SALT-FLOCCULATED ORGANIC MATERIAL AS A FOOD SOURCE IN ESTUARINE FOOD WEBS.

By

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ABSTRACT.

Large amounts of particulate material were found to flocculate from water collected from local, organic rich streams upon addition of salt water. This material was found to compose approximately 50% humic substances and to have a C:N ratio of about 50:1. Feeding studies using floc having various degrees of bacterial colonisation showed that bacterial abundance was an important determinant of the palatability of this material to the copepod grazer, *Tigriopus californicus*. Palatability of flocculant particles increased linearly with increasing bacterial abundances in the range of 0-10^6 cells $\cdot$ mL^{-1}, while increases in bacterial abundances above this concentration resulted in lower than expected grazing rates. Further inoculations with single strains of bacteria isolated from the floc showed that this increased palatability occurred with only certain components of the floc fauna. Ingestion of floc colonised by a "normal" bacterial fauna was found to be beneficial for the survival of C1 and adult stages of *Tigriopus* and was found to sustain egg production of inseminated females. The presence of floc did not, however, result in the improved survival of the earlier naupliar stages of *Tigriopus*. Mass spectrometric analyses suggest that significant amounts of carbon are assimilated from floc made from water collected during the winter months. As floc is ingested at very low rates in the absence of colonising microbes, benefit from the ingestion of floc is postulated to result from the conversion of floc carbon into microbial biomass and the subsequent assimilation of those microbes by the copepod grazer. The fact that few differences exist between the IR-spectra of floc food and the faecal material derived from copepods fed a floc diet suggests that little change is undergone by the bulk of the organic matrix during ingestion and passage through the copepod gut. Ingestion of floc may therefore work to protect the flocculant material from subsequent microbial degradation by compacting the material into pellets which will be rapidly delivered to the sediments as a result of high sinking rates. Feeding studies with a variety of species collected from local coastal

waters suggest that a wide spectrum of organisms may ingest flocculant material when that material is present in their environs. As flocculant material is primarily produced in the winter, a time when estuarine primary productivity is low, floc is postulated to play an important role in sustaining these organisms through periods of low food availability.

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GENERAL INTRODUCTION.

Every year rivers carry large amounts of organic material to the ocean. This organic export includes particles such as fragments of plant material and the remains of animals, as well as a large component of dissolved organic material (DOM); (Hedges et al. 1986; Romankevich 1984). Most of the organic material involved is derived from terrestrial production via leaching and physical transport of leaf litter and soil by rainfall, although a small portion of the material is produced *in situ* by photosynthesis of stream phytoplankton and submerged macrophytes and the death of associated grazers. The organic material carried by rivers represents about 1% of the total annual terrestrial primary production (Mantoura and Woodward 1983) and contributes significantly to the pool of organic material in both the estuarine and ocean environments (Romankevich 1984). An understanding of the behaviour and biological processing of this material is therefore of great interest to biologists and geochemists alike.

Of the total organic load carried by rivers, the majority is composed of very small particles and "dissolved" materials (Naiman and Sedell 1978; Mullholland 1981). The division between these two fractions is not easily determined, as a continuous spectrum of particle sizes exists. As a result, an arbitrary division based on passage through filters of a certain pore size is used to define these two fractions. The pore dimension utilised for this purpose varies among investigators and with the nature of the questions being addressed, but generally lies between 0.22μm (Meyer et al. 1987) and 1μm (Ogura 1977). The most frequent pore size used is 0.45μm (Boggs et al. 1985). Of the two fractions, the "dissolved" fraction is the more abundant, with the DOM:POM ratio lying in the range of 1.25 (Meybeck 1982). It is this "dissolved" component of the organic load of rivers that will be the focus of discussion.

To date, only a limited amount of information has been collected on the concentrations of DOM in rivers (Boggs et al. 1985). From the data sets that are available, the average concentration of DOM in rivers is generally calculated to lie in the range of 5mg · L^{-1} (Postma 1968; Van Bennekom and Salomons 1981; Stopinstev and Krylova 1955 quoted in Romankevich 1984), although estimates as high as 10mg · L^{-1} have been made (Sholkovitz 1976). The reasons for this wide range of calculated average DOM concentrations are several fold. Firstly, different estimates arise from differences in the nature of the areas drained by the rivers used by the authors in the calculation of their averages. For example, Mullholland and Kuenzler (1979) found that the export of organic carbon by streams with considerable swamp drainage was several times higher than that of streams of similar magnitude draining upland watersheds. Similarly, Vernadsky has calculated the range of organic material in river water to be as wide as 0.62-100mg · L^{-1} (Vernadsky 1960, cited by Romankevich 1984). Secondly, there is a strong seasonal component to the export of organic material. Mullholland and Kuenzler (1979) found organic carbon export to be greatest in winter and spring (times of maximum stream discharge) and noted particularly large export values associated with flood events in early fall. Finally, there is a wide variation in the concentration of dissolved material in rivers as a function of latitude, ranging from an average 3mg · L^{-1} in temperate and arid areas to 6mg · L^{-1} in the tropics and 10mg · L^{-1} in subarctic regions (Boggs et al. 1985). Calculated average values for DOM must thus be interpreted with caution.

To date, the composition of the DOM fraction in rivers has been poorly characterised (Meyer et al. 1987). However, the data sets that are available suggest that the DOM includes a wide range of compounds from molecules as small as methane to large, complex compounds such as hydrocarbons and humic substances. Also commonly encountered in natural waters are amino acids, fatty acids, polypeptides, lipids and

polysaccharides (Boggs et al. 1985). However, as these latter substances are generally only present in trace quantities and are rapidly utilised by microorganisms, the "dissolved" phase has usually been suggested to be predominantly refractory in nature (Mullholland 1981; Van Bennekom and Salomons 1981). Even this is in question though for, as Breger (1972) points out, dissolved material is added to rivers throughout their course, so at any point along the river there should be at least some fresh organic matter available for degradation. None the less, the labile compounds are often present in concentrations too dilute for effective utilisation by microbes. Thus, utilisation of DOM in rivers is not generally an important source of energy for riverine biota.

The transformation of DOM into particles has often been postulated as a mechanism for enhancing the availability of organic material to decomposers as well as to macroscopic grazers (Baylor & Sutcliffe 1963; Sieburth & Jensen 1970; Seki 1972; Mullholland 1981; Bowen 1984; Porter 1988). Evidence to support this hypothesis has recently been provided by Tranvik and Sieburth (1989) who have shown that a strong increase in bacterial abundance occurs when a dissolved organic fraction is converted to the particulate phase. One process which facilitates such a transformation is that of flocculation or aggregation, in which about 5-10% (Sholkovitz 1978), or perhaps as much as 25-30% (Mullholland 1981), of the riverborne organic material changes from a dissolved or colloidal state to a particulate one through interactions with marine cations. It should be noted that, throughout this paper the term flocculation will be used in the sense of Sholkovitz (1976) i.e. "a general term denoting a change from the dissolved to the solid phase" rather than in the sense of "particle transport" of O'Melia (1972) and Ezwald et al. (1974). As thus defined, the flocculation process takes place during the mixing of organic-laden fresh water with seawater in estuaries, with aggregation beginning at salinities near 0‰ and being complete by about 15-20‰ (Sholkovitz 1976).

The exact mechanism of the flocculation process is complex and poorly understood but, to the extent of our present understanding, can be summarised as follows.

Riverine organic materials are generally rich in negatively charged functional groups such as carboxylic groups and phenolic hydroxides. These groups are strongly hydrophilic and their abundance in aquatic organic materials allows even fairly large molecules to be in "solution" in fresh water. However, upon mixing with seawater in the estuary, several major reactions occur. The first of these is a compression of the "electrical double-layer" surrounding each molecule as a result of the high ionic strength of seawater. This double layer compression reduces the distances between molecules or particles at which repulsion by like charges occurs. Particles can thus approach close enough to one another for Van der Waals attractive forces to become strong enough to overcome all later repulsion by the fields of like charges surrounding each particle. Under such circumstances the two particles will coalesce and, if the resulting particle is large enough, they will enter the particulate phase (Mayer 1985). The second component of the flocculation process is one in which the charged groups of organic molecules are neutralised by interactions with seawater cations (primarily Ca^{2+} and Mg^{2+}) causing them to become increasingly hydrophobic. Such molecules will react by "balling up" upon themselves in what is known as the "Fuoss" effect and, in so doing will pass out of solution into the particulate phase (Crerar et al. 1981; Mayer 1982; Sholkovitz and Copland 1981; Smith and Milne 1981). This charge neutralisation also has the effect of reducing the repulsive forces between molecules, thus allowing them to coalesce as a result of Van der Waals interactions. In addition to causing charge neutralisation, multivalent cations can complex with more than one functional group and can, as a result, form bridges between several organic molecules, further increasing the aggregation effect (Mullholland 1981). Simple cation interactions are, however, not the only factors required to induce flocculation. This is illustrated by the fact that colloidal iron particles

are prerequisite to the occurrence of flocculation in estuaries, even at high cation concentrations (M. Preston, cited in Mantoura 1981). The exact role of iron in the flocculation process is unknown, but it has been suggested that the iron molecule acts as a nucleus around which flocculation is centered (similar to the action of dust particles in nucleating rain-drop formation).

The most rapid rate of particle formation in flocculation occurs in at salinities of 0-5‰ and the process is complete at salinities of 15-20‰. The process of flocculation is rather speedy, with 95% of all the material flocculated being formed within the first thirty minutes of mixing (Sholkovitz 1976). Resulting particles are amorphous and are generally dark brown in colour and several tenths of a micrometer in size (Mayer 1982). Over time these particles appear to aggregate with one another and also to incorporate other particles from the surrounding water column, resulting in large complexes reminiscent of the "marine snow" particles found in the open ocean (Prézlin and Alldridge 1983; Silver et al. 1986).

As mentioned, the solubility and complexing ability of organic material is primarily a result of the presence of negative charged functional groups such as carboxylic and phenolic groups associated with organic materials. As the degree of dissociation of such groups is strongly pH dependant, so the potential degree of cation complexation is also pH dependant. Above a pH of about 5, dissociation of carboxylic groups begins to occur and increases progressively until the pH reaches a level of about 10 (Bohn et al. 1985). As seawater frequently has a higher pH than does fresh water, due to the buffering capacity of the seawater components, increased levels of dissociation and thus of solubility and metal complexing ability would be predicted as rivers flow into the ocean. However, while increased dissociation inevitably occurs as acidic, organic

rich streams flow into an estuary, experiments have shown that pH is less important than salinity in controlling the flocculation of organic material in estuaries (Sholkovitz 1976).

An interesting aspect of the flocculation process is its intimate association with the transformation of many different kinds of metal from the filterable to the particulate phase. During the flocculation process, 80-100% of the riverine "dissolved" iron, 40% of copper and nickel, 25-45% of manganese and about 20% of the aluminium as well as minor amounts of other metals are transformed from the filterable to the particulate state, presumably by complexation with the flocculant organic material (Sholkovitz et al. 1978). This results in formation of metal enriched particles, the ingestion of which may result in the introduction of previously filterable metals into estuarine food chains (Huljev 1986). The importance of this "food vector" for metals is, however, strongly affected by the biological availability of that particle to the grazer (Luoma 1983). If floc is digested by organisms, the high concentrations of metals might become hazardous to the grazer, or to other organisms feeding upon it. If, alternately, the material passes through the gut of the organism unchanged, the threat of metal bioaccumulation occurring as a result of ingestion of the particle is much lessened. In contrast to this scenario of metal accumulation in the particulate phase in estuaries, other investigators (Cross and Sunda 1977; Duinker 1980) have found that, in some cases, exposure to seawater actually reduces the degree of complexation of metals such as copper with organic material, thus increasing the toxicity of the metal in the dissolved rather than the particulate phase. In such estuaries, the threat of bioaccumulation of metals through the ingestion of floc would presumably be less severe. It can thus be seen that changes in the degree and direction of metal complexation reactions in estuaries are a dynamic and important factor in determining the impact of the ingestion of flocculant material in estuaries.

The chemical identity of the material formed during the flocculation process is not well characterised. Humic substances unquestionably form a major portion of the flocculating materials (Sholkovitz 1976) although estimates as to the proportion of riverine organic material they compose vary widely. Sholkovitz et al. (1978) found the proportion to be 3-5%, Fox (1983a) estimated it at 0-20%, Sharp et al. (1982) at 7-20% and Aiken et al. (1985) and Malcolm (1985) both used estimates of 50%. Humic substances (HS) are defined as a group of heterogeneous organic substances having high molecular weights and yellow-black colouration. There are three major groups of humic substances. The first of these is the humic acids (HA) which are defined as that fraction of HS that is insoluble at pH 2, but is soluble at higher pHs. The second is the fulvic acids (FA) which are the fraction of the HS which is soluble at all pHs. The third is the humin fraction, which is the fraction of the HS that is not soluble at any pH and is therefore of little importance in aquatic systems. As mentioned, humic substances are generally very large molecules, having molecular weights of 500-1000 Daltons in the case of aquatic fulvic acids and molecular weights of 100,000 or more in the case of aquatic humic acids (Mayer 1985). In general humic substances have high concentrations of aromatic and long chained aliphatic groups and thus have rather low solubilities. Humic substances are thus obvious candidates for an active role in flocculation type processes. The study of the behaviour of humic substances during flocculation has thus received much attention and as a result, is one of the best understood portions of the flocculation process.

Estimates of the proportion of humic substances flocculating within the estuary range from 60-80% to 100% of the total "filterable" (passes through a 0.45μm filter) humic acid (Sholkovitz et al. 1978 and Fox 1983a respectively). However, as noted by Fox (1983b), there are instances where no significant removal of humic materials occurs during the mixing of riverine and ocean water under laboratory conditions. The

occurrence of humic acid removal is thus postulated by Fox to be strongly dependant upon either the chemical nature of the molecules making up the humic fraction and thus upon the characteristics of the watershed from which the humic substances originate, or else to be a process involving more factors than can be accounted for in the laboratory experiments.

When humic flocculation does take place, it generally represents the loss of only a small fraction (<20%) of the total dissolved materials, but results in a 30-60% reduction in the concentration of the high molecular weight organics within the estuary (Fox 1983a). This result reflects the findings of Sholkovitz et al. (1978) who found that approximately 43% of the material flocculated upon mixing with seawater was in the largest size fraction in his solution (filtered by 0.45μm filters, retained by 0.05μm filters), with decreasing amounts flocculating in the smaller sized fractions. From these results it can be implied that flocculation of riverine organic material selectively removes the larger molecular weight size fraction, leaving the smaller, more hydrophilic substances to continue on their passage into the ocean (Mantoura 1981). It is thus the larger humic substances (generally assumed to be humic acids) that are major components of salt-aggregated flocs in estuaries.

No estimates for the proportion of fulvic acids involved in flocculation are available, as it is usually assumed that these molecules are smaller and more hydrophilic than humic acids and thus do not contribute significantly to estuarine flocculation. However, as the distinction between humic and fulvic acids is arbitrary, based on differential solubilities in acid and base, it seems probable that many "fulvic" acids are in fact involved in estuarine flocculation. As fulvic acids are generally found in concentrations an order of magnitude higher than those of humic acids in aqueous

environments (Mayer 1985), these may contribute significantly to flocculation if even a small percentage of the total material aggregates.

Unfortunately, so much attention has been focused upon the flocculation of the humic and fulvic acid portion of the riverborne organic material, that the fact that many other organic molecules may also be involved (Fox 1983; Mayer 1985; Roman and Tenore 1984) is often obscured or completely overlooked. It should be recognised that, in addition to the fulvic and humic acid moieties, other substances such as carbohydrates and proteins are also present, although less abundantly in the flocculant material. Moreover, other substances such as polysaccharides which do not themselves aggregate, as well as larger particles such as bacteria, algae and faecal pellets may be scavenged by aggregates, resulting in a diverse chemical composition for the overall particle (Silver et al. 1986).

To look at the quantity of flocculant material produced in estuaries in terms of its potential global significance, some simple calculations of the amount of material produced annually can be made using literature values. These can then be compared with estimates of the annual primary production in regions where flocculation occurs. Postma (1968) uses a global average value of 5mL $\cdot$ L^{-1} of DOM for river input and a value of total runoff via rivers of 4×10^{13}g $\cdot$ $annum^{-1}$ to calculate the total supply of DOM to the ocean to be 2×10^{14}g $\cdot$ $annum^{-1}$. Other estimates are similar, ranging from 1.8 to 7 x 10^{14}g $\cdot$ $annum^{-1}$ (Bordovsky 1965b; Garrels and MacKenzie 1971). If we use a figure of 10% for the portion of this material flocculating in estuaries to convert Postma's DOM into estimates of POM produced by flocculation, this translates to 2×10^{13}g of new particulate material being formed in estuaries annually. Being about 50% by weight carbon (Steinberg and Muenster 1985), this converts to 1×10^{13}g $\cdot$ $year^{-1}$ new estuarine POC. Although this amount is at least an order of magnitude less than the estimated

global primary productivity in estuaries of 1.4×10^{14} to 1.4×10^{15}g C · year^{-1} (Williams 1981 and Woodwell et al. 1978 respectively), it still represents a substantial pool of organic material that may be available to grazers. Moreover, maximum flocculation in many rivers can be assumed to occur during the winter months, when rainfall and thus river runoff are highest. As this coincides with the time of minimum photosynthesis in the water column, there is a strong potential for flocculant material formed during this time to play an important role in sustaining organisms grazing upon it, if floc is indeed ingested.

As can be seen then, flocculation processes result in the production of a very large source of particulate carbon which may be available to estuarine grazers. This food source has been largely overlooked in the literature for several reasons. The first of these is that flocculant material has been assumed to consist mainly or entirely of humic acids, which are generally regarded as being refractory (Malcolm 1985). Thus the flocculant material has also been considered to be essentially immune to significant biodegradation (e.g. Mayer 1985). There are, however, several reasons to suggest that a re-evaluation of the assumed refractory nature of flocculant material should be made. The first is that there is ample evidence that humic molecules are in fact subject to significant biodegradation (e.g. de Haan 1972; Postma et al. 1978; Ramunni et al. 1987; Ryhannen 1968; Swift et al. 1987). The floc matrix may therefore provide a significant energy source to colonising microorganisms. Secondly, although humic substances do represent a major proportion of the flocculating materials, other, more labile materials, such as proteins and polysaccharides may also be incorporated into the floc matrix. These materials are a potentially important energy source for colonising microorganisms (Gjessing 1976; Means and Wijayaratne 1984; Roman and Tenore 1984). Such utilisation of lower molecular weight compounds complexed with larger, more refractory ones has been utilised by Meyer et al. (1987) to explain high rates of microbial growth

upon the largest fraction of the DOC from a blackwater stream. By contrast though, a similar study by Stabel et al. (1979) found no such degradation of the larger molecular weight size fraction of stream DOC. However, the largest size fraction analysed by Stabel's group corresponds closely with Meyer's "intermediate" size fraction which Meyer's group also found to be extremely unreactive. As the molecules that dominate the flocculation process are predominantly of high molecular weights, it seems possible that floc material incorporates the more bioactive components of the stream DOC and may, as a result, harbour a rich and active microfaunal population. Moreover, even if the floc matrix is completely inert, it may still exert a considerable influence on the growth of microorganisms by sorbing nutrients and organic substances from the surrounding medium and thus providing an enriched microzone in which rapid growth and cycling of the microbial fauna can occur (Jannasch and Pritchard 1972; Goldman 1984).

Another reason for the very small number of studies investigating the role of flocculant material in food webs again derives from the predominance of humic type molecules in such particles. Humic substances represent a complex spectrum of an almost infinite number of "random heteropolymers" (Malcolm 1985) the chemical identity of which is, as a result, extremely difficult to characterise. Advances in instrumentation such as those represented by Nuclear Magnetic Resonance (NMR) and Infra-Red (IR) spectrometry techniques have lead to many advances in the description of specified groups of humic molecules, particularly when those molecules can be brought into solution in organic solvents. However, these techniques are somewhat less satisfactory for the analysis of particulate materials such as floc. Working with a group of compounds that represent such a "black box" of unknown nature and composition has, perhaps understandably, been avoided by most investigators. None the less, many experiments can be carried out to examine the overall significance of ingestion of floc by

estuarine organisms, even in the absence of a complete characterisation of the floc material.

That estuarine flocculated material is ingested by copepods had previously been established (Lewis and McNee, unpublished data). However, little was known of the factors controlling the ingestion of this material. Moreover, whether any energetic benefit derived from this behaviour was also unknown. The object of this study then, was to investigate the energetic derived from ingestion of floc and to determine the mechanism by which that benefit was imparted. During the study, research centered upon several major areas each of which will be the focus of one chapter of this thesis. The first aspect of the food-web importance of floc examined was that of the factors which influence the ingestion of that material and thus the degree to which it can be of benefit to the grazer. The second aspect addressed was that of the extent of energetic benefit resulting from the ingestion of floc during specific developmental stages of the test organism. The third section of this paper presents experiments designed to determine which of three postulated routes is responsible for any benefit derived by the organism from grazing upon the flocculant material. The three roles proposed for the contribution of floc to estuarine food-webs are: that floc carbon may be directly assimilated by macro-organisms via digestion; that it may be assimilated by bacteria and the resulting microbial carbon utilised by macro-organism (i.e. a "microbial loop"); or that floc carbon itself may not be assimilated by the macro-organism. In this last scenario, organisms or particles otherwise too small to be utilised by that grazer, colonise or somehow become associated with the larger floc moiety and are thus converted into a particle that can be effectively utilised without the loss of energy associated with trophic transfer. This third mechanism has frequently been postulated for "marine snow", where again the food quality of the particle matrix is in question (e.g. Silver et al. 1986). In addition, the results of experiments to assess the potential extent of the ingestion of floc by zooplankton species

present in local coastal waters are presented and the findings of this study will be combined to present a comprehensive picture of the role of flocculant organic material in estuarine food webs.

I. FACTORS AFFECTING PALATABILITY OF FLOC.

Introduction.

Many factors may affect the palatability of flocculant materials. Primary among these are:

1) The degree and nature of the microbial colonisation of the floc;
2) The presence in the surrounding medium of materials that may be degraded or utilised in association with the floc; and
3) The nature of the flocculating molecules in particles.

With regard to the first of these, the fact that bacteria can play an important role in the nutrition of higher organisms via the "microbial food web" is now well established in the literature (e.g. Cammen 1980; Edwards 1987; Rieper 1978). In addition, the idea that the presence of bacteria upon particles enhances the nutritional quality of that particle has also been illustrated (Review by Fenchal & Jφrgensen 1977; Kirchman 1983; Tenore et al. 1982). Finally, the role of a particulate matrix in converting bacteria into a size fraction available to macrofaunal grazers has also been postulated (e.g. Paerl 1974; Prézlin and Alldridge 1983). It thus seems reasonable to hypothesise that bacterial colonisation of floc should enhance its palatability. This was tested by feeding copepods floc having different levels of microbial colonisation and measuring the resulting grazing rates.

That all bacteria may not be equal with regard to their food quality for copepods has been shown for a variety of marine harpacticoid copepods in a series of studies carried out by Rieper (1978, 1982 and 1984). If bacteria do indeed enhance the

palatability of flocculant materials, it is pertinent to ask whether the copepods react equally to all components of the microbial fauna, or whether only a portion of the microfauna is responsible for this increase. This question was addressed by inoculating "sterile" floc with different abundances of individual strains of bacteria and measuring the resulting grazing rates.

A source of nitrogen in the surrounding medium is often postulated to be of importance to microbial metabolism. This assertion arises from the very high C:N ratio characteristic of that material which is often equated with low biological availability (e.g. Wetzel 1975). Several studies have found increased degradation of detrital materials in the presence of an organic or inorganic source of nitrogen (Findlay & Tenore 1982; Tenore et al. 1979). As estuaries are sites of high concentrations of both inorganic and organic nutrients, this seems to represent a feasible pathway by which floc could be more actively colonised and metabolised and thus could become a richer food source for grazers. In order to test for possible priming effects from inorganic nitrogen on the floc fauna, an experiment was carried out to investigate the effect of enhanced nitrate levels upon the degree of floc colonisation and the palatability of the resulting particles to a copepod grazer.

The third factor postulated to play a role in determining the palatability of flocculating material is the nature of the compounds involved. As mentioned earlier, little is known about the nature of the salt-flocculated materials except that they consist, to a major degree, of humic substances. Degradation of these materials is generally a rather slow process (Strome and Miller 1978) and thus may not produce significant energy on a time scale relevant to copepod grazers. None the less, seasonal changes in the nature of humic materials that might influence the degradation of those molecules do exist (Johnsen et al. 1987; Steinberg & Muenster 1985). Moreover, the presence of other

molecules such as proteins and polysaccharides, even in trace concentrations, could be an important factor in changing the food value of the resulting particles.

Unfortunately, methods to characterise the humic acids once flocculated and to detect the presence of other components within the floc matrix are either unavailable, not sensitive enough for use with the small amounts of materials involved or else require such a high degree of sample handling before analysis that the relationship between the nature of the original substance and that of the material actually analysed is, at best, hazy. None the less, throughout this study attempts were made to gain information on the flocculant material and the stream water from which it was formed. In particular, measures of the C:N ratio and the proportion of the material flocculating that was humic in nature were made. Comparisons of the palatabilities of the floc in different seasons were also made, as was a comparison between the palatability of floc formed from natural water and that of floc made up solely of pure humic acids.

Materials and methods.

Sample sites.

Organic rich water was collected from several locations as shown in Figure 1.1. Of the locations shown, the primary sample site was Crescent Slough, an organic rich stream draining "Burns Bog" and the surrounding agricultural area of the Municipality of Delta. This stream empties into the Fraser River through sluice gates controlled by a small pumping station for which six years of flow were available (Municipality of Delta, Dale McTaggert, pers. comm.). These data were used to approximate the monthly flow

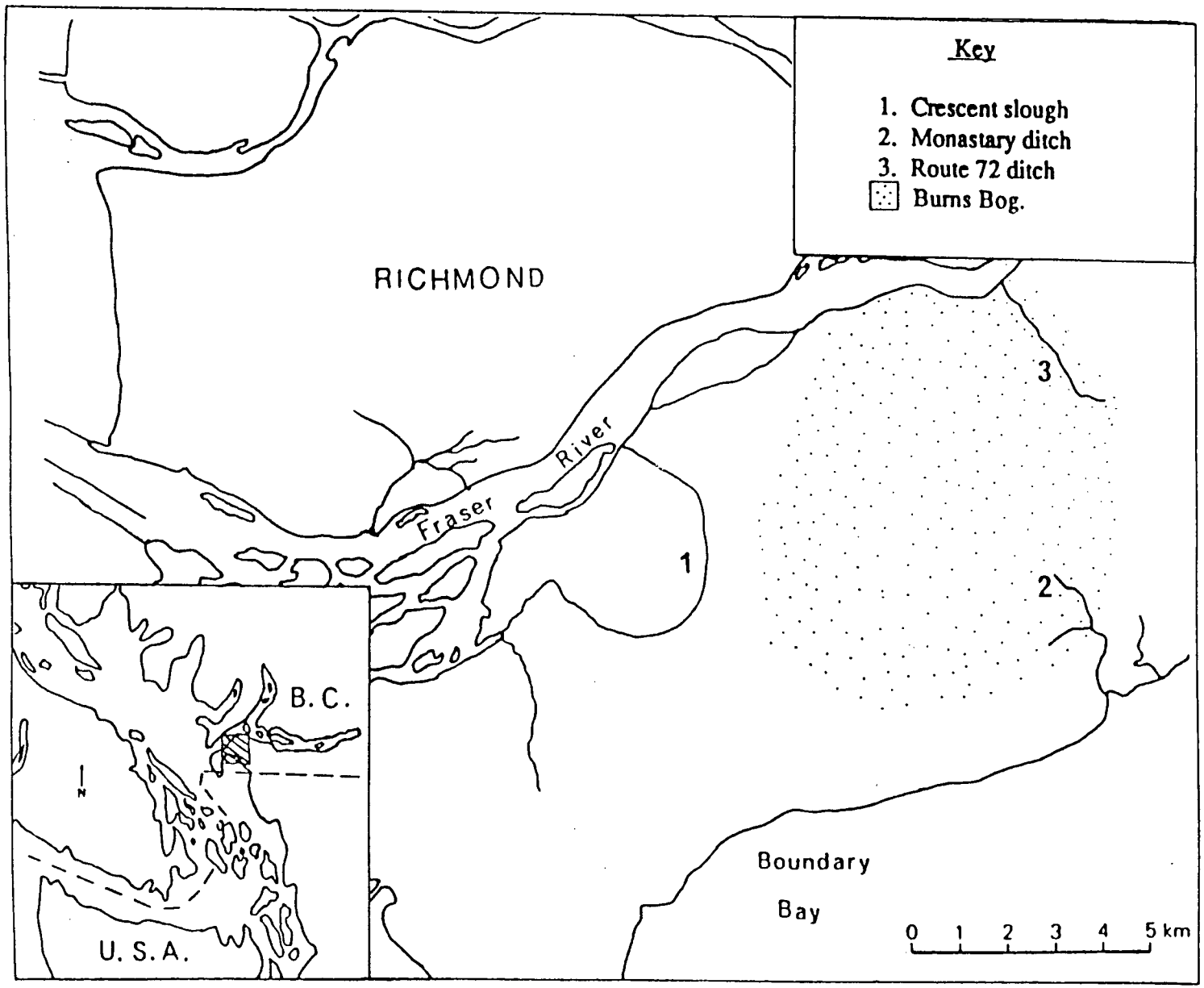


Figure 1.1. Location of the streams used as sources of organic rich water throughout this study.

of this stream by multiplying the hours each pump operated each month by the capacity of the pump. The resulting mean monthly flow estimates are recorded in Appendix 1, Table A1.1 and are presented graphically in Figure A1.2. Calculations using this data set give the yearly volume of water entering the Fraser River Estuary from this slough to be about 1 x 10^8 L (Table A1.1).

As can be seen from Figure A1.1, during the summer months this stream effectively ceases to flow. In fact, during this time organic poor Fraser River water is allowed to fill the stream bed for use in the irrigation of the surrounding fields. If collection of water samples for this study was required during the months in which no organic rich water was present in Crescent slough, collection was made either from "Monastery Ditch" at the point where it passes the East Delta Municipal Hall (site 2, Figure 1.1) or, when this too was impractical, from the "Route 72" drainage ditch, immediately upon its outflow from Burn's Bog, near River Road (site 3, Figure 1.1).

It should be noted that the primary slough used as a source of organic rich water in this study, Crescent Slough, flows into the Fraser River fairly high in the estuary. As salinities in these upper reaches of the estuary are fairly low, flocculation of the water from this particular source may not in fact take place. However, the intention of this study was not to measure the influence of this stream as a potential source of food within the estuary, but rather to provide a hypothetical model of what the consequences of flocculation of organic material in estuaries in general might be. As such, the selection of a source of organic rich water was a rather arbitrary decision with Crescent Slough and the other streams sampled being selected merely for their ease of access for sampling and their particularly rich organic load.

Sample collection.

Samples were collected from the slough with a bucket deployed by means of a 3m length of nylon rope. Water thus collected was placed into a 4L plastic bottles which had previously been washed with successive washes of 12N HCl, 1N HCl and rinsed several times with distilled water in order to remove organic and metallic contaminants. Dates of water sample collection and preliminary observations on the colour of the water sample and the degree of flocculation experienced when that sample was mixed with seawater are given in Appendix 1, Table A1.2. Upon return to the laboratory, the bottles of slough water were stored in the dark at 4°C until use.

Preparation of standard ocean water.

In order to ensure a constant composition for the seawater used in flocculation experiments, the salt solution Standard Ocean Water (SOW) (Morel et al. 1979) was used for all flocculation experiments unless otherwise stated. Solutions of SOW were prepared by dissolving appropriate amounts of all the major seawater salts (excluding $MgCl_2.6H_20$) in 12L of glass distilled water (GDW) in a 20L Pyrex vessel. Once completely dissolved, the $MgCl_2.6H_20$ which had been oven dried, was weighed out and added to the solution. The volume in the flask was then made up to 20L with GDW. Once all the salts had dissolved, the solution was bubbled with clean air until the pH of the SOW reached 8.0±0.05. The SOW was then passed through a resin column (Chelex 100, 100-200 mesh, Bio-Rad® Laboratories) to remove heavy metal contaminants.

When sterile SOW was required the medium was bubbled with clean CO_2 until the pH was approximately 5, at which point the SOW was placed in the autoclave. The

duration of the sterilisation period was volume dependant, with a 20 minute exposure to 121°C and 20 atmospheres pressure being utilised per litre. Reduction of pH resulting from bubbling with CO_2 minimised the precipitation of the magnesium salts during autoclaving and SOW returned naturally to a pH of 8 during the sterilisation process.

Description and maintenance of test organism.

The primary organism used in this study was *Tigriopus californicus*. This harpacticoid copepod was originally collected from splash pools on the West Coast of Vancouver Island in the mid 1960s and has been maintained in culture at U.B.C. since that time. The cultures used in this study were maintained in 2.8L Pyrex® flasks filled with SOW at 35‰ and pH 8 in a 16°C environment room having a 16:8 hour day-night cycle. Copepods were fed a diet of ground fish food (Wardley's® Basic Fish Food for Tropical Fish) and were placed in fresh medium approximately once every 21 days.

The life history of *Tigriopus californicus* involves twelve stages: six naupliar and six copepodite stages, the final (C6) of which is the adult form. In this species insemination of the female occurs only once, immediately after the moult between the C5 and C6 stages. The female will then proceed to produce a number of egg-sacs over a period of several weeks. As this copepod has a very rapid rate of growth the young produced will undergo an entire life cycle (from egg to egg) in approximately 20 days under laboratory conditions (Feldman 1986). During this development period the diet of *Tigriopus* changes considerably, with the diet of younger organisms being predominantly bacteria (Harris 1973) while the diet of the copepodite stages is dominated by detrital material and benthic algae (Dethier 1980). In this regard, it should also be noted that the first feeding stage of this organism appears to be the second nauplius (Feldman 1986).

Tigriopus is renowned to be an extremely tolerant organism (Burton et al. 1979; Dethier 1980), being able to withstand extreme changes in salinity and temperature and to survive long periods of starvation. Moreover, in its natural habitat of tide pools in the high intertidal (Dethier 1980) it would rarely, if ever, encounter flocculant material. As such then, *Tigriopus* was not the ideal test organism for use in this series of tests. Unfortunately, at the start of this project, no other copepod species were available in culture and attempts to culture the less tolerant calanoid copepod, *Acartia clausii*, were unsuccessful. Thus, out of necessity, *Tigriopus* was adopted as the test organism throughout the major portion of this study.

Preparation of copepods for use in experiments.

For the experiments on the feeding preferences of *Tigriopus*, C6 stage individuals of either gender were isolated from the main cultures, placed in polystyrene Falcon® tissue culture plates and rinsed with successive washes of clean SOW until all detritus had been removed. Copepods were then incubated in the laboratory for at least 24 hours to allow clearance of previously ingested food material during which time several further rinses with clean SOW were used to remove any faecal pellets produced. Clean organisms were then transferred into a SOW-antibiotic solution modified from Droop (1967), containing 800mg penicillin, 180mg dihydrostreptomycin, 40mg neomycin, 8mg of chloramphenicol and 20mg of dextrose per 100mL SOW. After 12-18 hours copepods were transferred from this solution into wells of a sterile Falcon® tissue culture plate containing autoclaved SOW and were allowed to recover for at least 12-36 hours before being used in an experiment. Such copepods were found to be axenic using the acridine orange direct count method of Hobbie et al. (1977). At this time any gravid females were

discarded as cannibalism of the earlier naupliar stages of *Tigriopus* is common (Burton et al. 1979 and pers. obs.) and prevents meaningful interpretation of the rates of pellet production observed during the feeding studies.

Experiments.

A. Preliminary analysis of organic rich stream water.

In addition to the observations on the colour and degree of flocculation made for each water collection, further analyses were carried out on a number of samples. The simplest of these was a characterisation of absorbance properties at 250, 365 465 and 665nm for the samples collected on Feb. 4, June 21 1988 and Jan. 23 and May 8 1989 using a Beckman DU-64 spectrophotometer. These measurements were made at natural pH values (around 8), as preliminary tests showed that absorbance of these samples was not significantly affected by pH variation within the range between pH 4 to 10, a range much wider than the natural variation at these sites. The ratio of the absorbances at 465 and 665nm have been used (primarily by soil scientists) to delineate molecular weight and the degree of condensation of the organic fraction (e.g. Kalinowski & Blondeau 1988; Summers et al. 1987). Essentially, this ratio is an expression of the chromatophore density of the compound under investigation. Increasing molecular size in organic molecules such as humic substances is associated with an increased density of chromatophores and thus with high absorbances in the red region of the spectrum. The E_4/E_6 ratio thus decreases with increased molecular size (Power and Langford 1988). This ratio and the E_{250}/E_{365} value, proposed by de Haan (1972) to be a superior delineator of the character of aquatic organic materials, were calculated for each sample.

An estimate of the amount of dissolved organic material (DOM), defined here as that material passing through a 0.8µm filter, was determined for the February 1987 water sample. (Throughout this study 0.45µm filtration was not utilised as it caused a significant reduction in the amount of flocculant material derived from that fraction, presumably by removing a portion of the larger colloidal organic molecules.) This analysis was made by lyophilising a known volume of water in a pre-weighed flask. Once all water had been removed the flask was re-weighed and the amount of "dissolved" material in the sample calculated by difference. The C:N ratio of this filterable material was determined using a CHN analyser (Carlo Erba Elemental Analyser, Model 1106).

Investigation of the nature and extent of the flocculation process was carried out using water from the May 1987 collection. Floc was made from this water by mixing exactly 500mL of 0.8µm-filtered slough water with exactly 500mL of autoclaved SOW. The mixture was stirred for 48 hours and the resulting particles were observed and measured at the microscope on two occasions, the first being immediately after stirring, the second five hours later. After observation samples were allowed to stand for 24 hours before being filtered onto pre-weighed 0.8µm Millipore® filters. They were then rinsed with two 150mL washes of 0.8µm filtered GDW to remove adsorbed salts and were dried at 65°C for twenty-four hours and the weight of newly flocculated material was calculated by difference after re-weighing the filters. In order to extract the humic portion of the flocculated material, filters were carefully re-wetted and 100mL of 1N NaOH was passed through the filters followed by two rinses of 50mL of GDW. Filters were then re-dried, re-weighed and the total amount of humic substances in the floc was calculated by difference. Finally, in order to determine the proportion of the humic acids in the "humic" fraction, the pH of filtrate and the water from the two rinses was reduced to 2, causing the humic acid fraction to flocculate. The humic acid was removed from

this solution by filtration onto a clean, pre-weighed 0.8μm filter and rinsed with two washes of 50mL of GDW. Filters were dried, re-weighed and the amount of humic acid was then calculated. The portion of mass dissolved from the initial floc not accounted for by the material flocculated in acid, was assumed to represent the fulvic acid fraction.

The CHN ratio of the flocculant material from the May 1987 sample collection was determined using a CHN analyser (Carlo Erba Elemental Analyser, Model 1106).

B. Importance of bacterial colonisation.

Preparation of floc.

"Normal" floc.

To make normal floc, slough water was filtered through a 0.8μm filter in order to remove the majority of suspended particulate material. 500mL of the filtered slough water was then placed into an autoclaved 1L erlenmeyer flask sealed with a cotton plug that had been wrapped in white paper to prevent shedding of cotton threads into the solution. 500mL of autoclaved SOW was added to the slough water and the mixture was then stirred for 24 hours to ensure maximum flocculation. An incubation period of at least one week was used to allow floc to develop a strong microbial fauna.

"Bacteria-free" floc.

In order to study the effect of microbial colonisation, it was first necessary to develop a microbe-free floc, the degree of colonisation of which could subsequently be

manipulated. Of the techniques tested, pasteurisation of the floc for one week at 65°C was adopted as the method that gave best sterilisation with least chemical changes to the resulting flocculant material. Pasteurised flocs were formed by mixing 500mL of 0.8µm filtered slough water with 500mL of sterile SOW in an autoclaved 1L flask sealed as for the normal floc. In this case though, a further barrier against evaporation and contamination was added in the form of an aluminium foil cover. Immediately after mixing, the sealed flask was placed into an oven at 65°C for one week, after which the sample was homogenised and a 15mL sub-sample was removed using sterile technique. This sub-sample was used to enumerate bacterial abundances on the floc using the acridine orange direct count technique of Hobbie et al. (1977). If bacteria were still present the flask was returned to the oven for a further two to three days after which time the sample was discarded if not sterile. Those samples that were successfully rendered "bacteria-free" were cooled and allowed to settle for several hours before use in any experiment.

"Reinoculated" floc.

The pasteurisation technique utilised in this study could potentially give rise to important changes in the nature of the compounds in the floc, such as denaturation of any associated proteins. In order to test for such changes, a reinoculated treatment was adopted in which pasteurised floc was reinoculated with the fauna found on normal floc. Palatability of this reinoculated floc could then be compared with that of floc that had never been treated. To this end floc that had been pasteurised for five days was removed from the oven, cooled and allowed to settle. 700mL of the overlying water was then decanted and replaced with the water overlying a sample of normal floc prepared at the same time as the floc that was sterilised. By this action the "bacteria-free" floc was reinoculated with the complete spectrum of microorganisms present in the normal fauna.

Subsequent to reinoculation the floc was allowed to stand in the laboratory for two days to allow the fauna to establish itself upon the floc. Longer periods for this incubation would have been preferable, but could not be used as the paired "bacteria-free" sample could not be allowed to remain in the oven for more than 1 week without risking serious heat damage to the floc.

1) Palatability changes with various degrees of bacterial colonisation.

These experiments were carried out to determine the importance of microbial colonisation upon the relative palatability of the different types of floc. Three floc types were tested:- normal floc, bacteria free floc and reinoculated floc (prepared as described above). Two control treatments were also employed:- a starved treatment to give an indication of any residual pellet production resulting from food remaining in the organism's gut and a treatment in which ground fish food was provided to allow comparison between experiments run at different times by testing for changes in the ingestion rates of a standard food source by the copepods used on each occasion. All feeding experiments were run in sterile polystyrene culture dishes (Falcon®) using autoclaved seawater and all transfers were made using sterile glassware. All floc treatments were allocated 2mL of concentrated floc suspension per well, an amount which ensured provision of an excess of material over that processed by the organisms during the experimental period. Fish food treatments were allocated a small amount (≈ 2mg) of ground fish food suspended in 1mL of sterile GDW and starved treatments were provided with 1mL of sterile GDW. Six replicates of each treatment were made for each 24 hour period run and a random design was used to assign treatments to wells. To ensure that observed grazing rates were a true indication of palatability, rather than an initial, non-selective response to the suspension of the preceding period of starvation it

was necessary to continue the experiment for longer than one 24 hour period. Thus, at the outset of the experiment, several hundred axenic copepods were allocated wells containing each of the five treatments tested. Containers containing these copepods were maintained at 16°C on a 16:8 hour day-night cycle and a five day sequence of grazing rates for each food source was obtained by isolating fresh copepods from the appropriate well for each 24 hour run.

To avoid the anomalous behaviour patterns that occur when individual *Tigriopus* are isolated for study (O'Brien 1987 and pers. obs.) five copepods were provided to each treatment well. Treatments were then incubated for 24 hours at 16°C with a 16:8 hour day-night cycle, after which copepods were removed using a sterile pipette and the number of pellets in each well was recorded. For each treatment, counts of the pellets produced per organism over a 24 hour incubation (as used by Gaudy 1974, Harvey et al. 1935, Lance 1964) were used as a measure of the grazing rates because particle count methodologies are inappropriate for use with amorphous and fragile materials such as floc. From these counts, average pellet production per organism was calculated. Upon the rare occasions upon which a mortality occurred during the 24 hours of the experiment, averages were calculated on the basis of the number of copepods surviving at the end of the run. Feeding experiments were carried out floc made with freshly collected water from three different seasons: May 1987 ("summer"), February 1988 ("winter") and April 1988 ("spring").

The value of precise estimates of bacterial abundance was recognised soon after the commencement of experimentation. Thus, for all experiments except that with summer floc, bacterial abundances were determined using the acridine orange direct count method of Hobbie et al. (1977). Bacterial abundances were enumerated both before each 24 hour run and after enumeration of faecal pellets at the end of the run with

an average "treatment" abundance being obtained for the latter by pooling the contents of all replicate wells before enumeration. However, as the similarity of both counts was very high, only the "after" counts will be reported.

Differences in the levels of pellet production rates and in bacterial abundances between treatments were determined using the appropriate t-test programmes on the statistics package, Epistat.

2) Palatability changes with various abundances of single strains of bacteria.

Preparation of agar plates and broths used in bacterial isolation.

Media utilised in this study were modified from those used by Weiner et al. (1980) for the culture of estuarine bacteria. For all isolations, plates of a simple marine peptone-yeast-extract (PYE) agar were prepared by autoclaving 12g of bacto-agar in 950mL SOW and 2mg of peptone and 1mg of yeast extract in 50mL of GDW. (Separation of these two media during heating prevented salt-precipitation of the protein.) After autoclaving the solutions were combined and poured into sterile 50mL petri dishes using sterile technique. Plates were allowed to cool and solidify before inoculation and any plates not used immediately were double bagged in plastic and refrigerated at 4°C until required.

PYE broth was prepared by autoclaving, separately, 950mL SOW and 2mg of peptone and 1mg of yeast extract in 50mL of GDW. After sterilisation, these solutions were mixed and poured into sterile 30mL screw capped test tubes using sterile technique.

Filled tubes were sealed and allowed to cool before inoculation and any tubes not utilised immediately were placed in a 4°C refrigerator until required.

Isolation of individual bacterial strains from floc.

Colonies of the strains composing the floc fauna were obtained by streaking floc material onto marine PYE agar plates. Plates were then incubated in the dark at 16°C until colonies were established (3-7 days) when bacteria from individual colonies were sampled and transferred to fresh PYE plates. Transfers continued to be carried out until plates contained pure colonies of the bacteria in question (2-3 transfers). Individual strains were inoculated into tubes of PYE broth and were incubated in the laboratory until exponential growth was observed (18-24 hours) at which time the culture was used in the inoculation of sterile floc.

All of the strains isolated above must have had their origin in fresh water, as sterile seawater medium was used in flocculating the material from which they were isolated. As a natural floc would also be colonised by marine microorganisms, three strains of bacteria of marine origin were obtained for use in palatability studies (J. Smit, Dept. Microbiology, U.B.C. pers. comm). *Pseudomonas atlantica*, *Deleya marina* and *Caulobacter* strain MCS6 were obtained as stab preparations on agar and were transferred from these to PYE broth before inoculation onto sterile floc.

Each strain isolated from floc was examined for its Gram staining properties, motility, ability to use various substrates and other properties by Dr.B. Ramey (Department of Microbiology, U.B.C., pers. comm.) in order to determine its taxonomic identity. A summary of some of the results of this analysis is given in Appendix 2, Table

A2.1. Tentative identities of each strain, where available, are given in Appendix 2, Table A2.2.

Inoculation of sterile floc.

All experiments using bacteria isolated from floc were made using floc made from water collected in January 1989, while those using known bacterial strains were carried out with floc from the May 1989 water collection. 125mL flasks containing sterilised floc from these dates were inoculated with different abundances of individual strains by the addition of various numbers of drops of the bacteria in PYE-broth prepared as described above. The number of bacteria in the initial inoculum usually varied from 10 to 150 million cells. Inoculated floc was incubated in the laboratory for two days to allow the fauna to establish itself upon the flocculant particles.

Feeding experiments.

Five replicates of each floc type were prepared by pipetting 2mL of concentrated floc suspension from the bottom of each flask into each well of the Falcon® tissue culture plates designated to receive that treatment. Five starved controls receiving 1mL of sterile GDW each were also employed for each experiment and all wells were topped up with autoclaved SOW. Treatments were allocated to wells according to a systematic experimental design and five copepods were placed in a controlled environment room at 16°C with a 16:8 hour day-night cycle and were allowed to feed in each for 24 hours. Pellet production and bacterial abundance in each treatment were enumerated as for experiment B1, this chapter, and these variables were related using their correlation

coefficient as calculated by the graphical package, Sigmaplot and determining their significance level from tables (Zar 1984).

3) Seasonal changes in the palatability of flocculant material.

As mentioned earlier, differences in the nature and composition of dissolved organic materials in fresh waters during different seasons have been reported by several authors (e.g. Johnsen et al. 1987; Steinberg and Muenster 1985). Differences in the nature and thus in the palatability of the flocculating materials were thus hypothesised to exist. To investigate this possibility, palatability of floc from each of the three seasons analysed (experiment B1) was compared using the standard, fish food treatment to control for differences in copepod grazing rates between experiments. Comparisons between pellet production levels for floc from each season and between bacterial abundances for the winter and spring data were made with the appropriate t-test programmes of the statistics package, Epistat.

C. Checks of the validity of the methods used during feeding experiments.

1) Effects of long term inoculation.

Reduced pellet counts from organisms fed reinoculated floc relative to those in the normal floc (Figs. 1.2a-c) might be interpreted as an indication of heat damage to the sterilised floc. However, this trend could also reflect the inadequacy of the short post-inoculation incubation period required by the limited period that the sterile floc could be allowed to remain in the oven. In order to determine which of these mechanisms was in

fact responsible for the observed effect, an experiment was run in which reinoculated floc was incubated for six weeks before execution of the feeding study. If heat damage is incurred during pasteurisation, the palatability of the reinoculated floc should remain lower than that of the normal floc, regardless of the duration of the incubation period. However, if the observed reduction in palatability merely reflects the inadequacy of the original re-colonisation period, grazing rates should be similar with both types of floc after the longer incubation.

For this experiment paired samples of normal and reinoculated floc were prepared daily for five days as before (Chapter 1, B1) except that a six week incubation period after reinoculation was used to ensure complete establishment of the fauna. At the end of this period, feeding experiments were carried out using these two floc types and a starved control following the same protocol as used for experiment B1, this chapter. Differences in palatability and degree of bacterial colonisation were tested with appropriate t-tests using the statistics package, Epistat.

2) Validity of flocculating with SOW rather than natural seawater.

Use of natural seawater was avoided throughout most of this study because of the potential variability in the metal concentrations, concentrations and nature of DOC and other such constituents of that medium. Unfortunately, this decision created the possibility that bacteria or some trace component, present in natural seawater but absent in the artificial medium had been overlooked during the feeding studies. To investigate this possibility, two experiments were carried out to determine whether the use of SOW had, in fact, made an important difference to the palatability of the flocculant material formed.

i) Comparison of the palatabilities and bacterial abundances in floc made with sterile SOW and that made with sterile natural seawater.

For this experiment floc was made by mixing 500mL of 0.8µm filtered slough water from the May 1989 collection with either 500mL of autoclaved SOW or with 500mL of autoclaved natural seawater. Four replicates of each floc type were prepared, stirred for 24 hours and allowed to stand for another six days to allow development of the microbial fauna. 2mL of concentrated floc solution from each flask were allocated to five replicate wells of Falcon® tissue-culture trays. Five starved control treatments were also run and all treatments were assigned to wells using a systematic design. Five antibiotic-cleaned *Tigriopus* were allocated to each well and pellet production and bacterial abundances in each replicate over a 24 hour period were determined. The floc treatments were compared with regard to these variables using paired t-tests with the statistics package, Epistat.

ii) Comparison of palatabilities and bacterial abundances in floc made with sterile SOW and that made with 0.8µm filtered natural seawater.

Bacterial strains removed by the sterilisation of seawater media might also play an important role in determining floc palatability in nature. To investigate this possibility, the previous experiment was repeated with the modification that seawater that had been 0.8µm-filtered rather than sterilised was used for flocculation. This resulted in flocs that had a microbial fauna as closely resembling that found in natural flocculant material as possible. Floc for this experiment was also formed from water from the May

1989 collection however, due to problems with early runs of the experiment, the water used for the run presented here had been in storage at 4°C for nearly two months. The importance of the seawater derived portion of the floc fauna was determined by comparison of the palatabilities and bacterial abundances of seawater and SOW flocculated floc using the paired t-test programme of the statistics package Epistat.

D. Other factors potentially influencing palatability.

1) Effect of elevated concentrations of inorganic nitrogen.

As mentioned earlier, increased biodegradation in the presence of enhanced levels of nitrogen has been postulated for detrital food sources (Findlay & Tenore 1982; Tenore et al. 1979). This experiment tested the ability of the inorganic nitrogen in the form of nitrate to enhance the palatability of and/or the microbial abundances on flocculant particles using levels of enrichment similar to those utilised for phytoplankton by Parslow et al. (1984).

For this analysis, exactly 500mL of 0.8µm filtered slough water was mixed with exactly 500mL of autoclaved SOW which had been enriched with nitrate in the form of $NaNO_3$. Three replicates of each of four nitrate enrichments: 0, 100, 500 and 1000µM were prepared. After a six day incubation period, each floc treatment was fed to five replicates of five antibiotic-cleaned *Tigriopus* and pellet production over a 24 hour period and bacterial abundances in each replicate treatment were determined and were tested for differences with paired t-tests using the statistics package, Epistat.

2) Comparison of floc formed from organic rich slough water with that from technical grade humic acid.

Comparison between the palatability of floc derived from a pure solution of humic acid with that formed natural seawater was felt to be of interest because of the high proportion of the flocculant material that is humic in nature (Mayer 1985; Sholkovitz et al. 1978; this chapter section A). In order to make such a comparison, a solution of pure humic acid of comparable concentration to natural slough water was prepared by mixing 25mg · L^{-1} of technical grade humic acid (TGHA) (Aldrich®) with autoclaved GDW. Two replicates of 500mL of an 0.8μm filtrate of this solution and one of 500mL of 0.8mm filtered natural slough water were poured into 1L erlenmeyer flasks, mixed with 500mL of sterilised SOW, stirred for 24 hours and incubated in the laboratory for two days. 700mL of the water overlying the floc in one of the SOW-TGHA mixtures were then decanted and replaced with 350mL of the water overlying the slough water floc in order to introduce the slough water fauna to the TGHA floc. After a further six days of incubation, six replicates of 2mL of concentrated floc solution from each flask and six starved controls were allocated to wells in Falcon® tissue culture plates according to a systematic experimental design. Five antibiotic-cleaned *Tigriopus* were then allocated randomly to each well and allowed to feed for 24 hours, after which time pellet production rates and bacterial abundances were enumerated and compared with paired t-tests using the statistics package, Epistat.

Results.

A) Preliminary analysis of slough water and flocculant material.

The E_{465}/E_{665} and E_{250}/E_{365} absorbance ratios for each water sample analysed are recorded in Table 1.1. It can be seen that the E_{465}/E_{665} ratios for these water samples are quite similar at all times, ranging from 6.6 to 7.7. Similarly the E_{250}/E_{365} ratios also have a fairly narrow range, with all values lying between 2.4 and 3.

Results from the lyophilisation of the February water showed that this water contains approximately 99.1 ± 19.5 mg · L^{-1} of filterable material (n=5). From analyses using the CHN analyser, this material was found to be only 9-16 weight percent carbon, giving a calculated range of DOC values for the slough water of 9 to 16 mgC · L^{-1}. Comparison of the carbon and nitrogen peaks from this analysis showed the filterable material from this slough to have a C:N ratio of 40:1.

The flocculant material produced by the mixing of slough water and SOW is a dark brown colour and consists of small amorphous particles 2-4μm in diameter. These aggregate over a period of hours to form large clumps, 10-200μm in diameter under non-turbulent conditions. Microscopic examination shows the flocculant particles to be strongly colonised by bacteria and micro-flagellates with a high proportion of the bacteria appearing to be associated with the floc rather than the surrounding water. The C:N ratio of flocculant material from February water was calculated to be 50:1 from CHN analysis.

Flocculation of water from May 1987 was found to produce 9.61 ± 2.31 mg · L^{-1} of new particulate material (n=12). Analysis of this flocculant material showed that

Table 1.1. Summary of the absorbance (E) ratios for water samples collected in various seasons.

	Feb. 1988	May 1988	June 1988	Jan. 1989	May 1989
E_{250}/E_{365}	3.0	3.0	2.8	2.5	2.4
E_{465}/E_{665}	6.9	7.1	6.6	7.2	7.1

about half of this material (4.07 ± 0.97 mg $\cdot$ L^{-1}) was humic in nature, and of the humic fraction about 50% (2.36 ± 0.85 mg $\cdot$ L^{-1}) had the solubility properties of a humic acid.

B. Importance of bacterial colonisation.

1) Palatability changes with various degrees of bacterial colonisation.

Pellet production data from the feeding experiments using floc made from summer, winter and spring water are plotted in Figures 1.2a, b and c. Paired t-tests between treatments on each experiment day show that, in all three seasons, "bacteria-free" floc is significantly less palatable than either normal or reinoculated floc at all times ($p < 0.01$). Normal floc is significantly more palatable than reinoculated floc on days 2, 4 and 5 with "summer" floc, at all times with "winter" floc and at all times except day 5 with "spring" ($p < 0.05$). Only on days 1, 2 and 5 of the summer experiment are the rates of pellet production in the starved treatments statistically different from zero. However, even on these occasions, pellet production is less than one pellet per organism per day, a level that is insignificant in comparison to the rates in any of the treatments in which food was provided. Anomalous results resulting from residual pellet production from material do not, thus, appear to be a problem in this experiment.

Pellet production over the five day run is similar within all treatments for the summer floc. With winter floc, pellet production essentially decreases over the five day period, with significant reductions in pellet production being found between day 5 relative to days 2,3 and 4 ($p < 0.001$) in the "bacteria-free" treatment. Decreased pellet production also occurred in the normal floc treatment between days 4 and 5 relative to

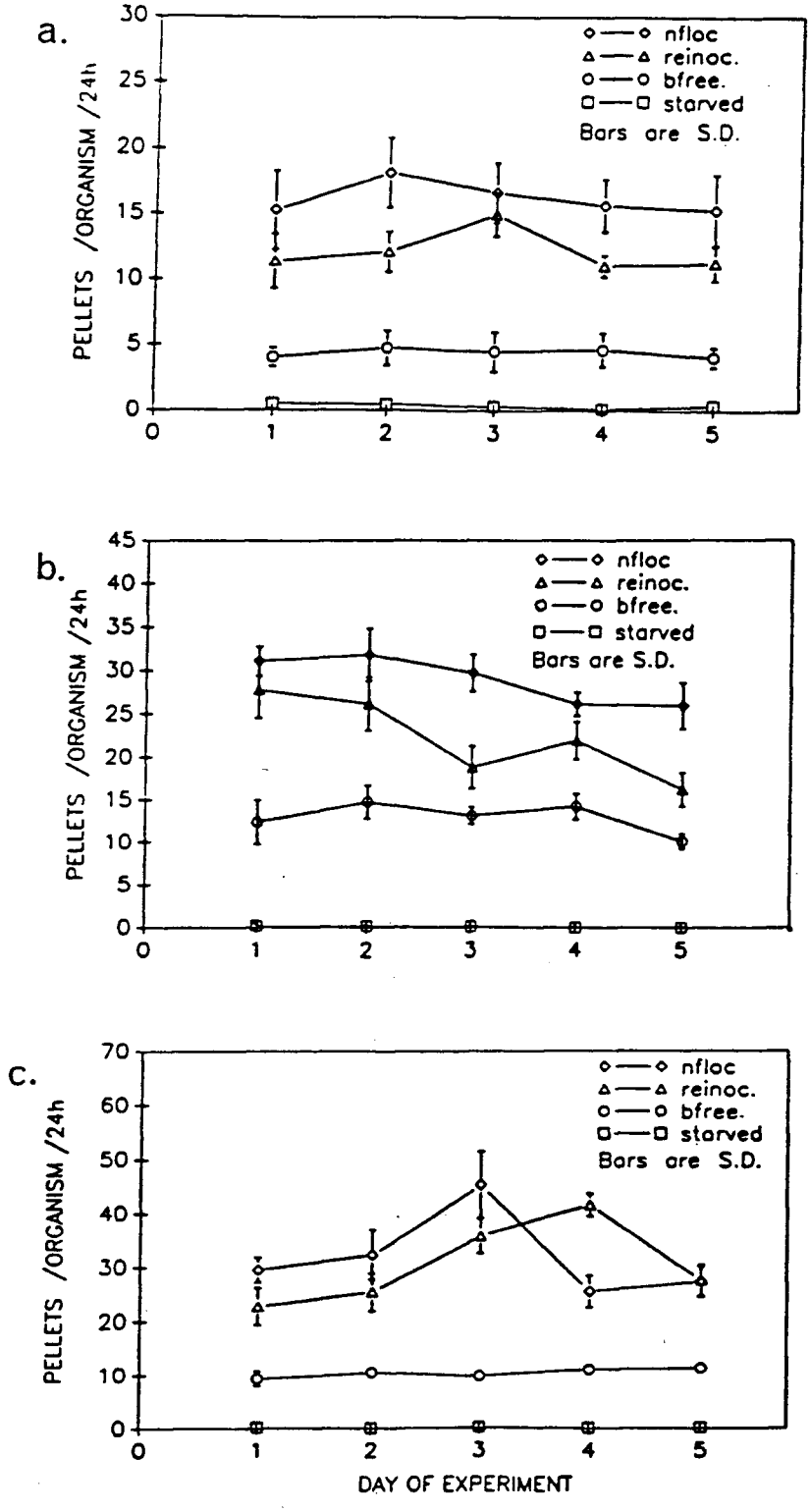


Figure 1.2. Comparison of pellet production of *Tigriopus* with different types of floc. a. Summer 1987. b. Winter 1988. c. Spring 1988.

days 1, 2 and 3 ($p < 0.05$) and in the reinoculated floc for days 4 and 5 relative to days 1, 2 ($p < 0.05$). In the reinoculated treatment the floc on day 3 also had very low palatability, significantly lower than that of floc on days 1, 2 and 4 ($p < 0.05$). For spring floc, palatability of the "bacteria-free" floc showed a slight but significant increase in pellet production on days 4 and 5 relative to days 1 and 2 ($p < 0.05$). Both normal floc and reinoculated floc underwent significant increases in palatability during the run. Pellet production with normal floc peaked on day 3, with palatability on that day being significantly higher than that of any other day ($p < 0.005$). This peak was followed by a strong decrease in pellet production on day 4 to a level significantly lower than that of days 1 and 2 ($p < 0.05$) before returning to levels similar to those of days 1 and 2 by day 5.

Bacterial abundance data from the winter and spring experiments are plotted in Figures 1.3a and b. Bacterial abundances in the starved and bacteria free treatments were similar at all times for floc from water of both seasons. Bacterial abundances in normal and reinoculated floc were significantly higher than those in the bacteria free treatment at all times for floc from both winter and spring water ($p < 0.0005$). In floc made from winter water, bacterial abundances on normal floc were significantly higher than those on reinoculated floc on days 1,3 and 4 ($p < 0.005$). For floc formed from spring water, bacterial abundances in the normal floc were significantly higher than those on reinoculated floc on all but the fifth day ($p < 0.05$).

Bacterial abundances in the winter "bacteria-free" floc decrease between day 1 and 2, peaks on day 3 when bacterial abundances are significantly higher than on all other days ($p < 0.0001$) and then decreases significantly on both days 4 and 5 ($p < 0.0005$). However, the bacterial abundance on the "bacteria-free" floc on the fifth day is still significantly higher than that of day 1 floc ($p < 0.0001$). Bacterial abundance

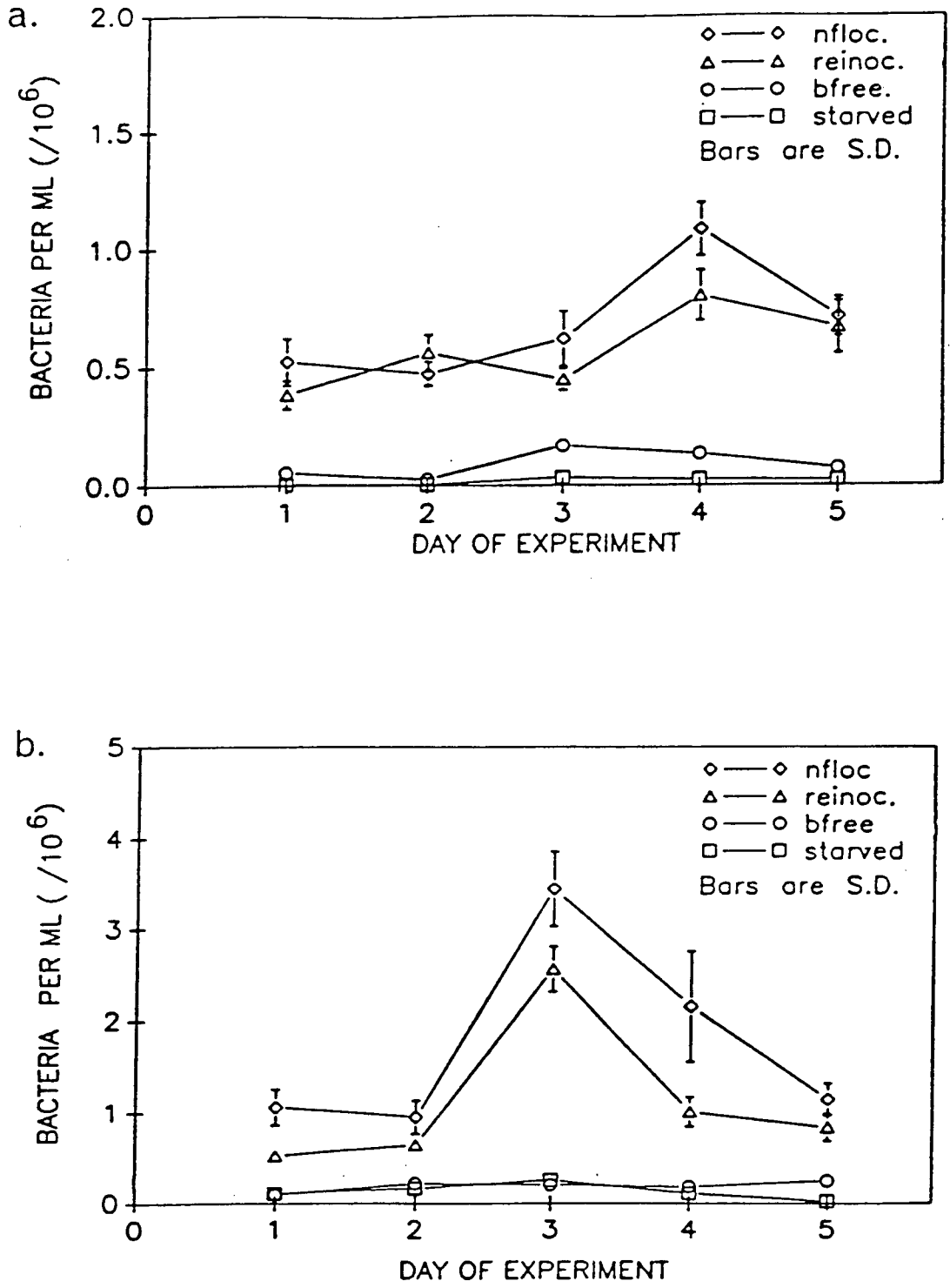


Figure 1.3. Comparison of bacterial abundances in floc and starved-control treatments after the completion of each 24h run. a. Winter 1988. b. Spring 1988.

increases over the five day run in both the normal and reinoculated floc for the winter floc with the exception of significant decreases in pellet production between days 1 and 2 in the normal floc, days 2 and 3 in reinoculated floc and between day 4 and 5 in both normal and reinoculated floc. In floc from spring water a remarkable peak in bacterial abundance occurred on day three in both the normal and the reinoculated floc treatments. In normal floc bacterial abundance on both days 3 and 4 are significantly higher than those on days 1, 2 and 5 ($p < 10^{-6}$) even though there was a significant decrease in abundance between days 3 and 4. By day 5 bacterial abundance had returned to the same level as that on day 1. The reinoculated floc from this season showed a similar pattern of abundance, except that bacterial abundances on both day 4 and 5 remained significantly higher than those at the start of the experiment.

Comparisons between the degree of bacterial colonisation and the level of pellet production for the winter and spring data are found in Figures 1.4a and b. Because both x and y variables are estimates, simple linear regression analysis may not be appropriate for analysis of this data set. Thus, for purposes of comparison only, the r values and slopes for each graph are reported (Figs 1.4 a & b). For both data sets significant correlations ($p < 0.01$) exist between pellet production and bacterial abundance. However, in the spring experiment bacterial concentrations higher than 10^6 cells $\cdot$ mL^{-1} were encountered on the floc. For these heavily colonised flocs levels of pellet production were lower than would be predicted from the relationship between bacteria and pellet production at lower bacterial abundances. Omission of the points resulting from this heavily colonised floc results in a higher correlation coefficient for this data ($r = 0.97$ rather than $r = 0.89$), however, both values are significant at the 0.01 level. Similarly, omission of these points results in an increase in slope corresponding in an improvement in pellet production of 28 pellets per 10^6 cell $\cdot$ mL^{-1} increase in bacterial abundance rather than the overall increase of 9 pellets for the same increase in bacterial abundance reported on Figure 1.4 b.

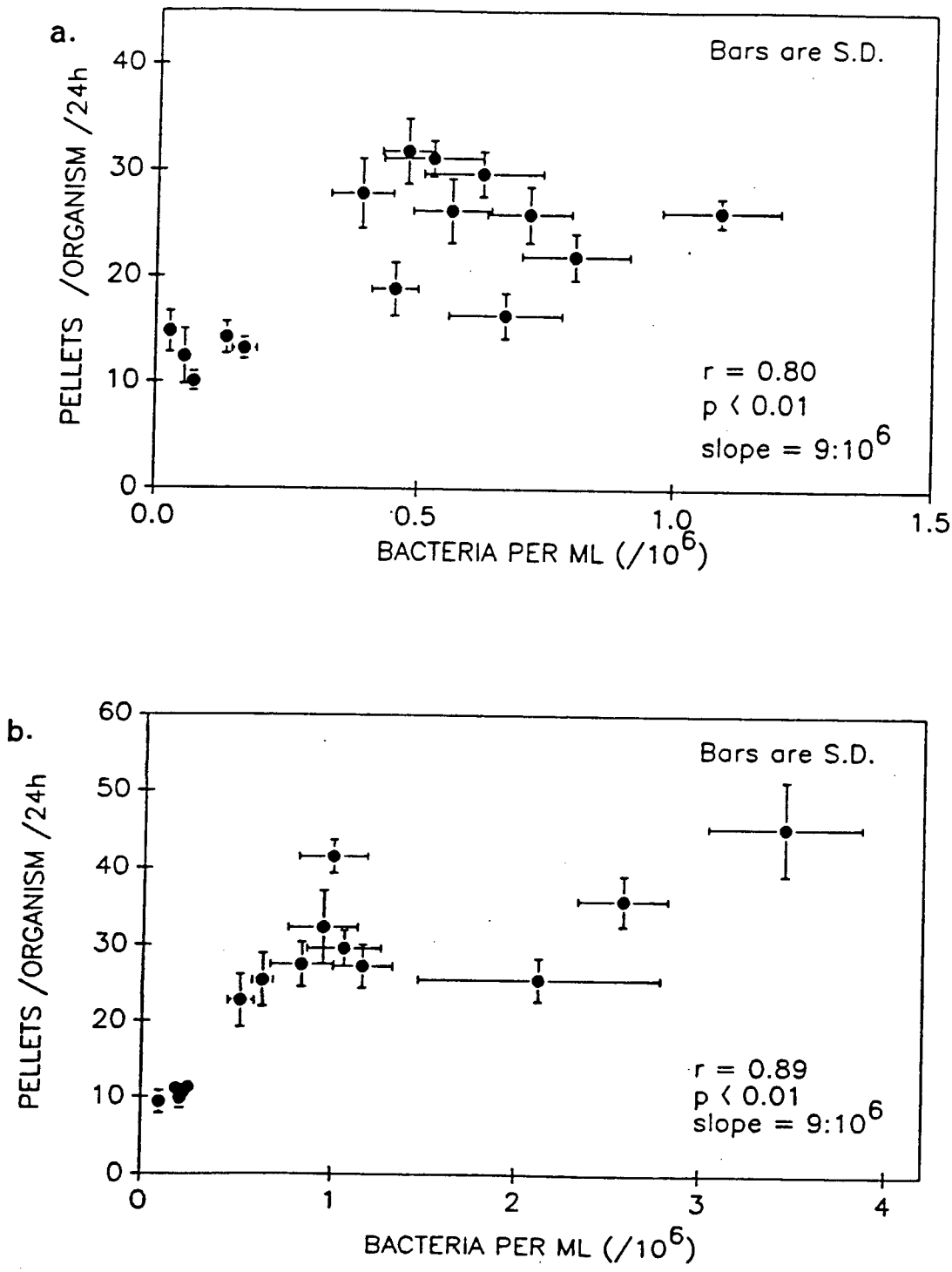


Figure 1.4. Comparison of pellet production with bacterial abundance on floc. a. Winter 1988. b. Spring 1988

2) Palatability changes with various abundances of single strains of bacteria.

Comparisons of bacterial abundance with pellet production are shown in Figures 1.5a-f for the six bacterial strains isolated from floc. Again, because both x and y variables are estimates, simple linear regression analysis may not be appropriate for analysis of this data set. Again, for purposes of comparison only, the r values and slopes for each graph are reported (Figs. 1.5a-i). From these it can be seen that although several strains of bacteria show significant correlations between bacterial abundances and pellet production, only in the cases of the "white" and "cream" floc strains (Figure 1.5a and b) and *Deleya marina* (Figure 1.5g) was a large increase in pellet production with increasing bacterial abundance observed (23, 7 and 22 pellets per 10^6 increase in bacterial abundance respectively). The presence of the strain of *Caulobacter* tested also appeared to result in some improvement in palatability of the floc material, particularly at very low bacterial abundances (Figure 1.5h). Overall though, the presence of this strain resulted in an overall increase in palatability corresponding to an increase in pellet production of only 0.5 pellets for each increase of 10^6 cells $\cdot$ mL^{-1}. All other strains gave rise to less than 1/10 of a pellet increase in palatability for each 10^6 cells $\cdot$ mL^{-1} rise in bacterial abundance, and the "pink" strain actually resulted in a decrease in pellet production of about 1 pellet for every 10^6 cells $\cdot$ mL^{-1} increase in bacterial abundances.

It is interesting to note that, as with the overall fauna, the correlation between pellet production and bacterial abundance for the "white" strain is best in the range of 0 to 10^6 cells $\cdot$ L^{-1}. If the points from floc with bacterial abundances higher than 10^6 are included in the correlation calculations the r value for this decreases from 0.95 ($p < 0.01$) to 0.55 ($p > 0.10$). Unfortunately, attempts to obtain cell densities higher than 10^6 cells $\cdot$

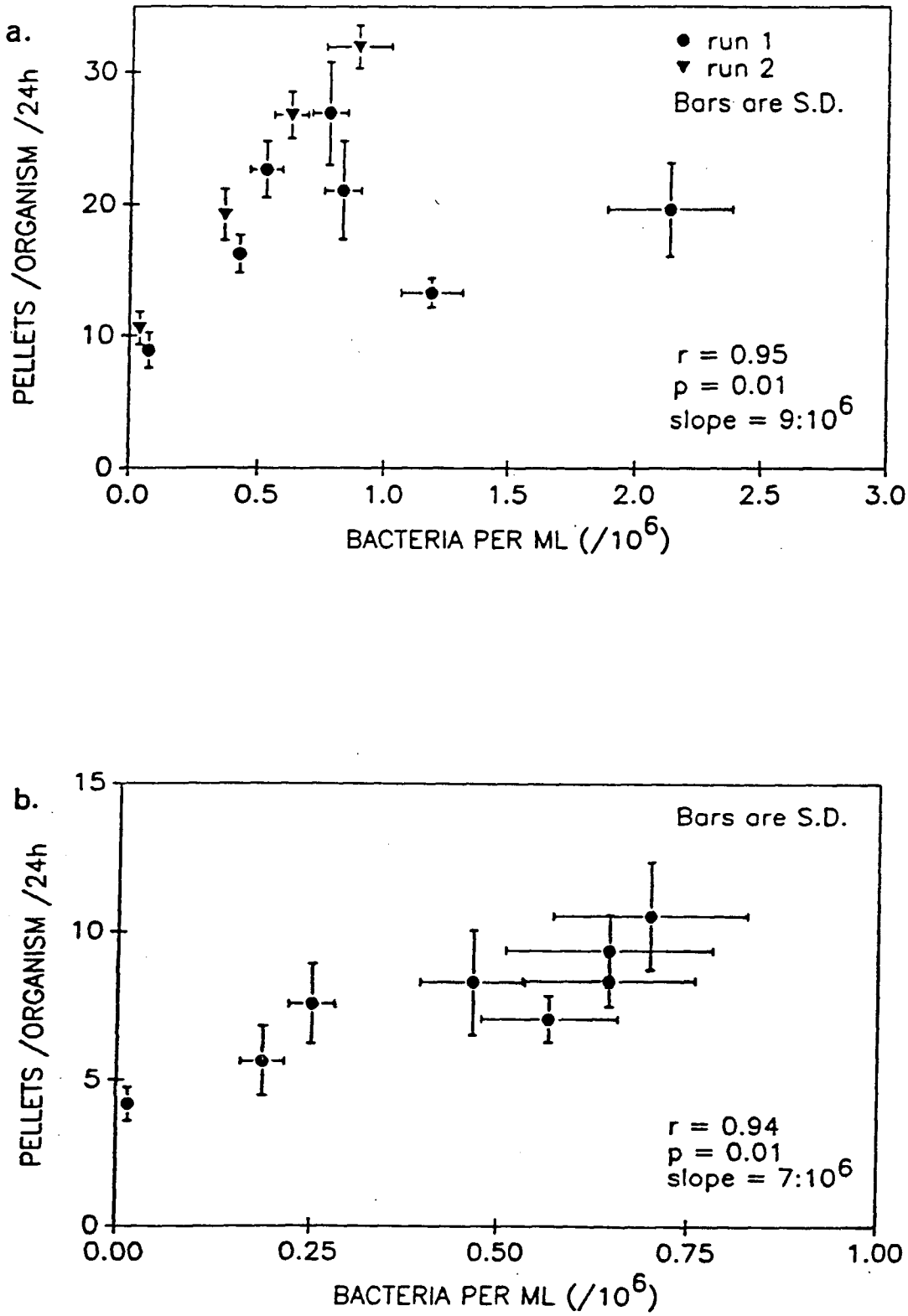
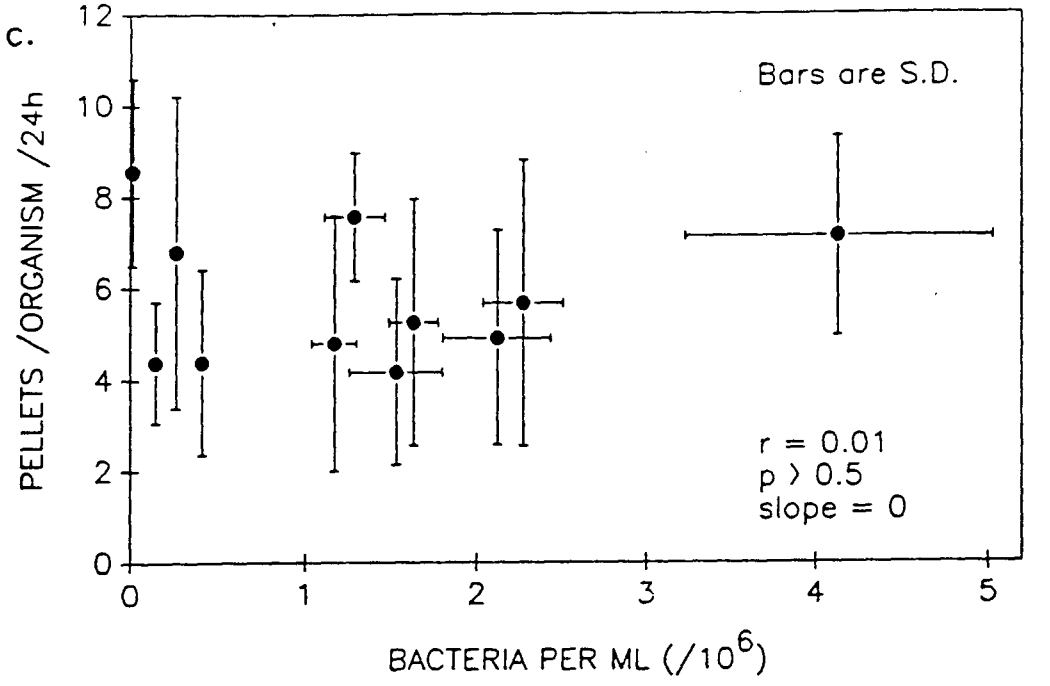


Figure 1.5. Comparison of pellet production with bacterial abundance on floc. a. "white" strain b. "cream" strain.



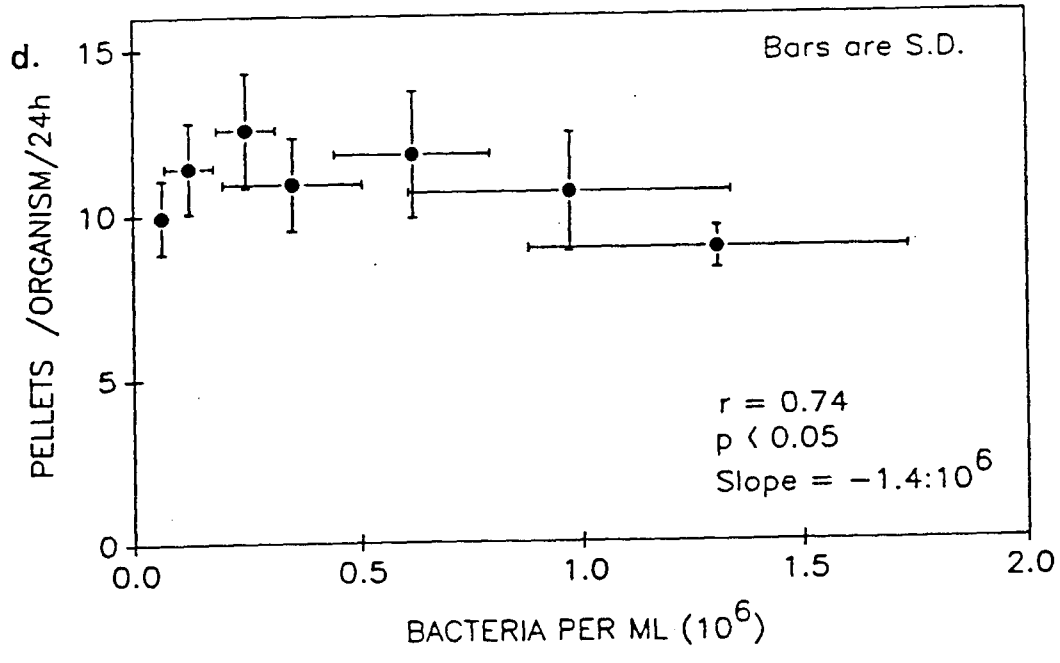
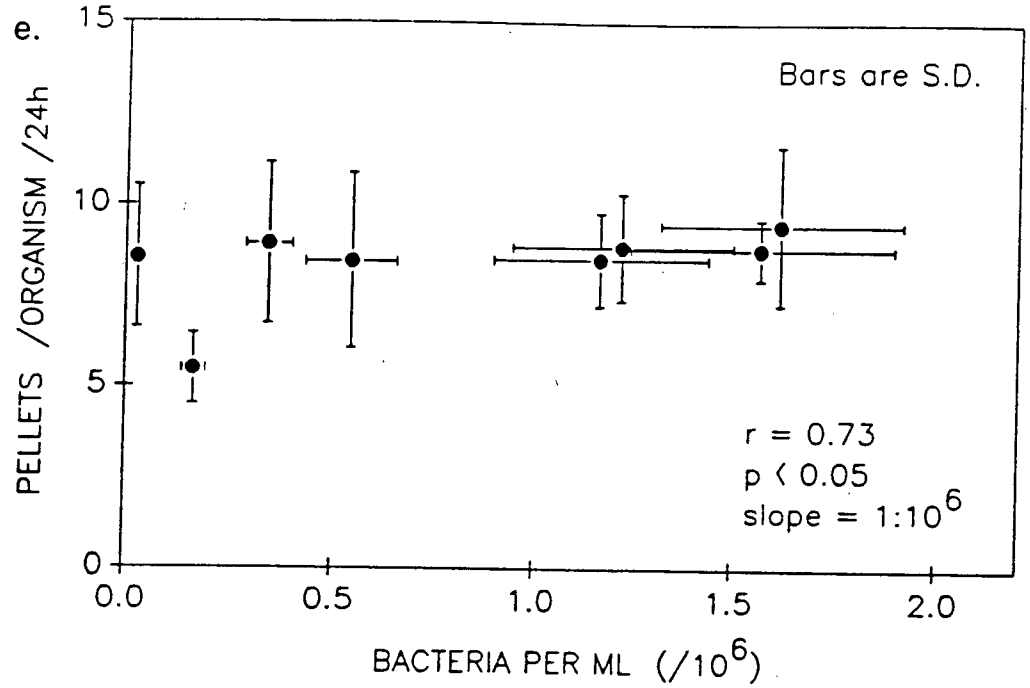


Figure 1.5 Cont'd. c. "smooth yellow" strain d. "pink" strain.



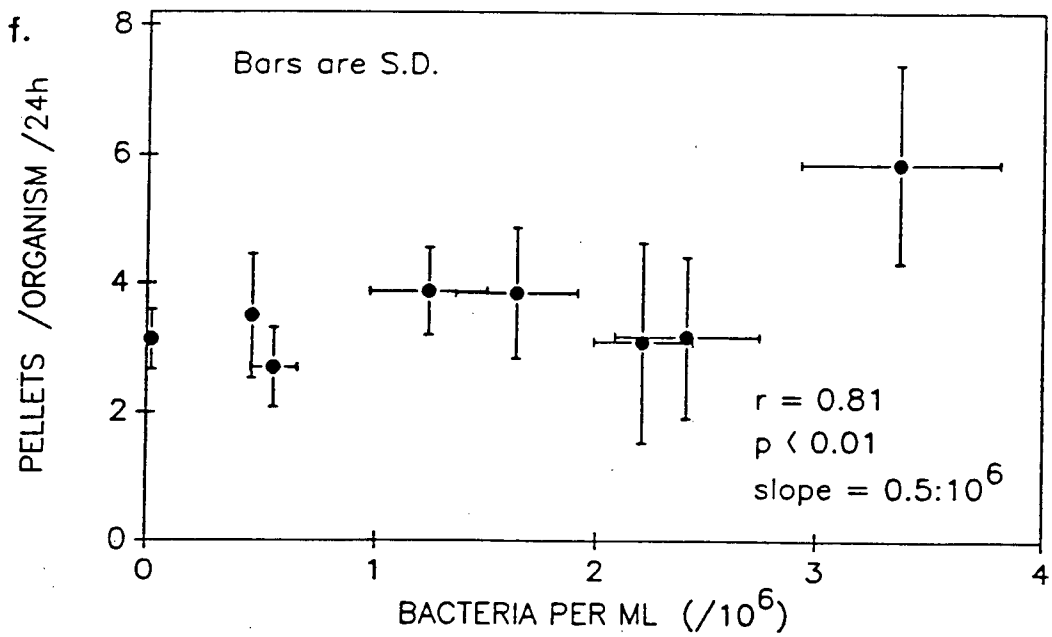


Figure 1.5 Cont'd. e. "hard yellow" strain f. amber strain.

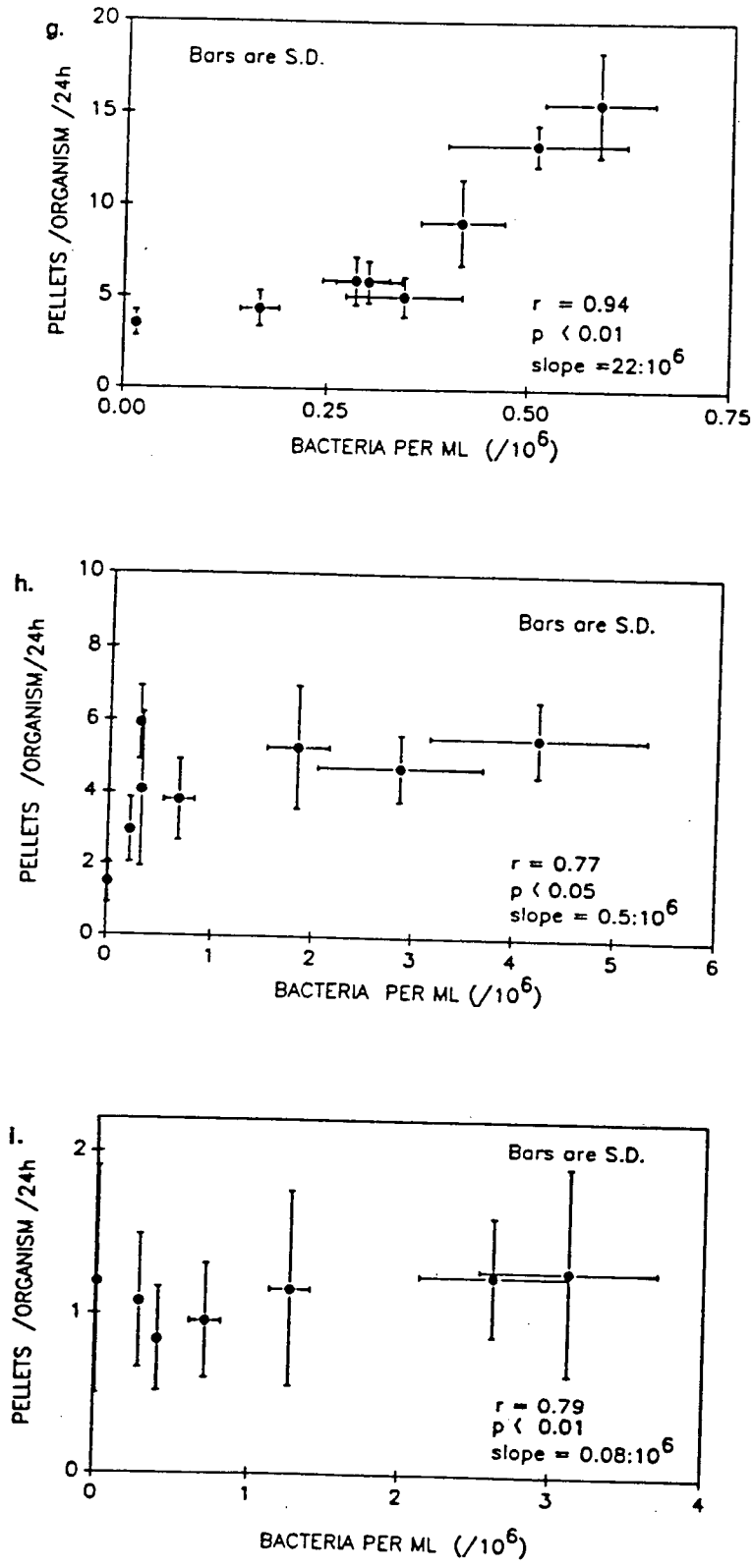


Figure 1.5 Cont'd. g. *Deleya marina* h. *Caulobacter* strain MCS6 i. *Pseudomonas atlantica.*

mL^{-1} in the "cream" strain and *Deleya marina* were unsuccessful, so it is unknown whether this decline in palatability is a ubiquitous response by these organisms to all palatable strains or whether it is unique to the "white" strain.

3) Seasonal changes in palatability of flocculant material.

Experiments utilising floc from water collected during different seasons showed very different levels of palatability. Comparisons for "bacteria-free" floc, normal floc and for the standard "fish food" treatments are found in Figures 1.6a, b and c. The basic palatability of the "bacteria-free" floc was found to be significantly higher for the winter and spring floc than for the summer floc ($p < 0.0001$), with winter floc being more palatable than spring floc in all but the final day of the experiments on which day the trends reversed ($p < 0.05$). In the case of normal floc, summer floc was again less palatable than floc from the other two seasons ($p < 0.0001$). However, no difference existed between the palatabilities of normal floc from winter and spring water except on the third day of the experiment where the level of pellet production with spring floc was significantly higher than for the winter floc ($p < 0.0005$). The number of pellets produced with a ground fish food diet was generally similar except on the first day of the spring experiment when pellet production was lower than that on the same day of the summer and winter experiments ($p < 0.05$) and on the second day of the summer experiment when pellet production was higher than that on the same day of the winter. Pellet production was also statistically higher on day 4 in the spring experiment than for the same day in the summer experiment and was higher on day 5 in both winter and spring experiments than in summer. These differences are, however, slight and would appear to reflect more an artifact of the statistical analysis than a real biological

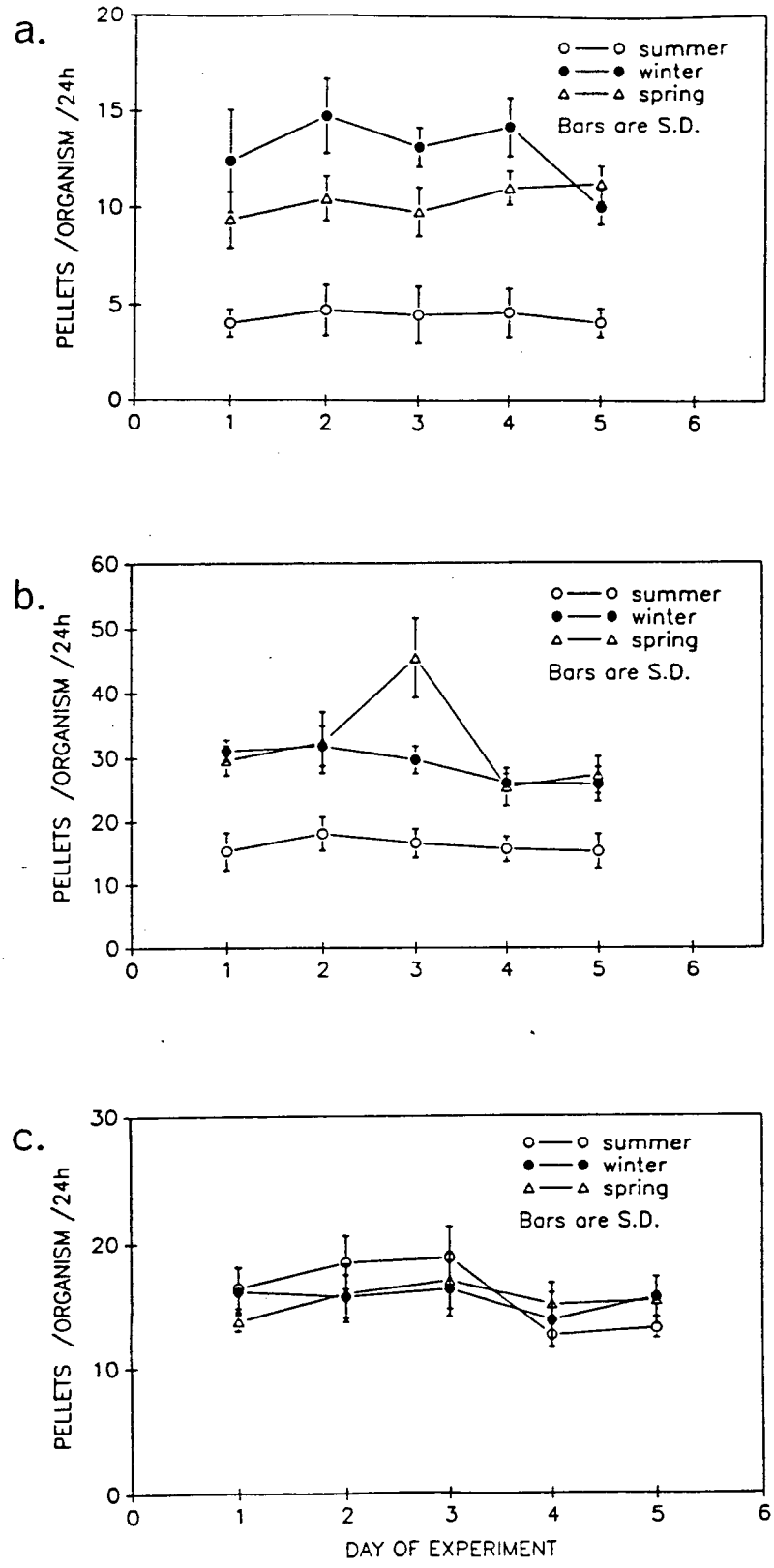


Figure 1.6. Comparison of pellet production in different seasons. a. bacteria-free floc b. normal floc c. fish food treatment.

difference. It thus seems that no important changes in the intrinsic grazing rates of the copepods occurred between each run of this experiment.

Although bacterial abundances were not enumerated during the experiment using floc made from summer water, comparison between pellet production with "bacteria-free" flocs made from water collected in each of the three seasons tested shows a much reduced palatability for summer floc relative to that in the other seasons (Fig. 1.6a). This reduced palatability is also seen in summer floc in which bacteria are present (Fig. 1.6b). Unfortunately, it is unknown whether bacterial abundances were also reduced in floc from this season. However, material flocculated from water collected in summer 1989 (Tables 1.2b and 1.3b) shows bacterial abundances similar to those found in the winter and spring experiments, and yet pellet production on this material (Tables 1.2a and 1.3a) was very low. This suggests that significant differences in bacterial abundances on floc made from water collected in different seasons do not occur in the laboratory.

C. Checks of the validity of the methods used during feeding experiments.

1) Effects of long term reinoculation.

Comparisons of pellet production levels and bacterial abundances for this experiment are presented in Figures 1.7a and b. Starved organisms in this experiment produced a number of pellets statistically greater than zero on days 4 and 5 of the experiment. However, at no time did the mean pellet production per starved organism exceed one pellet per 24 hour run. Levels of pellet production with normal floc were not significantly different to those found with reinoculated floc at any time. Bacterial abundances were higher in the normal floc than in the reinoculated floc on day 1 ($p <$

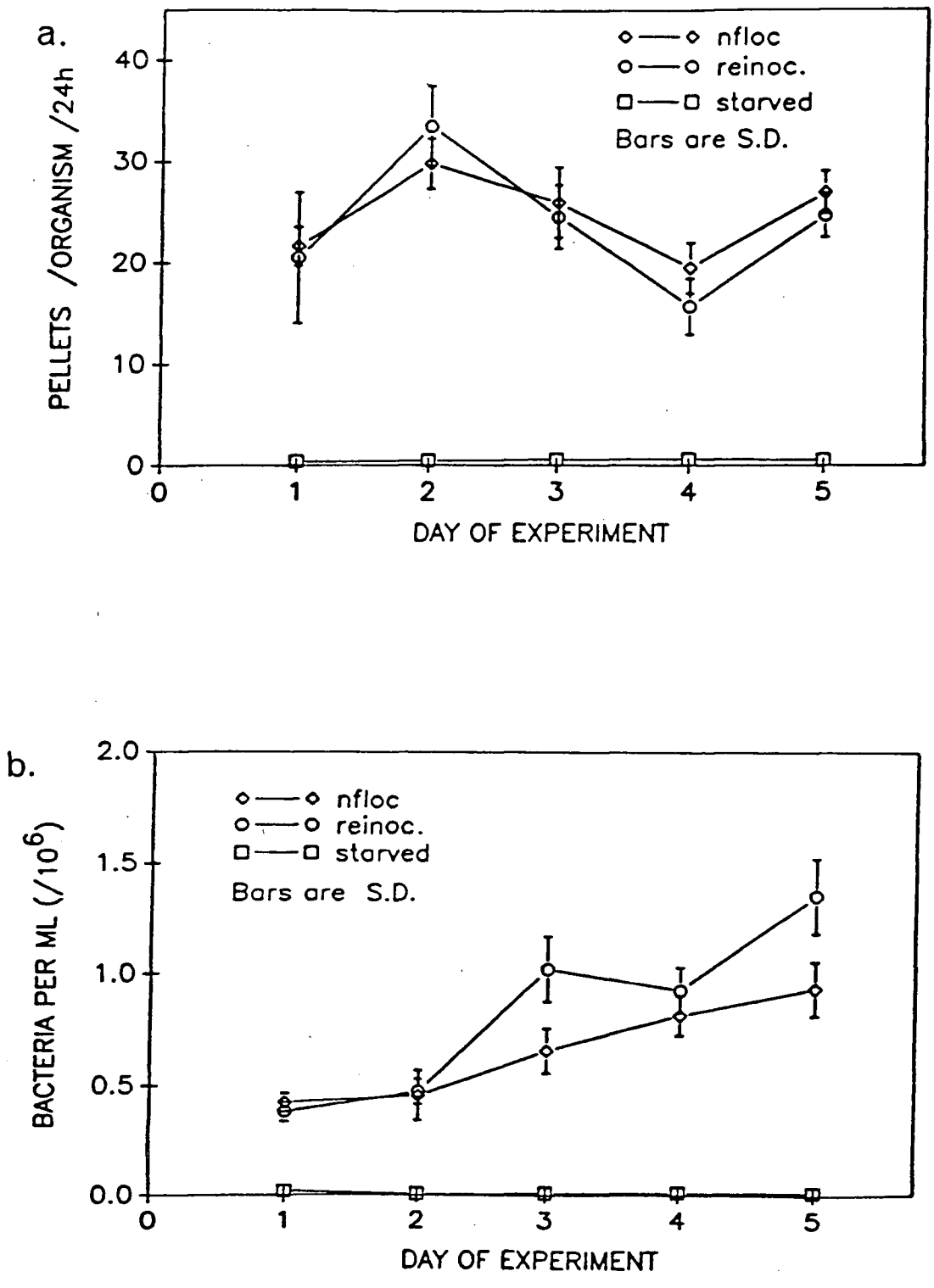


Figure 1.7. Comparison of pellet production (a) and bacterial abundances (b) on normal and six week inoculated floc

0.05), while those in the reinoculated floc were higher than those in the normal floc on days 3, 4 and 5 ($p < 0.01$). Bacterial abundances were not significantly different between the two floc treatments on day 2.

2) Validity of using SOW rather than natural seawater as a flocculating agent.

i) Comparison of palatabilities and bacterial abundances in floc made with sterile SOW and that made with sterile natural seawater.

Pellet production of organisms fed floc made with sterile seawater and with sterile SOW is compared in Table 1.2a. A test of the treatment means using a paired t-test show that there is no significant difference between pellet production of organisms in each treatment. Bacterial abundance on the floc in each replicate of both treatments was also enumerated and is presented in Table 1.2b. Again no significant difference between treatments was found.

ii) Comparison of palatabilities and bacterial abundances in floc made with sterile SOW and that made with 0.8µm filtered natural seawater.

Pellet production of organisms fed floc made with 0.8µm filtered seawater and with sterile SOW is presented in Table 1.3a. Bacterial abundance on the floc in each replicate of both treatments was also enumerated and is presented in Table 1.3b. Again, no difference between pellet production or bacterial abundances between the two treatments was found. Pellet production was, however, extremely low in all treatments.

Table 1.2a. Comparison of pellet production of organisms fed floc made with sterile natural sea water and that with floc made with sterile SOW.

Replicate	n	Pellet Number ± 1 S.D.	
		Seawater	SOW
1	5	13.5±3.3	11.2±2.2
2	5	15.6±2.9	12.4±2.5
3	5	15.0±3.1	15.5±3.4
4	5	16.1±2.0	16.0±3.5
Treatment mean (S.E)		15.1 (0.5)	13.8 (1.2)

Table 1.2b. Comparison of bacterial abundance ($x10^{-6}$) on floc made with sterile natural sea water and that on floc made with sterile SOW.

Replicate	n	Bacterial Abundance ± 1 S.D.	
		Seawater	SOW
1	5	1.00±0.15	1.08±0.19
2	5	1.08±0.16	0.97±0.15
3	5	1.14±0.21	0.84±0.13
4	5	1.12±0.23	1.07±0.20
Treatment mean (S.E)		1.08±0.03	0.99±0.06

Table 1.3a. Comparison of pellet production of organisms fed floc made with 0.8µm natural sea water and that with floc made with sterile SOW.

Replicate	n	Pellet Number ± 1 S.D.	
		Seawater	SOW
1	5	2.6±0.6	3.3±1.4
2	5	4.4±2.1	2.1±0.6
3	5	3.7±1.1	3.4±0.9
4	5	3.0±1.0	2.9±0.9
Treatment mean (S.E)		2.59 (0.4)	2.92 (0.3)

Table 1.3b. Comparison of bacterial abundance ($x10^{-6}$) on floc made with 0.8µm natural sea water and that on floc made with sterile SOW.

Replicate	n	Bacterial Abundance ± 1 S.D.	
		Seawater	SOW
1	5	0.77±0.10	0.58±0.09
2	5	0.77±0.12	0.87±0.14
3	5	0.77±0.07	0.68±0.12
4	5	0.72±0.11	0.88±0.09
Treatment mean (S.E)		0.76±0.01	0.75±0.14

D. Other factors potentially influencing palatability.

1) Effect of elevated concentrations of inorganic nitrogen.

Pellet production of organisms fed floc enriched with various concentrations of nitrate is presented in Table 1.4a. Tests of the treatment means using a paired t-tests show that none of the treatments results in significantly elevated pellet production relative to the control. Bacterial abundance on the floc was also enumerated and is presented in Table 1.4b. No significant difference between any of the treatments was found.

2) Comparison of floc formed from organic rich slough water with that made from technical grade humic acid.

The results of this experiment are found in Table 1.5. Technical Grade Humic Acid (TGHA) flocs were found to be consistently less palatable than those made from natural water, even after the addition of the fauna from natural floc ($p < 10^{-5}$). However, addition of the natural fauna did result in an increase in palatability relative to the uninoculated TGHA ($p < 0.005$). Bacterial abundances on both TGHA treatments were also significantly lower than those on natural floc ($p < 10^{-6}$) and those on the reinoculated TGHA are significantly higher than those in the untreated TGHA ($p < 10^{-6}$).

Table 1.4a. Pellet production of organisms fed floc enriched with various concentrations of inorganic nitrogen in the form of sodium nitrate.

Nitrate Enrichment	Pellet Production ± 1 S.D.			
	Replicate 1	Replicate 2	Replicate 3	Treatment mean (S.E.)
0	9.9 ± 2.5 (n=5)	9.5 ± 1.3 (n=5)	9.4 ± 2.4 (n=5)	9.6 (0.1)
0.0001M	8.6 ± 1.7 (n=5)	8.3 ± 3.3 (n=5)	10.5 ± 3.0 (n=5)	9.1 (0.6)
0.0005M	7.6 ± 1.7 (n=5)	14.0 ± 3.5 (n=5)	10.4 ± 1.8 (n=5)	10.67 (1.6)
0.001M	8.0 ± 0.5 (n=5)	8.8 ± 1.2 (n=5)	8.4 ± 1.2 (n=5)	8.40 (0.22)

Table 1.4b. Bacterial abundance ($x10^{-6}$) on floc enriched with various concentrations of inorganic nitrogen in the form of sodium nitrate.

Nitrate Enrichment	Bacterial Abundance ± 1 S.D.			
	Replicate 1	Replicate 2	Replicate 3	Treatment mean
0	0.96 ± 0.15 (n=5)	0.91 ± 0.15 (n=5)	0.87 ± 0.1 (n=5)	0.91 ± 0.05
0.0001M	0.78 ± 0.15 (n=5)	0.80 ± 0.14 (n=5)	0.85 ± 0.08 (n=5)	0.81 ± 0.04
0.0005M	0.70 ± 0.11 (n=5)	0.90 ± 0.14 (n=5)	0.79 ± 0.12 (n=5)	0.79 ± 0.10
0.001M	0.80 ± 0.11 (n=5)	0.95 ± 0.10 (n=5)	0.84 ± 0.13 (n=5)	0.86 ± 0.08

Table 1.5. Comparison of floc formed from natural, organic rich slough water with that formed from technical grade humic acid (TGHA) with regard to palatability and degree of bacterial colonisation. Bacterial abundances are given as X 10^{-6}.

Variable	normal floc	TGHA	RITGHA	starved
Pellet # ± 1 S.D.	16.5±1.3	8.4±1.1	11.1±1.0	0.7±0.4
Bacteria ± 1 S.D.	1.65±0.4	0.24±0.03	0.26±0.05	0.02±0.00

Discussion.

A. Preliminary analysis of organic rich stream water.

The E_{465}/E_{665} absorbance ratio values of 6.5-7.3 reported in this study (Table 1.1) are at the higher end of the range of 3-7.43 for these values quoted by Rashid (1985). As the E_{465}/E_{665} ratio decreases with increasing molecular size (Power and Langford 1988), these measurements imply that the molecules in these samples fall into the low range of molecular weights for humic substances. Moreover, as in general values of the E_{465}/E_{665} ratio decrease with increasing degrees of humification and condensation, it can be implied that the materials in "solution" (pass through a 0.8μm filter) in these streams have low levels of condensation and are relatively poorly degraded. Following de Haan (1983) the E_{250}/E_{365} ratios of about 3, found in this study, can be interpreted to indicate the presence of a large number of relatively high molecular weight (HMW) fulvic acids in the stream water analysed. It can therefore be concluded that the material dissolved in these streams is probably dominated by HMW, poorly degraded fulvic acids.

The use of the E_{465}/E_{665} ratio in aquatic environments has been questioned by de Haan (1972; 1983) on the basis of his assumption that aquatic humic substances are predominantly fulvic in nature and that fulvic acids have very low fluorescence at higher wavelengths. However, this assumption does not appear to be substantiated in this study where reasonably strong absorbances ($\approx$0.03) were measured even at the higher ranges of the spectrum. Thus, as both ratios give different information about the nature of the molecules, use of both ratios for analyses of waters having DOM with properties similar to that in the streams studied in this project would seem to be beneficial.

Whatever its nature, the amount of material involved in flocculation of water from organic-rich streams such as Crescent Slough is tremendous. Combining the calculated flow from this stream of 1 x 10^8 L (Table A1.1) with the amount of material found to flocculate from the February sample ($\approx$10mg $\cdot$ L^{-1}) gives a hypothetical contribution by flocculation of this material in the Fraser river estuary of about 1 metric tonne of new particulate material annually from this relatively tiny stream. As mentioned earlier though, this stream enters the Fraser fairly high up within the estuary (Fig. 1.1). where salinities are fairly low, and thus flocculation of the organic material from this particular source may not, in fact, take place. However, many much larger organic rich streams which drain the lowlands of British Columbia do flow directly into saline waters. Flocculation of the material from such rivers could therefore be an extremely important source of particulate material in the estuaries into which these rivers flow.

An interesting aspect of the behaviour of the flocculant material is that not all the darkly coloured samples showed significant degrees of flocculation upon addition of salt water (Table A1.2). Differential levels of flocculation had been reported previously by Fox (1983b) and have been postulated by that author to result from differences in the chemical nature of the organic load of the river. However, Fox also suggested that the absence of flocculation might reflect some inadequacy in the laboratory experiments used to study the process (Fox 1983b). As standardised techniques were utilised throughout this study and flocculation was observed only in certain samples, it seems likely that differences in the chemical identity of the molecules, rather than inadequacies in the laboratory techniques are responsible for this observation. It is interesting that darkly coloured, non-flocculant stream water was always associated with the first collection of water from the slough after the summer "no flow" period. It seems logical to postulate that a large proportion of the first molecules to be leached from the soil may consist of those with particularly high solubilities for which low levels of flocculation would be

expected. Similarly, slower moving (less soluble) molecules entering the stream after longer periods of leaching would be expected to show higher degrees of flocculation, as indeed is observed. It seems that, at least in the case of this slough, the chemistry of the molecules "dissolved" in the stream varies strongly throughout the year and has a strong effect on the amount and nature of the material flocculating upon contact with seawater.

Looking at the elemental composition of the floc material, it can be seen that the C:N ratio of the material flocculating is extremely high. This suggests that there are very few protein or amino-acid type moieties associated with the material flocculating from this water. Moreover, high C:N ratios are often found to correspond with a very low biological availability for the material in question (Wetzel 1975). Flocculant material from streams with similar properties to the ones utilised for this study may thus be less important as a food source than it is as a contributor of organic carbon to sediments.

The value of approximately 50% for the proportion of the flocculant material that is humic in nature is somewhat higher than the 33% found by Fox (1983b) for water from the Mullica river, and is significantly higher than the 7% found by the same author for the Broadkill river (Fox 1983b). This high humic content is probably a reflection of the highly peaty nature of the area drained by this stream (Burns Bog is in fact the location of a commercial peat mining company). However, in the light of the frequent assumption that only humic acids flocculate it is interesting, although perhaps not suprising, to note that fulvic acids are such an important component of the flocculating material. Analyses of the E_{250}/E_{365} ratios in this study implied that many of the dissolved organic molecules of the stream waters were fulvic acids of relatively high molecular weights. As the differences between humic and fulvic acids are much more theoretical than real, it seems reasonable that HMW molecules, operationally defined as fulvic acids, should be aggregated by salt-water. Moreover, it also seems likely that some of the coloured

material that does not flocculate may be composed of smaller, more soluble substances that would have the acid-base solubility properties of a humic acid. The latter has, in fact, been observed by Sholkovitz (1976).

B. Importance of bacterial colonisation.

i) Discussion of methods used.

Because of the amorphous nature of flocculant material, one of the major problems with this study was that of how to determine the degree of ingestion of that material. Particle counts were impossible as the floc particles break and reform randomly with manipulation. Measurements of the mass of material not incorporated into faecal pellets before and after a feeding experiment were also untenable because of the potential for damage to the material during the drying process required for such a measurement. Counts of faecal pellets were thus adopted for determinations of levels of ingestion throughout this study. Such counts have been utilised as indicators of grazing rates by many investigators (e.g. Gaudy 1974; Harvey et al. 1935; Lance 1964). Gaudy (1974) actually tested the effectiveness of such a method and concluded that "there is a significant correlation between daily food intake and faecal pellet production". This conclusion was modified by Marshall and Orr (1972) who suggest that numbers of pellets produced by one organism are only an approximate measure of the food ingested. However, they suggest that, when a number of organisms are taken together and an average pellet production is calculated, pellet counts can be a fairly effective delineator of grazing rates. As this study did use such a measure, the use of faecal pellet counts to measure grazing rates appears justified.

The question remains, though, as to whether ingestion rates for normal floc relative to the sterile material are indicative of increased palatability or whether they, in fact, indicate an increased feeding response to a food of lower quality. In support of the equation of elevated ingestion rates with high food quality, improved rates of ingestion for foods of superior quality and the rejection of inert particles (low nutritional value) has been demonstrated by Paffenhöffer and Van Sant (1985). In contradiction to this, the same authors suggest that material that does not provide a large energetic benefit to the grazer has a short period of residence within the animal's gut relative to one of better quality. The former would suggest that high pellet production is indicative of superior food quality while the latter suggests that high faecal pellet production may in fact be indicative of a lower food value. It is the assertion of this author that increased pellet production resulting from ingestion of different types of floc (e.g. normal versus "bacteria-free" floc) is indicative of the increased palatability of that material. This assertion is based on several observations. Firstly, as the floc matrix is the same in both treatments, it should be similarly digestible (or indigestible) in both cases. Increased pellet production is not then an indication of differing assimilabilities of the basic material and can thus be taken to indicate a difference in ingestion rates. Secondly, as floc is always unsuccessful in supporting the copepod through a lifecycle it an be assumed to be a fairly low energy food source. Thus, at no time should an organism feeding on either floc type experience an "energy-satiation" induced slow-down in the passage of food through its gut. Finally, as the presence of bacteria on floc should increase the energetic value of that particle, a slower rate of passage of that material through the gut would be predicted by Marshall and Orr (1972). As pellet production was higher in the normal floc, in spite of this fact, it seems likely that grazing rates on the normal floc are higher than those in the bacteria free floc. The use of faecal pellets to compare palatability between different floc types thus seems valid. Similarly, the assumption that increased rates of pellet production correspond with superior palatability

can be justified. By contrast, comparison between floc and, for example, a fish food diet using pellet production data is meaningless because of the different assimilation efficiencies and energy contents of these two food sources. The fish food controls are important however, in providing a food of standard quality, the pellet production from which can be used to monitor changes in the overall feeding rates of copepods between any two runs of the same experiment.

As mentioned earlier another concern with the methodology utilised was that of the extended duration of starvation prior to the use of the study organisms in any experiments, required by the processes used to render them relatively "bacteria-free". Most early feeding studies were thus run for a five day period to check for declines in pellet production over time that might indicate that increased feeding during the initial few hours of the experiments had taken place. A decline in pellet production not explicable in terms of bacterial abundances was observed only once during these experiments, on both the normal and reinoculated floc in the winter experiment (Fig. 1.2b). Although statistically significant, this decline is fairly small, particularly in the normal floc and at no time did grazing upon either floc type decline to the levels of the "bacteria-free" sample. It seems therefore, that grazing upon flocculant material is not a simple indiscriminate response to the addition of food after a long period of starvation. It seems likely that copepods like *Tigriopus* would ingest floc when that material is present in their natural habitat. This result is supported by the observation that, when *Tigriopus* is presented with an additional food source (e.g. *Isochrysis*, as in the mass spectrometer experiment, Chapter 3), the presence of both algal and flocculant material in that pellet can be observed.

With regard to the bacterial enumerations, it has been suggested that the use of acridine orange is inappropriate in humic rich waters because that stain precipitates with

the dissolved humic materials, resulting in such a high background fluorescence that no bacteria can be seen (Bergstrom et al. 1986). However, preliminary observations in this study found that once flocculated, humic materials do not appear to interact with this stain. The only occasions when problems were encountered with background fluorescence were when examining samples that had been autoclaved or allowed to remain in the oven for prolonged periods of time (10-15 days). At these times, floc began to stain a deep red colour and to take on a rather crystalline appearance. As such samples were never used for any experiments, there was no reason to avoid the use of acridine orange, particularly as the alternate fluorochrome, acriflavin, was found to cause significantly less fluorescence in the bacteria in the samples examined. None the less, counts made in this study were undoubtedly on the low side as a result of the particulate nature of the flocculant material which obscures bacteria on the side of the particle nearest the filter. However, the counts made were felt to be a reasonable estimate of the abundances of bacteria on floc in these fairly dilute samples.

Because the contents of all wells containing replicates of each treatment were pooled, the bacterial abundance values reported here are those of the overall treatment rather than of the specific well. Obviously under ideal conditions, separate counts would have been made for each well, allowing detection of local contamination, as well as giving a better estimate of the relationship between pellet production and bacterial abundance. Unfortunately, time and expense considerations made such an analysis untenable.

ii. Discussion of results.

Increased pellet production rates in normal floc relative to those in the bacteria free treatments in experiments from all seasons demonstrate the superior palatability of that material (Figs. 1.2a-c). Moreover, the degree to which the palatability is improved appears to be a function of the corresponding bacterial abundances (Figs. 1.3a and b). Plotting the data for bacterial abundances against the corresponding rates of pellet production, as in Figures 1.4a and b clearly demonstrates the very important role played by the degree of bacterial colonisation of particles in determining the palatabilities of that material. Particularly strong correspondence between bacterial abundances and palatability seem to exist in the range of bacterial concentrations between 0 and 10^6 cells $\cdot$ mL^{-1}. Higher levels of bacterial abundance were rarely observed, but upon the occasions that much elevated bacterial abundances were observed, they were associated with grazing rates much lower than would have been predicted from the data at lower concentrations. It thus seems that *Tigriopus* is able to detect the presence of the bacterial fauna and to react to it in a way that is proportional to the increase in abundance at lower levels of colonisation. The relatively low grazing rates at higher bacterial abundances are hard to interpret. It may be that above a bacterial abundance of 10^6 cells $\cdot$ mL^{-1}, copepods can no longer detect any difference in the quality of the particles. The variation in the pellet production rates measures would thus reflect variability in the grazing rates of the individual copepods in that treatment. Alternately, as bacterial abundances in the normal floc throughout all these experiments rarely exceeded concentrations of 10^6 cells $\cdot$ mL^{-1}, reduced feeding may reflect a negative response to these "un-naturally" elevated bacterial abundances. This result is particularly interesting in that the range in which changes in palatability occurred with changes in bacterial abundance corresponds closely with the range of abundances of 2.3 to 6.8 x 10^5 cells $\cdot$ mL^{-1} reported for the Strait of Georgia and Howe Sound (a local fjord); (Valdés and Albright 1981; Velji and Albright

1986 and L. Albright, Dept. Biology, Simon Fraser University, pers. comm.). The degree of bacterial colonisation may thus play an important role in determining the palatability of particles suspended in these waters.

From inoculations of sterile floc with individual bacterial strains, it became apparent that the improved palatability of the particles colonised by bacteria was based on a grazing response to only a small number of strains present on the initial floc material. *Tigriopus* grazers appear to respond to "white" and "cream" bacterial strains by increasing grazing rates (Figs. 1.5a & b). The other strains which appeared, from their frequent presence on agar plate preparations, to be equally ubiquitous on the floc material, induced no such response at any bacterial abundance (Figs. 1.5c-f). Moreover, similar selectivity was demonstrated with the three marine strains. Inoculation with *Deleya marina* resulted in strongly improved palatabilities with increasing abundance. Similarly, inoculation with the *Caulobacter* strain MCS6 may have given rise to improved palatabilities, particularly at very low abundances (Figs. 1.5g and h). By contrast, inoculation with *Pseudomonas atlantica* resulted in no change in palatability at any abundance of that organism (Fig. 1.5i). The similarities in the grazing responses elicited by the marine strains to those observed for floc isolates, suggest that the results of the laboratory experiments may indeed be applicable to the occurences within the natural environment.

Selective grazing by harpacticoid copepods on bacterial species has previously been reported by Rieper (1978; 1982; 1984). In agreement with the observations of Rieper (1982) all strains found to be preferred by the copepod grazer used in this study were gram negative strains with three out of four being Gram negative rods ("white", "cream" and *Deleya marina*) and the fourth a non-motile stalked bacterium which has a motile, rod-shaped swarm stage (*Caulobacter* strain MCS6). The results of this study

also paralleled those of Rieper (1982) in that both motile and non-motile forms were among those resulting in increased palatability (Table A2.1). Rieper however tested the palatability of her various bacterial strains by killing the bacteria and feeding them to copepods after drying and grinding whereas the experiments presented in this paper offered bacteria to the copepods in a form more closely resembling those encountered in nature. The results of this study therefore have the advantage of being free from any of the potential changes in the physical and chemical nature of the bacteria resulting from Rieper's methodology. Unfortunately, the reasons for the selective grazing of certain bacteria strains by *Tigriopus* were not specifically investigated during this study. From the data collected during characterisation of floc strains, no obvious biochemical traits specific to palatable species alone, can be found (Table A2.1). Moreover, the preferred species demonstrated highly variable rates of production of extra-cellular products on agar plates, varying from very low levels of production in the *Caulobacter* strain to very high levels for the "white" strain. By contrast, species such as *Pseudomonas atlantica*, not selected, showed extremely high levels of production of extra-cellular materials. The quantity of extra-cellular material produced cannot therefore account for the observed grazing responses. Thus it seems probable that it is the presence of some particular component within the capsular material of the bacterial strains or else some intrinsic property of the bacteria themselves that is responsible for promoting grazing. It would thus be very interesting to carry out a thorough biochemical analysis of the capsular materials of each strain and to test the ability of each constituent to cause a feeding response by incorporating them with the floc material. Unfortunately, such an analysis is beyond the scope of the present study.

Selectivity for certain strains of bacteria by harpacticoid copepods, as demonstrated in these experiments, has important implications for studies of microbial food webs in nature. In the past, studies on the availability of microorganisms for

macrofaunal organismal grazers have measured bacterial abundances on particles alone (Herndel and Peduzzi 1988; Silver et al. 1986). The results of this study suggest that the relative abundances and palatabilities of each bacterial strain colonising a particle may influence the degree to which that particle is grazed at least much if not more than the overall abundance of the bacteria present. Until recently however, information upon the composition of the microbial community in any sample could not be ascertained as no technique for the measurement of the *in situ* abundances of particular bacterial strains was available. However, rapid advances are now being made in this area, particularly as a result of the development of specific probes able to detect antigens (antibody probes), DNA sequences (DNA probes) or RNA sequences (RNA probes) (Lidstrom 1989). The use of these techniques allows enumeration of each strain within a sample and can thus be used to monitor species composition, microbial competition and perturbation effects. Future studies upon the potential value of any microbial food source must therefore make use of these new techniques in addition to the actual enumeration of the bacterial fauna, if meaningful interpretation of the data set is to be obtained.

As noted earlier, comparison between the basal palatabilities (those of sterile floc) show the presence of distinct differences in the palatability of the floc matrix at different times of the year (Figure 1.6a). The most obvious of these is the reduced palatability of floc formed from water collected in summer for *Tigriopus*. It thus appears that the chemical nature of the flocculating material changes in the summer in such a way as to discourage ingestion of the particles by grazers. Summer time is a time of low rainfall amd thus of low rates of water flow through the study streams. It is also a time when higher water temperatures prevail. Under such circumstances bacteria living within the streams would have higher activity rates and longer periods in which organic material in a particular parcel of water was available to them for degradation. The lower palatabilities of floc derived from such a water body might thus be expected to have

lower lability than that from the fast flowing cool streams sampled during the winter months.

The difference in palatabilities appears to continue even in the presence of a bacterial fauna (Figure 1.6b), with summer floc always having much lower palatabilities than the spring and winter types. The possibility that summer floc used in these experiments also had reduced bacterial abundances cannot be ruled out. However, other experiments using water collected in the summer of 1989 (Tables 1.2 and 1.3.) show that bacterial abundances on summer floc are generally similar to those on the winter and spring floc, yet the palatability of that material is still extremely low. Apparently, colonisation by bacteria, although important in determining palatability of the overall floc, is unable to completely compensate for the basic unpalatability of the underlying floc. Moreover, if changes in palatability of floc act in concert with changes in the composition or relative abundances of various strains of bacteria on the floc, very strong changes in palatability of particles in nature may be expected.

As it in winter and early spring that food is in shortest supply, the superior palatability demonstrated for floc during these times could have important implications for organisms within the estuary. Increased ingestion of flocculant material during these seasons may enhance survival of grazers exploiting this food resource. The presence, abundance and nature of flocculant material and its associated microfauna should not, then, be overlooked in studies of estuarine systems, particularly during times of low primary productivity.

C. Validity of the methods used during feeding experiments.

Although the potential for heat damage to the floc during the pasteurisation procedure cannot be ruled out, long-term reinoculation experiments show that the damage caused is essentially unimportant in terms of changing the palatability or degree of bacterial colonisation of that particle when the bacterial fauna is fully reintroduced (Figs. 1.7a and b). The fact that the staining properties of the pasteurised material (as described above) are similar to that of the natural floc for about the first ten days of pasteurisation and then change significantly also suggests that significant heat damage may not have taken place within the time-scale involved in these experiments.

These findings support the contention that reduced palatability of the reinoculated floc in the earlier experiments reflected the inadequacy of the two day reinoculation period used. It also suggests that the very low palatability of bacteria free floc is a result of the absence of the bacteria rather than a reflection of heat damage incurred by the floc during pasteurisation. Pasteurisation of floc thus appears to be a reasonably non-destructive method for producing sterile flocs for later manipulation.

In the light of the similarities in palatability over a long term inoculation, the fact that palatabilities of reinoculated floc differ from those of normal floc in the original two day inoculations, even when bacterial abundances are statistically similar (e.g. Day 3, Fig. 1.2b), suggests that bacterial conditioning may play a role in determining floc palatability. Shorter term inoculations may be adequate for the establishment of a bacterial fauna of similar density to that found in nature, but longer periods of time may be required for complete accumulation of capsular materials and exudates that are probably responsible for the observed increase in the palatability of the floc material.

Results from the experiment using both sterile SOW and sterile seawater as flocculating agents suggest that abiotic components present in seawater but absent from SOW are unimportant in determining either bacterial abundances on floc or the palatability of that material to *Tigriopus* grazers. Similarly, the similarity in the results from the experiments comparing the palatability and degree of colonisation of floc made from 0.8μm filtered seawater and that made from sterile SOW (Tables 1.3a and b) suggest that no significant artifacts have resulted from the use of the artificial medium in these experiments. Although, the fauna on the seawater flocculated material is most probably more diverse than that in the SOW treatments, the differences are apparently not strong enough to allow resolution by the techniques used in this study. It seems then that the use of SOW as a flocculating agent in these experiments did not significantly threaten the applicability of the results of this study to natural systems.

An interesting observation arising from this data set is the strong decrease in palatability noted for floc in this experiment during the second part of this experiment relative to that in the first (Tables 1.2a and 1.3a). As noted in the methods section, floc for the second experiment was formed from water from a summer collection that had been in storage for a significant period of time. It thus seems probable that the reduced palatability of the floc in the second part of this experiment can be attributed to slow degradation of the organic material during storage resulting in a flocculant particle of inferior food value. Why the bacterial abundances on this floc should be similar to those on the floc from the freshly collected water is, however, a mystery.

D. Other factors potentially influencing palatability.

From the similar bacterial abundances and palatabilities at all nitrate concentrations (Tables 1.4a and b), it appears that either the fauna is unable to utilise inorganic nitrogen, at least in the nitrate form, or else that the degradation of the floc material is not nitrogen limited. Tenore at al. (1979) have suggested that degradation of a particle is not in fact a simple function of the C:N ratio, but rather that it is strongly affected by the caloric value of the material available for degradation. As flocculant material probably has a very low caloric value, it seems likely that degradation is limited not only by the very low C:N ratio, but also by the poor quality of the floc material.

Floc made from TGHA was significantly less palatable to copepod grazers, even after reinoculation with the fauna from natural slough water, than the floc from the natural slough water itself (Table 1.5). This probably reflects the combination of a matrix of low palatability resulting from TGHA flocculation with the lower bacterial abundances found on these particles. The reason that bacteria should grow more poorly on TGHA (Table 1.5) than on natural flocculant material is an interesting area for speculation. For instance, this result might indicate that some non-humic portion of natural floc absent from the TGHA floc is particularly important in promoting bacterial growth and particle palatability. Alternately, this result may reflect damage caused to the TGHA molecules during the rigorous extremes used in their isolation, resulting in the material being less suitable to bacterial colonisation or copepod ingestion. Evidence for such damage has been found to occur during the extraction and purification of fulvic acids by Gregor and Powell (1987) and Visser (1985b). If this latter is the case, the result suggests that the many investigations of the bioavailability of humic substances that are carried out using base extracted humic substances (e.g. Mathur and Paul 1967; Swift et al. 1987; Visser 1985a; Tranvik and Sieburth 1989) should be treated with extreme

caution. Either way, this result emphasises the importance of carrying out experiments using natural, untreated organic matter if valid predictions of the palatability and food value of flocculant material are to be made.

II. SIGNIFICANCE OF INGESTION OF FLOC FOR GRAZERS.

Introduction.

Having determined that floc is ingested by copepod grazers, it is pertinent to ask whether such ingestion is beneficial, insignificant or even perhaps harmful to the organism involved. Strong evidence exists for the utilisation of humic-type particles as a food source in certain food chains. For example, Bowen (1984) found that amorphous particles formed from the slow precipitation in fresh water of organic matter from humic rich streams were considerably more digestible to larval toads (*Bufo americanus*) than were morphous detrital particles such as leaf fragments. Similarly, evidence for a food web based on peat carbon has been found in fresh waters by Schell (1983), where aquatic insect larvae were found to assimilate a great deal of peat detritus. However, in spite of a fairly high degree of fluvial export of organic carbon to saline waters, no evidence of the influence of peat detritus in the nutrition of marine organisms above the microbial level was found in Schell's study. Petipa et al. (1975) also suggested that humic substances were unimportant in the nutrition of marine organisms by showing that humic substances sorbed to a DEAE-type Sephadex anion exchanger were ingested more rarely by the copepod grazer, *Undinula darwinni* than was morphous detritus. Petipa et al. (1975) did however find a very high degree of assimilation for the small amount of humic carbon that was ingested. Given the abnormal mechanism used in their study for introducing humics into food webs, low levels of ingestion do not seem unreasonable. However, the combination of the high assimilation efficiencies found for humus by Petipa et al. (1975) this study and the evidence of ingestion of organic aggregates (Bowen 1984), suggest the potential for an important role in estuarine food webs for flocculant material.

Many analogies can be drawn between estuarine flocculated organic material and the amorphous organic particles formed from the aggregation of DOM in the open ocean. Both are composed of amorphous, fragile, flake-like particles of about the same size and both have a basic matrix which is of questionable food value. As marine snow type particles have been found capable of supporting growth of the brine shrimp, *Artemia salina* (Baylor and Sutcliffe, 1963; Seki 1972) it again seemed reasonable that flocculant material might also provide a food source for estuarine grazers. A specific investigation of the potential of estuarine flocculated organic material to form a food source for estuarine detritivores had not, however, been made until this study. The objective of the second phase of the study, then, was to determine the significance of ingestion of flocculant material for the grazer. As preliminary experiments showed that flocculant material was not capable of supporting *Tigriopus* through an entire lifecycle, experiments to determine the ability of floc to support certain key life-history events were chosen as a focus for this investigation. Each of these experiments was designed to focus upon phases that are particularly critical in the life history of the test organism, *Tigriopus californicus*.

The first experiment that will be described measured the duration of the period between insemination of the C5 female and the production of the first egg sac. This experiment was based on early observations upon *Tigriopus* that suggested that, under stressful conditions such as food limitation, this organism will slow its development until conditions improve (Kahan et al. 1988 and pers. obs.). Thus the rate at which the organism passes through various developmental phases will depend upon the degree to which the organism is experiencing food related stress. Such variations in development time can be utilised as a measure of the value of a food source of unknown quality, such as the flocculant material studied here.

The survival of females until the production of at least one egg sac is obviously a very important determinant of the continued survival of the species in the next generation and the fact that egg production in copepods can be limited by both food quantity and quality is well established (e.g. Ambler 1986; Checkley 1980). Moreover, observations made during preliminary studies, showed this period to be one particularly subject to high rates of female mortality when food was scarce or unavailable.

Mating in *Tigriopus* begins when C6 (adult) males clasp C5 females, using first antennae that are modified for this purpose. The pair then swims about together for approximately 48 hours, until the female moults from the C5 to the adult stage, at which point she is inseminated by the male and then released (Burton 1985). For the purposes of this experiment, the time between initial clasping and the actual insemination event was considered to be negligible and thus the observation of clasping was assumed to be synonymous with the insemination event. Although this may not be completely accurate, the large number of replications and random allocation of mating pairs to treatments used should compensate for the existing differences in the actual moment of insemination.

The success of gravid females in producing surviving young upon a certain diet is another important measure of the value of that food source. The second experiment described in this chapter examines this parameter for *Tigriopus* feeding upon a diet of normal flocculant material in comparison with those in the starved state and those fed a food source known to support the organism through an entire life cycle, Wardley's® Fish Flakes for Tropical Fish.

During the first few copepodite stages, *Tigriopus* displays particularly high growth rates relative to changes during the rest of the life history (Harris 1973). These stages are thus particularly vulnerable to starvation-induced mortality. As *Tigriopus* is

generally a rather hardy organism (Dethier 1980), these early copepodite stages represent perhaps the most suitable life history phase of this organism for use in testing for any benefit gained by ingestion of flocculant material. The third section of this chapter describes an experiment set up to examine the efficacy of various diets in sustaining *Tigriopus* through this relatively sensitive life history stage.

Materials and methods.

1.) Time between insemination and production of the first egg sac.

Organism.

Pairs of mating copepods were isolated, taking care to eliminate any males exhibiting precocious pairing with females younger than the C5 stage. These pairs were rinsed with SOW until all debris and faecal material had been removed, allowed to clear their guts for several hours and rinsed again several times before use. However, cleaning time was minimised to reduce separation of pairs before the initiation of the experiment. After cleaning, one pair of copepods was placed into each well of the Falcon® multiwell tissue culture plates in which various food types had been placed.

Experiment.

In an attempt to distinguish between the value gained by ingestion of the floc itself and that gained by ingestion of the floc fauna, two "floc" treatments were employed. The first of these was particulate floc material which includes both the floc

and the associated fauna. The second was a "floc water" treatment in which an equivalent volume of the water overlying the floc only was utilised. This water was assumed to supply the same fauna as the floc. Improved success in the presence of floc could then be taken to show that the presence of the flocculant particles impart an advantage to the organism.

The four food treatments utilised were:

1) Starved. 1mL of sterile glass distilled water (GDW) was provided to the tissue well receiving this treatment.

2) Fish food. A small amount (≈2mg) of ground Wardley's® Food for Tropical Fish was provided to each tissue well receiving this treatment and 1ml of sterile GDW was also added.

3) Floc. Approximately 2mL of the flocculant material prepared as for normal floc in Chapter 1, was pipetted from the bottom of the flask containing floc prepared as for normal floc above.

4) Floc water. Approximately 2mL of the water overlying the floc was pipetted from the same flask as in (3).

All wells were topped up with autoclaved SOW before addition of the copepods and the addition of a 1mL aliquot of GDW in the fish food and starved treatments was used to equalise the salinities of all treatments to that of the treatments receiving material from the flasks of flocculant material.

The four different treatments were assigned to individual wells of the Falcon® multiwell culture plates according to a systematic design. Upon addition of copepods the wells were incubated in a controlled environment room at 16°C and with a 16:8 hour day-night cycle. The copepods were observed every second day and the date of

appearance of the first egg sac was noted. When mortality occurred before production of an egg sac the date of mortality was also noted.

This experiment was run four times, twice using water collected in January 1989, once using the sample sample after a two month storage period at 4°C in the dark, and once using water from the May 1989 water collection. Various numbers of replicates of each treatment were utilised in each of these runs, depending on the availability of paired copepods at the time of the experiment. Mean time taken to become gravid was calculated for each treatment and compared using the independent t-test programme of the statistical package Epistat. For the two runs with the January water, mean mortality before production of the first egg-sac and mean percentages of the females in each treatment to produce at least one egg-sac were calculated and again were compared using an independent t-test.

2) Recruitment success of gravid females.

Gravid females were isolated from the main cultures and were cleaned as for the paired copepods above. Nine individuals were then allocated randomly to each of nine replicates of three treatments in 125mL erlenmeyer flasks topped up to 125mL with autoclaved SOW. The three treatments utilised were: starved, floc-fed and fish-food fed. About 15mL of concentrated floc material (prepared as for normal floc in Chapter 1) was provided to each flask receiving the floc treatment and about 7mL of sterile GDW were provided to each of the flasks receiving the starved and fish food treatments. The latter manipulation was carried out to equalise all treatments to the slight reduction of salinities experienced when suspensions of flocculant material are utilised. The flasks were then placed in an environment room at 16°C and with a 16:8 hour day-night cycle in random

order. The organisms were allowed to feed and reproduce for three weeks and the number of nauplii and copepodite stage offspring in each flask was then enumerated.

Two runs of this experiment were performed. During the second of these, survival of the adult females at the end of the three week period was monitored, as was the number of those females that were gravid at the end of the experiment. Recruitment success for nauplii and copepodites, survival and proportion of gravid females for each treatment were calculated and compared using an independent t-test using the statistical package Epistat.

3) Survival of the first copepodite.

C1/C2 stage *Tigriopus* were isolated from the main cultures and were cleaned with repeated rinses of SOW. To minimise premature moulting to later copepodite phases, copepodites were then utilised immediately rather than allowing an extra period for voiding of the gut. For this experiment ten individuals were assigned randomly to each well of a Falcon® multiwell tissue culture dish containing one of the thirty replicates of each of the four treatments: floc, floc water, starved and ground fish food. All treatments were prepared as for Experiment 1 (this chapter) and were allocated to wells according to a systematic design. Trays were incubated in an environment room at 16°C with a 16:8 hour day-night cycle. Survival of the copepodites over thirty days was monitored by removing six replicates of each treatment from the environment room every six days and enumerating the copepods remaining in each well. Each replicate was thus observed only once, resulting in statistically independent data points for all counts.

From these observations mean survival in each treatment for each sampling date was calculated and comparisons between each food treatment were made using an independent t-test with the statistical package Epistat.

Results.

1) Time between insemination and production of the first egg-sac.

The results of this experiment for floc made from January 1989 water soon after that collection date are shown in Figures 2.1a and b. The degree of mortality before production of at least one egg-sac in each treatment is summarised in Table 2.1. Proportions of the females becoming gravid during each experiment are reported in Table 2.2 and the mean time to become gravid with each diet is given in Table 2.3. The results of the feeding studies using aged water from this January collection and for floc made from the May water are summarised in Table 2.4.

A t-test of the data in Table 2.2 showed that both floc and fish food diets result in significantly lower mortality than that found in the starved treatment ($p < 0.05$). However mortality in the floc water treatment was not significantly different to that of the starved individuals. By contrast, mortality in the experiment using aged January floc and that from May 1989 water (Table 2.4) was complete in all but the fish food treatment. Analysis of the proportion of females producing at least one egg-sac in each treatment for the floc from freshly collected January water (Table 2.2) shows a significant increase in the percentage of the females producing at least one egg-sac in

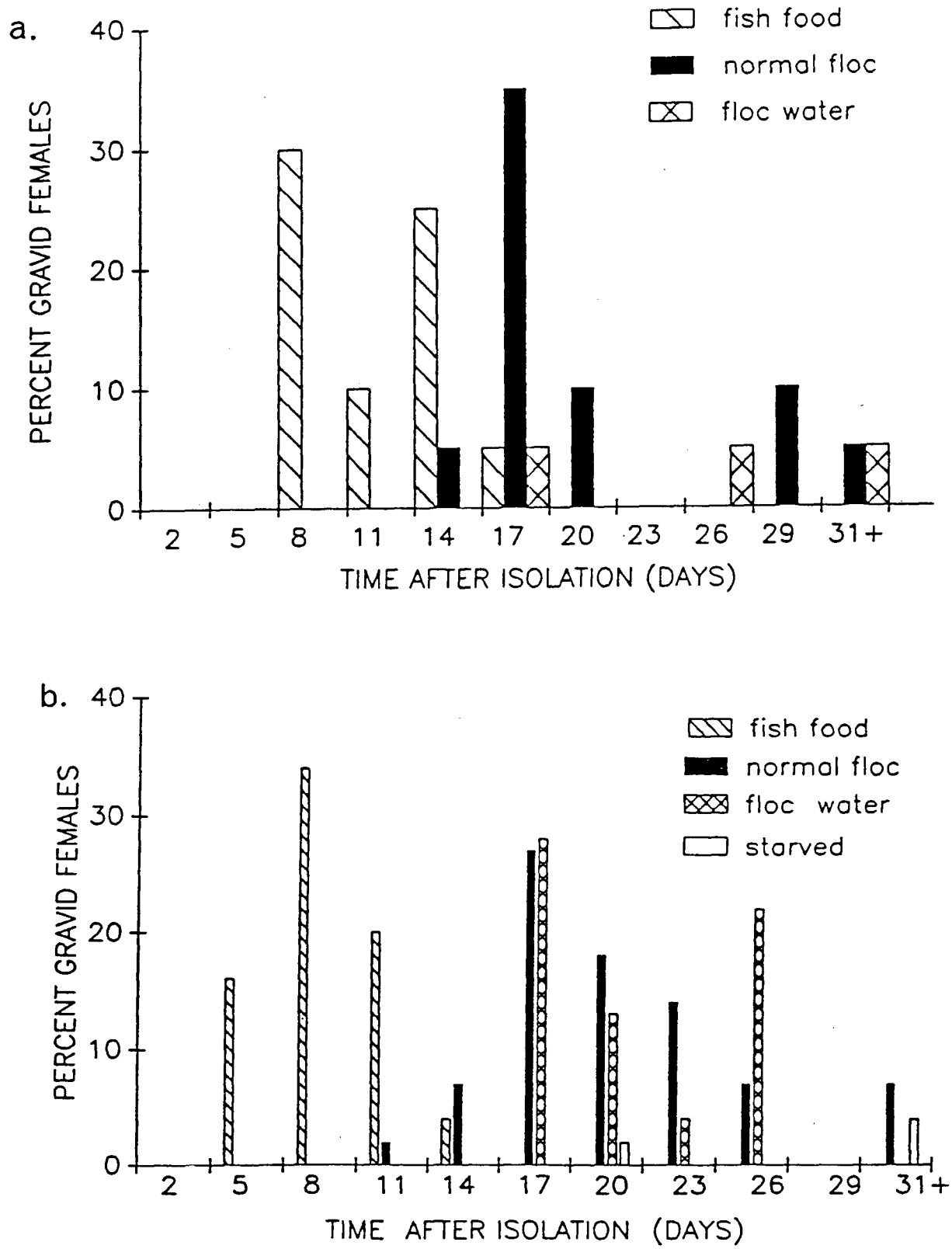


Figure 2.1. Plot of the duration of the period between insemination and production of the first egg-sac under different feeding regimes. a. run1 b. run 2.

Table 2.1. Degree of mortality before production of first egg-sac in the experiment using floc from freshly collected January 1989 water.

	Run 1 (%) (n=21)	Run 2 (%) (n=50)	Mean ± 1 S.D.
Floc	29	18	23.5±7.8
Floc water	95	53	74.0±30.0
Fish food	33	19	26.0±9.9
Starved	100	95	97.5±3.5

Table 2.2. Proportion of females producing at least one egg-sac in experiments using floc made from freshly collected January 1989 water.

	Run 1 (%) (n=21)	Run 2 (%) (n=50)	Mean ± 1 S.D.
Floc	52	78	65±18.4
Floc water	5	47.8	26.4±30.3
Fish food	66	71.2	68.6±3.6
Starved	0	5.4	2.7±3.8

Table 2.3. Mean time to become gravid with various diets for experiments using floc from freshly collected January 1989 water.

	Time (days) ± 1 S.D.		
	Run 1 (n=21)	Run 2 (n=50)	Overall Mean
Floc	19.2 ± 4.5	20.3 ± 5.2	19.8 ± 0.8
Floc water	21.5 ± 5.7	18.8 ± 2.4	20.2 ± 1.9
Fish food	11.6 ± 2.5	8.7 ± 2.3	10.2 ± 2.1
Starved	—	34.7 ± 13.3	—

Table 2.4. Summary of the degree of mortality before production of at least one egg-sac for floc from aged January water and water collected in May 1989.

	Mortality (%)		Successful Egg-sac Production (%)	
	January (n=24)	May (n=36)	January (n=24)	May (n=36)
Floc	91.7	100	8.3	0
Floc water	100	100	0	0
Fish food	4.4	8.3	95.6	91.7
Starved	0	100	0	0

both fish food and floc diets relative to the starved control ($p < 0.05$). By contrast, the number of females becoming gravid in the floc water treatment was statistically indistinguishable from that of the starved controls. For the floc made from aged January water and that from the May water collection (Table 2.4), only the females fed fish-food showed strongly increased success in egg-sac production relative to the starved controls.

An analysis of the duration of the period between insemination and production of the first egg-sac for the fresh floc from January water (Table 2.3) shows that production of an egg-sac takes significantly longer in the floc and floc water treatments than in the fish-food control ($p < 0.001$). Although only a small number of females became gravid in the floc water treatment, the period required for the production of the first egg-sac in those that did was similar to that required by organisms feeding on particulate floc. Only in the second run of the experiment with freshly collected January water did any individuals in the starved controls become gravid. In this case individuals in the starved treatment were significantly slower to produce an egg-sac than in any of the fed treatments ($p < 0.001$).

Despite the differences between the two runs of the experiment with freshly collected January water in terms of the time required to become gravid, an analysis of the mean values of this variable over the two runs shows essentially the same patterns as the individual experiments (Table 2.3). Overall, females fed fish-food become gravid significantly earlier than those in either the floc or floc water treatments ($p < 0.05$) but there is no significant difference between the development times of individuals in the floc and floc water treatments. A slow precipitation of flocculant material was observed in the floc water treatments throughout the second run of the experiment. The mass of material involved was very small, but none the less, small numbers of faecal pellets containing this material were noted in "floc water" wells during this run of the

experiment. Also, a low level of microbial colonisation of the walls of the wells in the floc and floc water was observed to develop about three weeks into the experiment. Observations of the starved treatments showed a similar development of microorganisms on the walls and floor of the wells in question.

2) Recruitment success of gravid females.

Results of the two runs of this experiment are summarised in Table 2.5. Clearly, the number of nauplii surviving in the fish food treatment is significantly greater than in either the starved or the floc treatments ($p < 0.01$) in both runs. However, in neither run nor in the overall mean survival, does the presence of floc result in enhanced survival of nauplii relative to the starved treatment. Similarly, in neither run, nor in the calculated mean, are the values for surviving copepodites in either the fish food or floc treatments significantly different from the survival of starved individuals.

A summary of the survival of females and proportions of those gravid in each treatment at the end of the three weeks is given in Table 2.6 for the second run of this experiment. Survival of females is significantly lower in the fish food treatment than in either the floc or starved treatments ($p < 0.005$). No significant difference in the survival of females exists between floc and starved treatments in this experiment. Significantly more females were found to be gravid in the floc treatments at the end of the experiment than in the starved condition. By contrast, no significant difference was found between fish food and starved or between fish food and floc conditions with regard to the proportion of females gravid at the end of the experiment.

Table 2.5. Recruitment success of gravid females under different feeding regimes..

	Mean # Nauplii/female ± 1 S.D.			Mean # Copepodites/female ± 1 S.D.		
	Run 1	Run 2	Mean	Run 1	Run 2	Mean
Floc	1.1 ± 3.3	0.0 ± 0.1	0.6 ± 0.8	0.1 ± 0.1	0	0.1 ± 0.2
Fish food	10.6 ± 7.9	9.7 ± 3.1	10.2 ± 0.6	8.8 ± 5.5	3.6 ± 1.9	6.2 ± 3.7
Starved	0	0	0	0	0	0

Table 2.6. Summary of the survival of females and proportion of these gravid at the end of a three week period.

	Starved	Floc diet	Fish food.
% Survival	72.8±17.7 (n=9)	83.9±15.8 (n=9)	17.2±23.6 (n=9)
% Gravid	1.2±3.7 (n=9)	31.8±27.2 (n=9)	26.6±38.3 (n=5)

3) Survival of first copepodite.

Results of this experiment are presented in Figure 2.2 with the omission of the observations from day 12 which, unfortunately, were lost. On day 6 of this experiment, none of the treatments were significantly different from the starved. However, mortality in the floc water treatment was significantly higher than in the floc and fish food treatments ($p < 0.05$). By day 18 mortality in the starved treatments was significantly greater than in all other treatments ($p < 0.05$). Mortality in both floc and floc water treatments on this date was significantly greater than that in the fish food treatment ($p < 0.01$). No significant difference could be detected between levels of mortality in floc and floc water treatments on this date. The pattern of mortality on day 24 was the same as that for day 18. However, by day 30 mortality in the floc water treatment had increased and was relatively greater than in the floc treatments ($p < 0.05$). Survival in both floc and floc and floc water treatments on this date was inferior to that in the fish food treatments ($p < 0.005$) and was superior to that in the starved treatments ($p < 0.05$).

Discussion.

As mentioned earlier, preliminary experiments found that flocculant material was shown to be incapable of supporting *Tigriopus* through an entire lifecycle. This is a situation often experienced with detrital food chains (e.g. Heinle et al 1977; Roman 1984), and was perhaps predictable in view of the poor food quality of the humic substances making up the bulk of the flocculant material (Chapter 1, Section A). In addition, it is often suggested that bacteria alone cannot form a complete food source for higher organisms because they lack certain essential fatty acids (Martin and Kukor 1984; Porter 1984). As a result, the presence of an additional source of food containing these

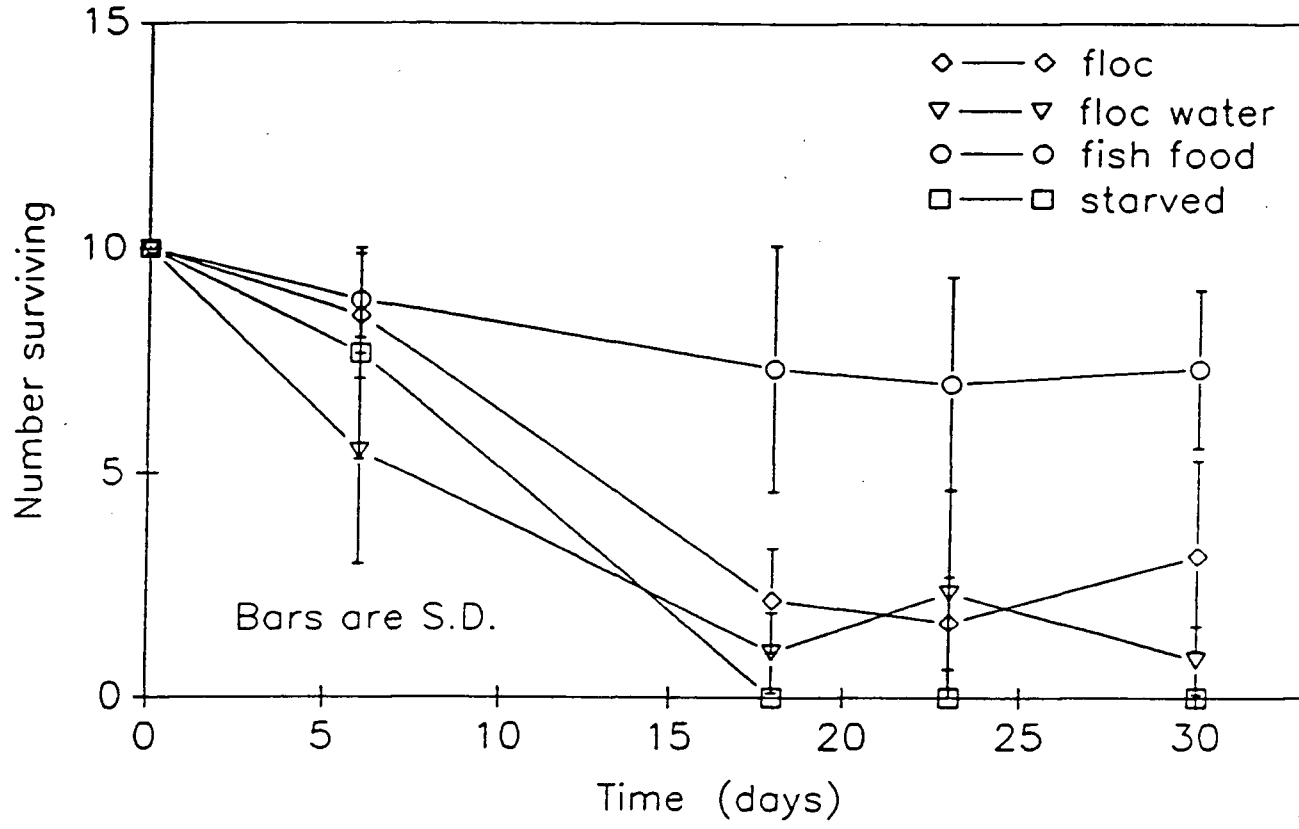


Figure 2.2. Plot of the survival of C1 stage *Tigriopus* under various feeding regimes.

essential compounds or the intervention of an intermediate grazer such as a protozoan or microflagellate is often postulated to be required. However, Rieper (1978; 1982) has shown that harpacticoid copepods are able to undergo a complete lifecycle on a diet solely composed of bacterial material. Thus it appears that the failure of the detrital food source to support the copepod throughout an entire lifecycle in these experiments reflects an inadequacy in the amount of bacterial biomass available for growth, rather than the absence of some essential dietary component.

Similar to the findings for other detrital food sources (Heinle et al. 1977; Roman 1984), this study has demonstrated that significant benefit is derived by *Tigriopus* from the ingestion of flocculant material, at least at certain times of the year. For example, floc formed from freshly collected water from the January 1989 collection significantly improved survival of the females between insemination and the production of the first egg-sac (Table 2.4 and Figs. 2.1a and b). In fact, survival and the proportion of females producing an egg sac is very similar in the floc and fish food treatments. Improved performance relative to the starved controls indicates that copepods must gain at least some benefit from the ingestion of floc. Improved success of copepods in the floc relative to the floc water treatments indicate that it is the presence of the flocculant material itself that is responsible for this benefit. Unfortunately it cannot be determined from this experiment whether benefit derives from assimilation of floc carbon or whether it results from the conversion of small organisms, not otherwise available to Tigriopus, into particles large enough to be utilised.

The strong improvement in the performance of organisms in the floc water treatment in the second run of this experiment (Table 2.2) is interesting. The presence of small amounts of flocculant material and the colonisation of the walls by microorganisms may explain this phenomenon as these factors could potentially provide a food source to

the organisms in these treatments. Similarly, improved success of organisms in the starved treatment during the second run of the experiment (Table 2.2) may be attributed to the appearance of a layer of microorganisms on the walls of the wells in question about three weeks into the experiment.

The fact that improvements in the survival of floc fed females relative to the starved controls in aged winter water and summer water did not take place (Table 2.4) is also interesting. It may be that the quality of the dissolved or flocculant materials available to the microfauna for metabolism is lower in these cases, ultimately resulting in less energy being available to grazers. Although refrigerated, some degradation of the more labile organic fractions in these samples would certainly take place over the three month storage period. Similarly, the water collected from the slough in May had been exposed to higher temperatures and longer residence times in the slough as a result of lower levels of rainfall. The more labile components of the water collected at that time may thus have already been utilised by the microfauna of the stream resulting in a predicted lower food value for the resultant floc particles.

Observations of *Tigriopus* throughout this study show that this organism consistently appears to slow its metabolism (evidenced by low levels of movement and extended duration of individual life history stages) in response to stress such as starvation and salinity and temperature extremes. Such behaviour has also been noted for a closely related *Tigriopus* species by Ranade (1957). Thus, the extended duration of the period between insemination and egg-sac production found for the floc diet relative to one of ground fish food in this study appears to indicate a lower food value for that material. Evidently, although some benefit is derived from the ingestion of floc, less energy provided by the ingestion of floc than is required for optimal growth rates. In addition, the rates of development determined in the second run of the experiment using freshly

collected January water are very similar for the organisms in the floc water treatment and those fed floc. The benefit derived by copepods grazing microorganisms on the walls of the wells and the very small amount of particulate material present in the well thus appears similar to that derived by those grazing upon the massive amounts of material provided in the floc treatment. This suggests that at least part of the benefit derived from ingestion flocculant material derives from the conversion of small organisms such as bacteria into particles large enough to be ingested by *Tigriopus*.

The benefits derived from the presence of flocculant material by adult copepods described above do not seem to extend to improved reproductive success. During the investigations of the reproductive success of gravid females with different diets (Table 2.5) it became obvious that the survival of the young in the floc treatments was no better than that of organisms in the starved treatments. Moreover, survival of the nauplii in both of these treatments was much inferior to that of the fish food fed organisms. Thus, though floc may be able to provide enough energy to adult copepods for maintenance, and perhaps for slow growth, it is possible that it does not provide enough energy to support the high energy needs required by the fast growth of the naupliar stages of *Tigriopus*. Alternately, the poor survival of these nauplii may reflect the unsuitability of the floc as a grazing substrate for these organisms. Although larger copepods can grasp the floc and manipulate the particles using various mouthparts, floc particles are too large for the nauplii to handle in this way, yet may not be large enough or firm enough to form a substrate upon which the animals can move and feed with ease. Floc may then, simply be an inappropriate medium for naupliar development. Selective cannibalism of the nauplii by adult copepods experiencing food stress can be suggested as another explanation for the poor success of the young in the floc treatments. Undoubtedly some degree of cannibalism does take place, particularly when adults are not receiving optimal nutrition from their diet. However, floc provides a large amount of particulate cover that

should provide a refuge from maternal predation for the nauplii and thus reduce the impact of this attrition. In addition, very low survival of nauplii was found in preliminary studies in which adult copepods were removed upon release of the nauplii from the egg-sacs.

The increased proportion of females found to be gravid in the floc treatments at the end of three weeks, relative to the starved controls (Table 2.6), suggesting that, not only does floc sustain copepods between insemination and egg-sac production, but it is also able to sustain the production of further egg-sacs. Thus, though the survival of the young is not improved while floc is the only food source, a diet of floc sustains the production of young that would then be able to rapidly exploit any new food reserves as soon as they became available.

The high degree of mortality for the adult females in the fish food treatments (Table 2.6) seems anomalous at first. However, this result might have been predicted on the basis of the prior observation that these copepods have faster development times with food of superior quality. It is possible that many females in the fish food treatments have produced all the egg-sacs possible for those organisms over the three week duration of the experiment and have thus completed their lifecycle and died. In contrast, copepods in the food treatments having lower food value (i.e the floc and starved treatments) have presumably slowed their metabolic rates in response to these conditions. These animals therefore have superior survival relative to those in the fish food treatments.

The lack of benefit derived from the presence of floc appears to be particularly acute in the naupliar stages of *Tigriopus*. As the copepods mature, improved survival with a floc diet relative to the starved treatments became apparent once more (Figure 2.2). Although survival of the C1 copepodites over the 30 day period was still

significantly lower than that observed with a fish food diet, copepods on both floc and floc water diets showed significantly improved survival relative to the starved condition. Moreover, copepods feeding on a floc diet showed significantly improved survival relative to those in the floc water treatments on the final day of the experiment. This again suggests an important role for floc in the conversion of microbial biomass into a size fraction available to the copepodite grazers. Moreover, increased survival of organisms in the floc treatments over those that had been starved suggests that flocculant material provides sufficient energy to sustain the earlier copepodite stages in spite of the fact that these are times of extremely rapid growth. This finding lends credence to the idea that poor survival of the naupliar stages is a result primarily of the inappropriate nature of that substrate for naupliar development, as opposed to the low energy content of the particles. Clearly then, although not an ideal food source, flocculant material could be important in sustaining organisms grazing upon it during periods when other food sources are in short supply.

III. SOURCE OF CARBON UTILISED BY GRAZERS, EFFECT OF INGESTION UPON CHEMICAL NATURE OF FLOC AND POTENTIAL IMPORTANCE OF INGESTION OF FLOC IN ESTUARINE AND COASTAL SYSTEMS.

Introduction.

The objective of this portion of the study was to distinguish which of these three pathways appears to be the one operating by combining observations from earlier experiments with the findings of an experiment using the natural abundance tracer method of carbon stable isotope analysis. An attempt was also made to investigate the changes in the chemical nature of the floc resulting from its ingestion and egestion by the grazer using IR-spectrometric techniques. Finally, an attempt was made to establish the potential importance of ingestion of flocculant material in estuarine and coastal waters by collecting a wide assortment of zooplankters and investigating whether or not such organisms ingest floc when it is provided as a sole food source under laboratory conditions.

Before describing the methods used in these experiments though, a brief introduction to the methodologies utilised will be given.

1) Mass spectrometric analyses.

The two natural isotopes of carbon (^{12}C and ^{13}C) are present in characteristically different ratios in marine plants relative to those found in terrestrial habitats. The

characteristic ratios of the two natural isotopes of carbon in these pools are often used as natural indicators of the pathways of carbon through food-webs (e.g. Schwinghamer et al. 1983; Rodelli et al. 1984 and Petersen et al. 1985). By convention (Craig 1953), isotope ratio data are expressed in terms of $\delta^{13}C$, which is the per mil difference between the isotope ratio of the sample and a reference standard material, such as PDB Limestone according to the following formula:

$$\delta^{13}C = \frac{(^{13}C/^{12}C)\ \text{sample} - (^{13}C/^{12}C)\ \text{standard}}{^{13}C/^{12}C\ \text{standard}} \times 1000$$

Using this designation, organisms from marine systems have a $\delta^{13}C$ of about -6 to -19 and C3 plants from terrestrial environments have $\delta^{13}C$ of -21 to -28 (Parker and Calder 1970).

Digestive selectivity by grazers selects the heavier isotope, resulting in a +1‰ fractionation of the animal's tissue relative to its food (Schoeninger & deNiro 1984). It follows that, if a grazer is exposed to two food sources with distinct isotopic ratios, the relative amount of carbon assimilated from each food source can be determined. In this study, the two food sources utilised were a marine flagellated algae *Isochrysis* sp. having a "heavier" isotopic ratio, and flocculant organic material of terrestrial origin with a "lighter" isotope ratio. If grazers are able to assimilate any floc carbon, the flesh of any organism eating floc should reflect this "terrestrial" source relative to one eating the marine *Isochrysis* food type.

2) Infra-Red Spectroscopy.

Absorbance in the infra-red portion of the spectrum is a result of periodic motions involving stretching or bending of the bonds making up the compound under analysis. It has been found that shorter and stronger bonds have a characteristic frequency of "stretching vibration" at shorter wavelengths (higher energy) within the IR spectrum, while longer bonds are characterised by "stretching frequencies" of longer wavelengths. This effect of bond length is modified by the fact that bonds with lighter atoms such as hydrogen have been found to vibrate at higher frequencies than those with higher molecular weight atoms (Sternhill and Kalman 1986). Thus certain functional groups and combinations of functional groups give rise to strong absorbtion at characteristic wavelengths which can be measured with an IR-spectrometer and used to obtain information about the chemical nature of the substance under investigation. For this study, IR-Spectroscopy has an additional advantage in that rather insoluble materials, such as those involved in flocculation, can be analysed without treatment with strong solvents such as NaOH, preventing unnecessary molecular degradation. This is possible because the absorbance frequencies of the important functional groups are essentially the same in both the solid and liquid state.

3) Investigation of the potential impact of the ingestion of floc in natural, coastal waters.

Recognising the ecological limitations of *Tigriopus*, described earlier, an attempt was made to relate the findings of the laboratory experiments using *Tigriopus* to the coastal systems in which flocculant material would naturally be encountered. To this end, a large number of different marine organisms were collected and were tested to

determine whether or not they would ingest floc, at least, under laboratory conditions. If floc is indeed ingested by these, more selective organisms, the benefits found to be derived by *Tigriopus* through the ingestion of floc would presumably also be gained by them.

Materials and Methods

1) Mass spectrometric analyses.

For this experiment copepods were cultured in 0.8μm filtered natural seawater (from the West Vancouver Laboratory Tanks), and were fed a diet of dried ground *Isochrysis*. The algae were obtained from batch cultures grown in 100L bags of natural seawater in a controlled environment room at 16°C with a 16:8 hour day-night cycle and were harvested during early senescence by filtration or centrifugation. The cells were then rinsed with several washes of GDW, dried in a 65°C oven, and ground finely in a mortar and pestle before being fed to *Tigriopus*. In the first run of this experiment, copepods were grown under these conditions in 2L erlenmeyer flasks on a windowsill in the laboratory. Under these favourable levels of daylight, strong growth of a blue-green alga contaminant which had a "lighter" isotopic ratio, resulted in the copepods used in this experiment having an initial body carbon signal more negative than that expected from a diet of pure *Isochrysis*. C1 stage copepodites for use in the first run of this experiment were isolated from these flasks and were rinsed several times with 0.8μm filtered seawater over a 24h period in order to remove all detrital material. Cleaned copepods were incubated in 100x15mm petri dishes (Fisher®) containing one of three diets:- *Isochrysis* alone, *Isochrysis* and floc mixed and floc alone. *Isochrysis* treatments received an addition of approximately 100μg of the dried algal material while for each

floc treatment, 6mL of concentrated floc was pipetted into fresh petri dishes from the bottom of flasks containing that material. For the mixed food treatment, a similar volume of floc was provided and an addition of about 100µg of *Isochrysis* was made. All dishes were filled to 50mL using sterile seawater and were incubated at 16°C with a 16:8 hour day-night cycle. Floc for this experiment was prepared from slough water collected in June 1988 as for normal floc in Chapter 1, section B.

Upon reaching maturity the surviving copepods were harvested, rinsed and allowed to clear their guts for six hours during which time several subsequent rinses with clean natural seawater were made to remove all detrital particles. Copepods were then killed by reducing the pH of their medium to 1 with 1N HCl, rinsed in GDW and re-isolated by centrifugation several times in order to remove inorganic salts. Cleaned copepods were then dried for 12 hours at 65°C, ground in a mortar and pestle and analysed for ^{13}C:^{12}C ratios using a V.G. Isogas "Prism" mass spectrometer coupled to a Carlo-Erba 1106 CHN analyser.

Because of the problems with contamination of the initial culture, this experiment was repeated with the modification that, in order to gain C1 stage copepodites having a more typical "marine" ^{13}C:^{12}C ratio, gravid copepods were isolated from the flasks described above and placed in 100 x 15mm petri dishes (Fisher®) in natural seawater. The female were again fed a diet of ground *Isochrysis*, but in this case the dishes were maintained in the dark in the laboratory, thus reducing the growth of the blue-green alga. Females were moved to fresh dishes every second day and the nauplii produced were allowed to grow to the C1 stage in the original dish, again in the dark. The resulting C1 stage copepodites which had a strongly marine body carbon signal against which any terrestrial carbon assimilation should be easily detected were then used in feeding studies

exactly as described above. Floc for this second run was prepared from water collected in January 1989.

2) IR-Spectroscopy.

Copepods utilised in this experiment were isolated from the main SOW based cultures and were cleaned by successive rinses with SOW over a six hour period, to allow removal of the material present in the gut of the organisms before commencement of experimentation. C5 and C6 stage copepods were then placed into 100x15mm petri dishes (Fisher®) containing autoclaved SOW. Floc used in this experiment was made from water collected in January 1989 and was then split into two portions. One of these was set aside as "food", the other was utilised in the feeding experiments. Copepods were fed 5mL of concentrated floc from this second portion per dish and were maintained on a dark shelf in the laboratory in order to reduce any growth of algae introduced by the copepods. The animals were allowed to feed for a week and dead organisms were removed daily to reduce cannibalism. Each week, remaining copepods were moved to a fresh dish of floc, and the pellets they had produced were isolated by repeated re-suspension of the floc-pellet mixture followed by removal of the excess floc once the heavier pelletised material had settled. The pellets were then washed by centrifugation and re-suspension in GDW and dried for five minutes at 65°C and for a further two days at room temperature. At the same time as the pellets were being recovered, a paired, un-grazed floc portion that had been set aside on the same shelf as the dishes containing the copepods was also centrifuged, rinsed and dried. The pellets from approximately five thousand copepods were then finely ground and made into a mull with DMSO as was a similar mass of the floc from the control bottle and the suspensions were analysed using a Perkin-Elmer 1600 Series FTIR spectrometer.

3) Investigation of the potential impact of the ingestion of floc in natural, coastal waters.

Organisms were collected during a series of three day cruises aboard the C.S.S. Vector between September 1988 and March 1989. Two vertical tows were made at the mouth of each of Jervis and Saanich inlets and the resulting samples were sorted immediately. Once isolated, members of the same species were placed in individual 2L thermos flasks filled with seawater collected from the surface at the sample site. These samples were then transported to the laboratory where they were maintained in a 4°C cold room in fresh seawater until use. In the laboratory the organisms were identified using the Identification Manual for British Columbia Pelagic Marine Copepods (Gardner and Szabo 1982) and the Laboratory Zooplankton Atlas for the Strait of Georgia (Fulton 1968).

Organisms utilised in tests were placed into Falcon® culture dishes or petri dishes, according to size and were either provided with floc or were left without added food in order to provide a control for pellets produced as a result of previously ingested material, still present in the gut. The dishes were incubated for 24 hours at 16 C with a 16:8 day:night cycle and the pellets produced per organism were quantified. Comparison was then made between the pellet production rates of the starved and floc fed organisms using the Student's t-test programme of the statistics package, Epistat in order to determine which species exhibited significant degrees of grazing upon flocculant material.

Results.

1) Mass spectrometric analyses.

The results of the mass spectrometric analyses are illustrated in Figure 3.1 a and b. In the first run of this experiment floc was found to have a $\delta^{13}C$ of -26.6 and *Isochrysis* of -12.3, while the residue in the bottom of the flasks in which the initial copepods were grown contained both the *Isochrysis* and a blue-green algal contaminant and had a $\delta^{13}C$ of -17.4. The initial copepodites were found to have $\delta^{13}C$ of -21.2, somewhat more negative than the residue upon which they were presumed to be feeding. Upon attaining the C6 stage with an *Isochrysis* diet, the copepods had a $\delta^{13}C$ of -16.2, demonstrating significant assimilation of the *Isochrysis* carbon. By contrast, copepods feeding upon floc had $\delta^{13}C$ of -21.6, a value similar to that of the original C1 stage specimens. Survival of the copepods in this treatment was very low, with much evidence of cannibalism being apparent. Copepods fed a mixture of floc and *Isochrysis* had a body signal of -19.8 suggesting either a lesser assimilation of the algal carbon, or else a slight influence of the floc carbon relative to those fed a diet solely consisting of *Isochrysis*. Faecal pellets of organisms in this mixed floc-algae treatment showed the presence of both green and brown components, often within the same pellet, indicating relatively non-selective ingestion of both floc and algal particles. Survival in the mixed floc-*Isochrysis* treatments was noted to be very good, with numbers of adults in these mixed treatments surviving to the C6 stage equalling or surpassing those observed in the pure *Isochrysis* treatments.

The two food types used in the second run of this experiment were found to have very similar $\delta^{13}C$ values to those in the first ($\delta^{13}C$ values of -27.0 and -12.5 were

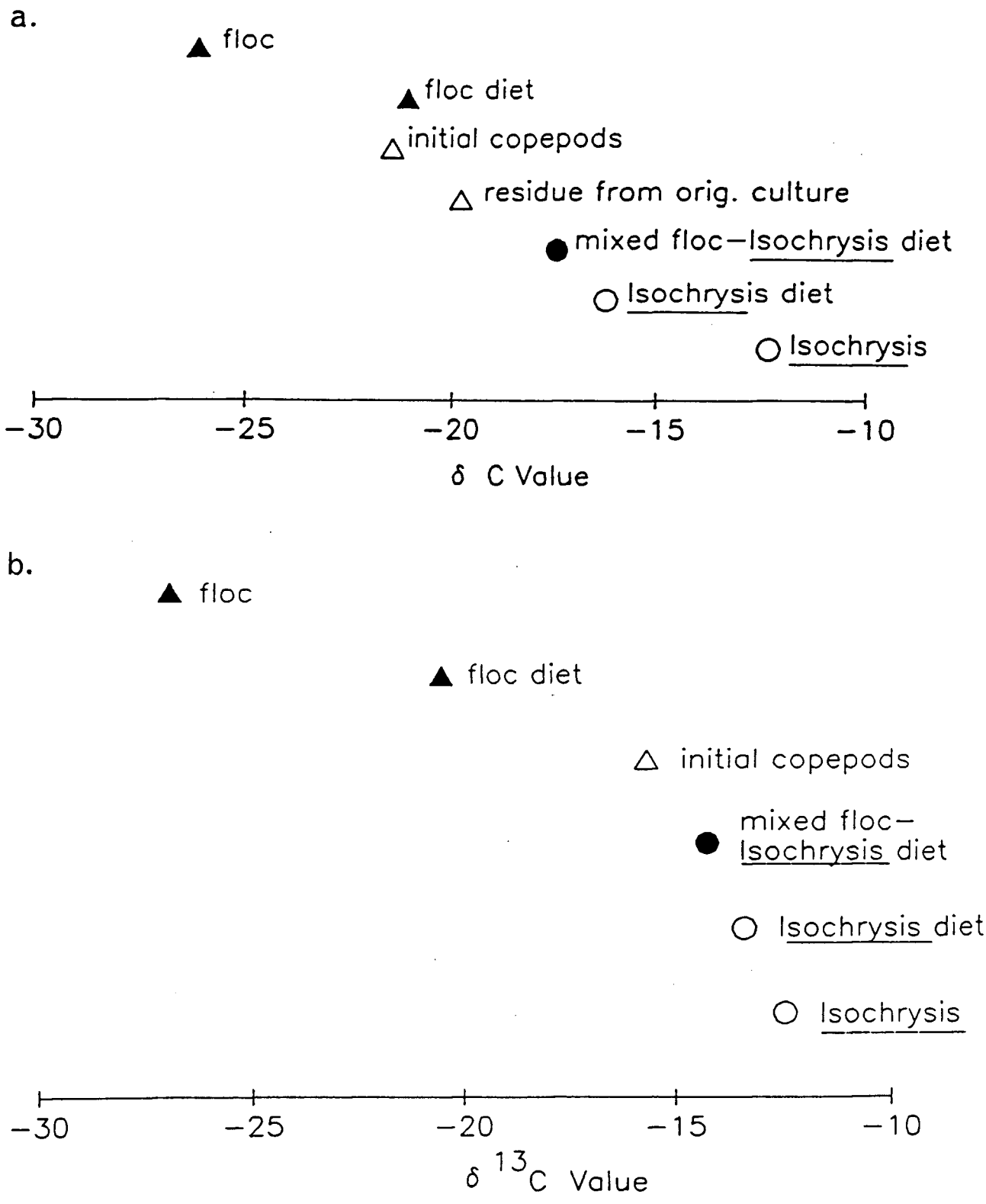


Figure 3.1. Mass spectrometric analysis of copepods fed upon different diets. a. first run b. second run.

measured for floc and *Isochrysis* respectively). The original C1 stage copepods however had a much more marine $\delta^{13}C$ of -15.7. At the end of the experiment the C6 stage copepods fed an *Isochrysis* diet had a strong $\delta^{13}C$ (-13.4) as did those fed a mixed *Isochrysis*-floc diet (-14.5). By contrast, copepods fed a diet of floc alone showed a strong influence of terrestrial carbon ($\delta^{13}C$ -20.6). Survival of copepods in all three treatments was very good and no blue-green algal growth was observed in any of the dishes during this run of the experiment.

2) Infra-red spectrometric analyses.

The results of this analysis are shown in Figure 3.2. It can be seen that the absorbance spectra of the floc and the pellet material are extremely similar. Stronger absorbances of the pellet material relative to the original floc in the range of 3200 cm^{-1} and 1100 cm^{-1} are the only differences observed. The former of these indicates an enrichment in -OH groups in the pellet material relative to the original floc, while the latter corresponds to the absorbance of the suspension medium, DMSO. The difference in the relative heights between the two floc types at this wavelength probably represents a simple subtraction error between the absorbances measured for DMSO during this run and those measured for the DMSO blank, run earlier, by the sensors within the machine.

3) Investigation of the potential impact of the ingestion of floc in natural coastal waters.

The results of these feeding experiments are given in Table 3.1. An asterisk after the name of any organism indicates that pellet production in the presence of floc was

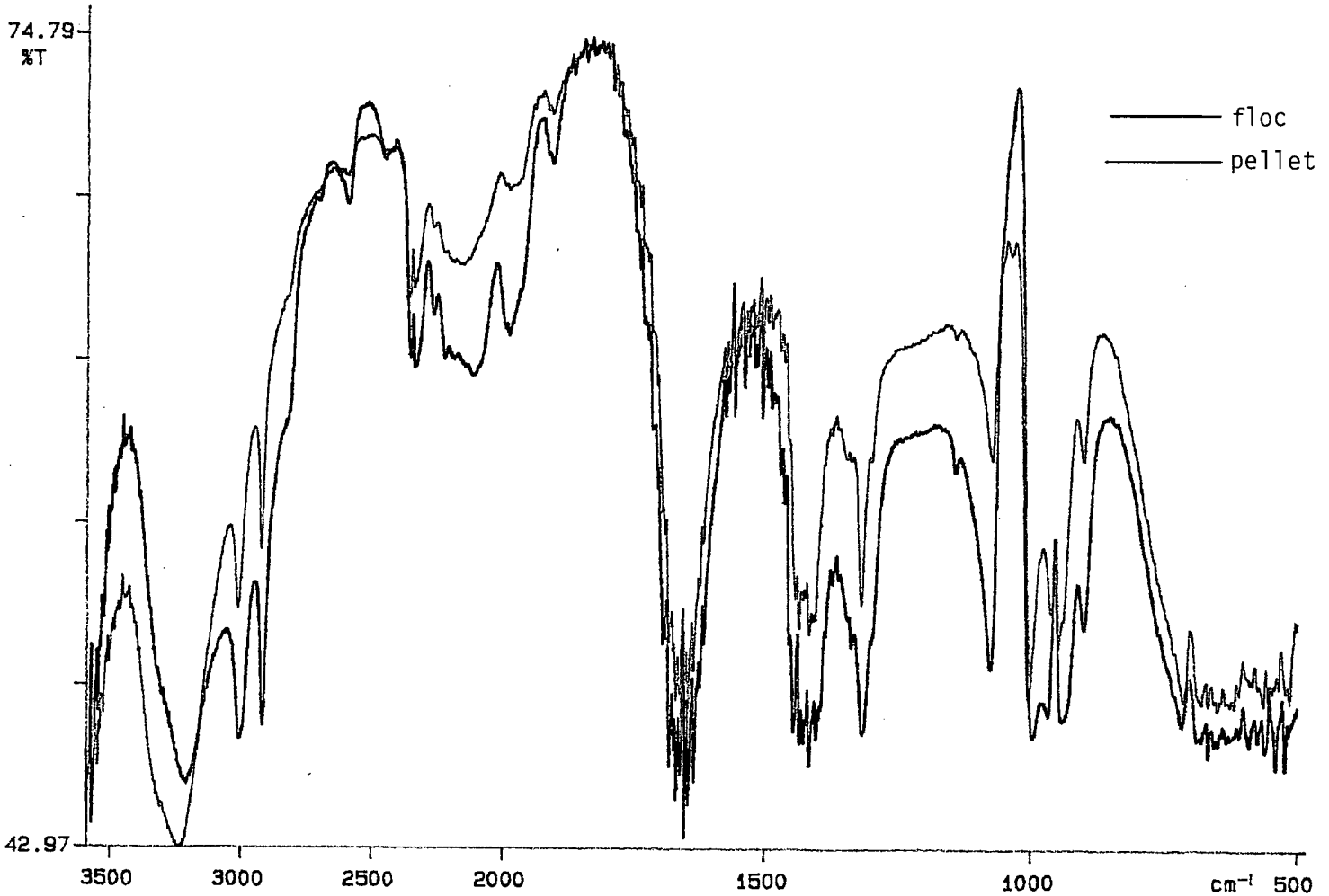


Figure 3.2. IR-Spectrometric comparison between floc material and the faecal material derived when *Tigriopus* is allowed to graze upon it.

Table 3.1. Comparison of pellet production by starved individuals with that of floc fed organisms for some common estuarine and coastal organisms.

Organism	n	Mean pellet number ± 1 S.D	
		Starved	Fed
*Amphiascus undosus**	9	1.0 ± 0.4	8.1 ± 1.0
Balanus sp.	9	28.1 ± 12.7	61.4 ± 30.2
*Calanus marshallae**	4 12	2.1 ± 0.5 0.3 ± 0.3	69.9 ± 14.5 59.7 ± 8.9
*Calanus pacificus**	12 6	0.3 ± 0.8 0.3 ± 0.4	14.6 ± 3.3 24.7 ± 9.0
Chiridius gracilis	6	0	0
Corycaeus anglicus	12	0.1±0.1	0.1±0.1
Eucalanus bungi	3	0	0
*Euchaeta japonica**	6	0.8±0.9	4.2±1.5
Hyperia sp.	9	3.9±3.2	5.1±3.5
Metridia pacifica	9 9	0.1 ± 0.2 0.4 ± 0.5	0.3 ± 0.4 1.8 ± 1.0
Neocalanus plumchris	6 12	0.1 ± 0.2 0.2 ± 0.2	1.3 ± 2.3 0.4 ± 0.4
Orchomenella sp.	6 14	3.0 ± 1.7 6.8 ± 6.4	5.5 ± 3.4 10.9 ± 9.2
*Pseudocalanus minutus**	9	0.1 ± 0.2	2.6 ± 0.9
Stilipes sp.	9 15	4.6 ± 3.4 4.0 ± 2.7	6.2 ± 2.8 5.1 ± 2.1
Thysanoessa rashii	10 12	4.2 ± 2.1 4.7 ± 3.72	116.5 ± 139.7 16.2 ± 23.5

significantly higher than that of the starved controls ($p < 0.05$). Of the fifteen species tested, five showed a definite tendency to graze floc carbon under laboratory conditions. In addition, several species (notably *Thysanoessa rashii* and the species of *Mytilus* tested) had individuals that displayed extremely high rates of grazing upon flocculant materials. However, the enormous variability between individual organisms resulted in no significant difference being found between the floc-fed individuals and the starved controls in terms of pellet production for these species.

Discussion

1) Mass spectrometric analysis.

Significant assimilation of the *Isochrysis* food source is evidenced by the considerable positive (more marine) shift in the carbon signal of the adult copepods in the first run of this treatment relative to the initial C1 stage *Tigriopus* (Fig. 3.1a). A similar, but less spectacular positive shift was also observed during the second run (Fig. 3.1b). This trend is in keeping with the fact that this alga would generally be regarded as a relatively high quality food for zooplankton grazers. High rates of assimilation of *Isochrysis* carbon would therefore be expected.

The very similar $\delta^{13}C$ ratios of the original organisms and those surviving to the C6 stage in the floc treatments in the first run of this experiment (Fig. 3.1a) appeared to indicate that little or no terrestrial carbon was being assimilated by *Tigriopus*. In fact, the similarities in the carbon ratios between the original C1 copepodites and the surviving C6 stage copepods in this treatment seemed to reflect the observed high levels of cannibalism in this treatment. By contrast the rather negative $\delta^{13}C$ value of copepods in

this treatment upon the second run, indicate significant assimilation of the "lighter" carbon isotope. It is possible that copepods in the first run of this experiment were also assimilating terrestrial carbon, but that this could not be detected against the rather negative signal of the initial copepodites. However, much evidence has been presented during this study to suggest that floc formed from water collected during the summer months is less valuable as a food source than that produced from water collected during the winter. As the floc used in the first run of this experiment was collected in the summer months, and was observed to sustain only a very few copepods to the C6 stage, it seems likely that this result reflects the low availability of the organic material derived from summer water collections to copepod grazers. The significant assimilation of stream carbon in treatments using floc made from water collected in the winter months suggests that the benefits found to derive from ingestion of flocculant material (previous chapter) can be attributed, at least in part, to utilisation of floc carbon either directly or through the mediation of the colonising microbes. As floc is ingested at very low rates in the absence of a microbial fauna (chapter 1), it appears that the mediation of the microfauna plays an important role in the flow of energy between the floc and the copepod grazer.

Unfortunately, these results cannot be conclusively interpreted to show utilisation of floc carbon, as floc for these experiments was delivered along with a significant volume of the overlying water from the flask. This overlying water may have provided a source or more labile "dissolved" terrestrial organic carbon that was being utilised by microorganisms colonising the floc and subsequently transferred to the copepod grazer. However, from the very low survival and development rates of organisms in the "floc water" treatments (previous chapter) and the very low concentrations of the stream "dissolved" fraction present in the experimental dishes, it seems that floc is the most probable source of the majority of the terrestrial carbon assimilated. It is thus postulated

that, of the three mechanisms proposed for the contribution of floc to estuarine food chains, the most probable is that in which floc carbon is broken down and assimilated by microbes, which are, in turn, assimilated by the copepod grazer.

Of course, it is possible that microbes use both particulate (floc) and dissolved carbon sources. Such a combination of carbon sources has been shown for flocculant organic matter in humic rich lakes (Tranvik and Sieburth 1989). In order to determine if this is also the case for estuarine flocculated organic matter, it would be interesting to run another mass spectrometric analysis in which the floc used had been rinsed several times in clean seawater before being fed to the copepods to completely eliminate all non-flocculant organic carbon. If terrestrial carbon is still assimilated in this treatment, the fact that floc carbon is metabolised could be definitively proven. If this experiment was combined with a study such as that of Travik and Sieburth (1989), in which activities of the microbes colonising floc with and without the addition of a source DOM were compared, the possibility of combined use of particulate and "dissolved" fractions could be investigated.

The results of the treatments in which both floc and *Isochrysis* were provided to copepods in both runs indicate strong preference for assimilation of the algal carbon. The absence of appreciable assimilation of floc carbon in the first run was not suprising in view of the absence of any evidence of it in the pure floc treatment. However, the absence of such assimilation during the second run of this experiment raises an interesting question; Why, if floc carbon is assimilated when presented as the only food source, is it not assimilated in addition to the *Isochrysis* carbon when both food sources are present? It might be suggested that the copepod is able to selectively reject the flocculant particles in favour of the algal material. However, the fact that both green and brown material was observed in the faecal pellets of organisms in this treatment appears

to refute this explanation. It seems then that this copepod may be able to acclimatize its digestive enzymes to the available food supply. In this way, when floc is the only available food supply, copepods could process it in such a way as to maximise the benefit derived from ingestion of that material. However, when *Isochrysis* is also present, enough energy may be derived from the algal material to prevent induction of the enzymes required to effectively utilise floc carbon. Such adjustments to the nature and supply of food have been shown to take place in other marine copepods, at least under certain circumstances (Mayzaud 1986 and references therein).

2) IR Spectrometric analysis.

The strong similarities between the absorbance spectrum of the pellet material and that of the floc food, suggests that little chemical change in the nature of the floc material during its passage through the gut of the copepod grazer. The only difference between the two treatments, i.e. the increase in the relative number of hydroxyl groups in the pellet material, is probably either an indicator of the high concentrations of this functional group in the material composing the faecal membrane (possibly chitin or some mucopolysaccharide) or an indicator of a higher degree of hydration of the material in the faecal pellets. Thus, it appears that although some assimilation of floc carbon by this grazer does take place, the bulk of the floc matrix is unaffected by passage through its gut. As the microbial biomass postulated to be the portion of the floc utilised by the grazer, constitutes only a very small portion of the overall floc particle, the removal of these organisms may not result in a large enough change in the bulk properties of the floc to be detected using this technique. Although the chemical nature of the majority of the flocculant material is little affected by passage through the copepod, grazing does have the effect of compacting the flocculant material into heavier particles which have higher sinking rates than that of the original floc. Grazing may thus significantly reduce the

residence time of the floc material in the water column. This would cause a similar reduction in the time available for further degradation of the flocculant material and might thus result in the delivery of the majority of the material to the sediments in a relatively unaltered form. Such a role for zooplankton pelletisation in the transport and preservation of organic material entering the sediments has been well documented in the open ocean (e.g. Bishop et al. 1986; 1987) and is likely to also apply to estuarine environments.

3) Investigation of the potential impact of the ingestion of floc in natural coastal waters.

In contradiction to the assertion by Paffenhöffer and Strickland (1970) that copepods are unable or unwilling to make use of "structureless detritus", the results of this study suggest that a number of organisms will eat floc, at least in the absence of an alternate food source, under laboratory conditions. Many of these species, such as the two species of *Calanus* and the euphausid *Thysanoessa*, were in fact the dominant species at the time of sampling. Moreover, of the species that were found not to ingest floc in this study, many are known to be carnivorous (e.g. *Corycaeus anglicus, Hyperia* sp. and *Chiridius gracilis*) and would not thus be expected to utilise a detrital food source such as floc. Others, such as *Neocalanus plumchris* and *Eucalanus bungii* were probably in non-feeding stages at the time of sampling [*Neocalanus* appears to suspend feeding during the winter months (Harrison et al. 1983) while *Eucalanus* appears to lack the mouth parts required for feeding in the C6 stage (Gardner and Szabo 1982)]. In essence then, this survey has shown that the potential for a number of organisms to graze flocculant material if that material is present in nature is very high. To overlook the presence of floc when investigating the survival and diet of estuarine organisms could

therefore result in a number of erroneous conclusions. Every effort should therefore be made to account for the presence this food source in future calculations of estuarine energy budgets, particularly during the winter months.

GENERAL DISCUSSION.

Flocculation of river-borne organic colloids in estuaries provides an enormous supply of organic material to organisms in that estuary, particularly in the winter months. In the past, it seems, this pool of organic carbon has been assumed to be fated to enter the estuarine sediments essentially unaffected by biological processes (Mayer 1985). This study has shown that this assumption, although it has some validity, must be made subject to certain provisos. Primary among these is that, although the chemical nature of the bulk of flocculant material may indeed remain essentially unaffected by the digestive processes of estuarine grazers, the same is not true in reverse. That is, ingestion of the floc may in fact have an important role in the sustenance of the grazer involved. Indeed, this study has shown that ingestion of flocculant material appears to result in improved survival and rates of development over those experienced by starved organisms. From mass spectrometric analyses it is apparent that this improved survival is, at least in part, a result of the assimilation of floc carbon presumably through the mediation of microbial decomposers. Additional advantage gained from the ingestion of flocculant material derives from the provision of a particulate matrix which converts the microbial fauna associated with floc from a size fraction too small to be exploited by the grazer into one that can be effectively utilised. By this action at least one trophic step is bypassed, allowing for a much more efficient transfer of the floc energy through the food-chain.

The discovery that floc carbon may be assimilated, at least at certain times of the year opens the possibility for bio-accumulation of metallic contaminants associated with the floc by estuarine grazers. This vector for trace metals in estuaries is one that has not been investigated to date. However, the findings of this study suggest that such an oversight may result in a serious under-estimation of the threat posed to estuarine food chains by metals associated with contaminated riverine organic materials, particularly in

the winter months. The potential for bio-accumulation of heavy metals and other contaminants as a result of the ingestion of flocculant material is a subject that should be addressed in future studies, as the implications for food web dynamics are very serious if indeed this does take place.

Extensive investigation of the factors influencing the ingestion of floc provide suggest that both the nature of the floc and the degree of microbial colonisation exert strong influences over the degree to which flocculant particles are grazed. Grazers appear to react to the presence of microbes upon a floc by increasing their rates of ingestion for particles with higher degrees of microbial colonisation. However, for unknown reasons, improved palatability with increasing bacterial abundances were not observed for cell densities higher than 10^6 cells $\cdot$ L^{-1}. This result is particularly interesting in the light of the observation that the bacterial abundances in which a strong influence upon palatability was observed correspond closely with the range of bacterial abundances in local waters (Valdés and Albright 1981; Velji and Albright 1986). Thus, it seems probable that changes of bacterial abundances within these waters may play an important role in determining the palatabilities of all particles, including those resulting from flocculation, suspended in those waters.

Further investigation into the role of bacterial colonisation in determining floc palatability showed that the increased palatabilities described above were a response to changes in the abundance of only certain strains of the overall bacterial fauna. Changes in the relative abundances of the "palatable" strains and the "unpalatable" strains such as might take place as a result of competition or even as a result of chance omissions or additions to the fauna colonising the floc in nature could, therefore, significantly change the palatability of the resulting particle. Enumeration of the overall abundance of particles in an experiment such as these may not, then, provide an adequate description of

the relative palatabilities of two different particles. Some knowledge of the composition and relative abundances of the different strains upon those particles would be advantageous. The techniques to make such a quantification accurately have only recently become available, and are in many cases still in the developmental stage. However, techniques such as DNA and RNA probes able to quantify the amount of such material from a particular strain that is present in a sample (Delong et al. 1988; Fuhrman et al. 1988; Lidstrom, 1989) hold much promise for the efficient practice of the type of microbial ecology described above.

The factors eliciting the increased grazing by copepods upon particles colonised with these species were not determined during this study. An analysis of the biochemistry of the preferred bacteria and the extra-cellular products exuded by them may provide some fascinating insights into the factors determining ingestion rates of particles in nature and would be much deserving of investigation.

The nature of the flocculant material may itself also has a strong influence upon the degree to which it is ingested by grazers. This is evidenced by the presence of important changes in the palatability of flocculant material during different seasons. In these experiments "bacteria-free" summer floc was found to elicit much inferior levels of grazing rates than floc made from water collected during any other season. The palatability of the basic floc material was thus postulated change from season to season, with that from summer water collections being less palatable to *Tigriopus* than that from winter or spring. Moreover, comparison between the palatabilities of these flocculant particles in the presence of bacteria showed that summer floc was still significantly inferior in its palatability, even though the degree of bacterial colonisation appears to be similar in all seasons. This latter observation may suggest that bacterial colonisation, although a powerful modifier of palatability, may not be able to overcome the basic

unpalatability of certain kinds of particle. However, if changes in the relative abundance of palatable and unpalatable strains also occur, these may act in concert with the changing palatabilities of the floc matrix in determining the overall palatability of that particle. The fact that less benefit is derived from the provision of floc that was made from summer water or from water that had been aged for several months and the absence of assimilation of "light" carbon from summer floc reflects the observed reduction in palatabilities of these particles.

It can be concluded then that maximum benefit from ingestion of flocculant material occurs during the winter months, when floc is most palatable and floc carbon is effectively assimilated by grazers. As it is during the winter months that flocculation is maximum and it is also during these months that other sources of food are less abundant, it would seem that flocculation has the potential to perform an important role in sustaining organisms grazing upon it until other food sources become abundant once more. In the future studies upon the energetics of estuarine organisms should pay close attention to the presence and nature of the flocculant material in the system under survey, particularly in the winter months.

The infrequency of sampling dates during this study precludes definitive conclusions regarding seasonal changes in the nature and bio-availability of flocculant material. Future studies using a more frequent sampling regime may provide important information about the potential extent of influence of flocculant material in estuarine food webs.

This study has provided several examples of changes undergone by organic material as a result of either the methods used in the extraction of humic fractions or as a result of the storage of organic rich water between its collection and use in experiments.

Care should be taken to carry out all investigations into the bio-availability of flocculant organic material using material as closely related to that found in nature as is possible. It is evident that this material is not as resilient to attack by microbes or strongly oxidising or reducing conditions as it was once believed to be.

CONCLUSIONS

Flocculation of organic material from streams with a large load of DOC upon addition of salt water was found to result in the provision of a large amount of new particulate material in the form of amorphous, brown flocs. About half of this material was found to consist of humic substances, with humic acids making up about 50% of the total humic fraction flocculating. The overall C:N ratio of the flocculant material was about 50:1.

Bacterial abundance was found to be an important determinant of the palatability of flocculant material to the copepod grazer, *Tigriopus californicus* using feeding studies with floc having various degrees of bacterial colonisation. Increasing bacterial abundances in the range of 0-10^6 cells/mL resulted in approximately linear increases in the palatability of flocculant particles. However, increases in bacterial abundances above this concentration resulted in little or no further increase in palatability. Inoculations with single strains of bacteria, isolated from the floc, showed that this increased palatability occurred with only certain components of the floc fauna, suggesting that some degree of grazing specificity was occurring in the behaviour of *Tigriopus*.

Ingestion of floc was found to be beneficial for the continued survival of C1 over a 30 day period. The presence of floc was also found to improve survival of adult

copepods and to promote and sustain egg production in inseminated females. The presence of floc did not however, result in improved survival of the earlier naupliar stages of *Tigriopus*.

Analysis of the δ13C values of organisms fed various diets suggest that floc carbon is primarily assimilated by copepod grazers when floc is the only available food source. In the presence of a more easily assimilable food source such as *Isochrysis* little or no floc carbon is assimilated. As floc is ingested at very low rates in the absence of colonising microbes, benefit from the ingestion of floc is postulated to result from the conversion of floc carbon into a more available form by the colonising microbes. Few differences exist between the IR-spectra of floc food and the faecal material derived from copepods fed a floc diet, suggesting that little change is undergone by the bulk of the organic matrix during ingestion and passage through the copepod gut.

Feeding studies with a variety of species collected from local coastal waters suggest that a wide variety of organisms may ingest floc when that material is available to them. As flocculant material is primarily produced in the winter, a time when estuarine primary productivity is low, floc is postulated to play a potentially important role in sustaining organisms grazing upon it through periods of low food availability.

REFERENCES CITED

Aiken, G.R. et al. 1985. Humic Substances in Soil, Sediment and Water. Wiley-Interscience. New York.

Ambler, J.W. 1986. Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est. Coast. Shelf Sci. 23:183-196.

Baylor, E.R. and W.H. Sutcliffe. 1963. Dissolved organic matter in seawater as a source of particulate food. Limnol. Oceanogr. 8:369-372.

Bergstrom, I., A. Heinänen and K. Salonen. 1986 Comparison of acridine orange, acriflavin and bisbenzimide stains for enumeration of bacteria in clear and humic acids. App. Environ. Micro. 51:664-667.

Bishop, J.K. et al. 1986. Particulate matter production and consumption in deep mixed layers. Observations in a warm core ring. Deep Sea Res. 33:1813-1841.

Bishop, J.K., J.C. Stepien and P.H. Wiebe. 1987. Particulate matter distributions, chemistry and flux in the Panama Basin: Response to environmental forcing. Prog. Oceanog 17:1-59.

Boggs, S., D.G. Livermore and M.G. Seitz. 1985. Humic macromolecules in natural waters. JMS-Rev. Macromol. Chem. Phys., C25:559-657.

Bohn, H.L., B.L. McNeal and G.A. O'Connor. 1985. Soil Chemistry. Wiley-Interscience. New York. 341pp.

Bordovsky, O.K. 1965a. Accumulation of organic matter in bottom sediments and its early diagenesis. Mar. Geol. 3:83-114.

Bordovsky, O.K. 1965b Accumulation of organic matter in bottom sediments. Mar. Geol. 3:33-82.

Bowen, S.H. 1984. Evidence of a detritus food chain based on consumption of organic precipitates. Bull. Mar. Sci. 36:440-448.

Breger, I.A. 1972. What you don't know can hurt you: Organic colloids and natural waters *in* (D.W. Hood ed.) Organic Matter in Natural Waters. Institute of Marine Science Occasional Publication No. 1.

Burton, R.S.W. 1985. Mating system of the intertidal copepod, *Tigriopus californicus*. Mar. Biol. 86:247-252.

Burton, R.S., M.W. Feldman and J.W. Curtsinger. 1979. Population genetics of *Tigriopus californicus* (Copepoda Harpacticoida): I. Population structure along the central California coast. Mar. Ecol. Prog. Ser. 1:29-39.

Cammen, L.M. 1980. The significance of microbial carbon in the nutrition of the deposit feeding polychaete *Nereis succinea*. Mar. Biol. 61:9-20

Checkley, D.M. 1980. Food limitation of egg production by a marine, planktonic copepod in the sea off Southern California. Limnol. Oceanogr. 25:991-998.

Craig, H. 1953. The geochemistry of the stable carbon isotopes. Geochim. Cosmochim. Acta 42:495-506.

Crerar, D.A. et al. 1981. Hydrogeochemistry of the New Jersey coastal plain II. Chem. Geol. 33:23-44.

Cross, F.A and W.G. Sunda. 1977. Relationship between bioavailability of trace metals and geochemical processes in estuaries *in* (M. L. Wiley, ed.) Estuarine Interactions. International Estuarine Research Conference, 4th, Mount Pocono, Pa., Academic Press, New York.

de Haan, H. 1972. The biological transformation of soluble humic substances in Tjeukemeer, the Netherlands: a preliminary report *in* Proc. Int. Meet. Humic Substances, Nieuwersluis. Pudoc, Wageningen.

de Haan, H. 1983. Use of ultraviolet spectroscopy, gel filtration, pyrolysis/mass spectrometry and numbers of benzoate-metabolising bacteria in the study of humification and degradation of aquatic organic matter *in* (R.F. Christman and E.T. Gjessing, eds.) Aquatic and Terrestrial Humic Materials. Ann Arbor Science.

DeLong, E.F. et al. 1988. Phylogenetic characterisation of organisms and populations using ribosomal RNA sequences. Abstact from ASLO-AGU Ocean Sciences Meeting 1988, New Orleans.

Dethier, M.N. 1980. Tidepools as refuges: Predation and the limits of the harpacticoid copepod *Tigriopus calfornicus* (Baker). J. Exp. Mar. Biol. Ecol. 42:99-111.

Droop, M.R. 1967. A procedure for routine purification of algal cultures with antibiotics. Br. Phycol. Bull. 3:295-297.

Duinker, J.C. 1980. Suspended matter in estuaries *in* (E. Olausson and I. Cato, eds.) Chemistry and Biogeochemistry of Estuaries. Wiley-Interscience. New York.

Edwards, R.T. 1987. Sestonic bacteria as a food source for filtering invertebrates in two Southeastern blackwater rivers. Limnol. Oceanog. 32:221-234.

Ezwald. J.K., J.B. Upchurch and C.R. O'Melia. 1974. Coagulation in estuaries. Env. Sci. Tech. 8:58-63.

Feldman, H.N. 1986. A detailed life history of the harpacticoid copepod *Tigriopus californicus*. B.Sc. Thesis in Zoology and Oceanography. University of British Columbia. Vancouver. Canada.

Fenchal, T.M. and B.B. Jφrgensen. 1977. Detritus food chains of aquatic ecosystems: The role of bacteria *in* (M. Alexander, Ed.) Advances in Microbial Ecology. Volume 1. Plenum Press, New York.

Findlay, S. and K. Tenore. 1982. Nitrogen source for a detritivore: Detritus substrate versus associated microbes. Science 218:371-373.

Fox, L.E. 1983a. The removal of dissolved humic acid during estuarine mixing. Est. Coast. Shelf Sci. 16:431-440.

Fox L.E. 1983b. Geochemistry of humic acid during estuarine mixing *in* (R.F. Christman and E.T. Gjessing eds.) Aquatic and Terrestrial Humic Materials. Ann Arbor Science.

Fulton, J. 1968. A laboratory manual for the identification of British Columbia marine zooplankton. Fish. Res. Bd. Can. Tech. Rpt. #55. 141pp.

Fuhrman, J.A. et al. 1988. Extraction from natural planktonic microorganisms of DNA suitable for molecular biological studies. Appl. Environm. Microbiol. 4:1426-1429.

Gardner, G.A. and I. Szabo. 1982. British Columbia pelagic marine copepoda: An identification manual and annotated bibliography . Can. Spec. Publ. Fish. Aquat. Sci. 62.

Garrels, R.M. and F.T. MacKenzie. 1971. Evolution of Sedimentary Rocks. W.W. Norton Co.

Gaudy, R. 1974. Feeding of four species of pelagic copepods under experimental conditions. Mar. Biol. 25:125-141.

Geller, A. 1983. Degradabilty of dissolved organic lake water compounds in cultures of natural bacterial communities. Arch. Hydrobiol. 99:60-79.

Goldman, J.C. 1984. Conceptual role for microaggregates in pelagic waters. Bull. Mar. Sci. 35:462-476

Gjessing, E.T. 1976. Physical and Chemical Characteristics of Aquatic Humus. Ann Arbor Science.

Gregor, J.E. and H.K.J. Powell. 1987. Effects of extraction Procedures on fulvic acid properties. Sci. Total Environ. 62:3-12.

Harris, R.P. 1973. Feeding, growth, reproduction and nitrogen utilisation by the harpacticoid copepod, *Tigriopus brevicornis*. Mar. Biol. Ass. U.K. 35:785-800.

Harrison, P.J. et al. 1983. Review of the biological oceanography of the Sstrait of Georgia: Pelagic environment. C. J. Fish. Aquat. Sci. 40:1064-1094.

Harvey, H.W. et al. 1935. Plankton production and its control. J. Mar Biol. Ass. 22:97-110.

Hedges, J.I. et al. 1986. Compositions and fluxes of particulate organic material in the Amazon river. Limnol. Oceanogr. 31:717-738.

Heinle, D.R., R.P. Harris, J.F. Ustach and D.A. Flemer. 1977. Detritus as food for estuarine copepods. Mar. Biol. 40:341-353.

Herndl, G.J. and P. Peduzzi. 1988. The ecology of amorphous aggregations (marine snow) in the Northern Adriatic Sea: General considerations. Mar. Ecol. 9:79-90.

Hobbie, J.E., R.J. Daley and S. Jasper. 1977. Use of Nucleopore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228.

Huljev, D.J. 1986. Trace metals in humic acids and their hydrolysis products. Environm. Res. 39:258-264.

Jannasch, H.W. and P.H. Pritchard. 1972. The role of inert particulate matter in the activity of aquatic micro-organisms. Mem. Ist. Ital. Idrobiol. 29(Suppl):289-308.

Johnsen, S. et al. 1987. Seasonal variation in composition and properties of aquatic humic substances. Sci. Total Environ. 62:13-25.

Kahan, D. Y. Berman and T. Bar-El. 1988. Maternal inhibition of hatching at high population densities in *Tigriopus japonicus* (Copepoda, Crustacea). Biol. Bull. 174:139-144.

Kalinowski, E. and R. Blondeau. 1988. Characterisation of sedimentary humic acids fractionated by hydrophobic interaction chromatography. Marine Chemistry 24:29-37.

Kirchman, D. 1983. Production of bacteria attached to particles suspended in a fresh water pond. Limnol. Oceanogr. 28:858-872.

Lance, J. 1964. Feeding of zooplankton in diluted sea-water. Nature 201:100-101.

Lewis, A.G. and J.J. McNee. 1986. Humic substances as a potential food source in estuaries. Unpubl.

Lidestrom, M.E. 1989. Molecular approaches to problems in biogeochemical cycling. A. van Leewen. 55:7-14.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms- A review. Sci. Total Environ. 28:1-22.

Malcolm, R.L. 1985. Geochemistry of stream fulvic and humic Substances *in* (G.R. Aiken et al., Eds.) Humic Substances in Soil, Sediment and Water. Wiley-Interscience. New York.

Mantoura, R.F.C. 1981. Organo-metallic interactions in natural waters *in* (E.K. Duursma and R. Dawson, Eds.) Marine Organic Chemistry. Elsevier Oceanography Series 31. Amsterdam.

Mantoura, R.F.C. and E.M.S. Woodward. 1983. Conservative behaviour of riverine dissolved organic carbon in the Severn estuary: Chemical and geochemical implications. Geochim. Cosmochim. Acta. 47:1293-1309.

Marshall, S.M. and A.P. Orr. 1972. The Biology of Marine Copepods. Springer Verlag New York.

Martin, M.M. and J.J. Kukor. 1984. Role of mycophagy and bacteriophagy in invertebrate nutrition *in* (M.J. Klug and C.A. Reddy, eds.) Current Perspectives in Microbial Ecology. Proceedings of the Third International Symposium on Microbial Ecology. Michegan State University 7-12 August 1983.

Mathur, S.P. and E.A. Paul. 1967. Microbial utilisation of humic acids. C. J. Microbiology. 13:573-580.

Mayer, L.M. 1982. Aggregation of colloidal iron during estuarine mixing: Kinetics, mechanism, and seasonality. Geochim. Cosmochim. Acta 46:2527-2535.

Mayer, L.M. 1985. Geochemistry of humic substances in estuaries *in* (G.R. Aiken et al. eds.) Humic Substances in Soil, Sediment and Water. Wiley-Interscience. New York.

Mayzaud, P. 1986. Digestive enzymes and their relation to nutrition. *in* (E.D.S. Corner and S.C.M. O'Hara eds.) The Biological Chemistry of Marine Copepods. Clarendon Press. Oxford. pp.165-225.

Means, J.C. and R.D. Wijayarante. 1984. Chemical characterisation of estuarine colloidal organic matter: implications for absorbtive processes. Bull. Mar. Sci. 35:341-356.

Meybeck, M. 1982. Carbon, nitrogen and phosphorus transport by world rivers. Am. J. Sci. 282:401-450.

Meyer, J.L., R.T. Edwards and R. Risley. 1987. Bacterial growth on dissolved organic carbon from a blackwater river. Microb. Ecol. 13:13-29.

Morel, F.M.M. et al. 1979. Aquil: A chemically defined phytoplankton culture medium for trace metal studies. J. Phycol. 15:135-141.

Mullholland, P.J. 1981. Formation of particulate organic carbon in water from a Southeastern swamp-stream. Limnol. Oceanogr. 26:790-795.

Mullholland, P.J. and E.J. Kuenzler. 1979. Organic carbon export from upland and forested wetland watersheds. Limnol. Oceanogr. 24:960-966.

Municipality of Delta; Dale Mc Taggert, Assistant Municipal Engineer. 4450 Clarence Taylor Crescent. Delta. British Columbia. Canada.

Naiman, R.J. and J.R. Sedell. 1978. Characterisation of particulate organic matter transported by some Cascade Mountain streams. J. Fish. Res. Board Can. 36:17-31.

O'Brien, P. 1987. The effect of copper on the life history stages of the harpacticoid copepod *Tigriopus californicus*. M.Sc Thesis. University of British Columbia.

Ogura, N. 1977. High molecular weight organic matter in seawater. Mar. Chem. 5:535-549.

O'Melia, C.R. 1972. Coagulation and flocculation *in* (W.J. Weber ed.) Physicochemical Processes For Water Quality Control. Wiley-Interscience.

Paerl, H.W. 1974. Bacterial uptake of dissolved organic matter in relation to detrital aggregation in marine and freshwater systems. Limnol. Oceanog. 19:966-972

Paffenhöffer, G.A. and J.D.H. Strickland. 1970. A note on the feeding of *Calanus helgolandicus* on detritus. Mar. Biol. 5:97-99.

Paffenhöffer, G.A. and K.B. Van Sant. 1985. The feeding response of a marine planktonic copepod to quantity and quality of particles. Mar. Ecol. Prog. Ser. 27:55-65.

Parker, P.L. and J.A. Calder. 1970. Stable carbon isotope ratio variations in biological systems *in* (D.W. Hood ed.) Organic Matter in Natural Waters. Institute of Marine Science Occasional Publication No. 1.

Parslow, J.S., P.J. Harrison and P.A. Thompson. 1984. Saturated uptake kinetics: transient response of the marine diatom *Thalassiosira pseudonana* to ammonium, nitrate, silicate or phosphate starvation. Mar. Biol. 83:51-59.

Peterson, B.J., R.W. Howarth and R.H. Garritt. 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food-webs. Science 227:1361-1363.

Petipa, T.S., A.V. Monakov, A.P. Pavlytin and Y.I. Sorokin. 1975. The significance of detritus and humus in the nutrition and energy balance of the copepod *Undulina darwini* Scott. Translated from Biologicheskaya Producktivnost' Yuzhnykh morei. pp.153-160, 1974 by the Translation Bureau (NDE) Multilingual Services Division. Department of the Environment, Fisheries and Marine Service. Marine Ecology Laboratory. Dartmouth. N.S.

Porter, K.G. 1984. Natural bacteria as food resources for zooplankton *in* (M.J. Klug and C.A. Reddy, eds.) Current Perspectives in Microbial Ecology. Proceedings of the Third International Symposium on Microbiology. Michegan State University. 7-12 August 1983. pp.340-345.

Porter, K.G. 1988. Phagotrophic phytoflagellates in microbial food-webs. Hydrobiologia 159:89-97.

Postma, H. 1968. Dissolved organic matter in the oceans. In. Advances in Organic Geochemistry. Pergamon Press. New York.

Postma, H., M.W. Manuels and J.W. Rommets. 1978. Breakdown and production of fluorescent substances in Dutch waters. Netherlands Journal of Sea Research 10:499-516.

Power, J.F. and C.H. Langford. 1988. Optical absorbance of dissolved organic matter in natural water studies using the thermal lens effect. Analyt. Chem. 60:842-846.

Prézlin, B.B. and A.L. Alldridge. 1983. Primary production of marine snow during and after an upwelling event. Limnol. Oceanogr. 28:1156-1167.

Ramunni, A. et al. 1987. Genetic relationships among the main classes of soil humic compounds. Sci. Total Environm. 62:419-422.

Ranade, M.R. 1957. Observations on the resistance of *Tigriopus fulvus* (Fischer) to changes in temperature and salinity. J. Mar. Biol. Ass. U.K. 36:115-119.

Rashid, M.A. 1985. Geochemistry of Marine Humic Compounds. Springer-Verlag. New York.

Rieper, M. 1978. Bacteria as food for marine harpacticoid copepods. Mar. Biol. 45:337-345.

Rieper, M. 1982. Feeding preferences of marine harpacticoid copepods for various species of bacteria. Mar. Ecol. Prog. Ser. 7:303-307.

Rieper, M. 1984. Relationship between bacteria and marine copepods. *in* (University Provence Eds.) Bacteriologie Marine. CNRS. Paris. pp.169-172.

Rodelli, M.R. et al. 1984. Stable isotope ratio as a tracer of mangrove carbon in Malaysian ecosystems. Oecologia 61:326-333.

Roman, M.R. 1984. Utilisation of detritus by the copepod, *Acartia tonsa.* Limnol. Oceanogr. 29:949-959.

Roman, M.R. and K.R. Tenore. 1984. Detritus dynamics in aquatic ecosystems: An Overview. Bull. Mar. Sci. 35:257-260.

Romankevich, E.A. 1984. Geochemistry of Organic Matter in the Ocean. Springer-Verlag. New York.

Ryhanen, R. 1968. Die bedeuteung der humussubsstanzen in soffhaushalt der gewasser Finnlands. Mitt. Int. Verein. Limnol. 14:168-178.

Schell, D.M. 1983. Carbon-13 and carbon-14 abundances in Alaskan aquatic organisms: Delayed production from peat in Arctic food-webs. Science 219:1068-1071.

Schoeninger, M.J. and M.J. DeNiro. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochim. Cosmochim. Acta 48:625-639.

Schwinghamer, P., F.C. Tan and D.C. Gordon. 1983. Stable carbon isotope studies on the Peck's Cove mudflat ecosystem in the Cumberland Basin, Bay of Fundy. Can. J. Fish. Aquat. Sci. 40(Suppl. 1):262-272.

Seki, H. 1972. The role of microorganisms in the marine food-chain with reference to organic aggregate. Mem. Ist. Ital. Idrobiol. 29 (Suppl.):245-259.

Sharp, J.H., C.H. Culberson and T.M. Church. 1982. The chemistry of the Delaware estuary. General considerations. Limnol. Oceanogr. 17:494-498.

Sholkovitz, E.R. 1976. Flocculation of dissolved organic and inorganic matter during the mixing of river water and seawater. Geochim. Cosmochim. Acta. 40:831-845.

Sholkovitz, E.R. 1978. The Flocculation of dissolved Fe, Mn, Al, Cu, Ni, Ca and Cd during estuarine mixing. Earth Planet. Sci. Lett. 41:77-86.

Sholkovitz, E.R., E.A. Boyle and N.B. Price. 1978. The removal of dissolved humic acids during estuarine mixing. Earth and Planet. Sci. Lett. 40:130-136.

Sholkovitz, E.R and D. Copland. 1981. The Coagulation and Absorbtion Properties of Fe, Mn, Cu, Ni, Cd, Co and Humic Acids in a River Water. Geochim. Cosmochim. Acta 45:181-189.

Sieburth J. McN and A. Jensen. 1970. Production and transformation of extracellular organic matter from littoral marine algae: A resume *in* (D.W. Hood, ed.) Organic Matter in Natural Waters. Institute of Marine Science Occasional Publication No. 1.

Silver, M.A., L. Shanks and J.D. Trent. 1986. Marine snow: microplankton habitat and source of small scale patchiness in pelagic populations. Science 201:371-373.

Smith, J.D. and P.J. Milne. 1981. The behaviour of iron in estuaries and its interaction with other components of the water *in* (J.-M. Martin, J.D. Burton and D. Eisma Eds.) River Inputs to Ocean Systems. Proceedings of a SCOR etc. Review and Workshop.

Stabel, H.H., K. Moaledj and J. Overbeck. 1979. On the degradation of dissolved organic molecules from Plussee by oligocarbophilic bacteria. Arch. Hydrobiol. Beih. 12:95-104.

Steinberg, C. and U. Muenster. 1985. Geochemistry of humic substances in lakewater *in* (G.R. Aiken et al. eds.) Humic Substances in Soil, Sediment and Water. Wiley-Interscience. New York.

Sternhill, S. and J.R. Kalman. 1986. Organic structures from spectra. Wiley and Sons New York.

Strome, D.J. and M.C. Miller. 1978. Photolytic changes in humic substances. Verh. Internat. Verein. Limnol. 20:1248-1258.

Summers, R.S., P.K. Cornel and P.V Roberts. 1987. Molecular size distribution and spectroscopic characterisation of humic substances. Science of the Total Environment 62:27-37.

Swift, R.S., G. Delisle and R.L. Leonard. 1987. Biodegradation of humic acids from New Zealand soils. Sci. Total Environ. 62:423-430.

Tenore, K.R., L. Cammen, S.E.G. Findlay and N. Phillips. 1982. Perspectives on research on detritus: Do factors controlling the availability of detritus to macroconsumers depend on its source? J. Mar. Res. 40:473-490.

Tenore, K.R. et al. 1979. The effect of organic nitrogen supplementation on the utilisation of different sources of detritus. Limnol. Oceanogr. 24:350-355.

Tranvik, L.J and J.McN. Sieburth. 1989. Effects of flocculated humic matter on free and attached pelagic microorganisms. Limnol. Oceanogr. 34:688-699.

Van Bennekom, A.J. and W. Salomons. 1981. Pathways of nutrients and organic matter from land to ocean through rivers *in* (J.-M. Martin, J.D. Burton and D. Eisma, Eds.) River Inputs to Ocean Systems. Proceedings of a SCOR etc. Review and Workshop.

Valdés, M. and L.J. Albright. 1981. Survival and heterotrophic activities of Fraser River and Strait of Georgia bacterioplankton within the Fraser River plume. Mar. Biol. 64:231-243.

Velji, M.I. and L.J. Albright. 1986. Microscopic enumeration of attached bacteria of seawater, marine sediment, fecal matter and kelp blade samples following pyrophosphate and ultrasound treatments. Can. J. Microbiol. 32:121-126.

Visser, S.A. 1985a. Effect of humic acids on numbers and activities of micro-organisms within physiological groups. Org. Geochem. 8:81-85.

Visser, S.A. 1985b. Viscometric studies on molecular weight fractions of fulvic and humic acids of aquatic, terrestrial and marine origin. Plant Soil. 87:209-221.

Weiner, R.J., D. Hussong and R.R. Colwell. 1980 An estuarine agar medium for enumeration of aerobic heterotrophic bacteria associated with water, sediment and shellfish. Can. J. Microbiol. 26:1366-1369.

Wetzel, R.G. 1975. Limnology. W.B. Saunders Co. Toronto.

Williams, P.leB. 1981. Incorporation of microheterotrophic processes into the classical paradigm of the planktonic food-web. Keiler Meers. Sonderh. 5:1-28.

Woodwell, G.M. et al. 1978. The biota and the world carbon Budget. Science 199:141-146.

Zar, J.H. 1984. Biostatistical Analysis. Prentice Hall, New Jersey.

APPENDIX 1.

SLOUGH FLOW DATA, SAMPLE COLLECTION DATES AND CHARACTERISATION OF WATER SAMPLES.

Table A1.1. Six year flow average and associated data for Crescent Slough, Delta. All data is in millions of Litres.

Month	Mean ± 1 s.d.	Maximum	Minimum
January	21.88 ± 19.42	52.27	1.15
February	15.54 ± 2.87	19.81	11.00
March	10.73 ± 6.63	22.06	0.33
April	8.37 ± 8.23	23.22	0.72
May	1.69 ± 1.72	4.75	0.14
June	1.92 ± 5.34	5.72	0
July	0.79 ± 1.18	3.05	0
August	0.47 ± 0.66	1.67	0
September	0.71 ± 0.65	1.52	0
October	2.73 ± 4.16	11.03	0.18
November	17.97 ± 17.37	40.96	0.70
December	17.64 ± 12.89	33.53	0.61

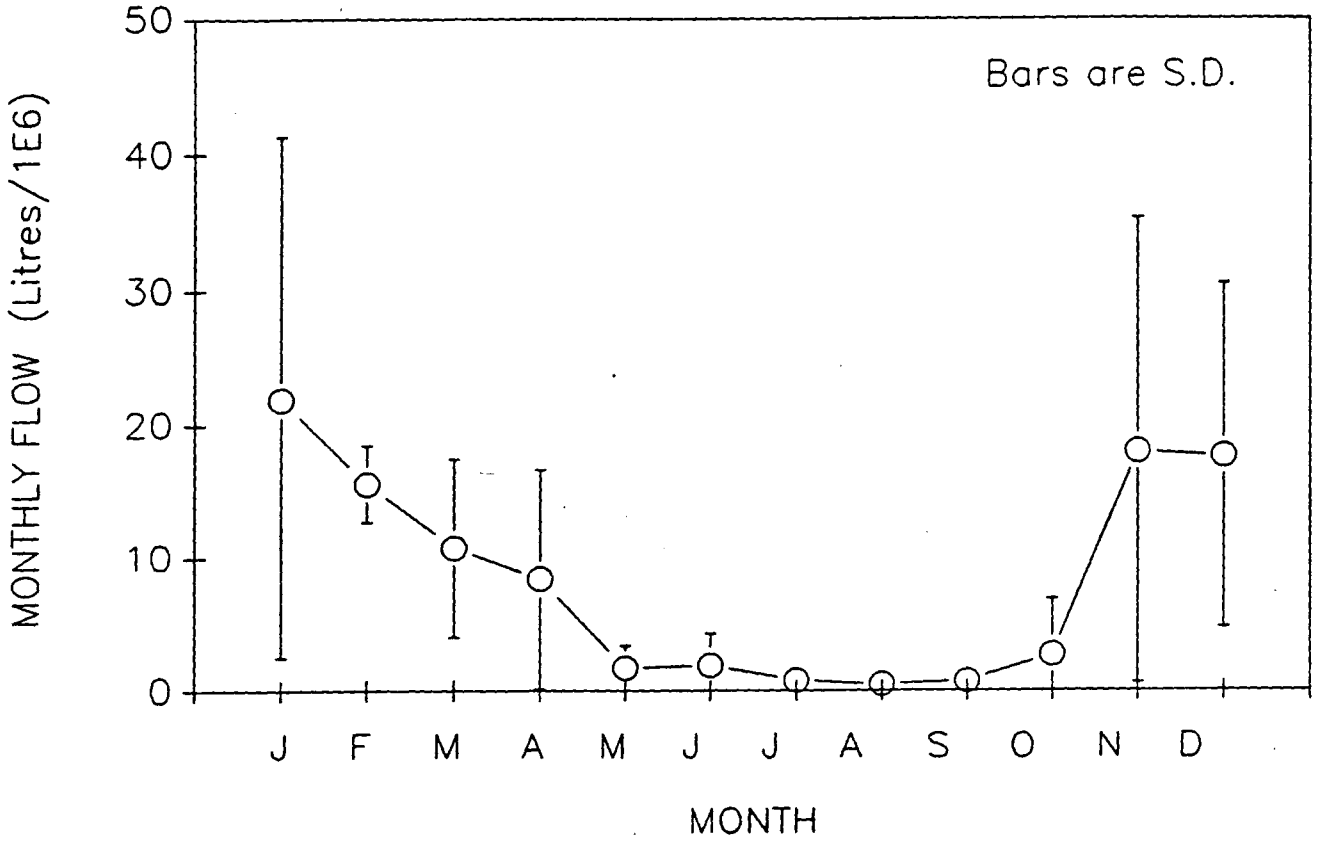


Figure A1.1. Plot of six year monthly flow averages of water entering the Fraser River Estuary from Crescent Slough, Delta.

Table A1.2. Collection dates and preliminary observations upon sample characteristics.

Collection date	Colour	Degree of Floc'n
May 28, 1987*. ("summer water")	Dark brown	strong
Oct 22, 1987*.	almost clear	none
Dec. 4 1987*.	dark brown	weak
Feb. 4, 1988*. ("winter water").	dark brown	very strong
April 5, 1988*. ("spring water").	dark brown	strong
May 17, 1988*.	dark brown	strong
June 21, 1988**.	dark brown	strong
January 11, 1989*.	dark brown	weak
January 23, 1989*.	dark brown	strong
May 8, 1989***.	very dark	very strong

* Designates sample from Crescent Slough.

** Designates sample from Monastry Ditch.

*** Designates sample from Route 72 Ditch.

APPENDIX 2.

CHARACTERISATION AND IDENTIFICATION OF BACTERIAL STRAINS ISOLATED FROM FLOCCULANT MATERIAL.

Table A2.1. Characteristics of bacterial strains isolated from floc used in single strain reinoculation experiments.

Character	Strain1 (pink)	Strain2.1 (hard yellow)	Strain2.2 (contaminant)	Strain3 (amber)	Strain4 (smooth yellow)	Strain5 (white)	Strain6 (cream)
cell shape	rod	rod	coccus	rod	rod	rod	rod
stain	Gram +ve	Gram -ve	Gram -ve	Gram +ve	Mixed	Gram -ve	Gram -ve
size(μm)	1x2-3	0.8x4	2	0.8-1x5	0.8-1x1.2-4	0.8-1x3-5	1x4
motility	Y/N	Y	N	Y	Y	Y	N
colony colour	pink	yellow	white	orange	yellow	white	cream
colony charact.	shiny opaque	shiny translucent undulate	shiny opaque entire convex	shiny opaque convex	shiny translucent entire raised	shiny translucent entire convex	shiny translucent entire raised
Nitrate	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	weak	-ve	-ve	-ve	-ve	+ve

Table A2.2. Tentative identities of bacterial strains

Strain	ID
1 (pink)	*Corynobacterium* sp.
2.1 (moist yellow)	*Flavobacterium* sp.
2.2 (white contaminant)	Cyanobacterium sp.
3 (amber)	*Corynobacterium* sp.
4 (hard yellow)	Unknown
5 (white)	*Pseudomonas* sp.
6 (cream)	*Pseudomonas* sp.