytochrome oxidase positive, even though the sewage sample plates incubated at 20C, still showed a predominance of cytochrome oxidase negative species. Two possibilities may explain this result:

1) MPP effluent (Fig. 1)

If cytochrome oxidase positive types were dominant in this effluent, then this might counterbalance the input of dominant cytochrome oxidase negative types via sewage, and account for our downstream results.

2) Selective die-off

Once introduced into the creek waters, cytochrome oxidase negative species may die-off more rapidly than cytochrome oxidase positive types, with the result that we would find a majority of cytochrome oxidase positive bacteria downstream. The study of Hoadley and McCoy (1966), mentioned in the introduction, supports this hypothesis. Their cytochrome oxidase positive type F pseudomonads (P. fluorescens), which constituted approximately 50% of their isolates, exhibited no die-off away from the sewage treatment plant outfall which they studied, while their other cytochrome oxidase negative isolates did. A possible explanation for the apparent survival of the cytochrome oxidase positive types is suggested by the observation of

Yamanaka (1964) that the cytochrome oxidase of P. aeruginosa functions as a nitrite reductase. This author has suggested that this enzyme participates in ATP formation during nitrite respiration under anaerobic conditions. The ability to use nitrate or nitrite as a terminal electron acceptor under anaerobic or partially anaerobic conditions, which is typical of cytochrome oxidase positive pseudomonads, could confer a definite advantage in polluted waters. During this quantitative study, damming of Brewery Creek was in effect. Reduction of flow rate may have produced anaerobic conditions in the creek water during this period. In fact, oxygen values, taken by Mr. Peter Rubec (graduate student, Biology Dept., U. of Ottawa) at our downstream station, showed the anaerobic conditions prevailing in Brewery Creek waters after damming occurred. Prior to damming, dissolved oxygen values were 8 ppm (surface) and 9 ppm (bottom) on June 26, 1971, and 8 ppm (surface and bottom) on July 26, 1971. After damming, O_2 values were 2 ppm (surface) and 0 ppm (bottom) on September 9, 1971, and 0.6 ppm (surface and bottom) on October 21, 1971. This selective advantage of cytochrome oxidase positive types over their cytochrome oxidase negative homologues may explain our downstream results.

Our results have also shown that more cytochrome oxidase positive bacteria are lipolytic at lower temperatures than at higher ones (Fig. 9). This would seem to indicate that cytochrome oxidase positive psychrophiles carry out

more lipid breakdown than their mesophilic counterparts, and for this reason, may play important roles in the degradation of fatty wastes in polluted aquatic systems.

During the same sampling period, 450 lipolytic isolates were classified and their lipolytic activities determined at various temperatures of incubation as a means of estimating whether a significant degradative activity can proceed under cold water conditions. The results of this investigation are discussed in the following section.

III) Classification of lipolytic psychrophiles

With the exception of a single Streptococcus isolate, all the lipolytic psychrophiles isolated from Brewery Creek were gram-negative rods. The results support the belief that the majority of psychrophiles are gram-negative rods (Witter, 1961), and more specifically that the majority of lipolytic psychrophiles are gram-negative (Breuil et Gounot, 1972).

Though the majority of the isolates were from the more polluted downstream site, all but one of the major groups (Aeromonas) were also present at the upstream site. Thus, even though the total number of lipolytic bacteria is much greater downstream, we cannot rule out the possibility that the increase is due partly to bacterial growth, stimulated by added nutrients. However, at least 7 storm sewer pipes, 15 sewage effluent pipes, and one effluent pipe from a meat packing plant dump untreated material into Brewery

Creek. In particular, Acinetobacter and Aeromonas, predominant in sewage, increased from 6% of the isolates at the upstream site to 42% downstream.

Both Acinetobacter and Pseudomonas are well known for their lipolytic (Baumann et al., 1968b; Gilardi, 1969; Stanier et al., 1966) and psychrophilic properties (Witter, 1961) and are often found in meat-packing operations (Ayres, 1955; Empey and Scott, 1939; Haines, 1933), sewage (Benedict and Carlson, 1971; Warskow and Juni, 1972), and both polluted (Druce and Thomas, 1970) and supposedly unpolluted (Bell et al., 1971) waters. Aeromonas has also previously been isolated from sewage (Chakrabarty et al., 1970) and from meat-packing operations (Eddy and Kitchell, 1959). The lipolytic properties of this group are well-known (Chakrabarty et al., 1970; Dempster, 1968).

With the exception of the single Streptococcus and Vibrio isolates, all the other groups, including Enterobacteriaceae, were relatively more frequently isolated at the less polluted upstream station. Though the Enterobacteriaceae, and more specifically both coliforms and fecal streptococci, are commonly used as indicators of sewage pollution, these are not psychrophiles, and it is possible that psychrophilic species come from different sources. In addition, lipolysis appears to be an exceptional characteristic for Enterobacteriaceae (Davis and Ewing, 1964; Lentsner et al., 1967). Alcaligenes has also previously been isolated from sewage (Benedict and Carlson, 1971), and from meat-packing opera-

tions (Ayres, 1955), but most of the species described in Bergey's Manual (1957) have higher temperature optima than our isolates, or are not lipolytic.

To our knowledge, Chromobacterium is not well known for its lipolytic properties and has not been reported in sewage or meat packing wastes. However, Breuil and Gounot (1972) have reported one isolate from a French river polluted with domestic and industrial wastes. Moraxella, at least according to Baumann (1968), is not commonly found in either soil or water. However, it was our fifth largest group (Table 8), and Bell et al. (1971) have recently obtained Moraxella isolates from the Qualicum River.

Taxonomic Problems

a) Pseudomonas

The majority of our Pseudomonas isolates (143 out of 202) were P. fluorescens or P. putida. The remaining 59 nonfluorescent isolates present some problem in classification, since Stanier et al. (1966) claim that only the fluorescent group grows at 4C. However, these workers also state that fluorescent pigment production is strongly influenced by nutritional factors. Two of our "non-fluorescent" proteolytic isolates were, in fact, fluorescent on Spirit Blue Agar, though not on King's B medium. The "non-fluorescent" isolates may, therefore, be other P. fluorescens and P. putida species which were incapable of pigment formation on the media provided.

b) Acinetobacter

The taxonomic status of the acinetobacters is less clear. Baumann, Doudoroff and Stanier (1968b) divided 106 strains of Acinetobacter into 7 sub-groups based on nutritional and physiological characteristics. In a separate study, Thornley (1967) divided 173 strains of Acinetobacter and related genera into 5 phenons, 3 of which she called Acinetobacter. However, 96% of phenon 3 and 45% of phenon 4 were cytochrome oxidase positive and therefore probably Moraxella.

All but one of four 4C isolates were, like Baumann's group B2, non-proteolytic and did not oxidize glucose. Only 5.4% of our isolates could utilize citrate, compared to 26% of Baumann's B2 isolates. However, none of the other subgroups showed any closer similarity. Our isolates also resemble the oxidase negative members of Thornley's phenon 4, only 16% of which were citrate positive. Baumann et al. (1968b) have proposed A. lwoffi as the species name for subgroup B2.

Of our 19 sewage isolates at 20C, 18 were non-proteolytic and did not oxidize glucose, but 12 could utilize citrate. Because of the latter characteristic they more closely resemble Baumann's A3 or B1 subgroups. Only two isolates oxidized glucose; both may belong to Baumann's A group.

Citrate utilization appears to be correlated with growth range and/or source. Most of our psychrophilic

acinetobacters, as well as Thornley's (1967) psychrophilic poultry isolates, were incapable of citrate utilization. In contrast, most Acinetobacter isolates from clinical specimens are citrate positive (Baumann et al., 1968b; Gilardi, 1969), as were our 20C sewage isolates. In another study, Breuillard, Michel and Lemoine (1970) found that citrate utilization was correlated with growth range characteristics and with GC content.

Our 4C Acinetobacter isolates, whether from sewage or meat packing plant wastes, are likely involved in a saprophytic spoilage role. However, mesophilic acinetobacters in raw sewage were as numerous as total coliforms (40,000,000 per 100 ml). Nineteen of our isolates, capable of growth at 37C, could conceivably be of human origin. Acinetobacters have been isolated from various human sources (Daly et al., 1962; Robinson et al., 1964; Taplin et al., 1963) and have been shown to be pathogenic.

c) Moraxella

Most of the strains classified by Baumann et al. (1968a) were clinical specimens. This may account for the difficulty in classifying our psychrophilic water isolates according to their scheme. Though there is some similarity between our isolates and their group I (M. osloensis) there are also important differences in citrate utilization, glucose oxidation, and production of lipase. These differences, and their psychrophilic nature, may warrant the majority being placed in a different species.

Degradative activities in the cold

Most of the isolates displayed stronger lipolysis at 4C, 10C and 20C than at higher temperatures (see Table 10). A small number, especially among the aeromonads (which also grew to higher temperatures), showed equally strong lipolysis at 30C and 37C. This trend has previously been noted for microorganisms involved in the spoilage of foods stored at low temperature (Witter, 1961). Since the water temperature of lakes and rivers in Canada seldom exceeds 20C, there would also be little advantage for bacteria found in aquatic habitats to possess lipolytic enzymes with a higher optimum.

Increased activity at low temperatures has also been reported for the proteolytic enzymes of P. fluorescens (Peterson and Gunderson, 1960). Though we did not determine the optimum temperatures for proteolysis, 170 of the 202 Pseudomonas isolates were proteolytic at 20C. The majority of Aeromonas, Vibrio and Chromobacterium isolates were also proteolytic, as were several members of other groups. In all cases, both gelatin and casein were hydrolyzed. In contrast, none of the Acinetobacter or Moraxella isolates were proteolytic.

With the exception of the 20C sewage isolates, all the strains characterized in this study were psychrophilic, as judged by their ability to grow at 4C. However, most were capable of growth at 30C; only 9 isolates out of 434 failed to grow at this temperature. In contrast, 107 grew

at 37C, and 14 (11 of them aeromonads) at 42C, the highest temperature tested. Other studies (Stokes, 1963; Werkman and Wilson, 1951) have also shown that many psychrophiles have relatively high maximum growth temperatures. Clearly, a wide growth range gives psychrophiles an advantage over their mesophilic counterparts since they can degrade usable substrates all year round.

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Appendix 1

Classification of bacterial genera mentioned in the introduction, according to Bergey (1957)

GENUS	ORDER	FAMILY
1) Gram-negative rods		
Achromobacter	Eubacteriales	Achromobacteriaceae
Acinetobacter[1]	Eubacteriales	Achromobacteriaceae
Aerobacter	Eubacteriales	Enterobacteriaceae
Aeromonas	Pseudomonadales	Pseudomonadaceae
Alcaligenes	Eubacteriales	Achromobacteriaceae
Arthrobacter	Eubacteriales	Corynebacteraceae
Caulobacter	Pseudomonadales	Caulobacteraceae
Chromobacterium	Eubacteriales	Rhizobiaceae
Comamonas[2]	Pseudomonadales	Spirillaceae
Cytophaga	Myxobacterales	Cytophagaceae
Escherichia	Eubacteriales	Enterobacteriaceae
Flavobacterium	Eubacteriales	Achromobacteriaceae
Moraxella	Eubacteriales	Brucellaceae
Pasteurella	Eubacteriales	Brucellaceae
Pseudomonas	Pseudomonadales	Pseudomonadaceae

Salmonella	Eubacteriales	Enterobacteriaceae
Serratia	Eubacteriales	Enterobacteriaceae
Shigella	Eubacteriales	Enterobacteriaceae
Sphaerotilus	Chlamydobacteriales	Chlamydobacteriaceae
Spirillum	Pseudomonadales	Spirillaceae
Vibrio	Pseudomonadales	Spirillaceae
Zoogloea	Pseudomonadales	Pseudomonadaceae
2) Gram-negative cocci		
Hyphomicrobium	Hyphomicrobiales	Hyphomicrobiaceae
3) Gram-positive rods		
Actinomyces	Actinomycetales	Actinomycetaceae
Bacillus	Eubacteriales	Bacillaceae
Brevibacterium	Eubacteriales	Brevibacteraceae
Corynebacterium	Eubacteriales	Corynebacteraceae
Lactobacillus	Eubacteriales	Lactobacillaceae
Microbacterium	Eubacteriales	Corynebacteraceae
Mycobacterium	Actinomycetales	Mycobacteraceae
4) Gram-positive cocci		
Diplococcus	Eubacteriales	Lactobacillaceae
Micrococcus	Eubacteriales	Micrococcaceae
Sarcina	Eubacteriales	Micrococcaceae
Streptococcus	Eubacteriales	Lactobacillaceae

5) Non-gram stainable coiled rod

Leptospira	Spirochaetales	Treponemataceae

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[1] Described as a non-motile Achromobacter in Bergey (1957). In the French classification, it is known as Acinetobacter (Order: Bacteriales, Family, Pseudomonadaceae), according to Brisou and Prévot (1954).

[2] This genus was proposed by Davis and Park (1962) to characterize certain non-curved rods apart from the genus Vibrio.

Appendix 2

Detailed characteristics of Spirit Blue Agar plates from which lipolytic bacteria were isolated for classification purposes

Sample reference number[1]	Dilution of isolation plate	Total colonies	Total COP colonies	COP lipol. colonies	lipol. col. numbers	Numbers isolated
F 52-A	1/500	66	12	10	51	10
F 52-B	"	62	36	22	47	5
F 52-C	"	289	141	112	183	15
F 53-A	"	64	37	7	21	6
F 53-B	"	136	71	9	47	13
F 53-C	"	109	35	10	50	9
F 54-A	"	86	44	36	74	9
F 54-B	"	85	40	27	65	9

F 54-C	"	96	62	58	84	6
F 55-A	"	52	24	3	17	6
F 55-B	"	108	29	17	40	9
F 55-C	"	105	27	10	30	10
F 56-A	"	94	60	29	56	9
F 56-B	"	68	31	16	43	8
F 56-C	"	80	48	23	51	9
F 57-A	"	116	70	67	113	10
F 57-B	"	136	76	66	126	10
F 57-C	"	78	43	42	74	10

F 58-A	"	64	39	39	62	9
F 58-B	"	74	50	50	74	9
F 58-C	"	84	52	52	84	12
F 59-A	"	65	44	44	64	12
F 59-B	"	55	43	43	54	11
F 59-C	"	54	39	39	54	11
F 60-A	"	100	52	52	100	10
F 60-B	"	175	90	90	175	11
F 60-C	"	119	70	70	119	10
F 61-A	"	82	44	36	73	13
F 61-B	"	66	43	41	64	14

F 61-C	"	61	47	42	58	11
F 62-A	"	152	80	80	152	11
F 62-B	"	156	82	82	156	10
F 62-C	"	105	59	59	105	13
W 59-A	1/10	26	20	19	24	14
W 59-B	"	11	7	5	9	5
W 59-C	"	28	21	20	26	14
W 62-A	"	111	74	74	111	9
W 62-B	"	126	81	81	126	11
W 62-C	"	121	73	73	121	11
B-200 -A	$1/10^5$	113	37	13	47	11

F 61-C	"	63	47	42	58	11
F 62-A	"	152	80	80	152	11
F 62-B	"	156	82	82	156	10
F 62-C	"	105	59	59	105	13
W 59-A	1/10	26	20	19	24	14
W 59-B	"	11	7	5	9	5
W 59-C	"	28	21	20	26	14
W 62-A	"	131	74	74	131	9
W 62-B	"	126	83	83	126	11
W 62-C	"	121	72	72	121	13
S-20C -A	$1/10^5$	113	37	12	47	11

S-20C -B	"	50	12	5	30	8
S-20C -C	"	34	5	3	21	8
S-4C -A	$1/10^4$	64	17	9	37	13
S-4C -B	"	50	21	16	24	7
S-4C -C	"	22	2	1	11	7

[1] Numbers 52 to 62 inclusively refer to the bi-monthly sampling dates when isolations were performed. This covered the Nov. 23rd, 1971 to May 1st, 1972 period. Sewage isolates were obtained from a sample taken on July 17, 1972. F-Fournier. W-Wright. S-sewage. Triplicate samples (A, B, C) were taken at each station.

Appendix 3

Group differentiation of the 6 psychrophilic lipolytic *Enterobacteriaceae* isolated, using the 20 biochemical tests of the Analytab system

Test	*Enterobacteriaceae* reference number					
	F56A-1	F59A-6	W59B-4	W59C-3	W62B-4	S(20C)-C-1
Lactose	+	+	+	-	+	+
Arginine dihydrolase	-	-	-	-	-	-
Lysine decarboxylase	+	-	-	-	-	+
Ornithine decarboxylase	+	-	+	-	-	-
Citrate	+	-	-	-	-	+
H_2S production	-	-	-	-	-	-
Urease	+	+	+	+	-	+
Tryptophan deaminase	-	-	-	-	-	-
Indole	+	+	+	-	-	-
Acetoin	-	-	-	-	+	+
Gelatin	-	+	+	+	-	-
Glucose	+	+	+	+	+	+
Mannitol	+	+	+	-	+	+
Inositol	-	(±)	+	-	-	+
Sorbitol	+	(±)	+	-	-	+
Rhamnose	(±)	-	-	(±)	-	+
Saccharose	+	+	+	+	+	+
Melibiose	-	-	-	+	+	+
[illegible]	+	+	+	(±)	+	+