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Effect of salmon cage aquaculture on the pelagic environment of temperate coastal waters: seasonal changes in nutrients and microbial community

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ABSTRACT: The effects of salmon farm inputs on pelagic nutrient concentrations and planktonic microbial abundance and biomass were investigated in Loch Fyne, a temperate fjordic environment off the west coast of Scotland. The concentration of photosynthetic pigments and inorganic and organic nutrients, and the abundance and biomass of the autotrophic and heterotrophic microorganisms, were determined over a complete annual cycle from 3 depths (5, 15 and 25–30 m) at 4 stations located at differing proximities to the fish farm. Ammonium and dissolved organic nitrogen concentrations and heterotrophic microbial abundance and biomass were significantly higher at the stations nearest to the fish farm, suggesting that these and other nutrients derived from the fish farm may be directly or indirectly enhancing heterotrophic microbial activity. This in turn suggests that the heterotrophic microbial food web was responsible, at least in part, for processing matter and energy released into the pelagic environment from the salmon farm. By contrast, pigment concentrations, including chlorophyll *a*, tended to be similar at all stations, supporting the conclusions of previous studies that failed to establish a clear relationship between fish farm inputs and phytoplankton biomass. As such, the response of the heterotrophic microbial community is probably a more appro-priate indicator than chlorophyll concentration of local ecological effects of fish farms in temperate coastal waters.

KEY WORDS: Fish farm · Plankton community · Bacteria · Nutrients · Chlorophyll

INTRODUCTION

Intensive mariculture is increasingly promoted as an alternative source of protein and polyunsaturated lipids to meet societal demand, as marine fisheries continue to be exploited at close to or beyond sustainable rates. It is predicted that aquaculture production will become as important as production from wild fisheries within the early decades of this century (Brugère & Ridler 2004); however, mariculture is not without its effects on the environment (reviewed in Black 2001). Marine finfish cage culture is an open system where inputs of juvenile fish and their feed are balanced by outputs of harvested and escaped fish, excretory products that enter the local pool of dissolved nutrients, and particulate organic material in the form of waste feed and faecal material. Inputs which are not recovered during harvest must be assimilated by the environment. Matching the capacity of the environment to absorb these waste materials with the scale of mariculture activity is important in order to avoid both local and wider-scale disturbances to ecosystem function (e.g. bottom water anoxia and eutrophication).

Several studies have addressed the impact of mariculture on sediment chemistry and the ecology of benthic communities (e.g. Gowen & Bradbury 1987, Kaspar et al. 1988, Holmer & Kristensen 1992, Findlay et al. 1995, Beveridge 1996, Karakassis et al. 1998, Brooks & Mahnken 2003, Brooks et al. 2003, Pereira et al. 2004, Holmer et al. 2005). As a result, it is now well established that the addition from fish farms of reduced organic material to the seabed causes increased

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sediment oxygen demand, leading to increases in sulphate reduction, increased inorganic nutrient fluxes and often profound, if spatially limited, effects on the benthic ecosystem. By contrast, relatively few studies have examined the effects of fish farm inputs on the pelagic environment and planktonic ecosystem structure and function (see review in Beveridge 1996, Pitta et al. 1999, Alongi et al. 2003).

The most likely means by which inputs from fish farm cages may influence planktonic ecosystems is via alteration of inorganic and organic nutrient pools; although reduced light attenuation and the presence of pollutants (e.g. metals, antibiotics) may also play a role. Dissolved inorganic nutrients are released into the pelagic environment from fish farm cages either directly, as fish excretory products (ammonium and urea), or indirectly as a result of remineralisation of particulate organic waste. Such inorganic nutrient inputs have the potential to enhance the growth of phytoplankton and the formation of harmful phytoplankton blooms has been attributed to aquaculture inputs (e.g. Sorokin et al. 1996). They may also lead to changes in phytoplankton community composition via altered nutrient ratios with, for example, an increased N:Si ratio favouring the growth of flagellates rather than diatoms (Officer & Ryther 1980). Dissolved organic nutrients, by contrast, are released from fish farms indirectly via dissolution of particulate organic waste and are likely, due to the high nutritional content of waste food and faeces, to represent a highly labile source of nutrients for heterotrophic bacteria.

To our knowledge, few studies to date have examined the effect of fish farm inputs on the phytoplankton community composition or production (Wallin & Hakanson 1991, Pitta et al. 1999, Alongi et al. 2003); however, several studies have indirectly examined the effect of fish farm inputs on the autotrophic microbial community by using chlorophyll concentration as a proxy for phytoplankton biomass (e.g. Beveridge et al. 1994, Wu et al. 1994, Pitta et al. 1999). The majority of these studies concluded that chlorophyll concentrations are not enhanced by nutrient inputs from fish farm cages (e.g. Beveridge et al. 1994, Wu et al. 1994, Beveridge 1996, Pitta et al. 1999), although a few concluded the contrary (e.g. Wallin & Hakanson 1991, Nordvarg & Johansson 2002). The lack of a response of chlorophyll concentration to fish farm inputs may not necessarily indicate a lack of response by the phytoplankton, as increases in cell growth may have been balanced by mortality due, for example, to predation by protozoan or metazoan zooplankton. Rapid water exchange in coastal waters may also disperse phytoplankton, and in this respect it is significant that macrophyte growth has been shown to be stimulated by fish farm effluents (Neori et al. 2004). Hydrographic conditions are clearly important in dictating the degree of impact on fish farm inputs on pelagic ecosystems.

The response of the heterotrophic planktonic microbial community to fish farm inputs has received even less attention than that of the autotrophic community. Of the few, mostly seasonal, published studies, some have recorded enhanced bacterioplankton abundance near fish farms (La Rosa et al. 2002, Sakami et al. 2003, Pitta et al. 2006) while others have not (Alongi et al. 2003, Maldonado et al. 2005). As with phytoplankton, a lack of increase in bacterioplankton abundance and biomass may not necessarily preclude a response if bacterioplankton biomass is dispersed or cell growth is balanced by mortality due to protozoan grazing or viral-induced lysis (Ducklow 1992). The effect that nutrient enrichment might have upon the bacterioplankton in the vicinity of fish farms, and the consequences that increased bacterioplankton production might have for the flow of energy and materials through the pelagic ecosystem, have therefore to be fully investigated.

The present study examined the effect of inputs from salmonid mariculture activities on the pelagic environment of a temperate coastal water site over a complete annual cycle. In particular, the study focused on nutrient concentrations, and autotrophic and heterotrophic microbial abundance and biomass, in order to identify the chemical and microbiological components of the pelagic environment exhibiting a significant response to fish farm inputs throughout the year. The study was undertaken in Loch Fyne, a 64 km long deep-silled sea loch opening into the Firth of Clyde on the west coast of Scotland. Many Scottish sea lochs are fjordic estuaries, located in relatively pristine environments, within which are basins that may be characterised by low flushing rates (Edwards & Sharples 1986). They therefore provide a clean, sheltered and accessible environment for locating mariculture cages but, due to restricted exchange, may be sensitive to inputs resulting from the mariculture activities (Levings et al. 1995); Loch Fyne is one such sea loch. The study forms part of an ongoing programme of research into the effects of inputs from salmonic mariculture on the pelagic environment of Loch Fyne. Effects on bacterioplankton production and community composition during the summer months will be published elsewhere (R. J. G. Leakey et al. unpubl., M. C. Hart et al. unpubl.).

MATERIALS AND METHODS

Study site and sampling regime. Sampling was undertaken at approximately monthly intervals from August 1998 to August 1999 in the vicinity of salmonid mariculture cages located near Quarry Point (Ord-

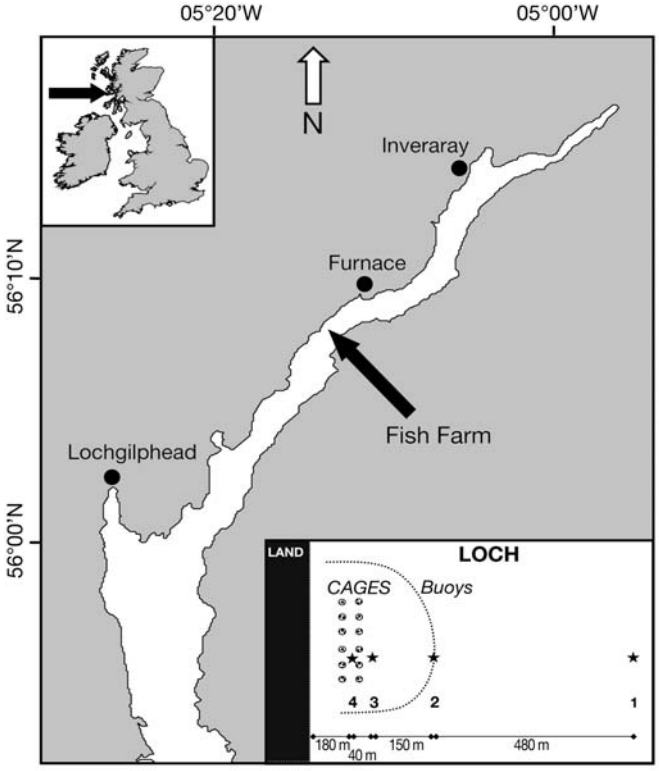

Fig. 1. Location of the fish farm near Quarry Point in the upper basin of Loch Fyne. Insert shows locations of sampling stations (★) relative to the fish farm cages

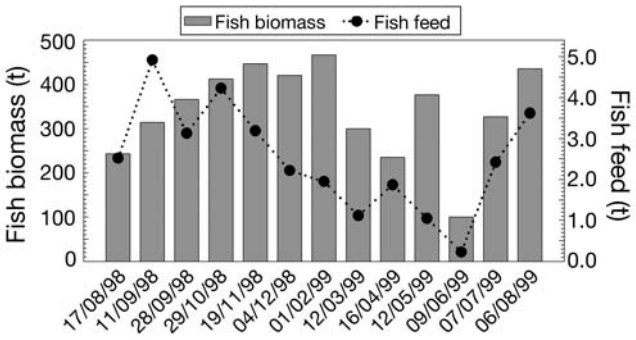

Fig. 2. Quantity of fish biomass in cages, and weight of fish feed added to cages on sampling days at the fish farm. Dates are dd/mm/yy

nance Survey grid reference NR997982) in the upper basin of Loch Fyne (Fig. 1). Loch Fyne is the longest and deepest of the Scottish sea lochs with a relatively low tidal range (~3 m at spring tides) and a small intertidal area (4% of total high water area). The upper basin, in which this study was carried out, has a maximum depth of 135 m and is separated from the lower reaches of loch by a 42 m sill (Edwards & Sharples 1986). A mean turnover time of 10.5 d (range 3 to 16 d) has been estimated for the water column above sill depth within the upper basin, with turnover here defined as the time needed replace ~60% of the original water mass (Gillibrand 2001). The fish farm was located within 200 m of the western shore of the loch and comprised 12 to 15 cages containing salmon *Salmo salar*. Fish production at the site was difficult to determine owing to several movements of stock between sites but monthly biomass and feed input data are given in Fig. 2.

Water samples and measurements were undertaken at 4 stations located at differing proximities to the fish farm (Fig. 1). Stn 1 (referred to hereinafter as the reference station) was ~650 m to the southeast of the farm in a water column depth of ~60 m (56° 07.69′ N, 05° 12.95′ W). Stns 2, 3 and 4 were situated progressively closer to the farm, with Stn 4 (referred to hereinafter as the fish farm station) located within 5 m of a cage within the mariculture complex and in a water column depth ranging from 22 to 25 m.

At each station, measurements of temperature and salinity were made at 1 to 5 m intervals throughout the water column (Braystoke Series 600 temperature-salinity meter). A single 2 l NIO water bottle sample was then collected from 5, 15 and either 25 (Stns 3 and 4) or 30 m (Stns 1 and 2) depth for the determination of dissolved nutrient concentrations, photosynthetic pigment concentrations, and microbial abundance and biomass. On one occasion during the study (9 June 1999) measurements of current velocity were made at the reference and fish farm stations using Model S4 Current Meters (InterOcean Systems) placed for 24 h at 5 and 15 m depths.

Analytical methods. To determine dissolved inorganic and organic nutrient concentrations, a 250 ml subsample was filtered through an ashed glass microfibre filter (Whatman GF/F). The filtrates were kept frozen at –20°C and analysed subsequently for dissolved ammonium (NH_4), nitrate (NO_3), phosphate (PO_4) and reactive silica (SiO_2) by colorimetric flow injection analysis using a Lachat Quickchem 8000 FI analyser and the methods recommended by the manufacturer. These methods are adaptations from standard seawater analyses given by Grasshoff et al. (1999). Dissolved organic nitrogen (DON) and phosphorus (DOP) were determined by subtraction of inorganic nitrate and phosphate concentrations measured on filtrate which had been oxidised using hydrogen peroxide as oxidant supported by UV irradiation (Strickland & Parsons 1972).

To determine photosynthetic pigment concentration, a 300 ml subsample was filtered through a glass microfibre filter (Whatman GF/F). Following extraction in 90% acetone with sonication, chlorophyll (chl) *a*, *b* and c_{1+2} concentrations were measured by 2-eluant stepwise isocratic HPLC (Mantoura et al. 1997) and carotenoid concentration was measured by spectrophotometry (Lorenzen 1967).

To determine the abundance and biomass of bacterioplankton and nanoplankton, a 250 ml subsample was preserved in 1% glutaraldehyde solution and stored at 4°C in the dark for analysis by epifluorescence microscopy within 2 d (Hobbie et al. 1977, Porter & Feig 1980). For bacterioplankton, a 5 ml aliquot was stained with 5 µg ml^{-1} DAPI, concentrated onto a 0.2 µm pore size black polycarbonate membrane filter, and examined at ×1000 magnification under fluorescence illumination using a Zeiss Axiovert 100S microscope equipped with 09 (blue excitation) and 02 (UV excitation) filter blocks. Bacterioplankton were identified by their blue fluorescence observed under UV light illumination and ≥400 cells in ≥10 fields of view counted. Bacterioplankton population carbon biomass was calculated from cell abundance using a conversion factor of 30.2 fg C $cell^{-1}$ (Fukuda et al. 1998). For nanoplankton, a 20 ml aliquot was stained with DAPI, concentrated onto a 1 µm pore size standard polycarbonate filter, and examined at ×1000 magnification under fluorescence illumination. Nanoplankton were identified by their blue fluorescence observed under UV light illumination, and heterotrophs (HNAN) were distinguished from phototrophs (PNAN) by the absence of chl *a* autofluorescence observed under blue light illumination. At least 30 cells in ≥30 fields of view were counted. To determine nanoplankton cell volume, the linear dimensions of 30 cells from each filter were measured by calibrated ocular micrometer and converted to volume using a standard geometric configuration. Nanoplankton population volume was calculated by multiplying mean cell volume by abundance. HNAN population volume was converted to carbon biomass using a conversion factor of 0.22 pg C $µm^{-3}$ (Børsheim & Bratbak 1987). PNAN population carbon biomass (pg) was calculated from cell volume ($µm^3$) using the equation:

$$\text{Cell carbon} = 0.109\ (\text{live cell volume})^{0.991}$$

(Montagnes et al. 1994), where live cell volume is 1.41 times preserved volume for cells fixed in glutaraldehyde (Verity et al. 1992).

To determine the abundance of ciliate microplankton, a 250 ml subsample was preserved with Lugol's iodine solution (1% final concentration) and stored at 4°C in the dark for analysis by inverted microcopy (Utermöhl 1958). A 50 ml aliquot was concentrated by settling for 24 h and ciliates identified, where possible to species level, and enumerated at ×200 to ×400 magnification using a Zeiss Axiovert 100S inverted microscope. On occasion, assessments of diatom and dinoflagellate species composition and relative abundance were also undertaken on the same Lugol's iodine-preserved subsample.

Statistical analyses. Significant differences in biological variables and nutrient concentrations between stations were analysed by 3-way ANOVA and, when appropriate, by Tukey's multiple comparison tests (Zar 1999). Data were log_{10}-transformed prior to analysis to reduce heteroscedasticity.

Community structure differences between stations were also analysed by multivariate approaches, using the software PRIMER 5b (Plymouth Routines in Multivariate Ecological Research). Multivariate data analysis was by hierarchical clustering with group-average linking and nonmetric multidimensional scaling (MDS) ordination based on the Bray-Curtis similarity measure calculated on square-root-transformed data. Cluster analysis finds 'natural groupings' of samples such that samples within a group are more similar to each other than samples in different groups; MDS ordination constructs a 'map' or configuration of the samples in which the closest sample points have the highest similarity with the goodness-of-fit of the regression measured by calculating the stress value (stress < 0.05 gives excellent representation with no prospect of misinterpretation). Further verification of groups identified as significantly different from each other was attempted using the nonparametric permutation analysis of similarities (ANOSIM) applied to the similarity matrix underlying the ordination or classification of samples. The PRIMER program SIMPER ('similarity percentages') was implemented to determine the contribution of individual groups to dissimilarity between the communities at each station and depth for each date. The PRIMER program BIOENV was used to determine which combination of the environmental variables best matched the observed community structure. The environmental variables tested were nutrient concentrations, water temperature and depth.

RESULTS

Most of the observed differences among variables recorded during the study could be accounted for by data from the reference and fish farm stations (Stns 1 and 4); therefore only raw data from these 2 stations are presented in figures in order to aid interpretation. By contrast, summary data are presented for statistical analyses undertaken using data from all stations.

Physical environment

The physical properties of the upper basin of Loch Fyne showed a typical pattern of seasonal stratification caused by the interaction of heat and freshwater inputs moderated by the restricted exchange with coastal

waters that is typical of silled temperate fjords. Water temperature at the sampling stations ranged from 6°C in February to 15°C in August 1998 and 1999, within the normal temperature range for this coastal area (Edwards & Sharples 1986). Lowest salinities (minimum 17) were recorded in surface waters in winter and highest values (maximum 33) recorded in deeper waters during summer. Current velocities at the fish farm, recorded over a 24 h period during June, ranged from 2.5 to 5 cm s^{-1} at 5 m depth and 5 to 7.5 cm s^{-1} at 15 m depth. By contrast, velocities at the reference station ranged from 5 to 7.5 cm s^{-1} at both 5 and 15 m depth. The reduction in surface current speed within the mariculture complex was probably caused by frictional losses from cage structures. The prevailing tidal currents at the fish farm and reference stations ran parallel to the shoreline, as would be expected in highly stratified sea loch systems, where currents are topographically constrained by the coast and where vertical water exchange is limited. The locations of the sampling stations were therefore perpendicular to the flow of water, with material released from the fish farm cages tending to be carried away from the reference station.

Chemical and biological variables

Inorganic and organic nutrients

Respective NH_4 and DON concentrations, averaged across all dates and depths throughout the study, were significantly different between stations (Table 1), with concentrations 2.2 and 1.2 times higher at the fish farm station than at the reference station. Highest NH_4 and DON concentrations were observed in August 1998, September and June, with lowest concentrations in winter. When averaged across all dates, NH_4 and DON concentrations were found to be significantly higher (Tukey's test, $p < 0.05$) at 5 m depth at both Stns 4 and 3, with respective NH_4 maxima of 3.55 and 5.39 µM, and DON maxima of 12.94 and 16.04 µM, recorded in August 1998 (Fig. 3). No significant differences in DOP concentration were detected among all 4 stations, although the lowest concentrations were recorded at the reference station, with a mean across all dates and depths of 0.21 µM. DOP concentrations, averaged across all dates, were significantly higher (Tukey's test, $p < 0.05$) at 5 m, with maximum values of 0.34 µM recorded in summer and lowest concentrations observed in winter. There were no significant differences in PO_4, NO_3 and SiO_2 concentrations between stations, although the highest PO_4 concentration was observed at the fish farm station (1.37 µM). By contrast, there were highly significant differences (Table 1) in PO_4, NO_3 and SiO_2 concentrations between depths and dates, with significantly higher values recorded at depth (25–30 m) and from autumn until early spring.

Table 1. Results of 3-way ANOVAs for variables undertaken using sample data collected at different stations (1, 2, 3 and 4), depths (5, 15, 25–30 m) and dates (13 sampling events). DON: dissolved organic nitrogen; DOP: dissolved organic phosphorus; PNAN: phototrophic nanoplankton; HNAN: heterotrophic nanoplankton. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant

Variable	Source of variability		
	Station	Depth	Date
NH_4 concentration	***	*	***
NO_3 concentration	ns	***	***
DON concentration	***	***	***
PO_4 concentration	ns	***	***
DOP concentration	ns	**	***
SiO_2 concentration	ns	***	***
Ratio N:P	ns	***	***
Ratio N:Si	ns	ns	***
Ratio P:Si	ns	**	***
Chl *a* concentration	ns	***	***
Chl *b* concentration	ns	*	ns
Chl c_{1+2} concentration	ns	**	***
Carotenoid concentration	ns	***	***
PNAN abundance	**	***	***
PNAN biomass	*	***	***
Mesodinium sp.	ns	**	**
Bacterioplankton abundance	***	***	***
Bacterioplankton biomass	***	***	***
HNAN abundance	ns[a]	***	***
HNAN biomass	***	***	***
Ciliate abundance[b]	ns	***	***

[a] $p = 0.056$
[b] *Mesodinium* sp. not included

No significant differences were observed in the ratios of dissolved N:P, N:Si and P:Si among all 4 stations. The N:P ratio on most sampling dates varied between 10 and 17 but was >17 at 5 m depth in August 1998 and 1999 and early September, and never <9 (Fig. 3). The stoichiometric N:Si ratios remained inside the range 1 to 3.3, except in June, when a high N:Si ratio of 4.4 was recorded at 5 m depth at the reference station. The stoichiometric P:Si ratios were within the range 0.05 to 0.33 on all occasions.

Photosynthetic pigments

No significant differences were detected in chl (*a*, *b*, c_{1+2}) and carotenoid concentrations among all 4 stations, although there were highly significant differences between depths (Table 1), with the highest concentration of all pigments at 5 m (Fig. 4). There were several periods of maximum chl *a* concentration at each of the stations. One maximum occurred in late September, mainly at 5 m, dominated by the diatom *Pleurosigma normanii* and associated with salinity

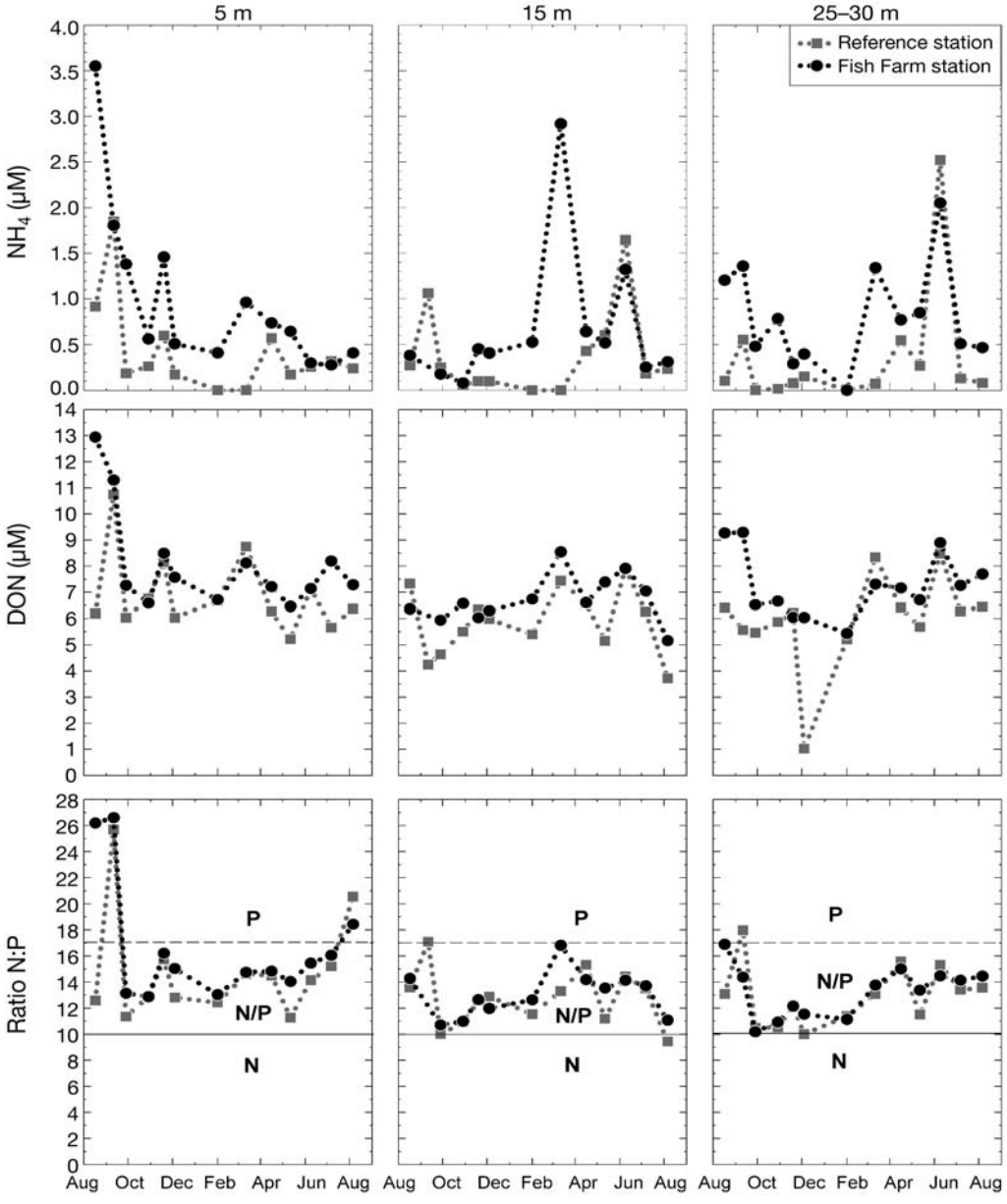

Fig. 3. NH_4 and DON concentrations and N:P ratios at 5, 15 and 25–30 m depth at the fish farm and reference stations. The solid horizontal line equates to an N:P ratio of 10, the theoretical upper limit for nitrogen limitation of phytoplankton growth according to Forsberg et al. (1978). The dashed horizontal line equates to an N:P ratio of 17, the lower theoretical limit for phosphorous limitation of phytoplankton growth according to Forsberg et al. (1978)

stratification; chl *a* concentrations at this maximum ranged between 6.25 µg l^{-1} at the fish farm station and 7.08 µg l^{-1} at the reference station. During May and early June there were high chl *a* concentrations at all depths, with maximum concentrations ranging from 3.37 µg l^{-1} at the fish farm station to 5.84 µg l^{-1} at the reference station, associated with salinity and temperature stratification. In July and especially in August 1999 there was a maximum chl *a* concentration at 5 m dominated by the dinoflagellate *Ceratium* (mainly *C. lineatum*) in a thermally stratified water column; chl *a* concentrations varied between 12.25 µg l^{-1} at the fish farm station and 26.17 µg l^{-1} at the reference station. Chl *b* concentrations were similar at all the stations and exhibited higher values in spring and early summer, with maximum values of 0.46 µg l^{-1} at the fish farm station and 0.43 µg l^{-1} at the reference station. Chl c_{1+2} concentrations were only significantly different in August 1999 (Tukey's test, $p < 0.05$) with maxima of 0.05 µg l^{-1} at the fish farm station and 0.13 µg l^{-1} at the reference station. Carotenoid concentrations showed a similar distribu-

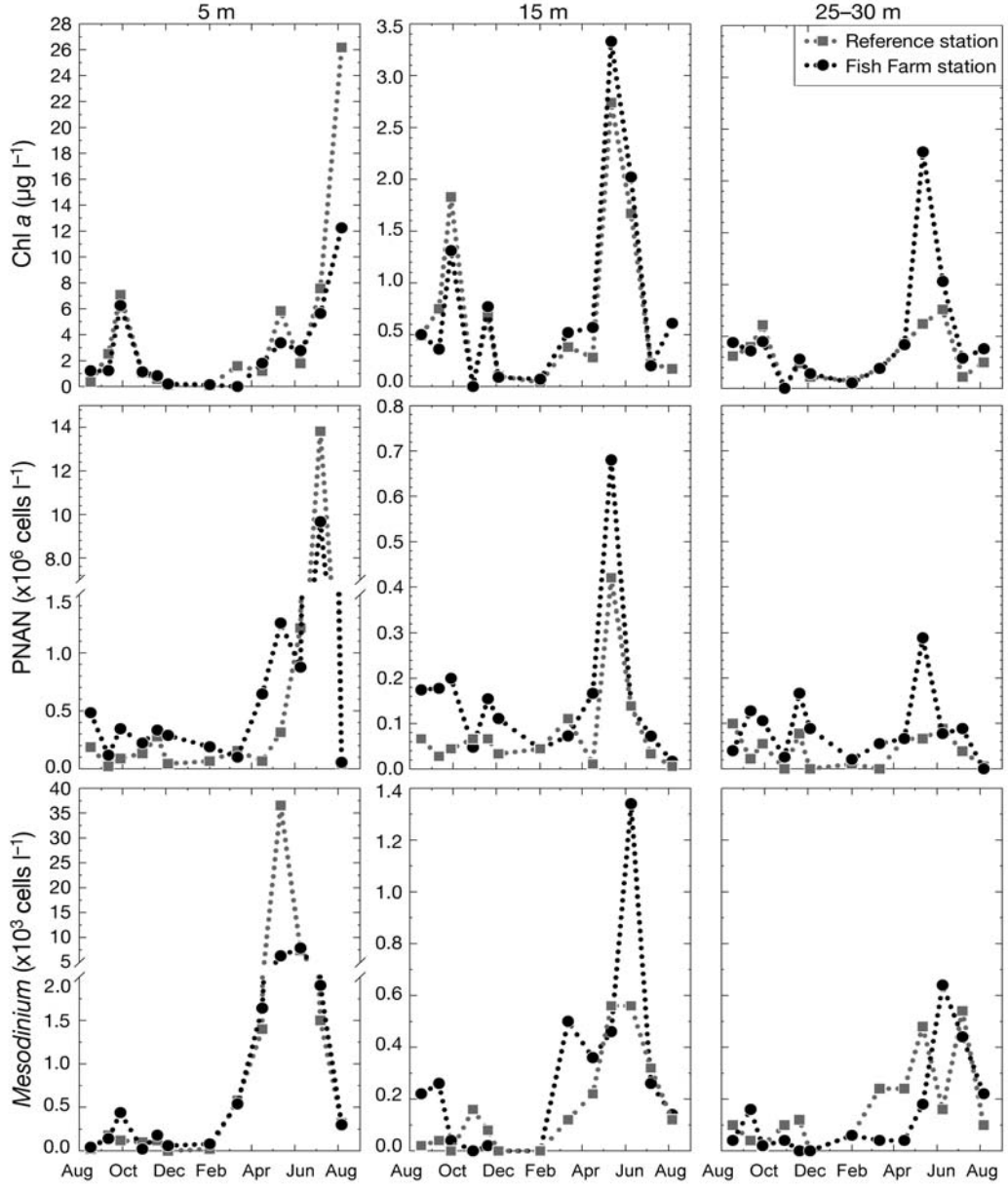

Fig. 4. Chlorophyll *a* concentrations, PNAN abundance and *Mesodinium* sp. abundance at 5, 15 and 25–30 m depth at the fish farm and reference stations. Note different *y*-axis scales for different depths

tion to chl *a* with maximum values in August 1999 of 10.86 µg l^{-1} at the fish farm station and 28.20 µg l^{-1} at the reference station.

Autotrophs

PNAN abundance and biomass increased from Stns 1 to 4, with significantly (Tukey's test, $p < 0.05$) higher abundance and biomass at Stns 3 and 4 (Fig. 4). PNAN abundance and biomass, averaged over all dates and depths, were 6.0×10^5 cells l^{-1} and 5.6 µg C l^{-1} at the fish farm station, and 4.1×10^5 cells l^{-1} and 4.2 µg C l^{-1} at the reference station, respectively. Abundance and biomass averaged across all dates decreased significantly with depth at all stations (Tukey's test, $p < 0.05$). The highest abundances and biomass were in July. PNAN were classified into 3 size groups: small (2 to 6 µm), medium (7 to 11 µm) and large (12 to 15 µm). Size distribution did not show any significant differences between stations but there were significant (Tukey's test, $p < 0.05$) differences between depths, with a greater proportion of small PNAN at 5 m, medium PNAN at 15 m and large PNAN at 25 to 30 m.

For the purposes of this study, all ciliates were considered to be primarily heterotrophic, except *Mesodinium* sp. which was classified as an autotroph. The abundance of *Mesodinium* sp. did not show any significant differences between stations but there was a highly significant ($p < 0.05$) decrease with depth, with mean abundances, averaged over all dates, of 1.9×10^3 cells l^{-1} at 5 m, 0.2×10^3 cells l^{-1} at 15 m and 0.1×10^3 cells l^{-1} at 25 to 30 m (Fig. 4). *Mesodinium* sp. abundance was highest in spring with a maximum abundance, averaged over all depths, of 4.9×10^3 cells l^{-1} recorded in May, and lowest in winter with a minimum abundance, averaged over all depths, of 16.7 cells l^{-1} recorded in December. There was a bloom in May with the highest concentrations of 36.5×10^3 cells l^{-1} recorded at the reference station at 5 m depth.

Heterotrophs

Bacterioplankton abundance and biomass, averaged over all dates and depths throughout the study, were 9.8×10^8 cells l^{-1} and 29.6 µg C l^{-1} at the fish farm station, and 7.7×10^8 cells l^{-1} and 23.4 µg C l^{-1} at the reference station, respectively (Fig. 5). Taken as a whole, abundance and biomass values were highly significantly different between stations, depths and dates (Table 1). When averaged over depths and dates, values were significantly higher (Tukey's test, $p < 0.05$) at the fish farm station than at the reference station. When averaged over dates and stations, values were significant higher (Tukey's test, $p < 0.05$) at 5 m than at 15 m and 25 to 30 m, with no differences between the latter 2 depths. Abundance and biomass were higher during summer and lower in winter.

HNAN abundance and biomass were almost twice as high at the fish farm station compared to the reference station with mean values, averaged over all depths and dates, of 3.4×10^5 cells l^{-1} and 5.2 µg C l^{-1}, and 1.7×10^5 cells l^{-1} and 2.7 µg C l^{-1}, respectively. The highest values of abundance and biomass were in July while the lowest values were in winter at all stations (Fig. 5). HNAN biomass was significantly different between stations, while abundance was close to significantly different ($p = 0.056$) between stations (Table 1). HNAN abundance and biomass, averaged over dates and stations, were significantly higher (Tukey's test, $p < 0.05$) at 5 m than at 15 m and 25 to 30 m, with no differences between the latter 2 depths. HNAN were classified into the same 3 size groups as PNAN. The proportions of large (12 to 15 µm) HNAN were significantly (Tukey's test, $p < 0.05$) higher at all depths at the fish farm station (mean 6.5 and 1.8% at the fish farm and reference stations, respectively). The proportion of small (2 to 6 µm) HNAN was significantly higher at 5 m (Tukey's test, $p < 0.05$) but no other differences in size distribution were observed between depths.

Ciliate abundance (excluding *Mesodinium* sp.) did not show any significant differences between stations, but there was a highly significant decrease with depth, with mean abundances averaged over all dates and stations of 4.1×10^3 cells l^{-1} at 5 m, 1.1×10^3 cells l^{-1} at 15 m and 0.7×10^3 cells l^{-1} at 25 to 30 m. Ciliate abundance was highest in spring, with a maximum value, averaged over all depths and stations, of 7.6×10^3 cells l^{-1} recorded in May, and lowest in winter with a minimum abundance, averaged over all depths and stations, of 0.1×10^3 cells l^{-1} recorded in December (Fig. 5). There was a bloom in May, with the highest concentrations of 22.5×10^3 cells l^{-1} and 20.9×10^3 cells l^{-1} recorded at 5 m depth at the fish farm and reference stations, respectively.

ANOVA

The results of the 3-way ANOVAs performed to test the effects of station location, depth and date on biological and chemical variables are shown in Table 1. Most variables exhibited significant differences between depths and/or dates. Of more interest were the significant differences recorded between stations in the concentration of NH_4 and DON, and the abundance and/or biomass of bacterioplankton, HNAN and PNAN.

Multivariate analyses

MDS ordinations and hierarchical cluster classifications, conducted on the combined photosynthetic pigment concentration and microbial abundance and biomass data for each sampling date, displayed a gradation in planktonic microbial community composition across the stations related to their proximity to the fish farm. This apparent effect of the fish farm on the planktonic community is more evident in the 5 m data (black arrows in Fig. 6). The stress was very low for all the plots (<0.05) indicating that the 2-dimensional plots are an excellent representation of the sample relationships. The cluster classification at various levels of similarity revealed 2 different groups for most sampling dates: 1 group comprising the variables recorded near the fish farm cages at Stns 4 and 3 (especially the 5 m data), and the other group comprising the variables recorded furthest from the fish farm at Stns 1 and 2 (see example in Fig. 6). The results of the 1-way ANOSIMs supported this conclusion with R-values close to the maximum (1) for differences between variables recorded at the 2 stations near the fish farm and the 2 stations further away.

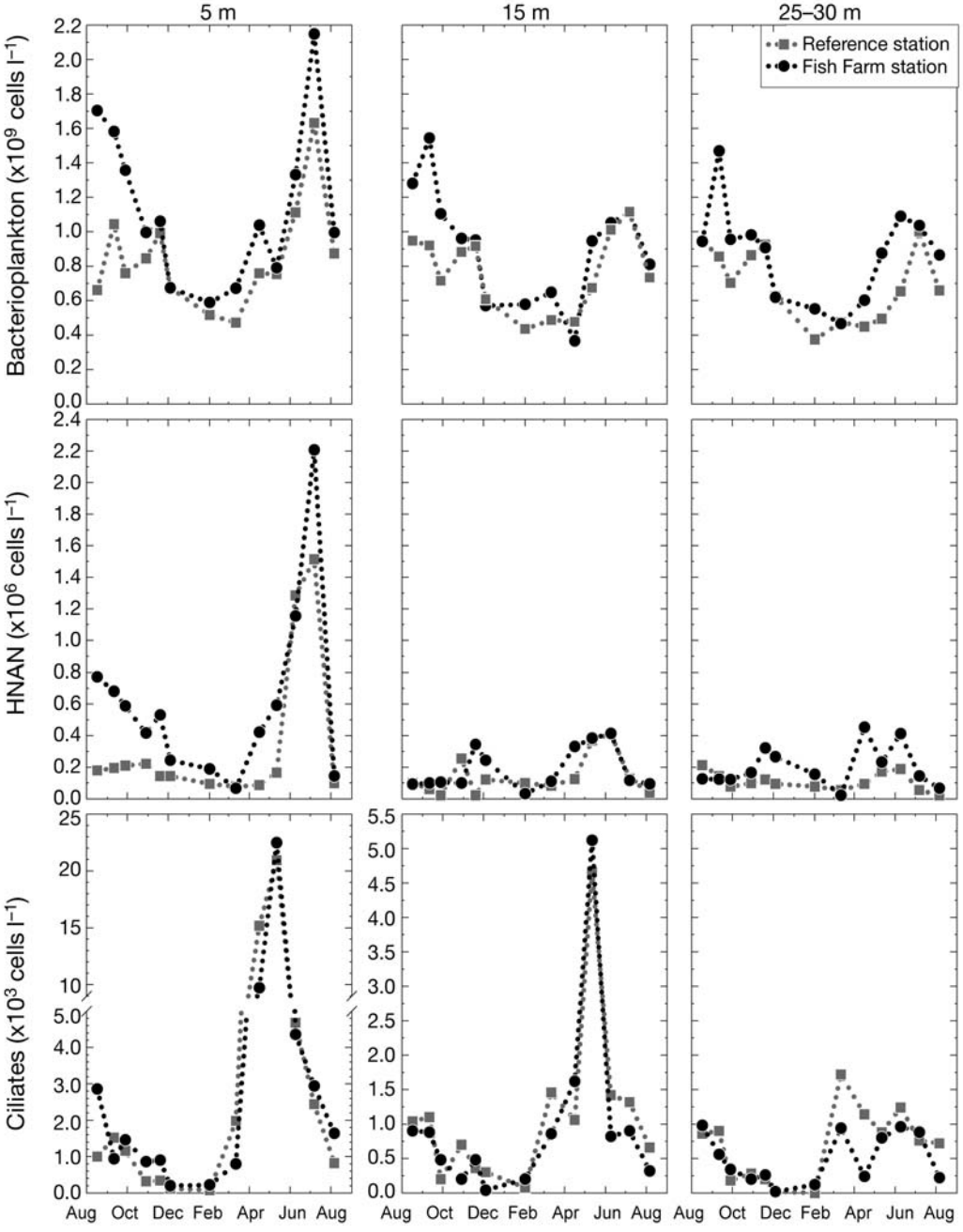

Fig. 5. Bacterioplankton, HNAN and ciliate abundance (excluding *Mesodinium* sp.) recorded at 5, 15 and 25–30 m depth at the fish farm and reference stations. Note different *y*-axis scales for different depths

The SIMPER program analysis identified the contribution of individual groups to dissimilarity between communities at each station and depth on each sampling date (where dissimilarity is expressed as the percentage of the total dissimilarity contributed by the *i*th group). Results of this analysis conducted on the microbial abundance and chlorophyll concentration data revealed that bacterioplankton abundance was primarily responsible for differences observed between stations and between depths with values of 96 to 98%.

The BIOENV program analysis identified the environmental combination which best explained the differences in the biological data between stations on each sampling date. For surface (5 m depth) waters, NH_4, DON and PO_4 were the best explanatory variables, with maximal Spearman rank correlation values (ρ_w) equal or close to 1 (Fig. 7).

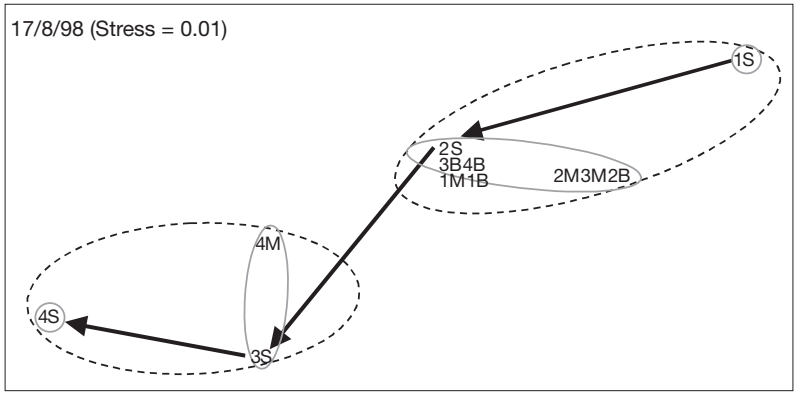

Fig. 6. Example MDS ordination plot of the selected biological and chemical data (bacterioplankton, HNAN, PNAN and ciliate abundance and chl *a*, *b*, c_{1+2} and carotenoids concentration) recorded at 5, 15 and 25–30 m depth at the fish farm and reference stations on 17 August 1998. Clusters are superimposed at similarity levels of 90% (dashed line) and 95% (continuous grey line). Arrows indicate changes in the planktonic community from Stns 1 to 4 (S = 5 m, M = 15 m, B = 25–30 m)

DISCUSSION

This study investigated the effects of fish farm inputs on the dissolved nutrient pool, and the autotrophic and heterotrophic microbial communities, at different depths within the water column of Loch Fyne over a full annual cycle. Several variables exhibited significantly higher values at stations nearer to the fish farm cages: specifically, NH_4 and DON concentration, bacterioplankton and PNAN abundance and biomass, and HNAN biomass. These results suggest that, over the entire year, fish farm inputs were altering natural nutrient concentrations and eliciting a response by major components of the heterotrophic planktonic microbial community, with only a partial response by the autotrophic microbial community. Almost all variables exhibited significant differences between depths and between seasons, reflecting the expected influences of water column stratification and seasonality on biological and chemical processes within the sea loch environment. In most cases, these depth and seasonal effects were of a greater magnitude than station effects, indicating that natural perturbations rather than fish farm inputs were responsible for much of variability observed.

Higher concentrations of NH_4 and DON were observed throughout most of the year at stations nearer to the fish farm. These observations are in good agreement with the higher concentrations in waters near fish farms of NH_4, NO_3 and NO_2 observed by Wu et al. (1994), NH_4 and PO_4 observed by Pitta et al. (1999) and dissolved inorganic phosphorus (DIP) and dissolved organic carbon (DOC) observed by La Rosa et al. (2002). Although the N:P, N:Si and P:Si ratios did not exhibit any significant differences between the stations, the N:P ratio ranged from 10 to 17 during most of the year, suggesting alternating nutrient limitation (Forsberg et al. 1978). The ratio was >17 (indicative of P limitation) in August 1998 and 1999 and early September at 5 m depth (corresponding with higher concentrations of NH_4 and DON) but was almost never <10 (indicative of N limitation). The N:Si and P:Si ratios were generally within the respective ranges of 1:1 to 3.3:1 and 0.05:1 to 0.33:1, which are indicative of pelagic environments characterised by the coexistence of diatom and non-diatom phytoplankton; diatoms tend to out-compete non-siliceous algae at low N:Si and P:Si ratios (Sommer 1999).

The chlorophyll concentration data confirm previous failures to establish a clear relationship between farm inputs and phytoplankton abundance and biomass, even where large inorganic nutrient inputs have been observed (Beveridge 1996, Pitta et al. 1999). Chlorophyll concentrations did not seem to be significantly affected by nutrient release from the fish farm; however, it is possible that phytoplankton cellular growth rates may have been higher closer to the fish farm, with all new production either dispersed by currents or grazed by protozoan and metazoan zooplankton, thereby preventing an increase in biomass. In contrast to the chlorophyll concentration and *Mesodinium* sp. abundance data, PNAN abundance and biomass were significantly higher in the vicinity of the fish farm, suggesting that at least some components of the autotrophic microbial community may have responded to nutrient inputs; indeed, the growth of nanoflagellates may be favoured over that of diatoms near fish farms

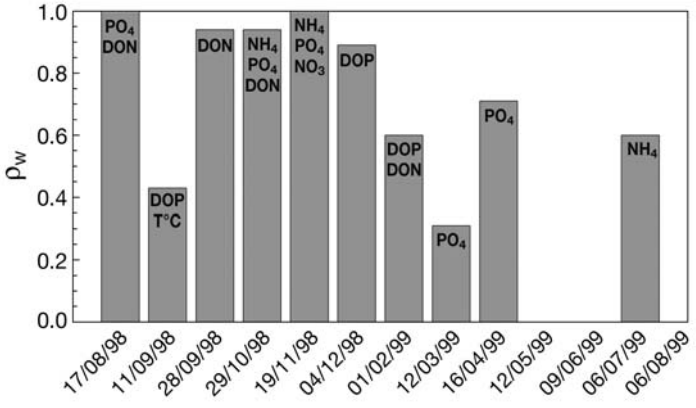

Fig. 7. Combination of the environmental variables (nutrient concentration, depth and water temperature) which best explained the differences in the biological data between stations on each sampling date (dd/mm/yy) at 5 m depth, as measured by weighted Spearman rank correlation ρ_w

where N and P may increase in the absence of concurrent increases in Si (Harrison & Davis 1979). On the other hand, some of the PNAN recorded may have been mixotrophic and responding to enhanced bacterioplankton abundance in the vicinity of the fish farm.

The evidence of a response of the heterotrophic planktonic microbial community to fish farm inputs in Loch Fyne was more compelling than that of the autotrophic community, with bacterioplankton abundance and biomass and HNAN biomass significantly higher at stations nearer to the fish farm. The higher bacterioplankton biomass recorded in the vicinity of the fish farm is to be expected given the labile nature of much of the particulate and dissolved organic input associated with the fish farm and the fast cellular growth rate potential of bacteria. Indeed, a concurrent study undertaken in Loch Fyne during May, June and July 1999 observed higher bacterioplankton production at the fish farm station than at the reference station (R. J. G. Leakey et al. unpubl.). Our data are consistent with previous observations of increased bacterioplankton abundance recorded near fish farm cages in Italian and Greek coastal waters (La Rosa et al. 2002, Pitta et al. 2006) and increased bacterioplankton abundance and production in a eutrophic embayment containing fish net pens off the Japanese coast (Sakami et al. 2003); however, studies undertaken in Malaysian mangrove estuaries and western Mediterranean coastal waters have found no response by bacterioplankton to fish farm inputs (Alongi et al. 2003, Maldonado et al. 2005). In the present study, DON concentrations were significantly higher in the vicinity of the fish farm but DOP concentrations were generally similar at all stations. The similarity in the DOP pool across all stations may be due to enhanced bacterioplankton uptake near the fish farm or may reflect the presence of large concentrations of refractile organic matter within Loch Fyne effectively masking any changes in the more labile constituents introduced from the fish farm.

HNAN bacterivory was also observed during summer months (R. J. G. Leakey et al. unpubl.) and it is probable that the higher HNAN biomass recorded at stations near the fish farm in the present study was a consequence of the higher bacterioplankton abundance and biomass in the vicinity of the fish farm. There was also a higher abundance of large (12 to 15 µm) HNAN at stations near the fish farm, indicating a modification of nanoplankton species composition. Both HNAN and PNAN are subject to grazing by ciliates (Capriulo 1990) but no significant differences in ciliate abundance were detected between stations. If grazers of nanoplankton were resource limited then their growth should be influenced by changes in nanoplankton biomass; however, the complex predator–prey relationships and weak nature of biological coupling in planktonic food webs renders identification of such changes difficult (Tett 1992).

The differences in components of the planktonic microbial community associated with the fish farm are also confirmed by the MDS ordinations and hierarchical cluster classifications, especially with respect to analyses conducted on data collected from 5 m depth. The differences reflect the higher abundance and biomass of bacterioplankton, HNAN and PNAN in the vicinity of the farm, as identified by the SIMPER program.

CONCLUSIONS

This study has shown that, over a complete annual cycle, salmon farm inputs enhance the local concentrations of NH_4 and DON in the upper basin of Loch Fyne. The higher abundance and biomass of heterotrophic microorganisms near the fish farm suggests that nutrients derived from the fish farm may be directly or indirectly enhancing heterotrophic microbial activity. This in turn suggests that the heterotrophic microbial food web was responsible, at least in part, for processing matter and energy released into the pelagic environment from the salmon farm. By contrast, autotrophic biomass measured as chlorophyll concentration tended to be similar at all stations, supporting the conclusions of previous studies that failed to establish a clear relationship between fish farm inputs and phytoplankton biomass. As such, the response of the heterotrophic microbial community is probably a more appropriate indicator than chlorophyll concentration of local ecological effects of fish farms in temperate coastal waters.

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