The effects of a single freshwater release into the Kromme Estuary. 2: Microalgal response

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Abstract

The release of freshwater from the Mpofu Dam in November 1998 resulted in a short-term (6 d) longitudinal and vertical gradient of both salinity and nitrate in the estuary. There was no significant increase in phytoplankton chlorophyll *a* during the period of the study. The phytoplankton communities were dominated (> 10%) by diatoms and flagellates throughout the study. The average number of diatoms increased significantly from 301 cells·ml⁻¹, prior to the release, to a maximum of 3 856 cells·ml⁻¹ by the end of the first spring tidal cycle. The average number of flagellates increased from 1 903 cells·ml⁻¹, before the release, to a maximum of 3 300 cells·ml⁻¹ after two spring tidal cycles. There was no change in subtidal benthic chlorophyll *a* biomass but intertidal benthic chlorophyll *a* increased from 35.6 mg·m⁻², before the release, to 63.3 mg·m⁻² by the sixth day. The conclusion is that the amount of freshwater released was insufficient to increase the nutrient content of the water to a level that resulted in a significant increase in primary productivity. The length of time that the freshwater influence was present also prevented a significant increase in microalgal growth.

Introduction

The Kromme Estuary is narrow and extends for 14 km from a permanently open mouth to a rock sill that forms the tidal head of the estuary. The mouth stays open by virtue of a large tidal prism. An average channel depth of 1.5 m characterises the lower reaches of the estuary (6.6 km upstream of the mouth). Depths of between 3 to 5 m are common in the middle and upper reaches. A sandbar extends across the estuary between Sites 2 and 3 (Fig. 1) and is exposed during spring low tide.

There are two dams above the estuary and their combined holding capacity (Mpofu Dam 107 x 10⁶ m³ and the Churchill Dam 33.3 x 10⁶ m³) exceeds the mean annual runoff (MAR) of the Kromme River (105 x 10⁶ m³). The Mpofu Dam is 4 km upstream from the tidal head of the estuary, so that practically the entire runoff from the Kromme River catchment area is retained in the dam in a normal season (Reddering, 1988).

The Department of Water Affairs and Forestry (DWAF) annually releases 2 x 10⁶ m³ of water from the Mpofu Dam for the "ecological requirements" of the Kromme Estuary and particularly to maintain the salinity at, or less than, 35‰. If the 2 x 10⁶ m³ of water were to be discharged continuously, it would flow at 0.063 m³·s⁻¹. However, in the recent past, the water has been released as equal monthly amounts of almost 167 000 m³. As a result of this small amount of freshwater entering the estuary, the water column has generally been well mixed with the salinity averaging 32‰ or more, throughout the estuary (Bickerton and Pierce 1988). The present state of the estuary is considered by some ecologists as a sheltered arm of the sea rather than a fully functional estuary. Because of this, it has a low productivity relative to other permanently open estuaries that receive a constant freshwater flow.

The aim of this project was to determine the biomass and biodiversity response of estuarine microalgae to a single 2 x 10⁶ m³ release of water from the Mpofu Dam into the freshwater-starved, but permanently open, Kromme Estuary. This volume represented the full annual water allocation being released on a single occasion.

The intention was to recreate a normal estuarine salinity profile to determine whether there would be an increase in the biomass of pelagic and benthic microalgae. Three hypotheses were proposed before the study commenced. These were tested using data collected for a period starting 5 h before the water release and ending 50 d after the start of the release.

Hypotheses

The release of 2 x 10⁶ m³ of water from the Mpofu Dam:

(i) Will result in a fourfold increase (5 to 20 µg·l⁻¹) in average water column (pelagic) chlorophyll a *after three spring tidal cycles (42 d).*

Average water column chlorophyll *a* concentrations in the estuary from previous sampling sessions in 1997 and 1998 were 5.6 µg·l⁻¹ (average salinity of 21.5 g·l⁻¹) and 2.1 µg·l⁻¹ (average salinity of 33.2 g·l⁻¹) respectively. Hilmer (1990) working in the nearby Sundays Estuary showed that three spring tidal cycles were required after a freshwater pulse to produce the maximum chlorophyll *a* content in the water column.

(ii) Will change the structure of the phytoplankton groups from being flagellate-dominated, to a condition where diatoms will be dominant. Later, as the freshwater input dissipates and the water column once again becomes well mixed, flagellates will regain their dominance. As a result, phytoplankton group diversity (H') and evenness (J') will increase to above 0.1 and 0.03 respectively for the period when diatoms are dominant.

Diatom counts and diversity indices, in brackets, of the 1997 and 1998 sampling sessions were 60.7 cells·ml⁻¹ (H' = 0.085, J' = 0.027) and 110 cells·ml⁻¹ (H' = 0.091, J' = 0.025) respectively. Flagellate counts during the same sessions were 1 381 and 3 833 cells·ml⁻¹ respectively.

(iii) Will result in a twofold increase in average benthic microalgal chlorophyll a.

This would be the result of the increase in mineral nutrient concentration within the water column. Average benthic chlorophyll *a* concentrations during previous sampling sessions had been 32.2 (subtidal) and 41.8 mg·m⁻² (intertidal) in 1997 and 53.3 (subtidal) and 82.4 mg·m⁻² (intertidal) for 1998.
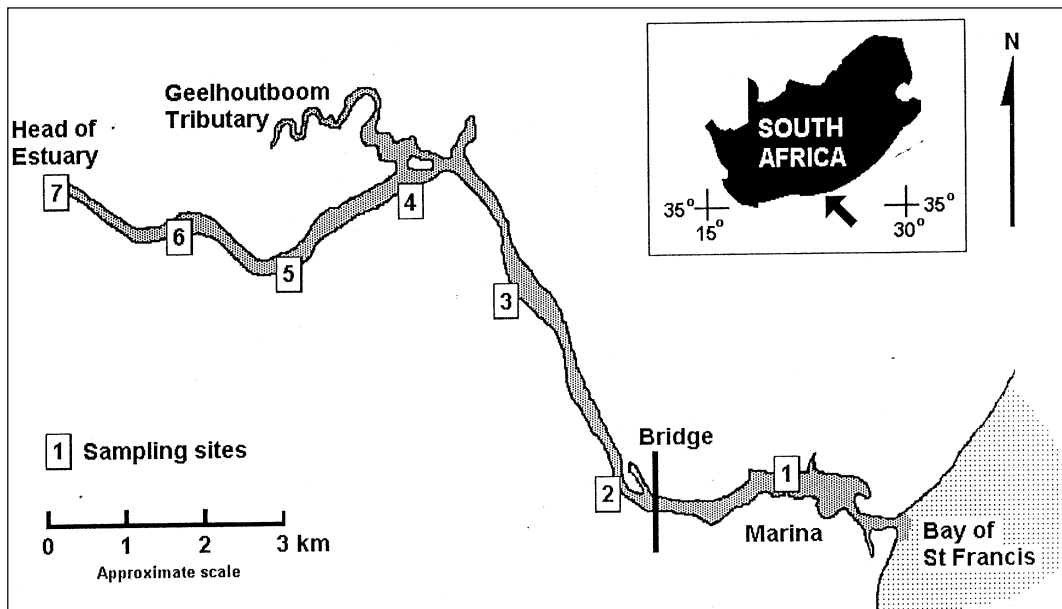
Materials and methods

Seven sites were selected along the length of the Kromme Estuary (Fig. 1). Sampling took place during spring high tides where possible, the first of which was five hours before the release of water from the Mpofu Dam (16/11/1998). Subsequent sampling took place 2, 4 and 6 d after the start of the release (18, 20 and 22/11/1998 respectively) and then at spring high tide for the following three spring tidal cycles (3, 17/12/1998 and 05/01/1999).

Salinity and temperature were recorded using a WTW conductivity/temperature meter (CTD) for the first three sampling sessions at 0, 0.5 and 1 m and, if possible, at 1 m intervals to the bottom. Later salinity measurements were recorded using a refractometer (Atago Opticals) after the CTD became non-functional. The salinity values measured by the refractometer are similar to those obtained using a CTD except that the former is less sensitive; i.e. it is only possible to estimate the decimal units.

Light attenuation was determined using a secchi disc. The vertical attenuation coefficient was determined as described by Dawes (1981): K (m^{-1}) = (1.7 / secchi depth).

Nitrate concentration was determined using the reduced copper-cadmium method described by Bate and Heelas (1975). Nitrate was collected from surface and bottom water at each site using a weighted pop-bottle. The nitrate in the filtered (Whatman GF/C filters) water samples was reduced to nitrite then determined as NO_2^--N plus NO_3^--N. Nitrite was quantitatively incorporated into a diazo-couple (red/purple) compound and then the concentration determined at 540 nm using a GBC UV-VIS spectrophotometer. All results were expressed in μM values as these units obviate any problems of interpretation between N and other oxidised nitrogen (NOx) compounds.

On all occasions, water samples taken to identify phytoplankton groups and measure pelagic chlorophyll a were obtained using a 500 ml weighted pop-bottle. This was opened *in situ* by removing a stopper at 0, 0.5, 1 m depths and, if possible, at 1 m intervals to the bottom.

For phytoplankton chlorophyll a analysis, 500 ml water samples were gravity-filtered through plastic Millipore filter towers with Whatman (GF/C) glass-fibre filters. The chlorophyll a was
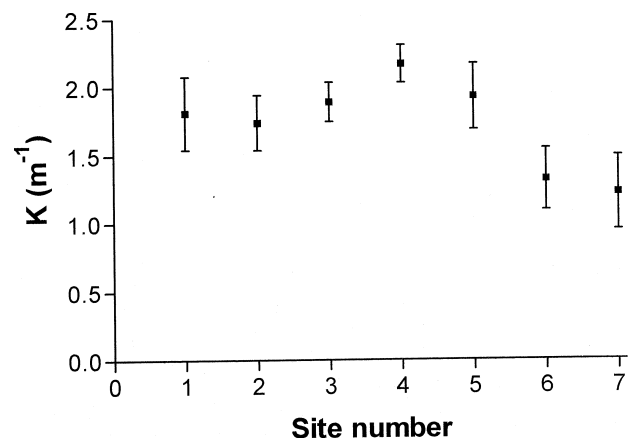

Figure 2
Average light attenuation (K) through the water column (m^{-1}) at each sampling site of the Kromme Estuary

extracted by placing the filters into glass vials with 10 ml of 95% ethanol (Merck 4111). The samples were then stored overnight in a freezer. The contents of the vials were filtered and the light absorbance at 665 nm of the supernatant was determined, before and after adding two drops of 0.1 N HCl, using a GBC UV-VIS spectrophotometer. Chlorophyll a was calculated using the equation of Hilmer (1990) that had been derived from that of Nusch (1980):

Chlorophyll a biomass (μg·l^{-1})
= $(E_{b665} - E_{a665})$ x 29.6 x (v/(V x l))

where:

E_{b665}	=	absorbance at 665 nm before acidification
E_{a665}	=	absorbance at 665 nm after acidification
v	=	volume of solvent used for the extraction (ml)
V	=	volume of sample filtered (l)
l	=	path length of spectrophotometer cuvette (cm)
29.6	=	constant calculated from the maximum acid ratio (1.7) and the specific absorption coefficient of chlorophyll a in ethanol (82 g·l^{-1}·cm^{-1}).

Benthic microalgal biomass was determined by measuring the concentration of their photosynthetic pigment, chlorophyll a. To measure this benthic chlorophyll a, two 14 mm internal diameter cores of sediment surface were taken from each of two subtidal and two intertidal sites using a coring device developed by Rodriguez (1993). The chlorophyll a from the top 10 mm of sediment from the two combined samples was extracted using 30 ml of 95% ethanol. The samples were then stored in a cooler box with ice bricks. After extraction overnight at 4°C, the benthic chlorophyll a content of the sediment was determined by first centrifuging and then filtering the samples through glass-fibre filters (Whatman GF/C). The extract was analysed on a high performance liquid chromatograph (HPLC) attached to a Waters Lambda-Max 481 LC spectrophotometer and Waters LM-45 solvent delivery system. A 30% methanol and 70% acetone mixture was used as a carrier. The system was calibrated using the chlorophyll a of red seaweed (*Plocamium corallorhiza*) because it contains no chlorophyll b to interfere with the chlorophyll a reading at 665 nm (Du Preez, 1998). *P. corallorhiza* was crushed using a mortar and pestle in 20 ml of 95% ethanol and filtered through a glass-fibre filter (Whatman GF/C). The chlorophyll a concentration was determined from the absorbance reading using the equation (Nusch, 1980):

Chlorophyll a biomass (mg·m^{-2})
= $(E_{b665} - E_{a665})$ x 29.6 x (v/A) x 1 000

where:

E_{b665} = absorbance at 665 nm before acidification

E_{a665} = absorbance at 665 nm after acidification

A = area of the sample (mm^2)
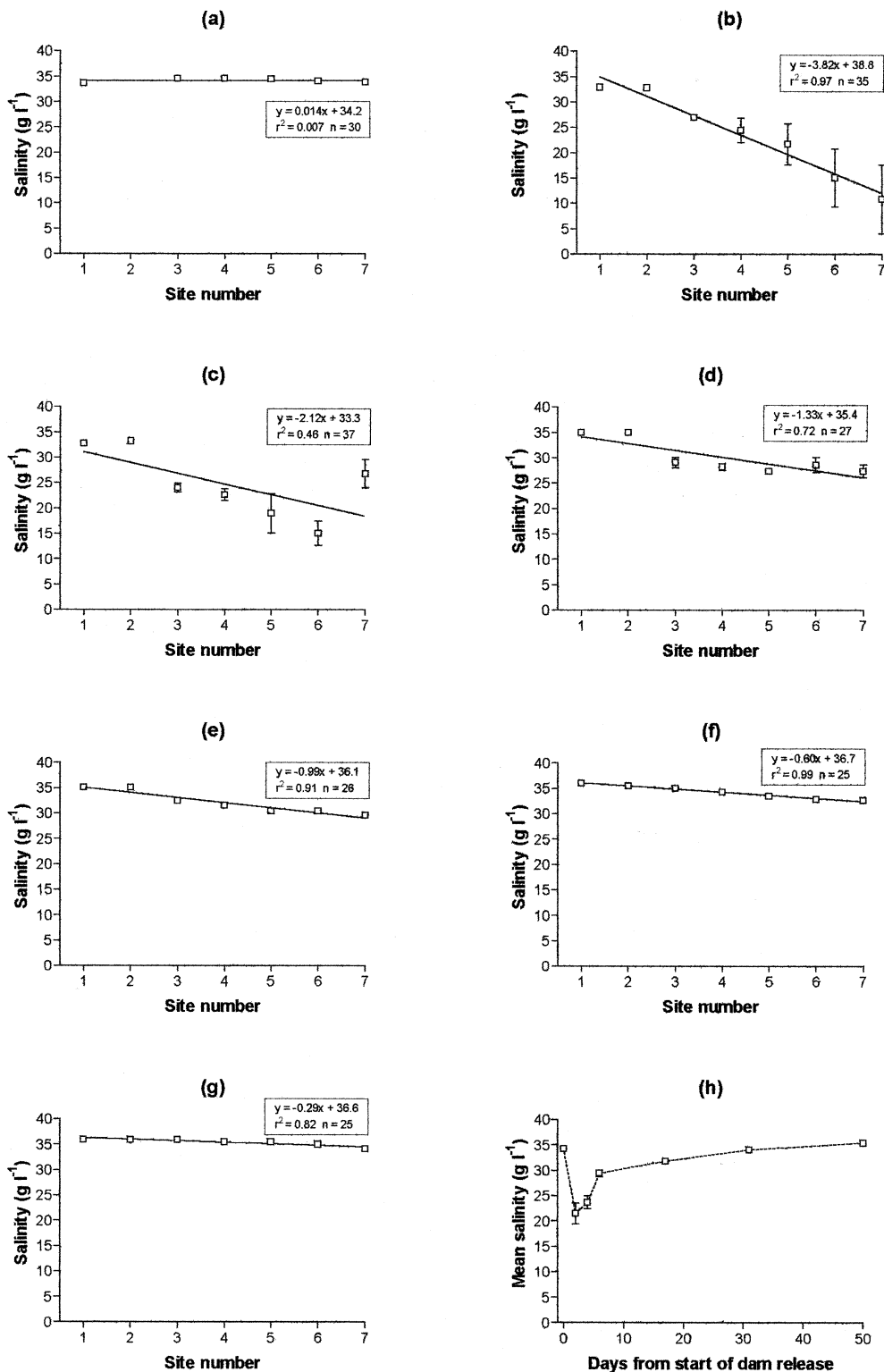
v = volume of solvent used for the extraction (ml)

Figure 3
Mean longitudinal salinity distributions prior to the 2 x 10^6 m^3 Mpofu Dam release [Day 0, (a)] and for three spring tidal cycles after the release [Days 2, (b); 4, (c); 6, (d); 17, (e); 31, (f) and 50, (g)]. Figure (h) represents the mean salinity in the estuary relative to the start of the release (n = 205). Vertical bars represent ± SE mean.
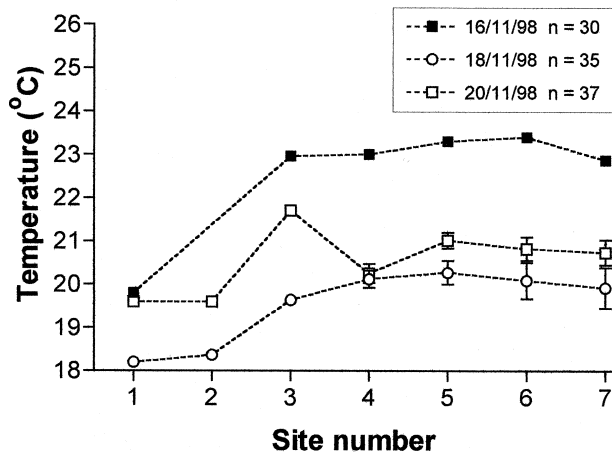
Figure 4
*Mean longitudinal temperature distributions [16 (Day 0), 18
(Day 2) and 20 (Day 4) November 1998, all depths included].
Vertical bars represent ± SE mean.*

29.6 = constant calculated from the maximum acid ratio (1.7)
and the specific absorption coefficient of chlorophyll *a*
in ethanol (82 g·l⁻¹·cm⁻¹).

1 000 = correction factor (μg·mm⁻² to mg·m⁻²)

Samples used for phytoplankton identification were preserved by
adding 1 ml of 25% glutaraldehyde solution to 150 ml of sample.
Sixty millilitres of preserved sample was stained with Rose Bengal
and settled overnight in a 27.8 mm internal diameter Utermöhl
settling chamber (Hilmer, 1990). Identification and cell counts
were done using a Zeiss IM 35 inverted microscope at maximum
magnification (630x). The numbers of flagellate, diatom,
dinoflagellate, chlorophyte and cyanophyte microalgal cells in
1 ml were calculated using the formula:

$$\text{Cells·ml}^{-1} = ((\pi r^2)/A) \times C/V$$

where:

A = area of each frame (mm²)
C = number of cells in each frame
V = volume sample in the settling chamber (ml)

Phytoplankton group diversity (H') was estimated by inserting the
average number of cells per ml of each of the five phytoplankton
groups into the Shannon-Wiener diversity equation (Zar, 1984). In
addition, the evenness of spread of cell numbers between the five
phytoplankton groups was included using the evenness index (J').
The Student's t-test and one way ANOVA (Tukey's Multiple
Comparison Test) were used to compare different sampling sites
and dates for significant differences.

Results

Water column biotic and abiotic variables

There was heavy rainfall that persisted for a few days soon after the
start of the release of water from the Mpofu Dam causing tributar-
ies, the Geelhoutboom included, to flow. However, there was a
strong·longitudinal salinity gradient during the study with no
evidence that the additional freshwater had a significant influence
on the results of the study.

Average light attenuation through the water column (Fig. 2) for
the period of the study was greatest in the middle reaches of the
estuary (Site 4). Subtidal sediments were extremely fine in this
region where the Geelhoutboom Tributary enters the Kromme
Estuary. However, there were no significant differences between
light attenuation coefficients at any of the sites. There was no
correlation between vertically averaged phytoplankton chloro-
phyll *a* and light attenuation during any of the sampling sessions.

Pre-release; 16 November 1998

Two sampling sites were selected within the Mpofu Dam to
determine water quality as well as phytoplankton chlorophyll *a* and
group diversity. The first site was located near the outlet at the dam
wall while the second site was approximately 1 km from the wall.
Temperature recorded in the dam was highest near the surface at
both sites (21.3°C at Site 1 and 20.8°C at Site 2). Temperature
gradually decreased with depth so that bottom water was approxi-
mately 2°C cooler than surface water (19°C at Site 1 and 18.5°C at
Site 2). Water in the dam was turbid with a Secchi depth of 0.45 m
(attenuation coefficient K = 2.22 m⁻¹) being recorded at both sites.
Nitrate concentration was even throughout the water column and
ranged from 24 to 26 μM (Scharler, 1998). The average phyto-
plankton chlorophyll *a* for both sites was 2.5 μg·l⁻¹ ± 0.3 SE.
Diatoms (71.0%) and flagellates (26.2%) dominated the phyto-
plankton. The most abundant diatom present in the dam was a
chain-forming species of *Aulacoseira*. Chlorophytes (2.8%) were
the only other phytoplankton group recorded.

Salinity was constant throughout the estuary (range 33.7 to
34.7 ‰) prior to the release of water from the Mpofu Dam
(Fig. 3(a)). There was also little difference in temperature between
Sites 3 to 7 (23.1°C ± 0.04 SE) but a 3°C lower temperature was
measured near the mouth of the estuary at Sites 1 and 2 (Fig. 4).
There was no vertical gradient of salinity, temperature or nitrate
concentration, which is indicative of a well-mixed estuary. In
addition, there was no longitudinal gradient of nitrate (Fig. 5(a)).
Mean phytoplankton chlorophyll *a* (all depths included) reached a
maximum of 7.7 μg·l⁻¹ ± 1.1 SE at Site 4 (Fig. 6(a)). Chlorophyll *a*
concentrations, particularly in the middle reaches (Sites 3 to 5),
displayed high variability with depth. Average phytoplankton
chlorophyll *a* for the estuary was 4.3 μg·l⁻¹ ± 0.7 SE (Fig. 6(h)).
Flagellates (average 1 903 cells·ml⁻¹ ± 65 SE) and diatoms (average
301 cells·ml⁻¹ ± 61 SE) dominated the phytoplankton (85% and
13% respectively) (Fig. 7). Over the full period of the study
dinoflagellates, chlorophytes and cyanophytes were never domi-
nant and none of these phytoplankton groups exceeded 2% of the
total number of cells making up the phytoplankton.

Post-release; 18 November 1998

A strong longitudinal salinity gradient (32.9‰ near the mouth and
10.9‰ at the head of the estuary) had developed by the second day
after the start of the release (Fig. 3(b)). A strong halocline had
developed at the four uppermost sites. The strongest halocline,
0.1‰ at the surface and 33.9‰ at 2.5 m, was located at the head of
the estuary. The water at the mouth of the estuary (Sites 1 and 2) was
significantly cooler than in the rest of the estuary (t = 4.88, DF = 33,
p < 0.01) (Fig. 4) but there was no significant difference in
temperature between the upper five sites. The similarity in tem-
perature between all seven sites indicates that the dam water had no
effect on the temperature of the estuary water.

The freshwater release contributed nitrate to the estuary creat-
ing a strong positive linear gradient in nitrate, particularly on the

surface (Fig. 5(b)). Nitrate at the surface at the upper four sites was significantly higher than nitrate in the bottom water (t = 2.85, DF = 6, p = 0.03). The average phytoplankton chlorophyll a in the estuary (1.8 $\mu g \cdot l^{-1}$ ± 0.2 SE) was significantly lower than the pre-release average of 4.3 $\mu g \cdot l^{-1}$ ± 0.7 SE (Tukey's q = 2.52 > $q_{0.05, 169, 7}$ = 1.78) (Fig. 6(h)). However, phytoplankton chlorophyll a was still highest in the middle reaches of the estuary and reached a maximum vertical average of 3.0 $\mu g \cdot l^{-1}$ ± 0.6 SE at site 5 (Fig. 6(b)). There was a slight increase in the average numbers of flagellates and diatoms (2 282 cells·ml^{-1} ± 200 SE and 337 cells·ml^{-1} ± 52 SE respectively) after the release (Fig. 7).

Post-release; 20 and 22 November 1998

During the following four to six days, the longitudinal salinity gradient gradually weakened (Fig. 3(c) and 3(d)). The temperature on Day 4 was again significantly lower at the mouth of the estuary (Sites 1 and 2) when compared to the five upper sites (t = 5.19, DF = 35, p < 0.01) (Fig. 4). This lower temperature was caused by seawater intrusion. Although the longitudinal nitrate gradient on Day 4 was not as strong as it was on Day 2 (Fig. 5(c)), there was still a significantly higher concentration of nitrate in the surface water of the upper five sites compared to the bottom water (t = 3.59, DF = 8, p < 0.01). However, this was not the case on the Day 6 by which time nitrate concentration was similar at all sites along the length of the estuary as well as between top and bottom water.

Average phytoplankton chlorophyll a on Day 4 (2.1 $\mu g \cdot l^{-1}$ ± 0.2 SE) was still significantly lower than prior to the release (Tukey's q = 2.20 > $q_{0.05, 169, 7}$ = 1.78). Average phytoplankton chlorophyll a on Day 6 (4.9 $\mu g \cdot l^{-1}$ ± 0.5 SE) had
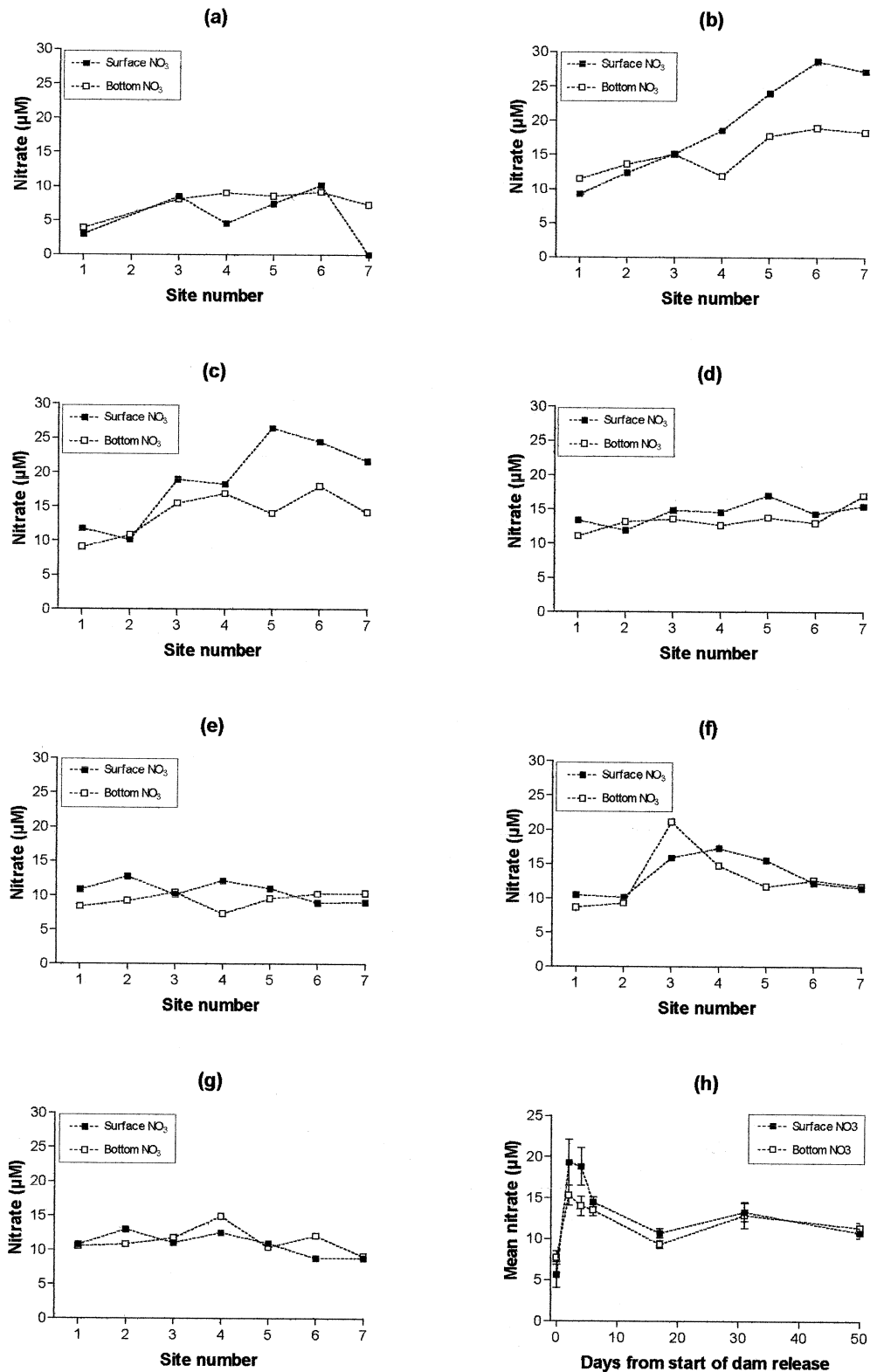

Figure 5

Longitudinal nitrate distributions prior to the 2 x 10⁶ m³ Mpofu Dam release [Day 0, (A)] and for the following three spring tidal cycles [Days 2, (b); 4, (c); 6, (d); 17, (e); 31, (f) and 50, (g)]. Figure (H) represents the mean nitrate in the estuary relative to the start of the release (n = 96). Vertical bars represent ± SE mean.
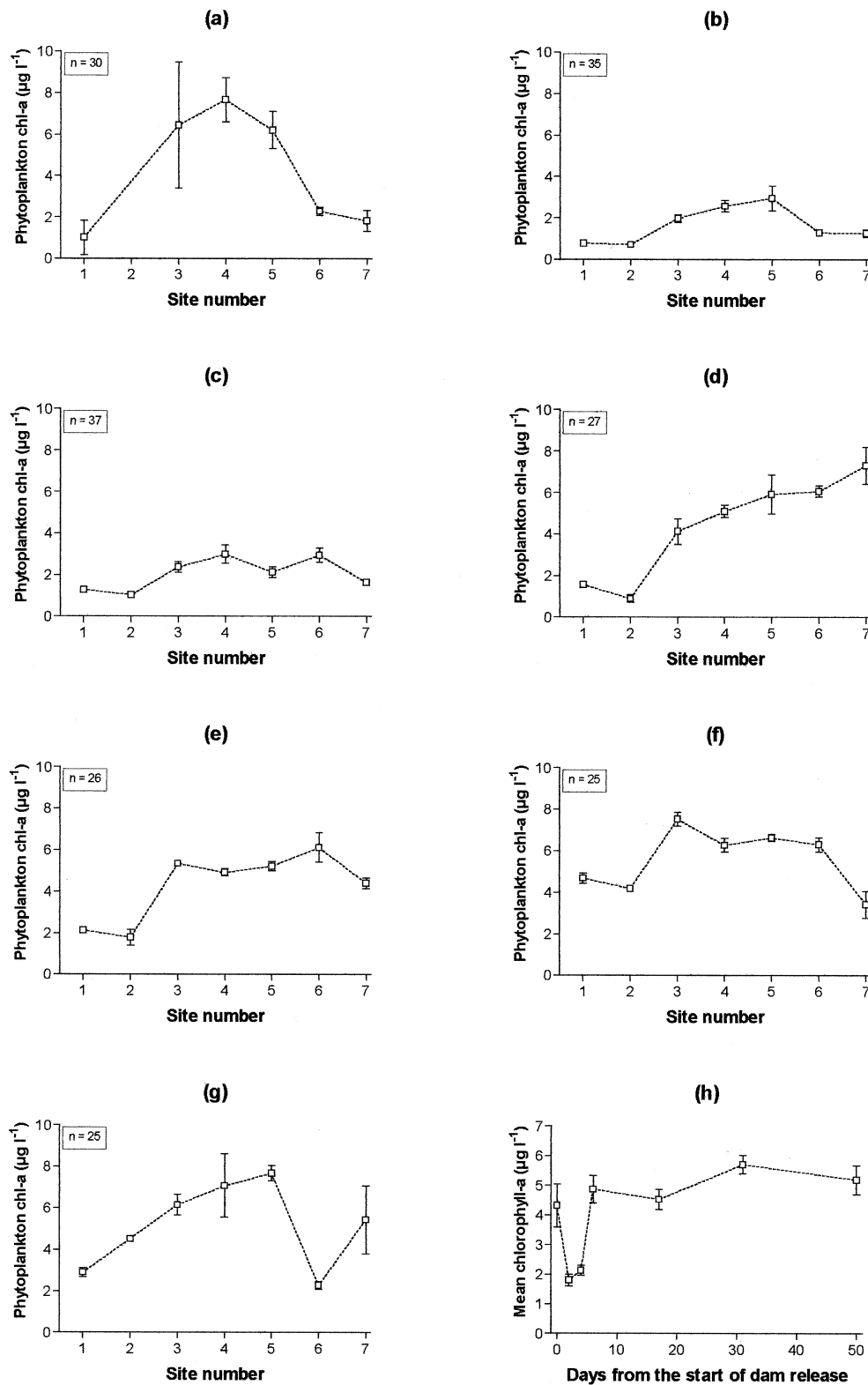
Figure 6

Mean longitudinal phytoplankton chlorophyll a distributions prior to the 2 x 10⁶ m³ Mpofu Dam release [Day 0, (a)] and for the following three spring tidal cycles [Days 2, (b); 4, (c); 6, (d); 17, (e); 31, (f) and 50, (g)]. Figure (h) represents the mean phytoplankton chlorophyll a in the estuary relative to the start of the release (n = 205). Vertical bars represent ± SE mean.

increased significantly since Days 2 and 4 (Tukey's $q = 3.07$ and 2.74 respectively $> q_{0.05, 169, 7} = 1.78$). However, there were no significant changes in the numbers of flagellates (20/11/1998: 2 053 cells·ml^{-1} ± 158 SE and 22/11/1998: 2482 cells·ml^{-1} ± 153 SE) or diatoms (20/11/1998: 152 cells·ml^{-1} ± 22 SE and 22/11/1998: 721 cells·ml^{-1} ± 93 SE) (Fig. 7).

Post-release; 3, 17 December 1998 and 5 January 1999

During the following three spring tidal cycles, salinity in the estuary became well mixed longitudinally and vertically (Figs. 3(e) to 3(g)). The longitudinal salinity gradient had dropped to 1.5‰ by the third spring tidal cycle (Day 50). Nitrate had also become well mixed into the water column along the longitudinal and vertical axes of the estuary (Figs. 5(e)-5(g)). By the second and third spring tidal cycles, the highest nitrate concentrations were in the middle reaches of the estuary and concentrations in the bottom waters often exceeded those in the surface water.

There was no significant increase in average phytoplankton chlorophyll a when these three days were compared to the Day 6, but a maximum of 5.7 µg·l^{-1} ± 0.3 SE was measured on the 31st day after the release (2 spring tidal cycles later). In addition, the average phytoplankton chlorophyll a values on Days 6, 17, 31 and 50 were not significantly greater than prior to the release (Day 0) (Tukey's q in all cases $< q_{0.05, 169, 7} = 1.78$). There were a number of major changes in the numbers of cells over the three spring tidal cycles. A bloom of diatoms, made up of *Extubocellulus* and *Leptocylindrus* spp., occurred one spring tidal cycle after the start of the release. The peak in cell numbers was most apparent at Site 6 (Fig. 7) where there were approximately 11 150 cells·ml^{-1}

throughout the water column. As a result, the average number of diatoms in the estuary was significantly higher on Day 17 than on Days 0, 2 and 4 after the release (Tukey's q in all cases $>q_{0.05, 41, 7} = 2\,940$). The average number of diatoms then gradually decreased by the second (2 602 cells·ml⁻¹ ± 332 SE) and the third spring tidal cycle (2 107 cells·ml⁻¹ ± 307 SE). The average number of flagellates increased significantly by the second and third spring tidal cycles when compared to Days 0, 4, 6 and 17 (Tukey's q in all cases $> q_{0.05, 41, 7} = 1\,205$). The average number of flagellates was 1 983 cells·ml⁻¹ ± 90 SE, 3 300 cells·ml⁻¹ ± 269 SE and 3 292 cells·ml⁻¹ ± 332 SE on Days 17, 31 and 50 respectively after the start of the release (Fig. 7).

Benthic microalgal chlorophyll *a*

Site 7 was not sampled for benthic microalgae due to the rocky substrate. Intertidal benthic chlorophyll *a* was significantly higher during the study than subtidal sites (t = 3.93, DF = 80, p < 0.01) (Fig. 8).

When the average chlorophyll *a* of intertidal sites was compared, Site 1 was significantly higher than Sites 4 and 5 (Tukey's q = 39.8 and 65.5 respectively > $q_{0.05, 35, 6}$ = 39.5), and Site 2 was higher than Site 5 alone (Tukey's q = 57.5 > $q_{0.05, 35, 6}$ = 39.5). The results of the subtidal site comparison exhibited a stronger trend. The average chloro-
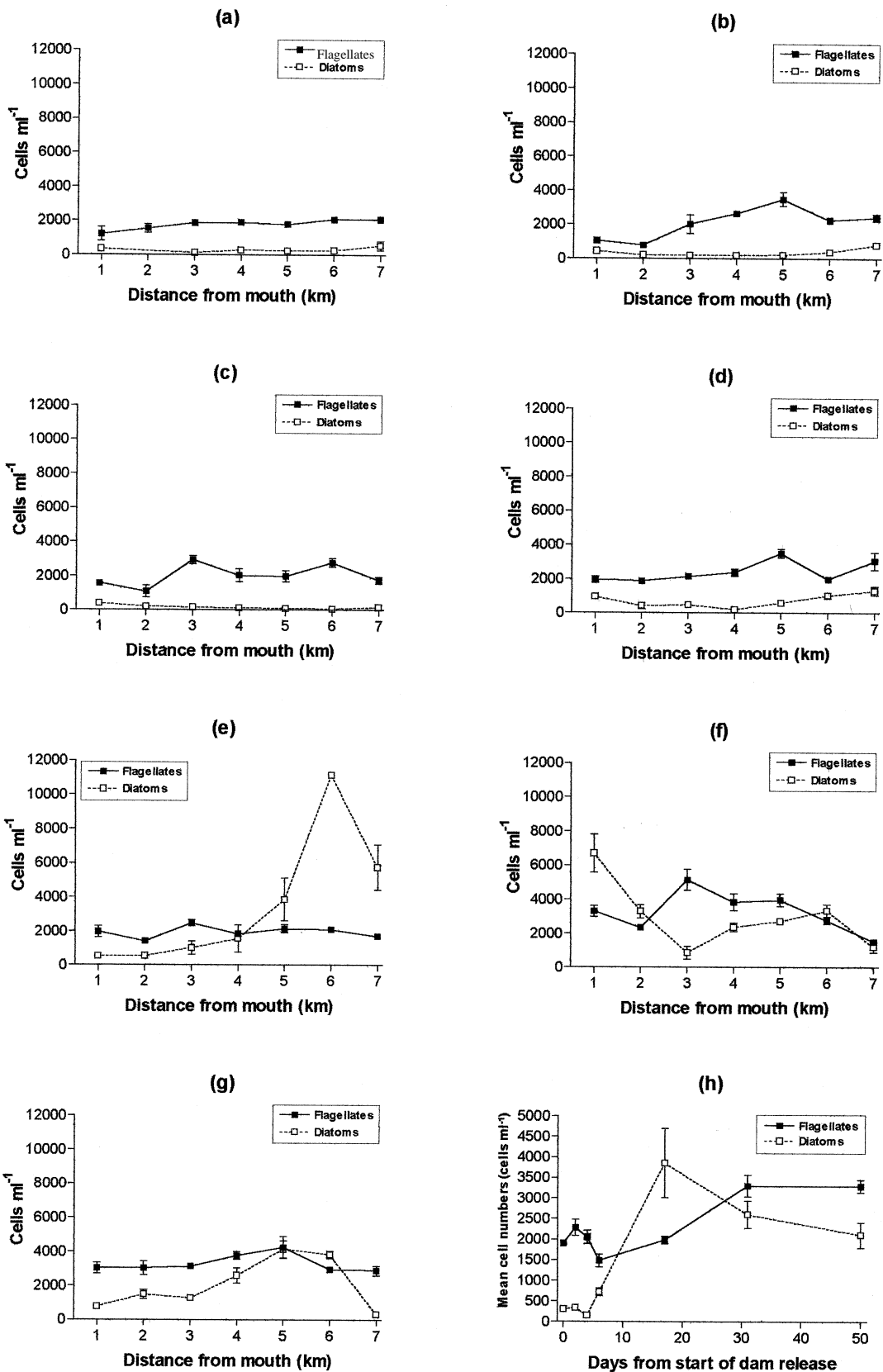
Figure 7

Mean Longitudinal flagellate and diatom distributions prior to the 2 x 10⁶ m³ Mpofu Dam release [Day 0, (a)] and for the following three spring tidal cycles [Days 2, (b); 4, (c); 6, (d); 17, (e); 31, (f) and 50, (g)]. Figure (h) represents the mean flagellate and diatom numbers in the estuary relative to the start of the release (n = 205). Vertical bars represent ± SE mean.
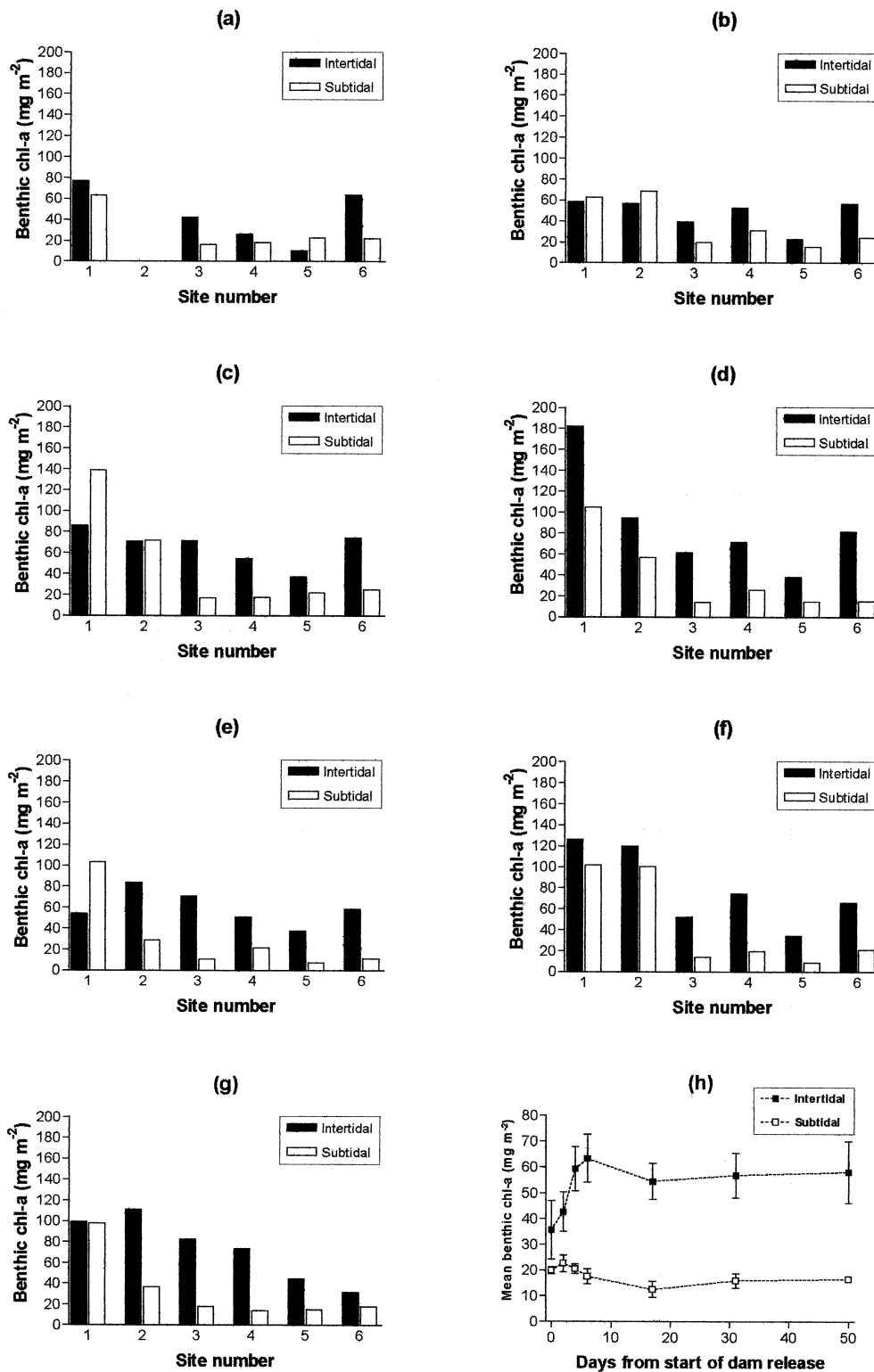
Figure 8

Mean longitudinal benthic chlorophyll a distributions from prior to the 2 x 10⁶ m³ Mpofu Dam release [Day 0, (A)] and for the following three spring tidal cycles [Days 2, (b); 4, (c); 6, (d); 17, (e); 31, (f) and 50, (g)]. Figure (h) Represents the mean benthic chlorophyll a (vertical bars = ± SE mean) of the four uppermost sites relative to the start of the release (n = 140).

phyll a concentrations of Sites 1 and 2 were higher than all other sites (Tukey's q > $q_{0.05, 35, 6}$ = 25.0) but Site 1 was significantly higher than Site 2 (Tukey's q = 35.6 > $q_{0.05, 35, 6}$ = 25.0). There were no significant differences between any of the other sites. Averages of intertidal and subtidal chlorophyll a measurements for each sampling date were compared using one-way ANOVA to determine whether there was any change in chlorophyll a over the entire 50 d sampling period. The results of the analysis showed that there were no significant changes in benthic chlorophyll a (Tukey's q < $q_{0.05, 34, 7}$ = 66.3 (subtidal) and 55.0 (intertidal)) over the study period.

Discussion

Impounding water has pronounced effects on the timing, magnitude and quality of water moving between watersheds and the coastal zone. In addition to the hydrological effects, impounding water traps sediments, accumulates organic matter and converts inorganic nutrients into organic forms (Hopkinson and Vallino, 1995). The annual allocation of 2 x 10⁶ m³ of water from the Mpofu Dam has been released into the Kromme Estuary, as monthly pulses of 1.67 x 10⁵ m³ of water. Only the head salinity was affected by those releases (CERM, 1995).

The earlier pre-release monitoring of conditions in the Kromme Estuary during the monthly water release pattern showed the average salinity in the estuary to be relatively high (up to 33.2 ‰) and the average phytoplankton chlorophyll a only reached a maximum of 5.6 µg·l⁻¹. Flagellates always dominated the phytoplankton resulting in a low average phytoplankton group diversity (H' = 0.088) relative to the nearby Gamtoos Estuary. The Gamtoos Estuary is also a permanently open system but the Gamtoos River still delivers a

substantial amount of the mean annual runoff (50%) and the average base flow was estimated to be just less than 1 m³·s⁻¹ (Schumann and Pearce, 1997). Both diatoms and flagellates dominate the phytoplankton in the Gamtoos Estuary and a much higher group diversity (H' = 0.53) is present.

Just prior to the release of the 2 x 10⁶ m³ from the Mpofu Dam, salinity in the estuary was high, with 33.9‰ recorded at the tidal head of the estuary. The average phytoplankton chlorophyll *a* was similar to the 1997 and 1998 measurements (4.3 μg·l⁻¹) but the phytoplankton group diversity (H' = 0.22) was considerably higher as a result of a higher proportion of diatoms being present. The pulse of freshwater created a strong salinity gradient and a halocline became well developed in the upper reaches of the estuary. This was short-lived and the water in the estuary was mixing again by the sixth day after the release. There was very little salinity gradient by Day 17 after the release. There was still a slight longitudinal salinity gradient 50 d after the release, so it would be expected that under natural flow conditions, a return to a mixed Kromme Estuary takes much longer time than was recorded in the study. A report on a study of a flood event in the Richmond Estuary in New South Wales, Australia, described the gradual change from a small, highly stratified estuary, through a moderately stratified, to a large vertically homogenous regime. It took the saline wedge more than 283 d to penetrate the full 50 km upstream of the mouth (Eyre and Twigg, 1997).

The freshwater release from the Mpofu Dam did introduce some nitrate (up to 28 μM) but this was only measurable for the first 4 d. Average phytoplankton chlorophyll *a* decreased significantly over the 4 d after the release but recovered to just slightly higher than the pre-release concentration by the sixth day. There was no further significant increase in phytoplankton chlorophyll *a* over the rest of the three spring tidal cycles.

Another freshwater-deprived estuary in the Eastern Cape, the Kariega, experiences limited base flow and short-lived flooding events. As a result of the reduced river flow, the estuary is nitrate limited, which has resulted in reduced algal growth (Allanson and Read, 1995). It is possible that the decrease in nitrate after Day 4 in the Kromme Estuary was the result of its being taken up by phytoplankton. There was an increase in phytoplankton biomass from Day 4 to Day 6. Nitrate concentrations were low subsequent to Day 6 and this may have prevented further phytoplankton growth. Wooldridge and Callahan (2000) found no significant increase in endemic copepod populations during the study. It is unlikely that grazing by zooplankton significantly reduced phytoplankton biomass. A more likely possibility is that low phosphate concentrations limited biomass. Scharler and Baird (2000) highlighted this. Phosphate concentrations in the estuary were low throughout the study (<0.5 μM) and were reduced further during the release (18/11/1998 and 20/11/1998) to <0.2 μM. They then increased steadily until 7 days after the start of the release (23/11/1998) when they were once again back to former levels. Average phytoplankton chlorophyll *a* biomass followed a very similar trend to phosphate.

Diatoms increased significantly just one spring tidal cycle after the release and bloomed in the middle-upper reaches of the estuary. Flagellates increased significantly by the second and third spring tidal cycles after the release. Phytoplankton group diversity (H') increased from 0.22 prior to the release to a maximum of 0.38 by the end of the study (50 d). This is the result of the significant increase in flagellate (1 903 cells·ml⁻¹ ± 65 SE to 3 300 cells·ml⁻¹ ± 27 SE) and diatom numbers (301 cells·ml⁻¹ ± 61 SE to 3 856 cells·ml⁻¹ ± 845 SE), as well as the more even spread of cell numbers between these two phytoplankton groups.

There was no significant increase, using Tukey's Multiple Comparison Test, in either average subtidal or intertidal benthic chlorophyll *a* as a result of the release of freshwater. However, intertidal benthic chlorophyll *a* of the upper four sites did show a non-significant rise from before the release (35.6 mg·m⁻² ± 11.4 SE) to a maximum (63.3 mg·m⁻² ± 9.3 SE) within six days after the release. Benthic microalgae have a tendency to grow in patches (Rodriguez, 1993) and this raises the coefficient of variation of benthic chlorophyll *a* data. Hence, although the increase from 35.6 to 63.3 mg·m⁻² was not significant, it is worthy of note. This suggests that the microphytobenthos did respond to increased flow, reduced salinity or the higher concentration of nutrients in the water column. The chlorophyll *a* of benthic microalgae in the Gamtoos Estuary has been shown to reach a maximum at a flow rate of 1 m³·s⁻¹ (Snow et al., 2000). This maximum in biomass at a specific flow rate suggests that in the Gamtoos Estuary benthic microalgae respond primarily to water column nutrients. This is similar to the data of Lukatelich and McComb (1986) who found a significant, positive correlation between benthic microalgal chlorophyll *a* concentration with ammonium, nitrate and organic nitrogen concentrations in the water column.

Conclusions

The original hypotheses addressed were as follows:
The release of 2 x 10⁶ m³ of water from the Mpofu Dam:

(i) *Will result in a fourfold increase (5 to 20 mg·l⁻¹) in average water column (pelagic) chlorophyll a after 3 spring tidal cycles (42 d).*

The highest average phytoplankton chlorophyll *a* biomass was just 5.7 mg·l⁻¹ ± 0.3 SE, measured two spring tidal cycles after the start of the release. The increase was not significant so the hypothesis was rejected. The low water column nitrate, and phosphate concentrations after Day 4 may have restricted further phytoplankton growth.

(ii) *Will change the structure of the phytoplankton groups from being dominated by flagellates, to one where diatoms will be dominant. Later, as the freshwater dissipates and the water column becomes well mixed, flagellates will regain their dominance.*

Both diatoms and flagellates were dominant (>10%) prior to and after the release except on the fourth day when diatoms made up only 7% of the phytoplankton. However, the proportion of flagellates was much higher (85%) than diatoms (13%) before the freshwater release. One spring tidal cycle after the release, diatoms became the most abundant group (66%) as a result of a bloom (*Extubocellulus* and *Leptocylindrus* spp.) but by the second and third spring tidal cycles, flagellates became more abundant (55 and 57% respectively). Phytoplankton group diversity (H') and evenness (J') were greater than 0.16 and 0.048 respectively throughout the study period. Dominance in this study was defined as the group/s that contributed >10% of the phytoplankton. Flagellates were a dominant phytoplankton group throughout the study so the hypothesis was rejected.

iii) *Will result in a twofold increase in average benthic microalgal chlorophyll* a.

Average subtidal benthic chlorophyll *a* did not increase over the sampling period. Intertidal benthic chlorophyll *a* did increase, although not significantly, from 35.6 mg·m⁻² ± 11.4 SE (Day 0) to 63.3 mg·m⁻² ± 9.3 SE (Day 6). The hypothesis was rejected for subtidal chlorophyll *a* but was accepted for intertidal chlorophyll *a*. The intertidal benthic microalgae increased

in biomass during the period that nitrate concentration was highest in the fresher surface water.

The release of 2 x 10⁶ m³ freshwater from the Mpofu Dam did create a salinity gradient and increase mineral nutrients in the estuary. However, the less dense freshwater did not mix well with the deeper saline water, so the effect of the release was short-lived (6 d) and could have been why average phytoplankton chlorophyll a did not increase significantly during the period of the study. Continuous river flow is important to maintain high phytoplankton biomass in estuaries, particularly in the freshwater-seawater interface region (Snow, 2000).

There was a positive and significant response in the numbers of flagellates and diatoms. This resulted in an increase in phytoplankton group diversity. Subtidal benthic microalgae did not respond to the freshwater pulse but the intertidal benthic chlorophyll a did increase, although not significantly, during the first 6 d of the study. The magnitude of the change in estuarine microalgae in response to the release was small and the persistence of change was very short-term, particularly from a salinity gradient and nutrient point of view.

To increase the level of microalgal productivity in the Kromme Estuary, a base flow similar to the nearby Gamtoos estuary appears to be required. It seems that it is essential to introduce nutrients over an extended period, to allow for the highly productive river-estuary interface (REI) region to develop. However, bearing in mind the similar sizes of the two estuaries, this could require up to 32 x 10⁶ m³ (i.e. 1 m³·s⁻¹ or 30% of the MAR) of water to be released from the dam each year.

Acknowledgements

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