Phytoplankton blooms in the Huon Estuary, Tasmania: top-down or bottom-up control?

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The roles of “top-down” and “bottom-up” factors were investigated in terms of their influence on the diatom and dinoflagellate abundances in the microtidal, salt wedge Huon Estuary, Tasmania, Australia. Long-term (1996–2005) changes in Chl a, the peridinin:Chl a ratio and the abundance of autotrophic dinoflagellates were observed to coincide with the warming of regional surface waters. There were significant seasonal differences in pigment-specific net growth rates for Chl a, peridinin and fucoxanthin. Diatoms dominated the spring bloom when species such as Skeletonema costatum had the highest net growth rates and fucoxanthin-specific gross growth rates were 10^0.9 day^{-1}. During late summer, the peridinin-specific grazing mortality was significantly less than the fucoxanthin-specific grazing mortality and dinoflagellates increased their dominance of the phytoplankton community. This late summer relaxation of grazing pressure on dinoflagellates was associated with a decline in the overall abundance of microheterotroph (MH) grazers and a peak in the abundance, biomass and estimated grazing rates of mesozooplankton. We suggest the composition of the autumn phytoplankton community was dependent upon a trophic cascade where mesozooplankton, such as Noctiluca scintillans, preyed upon MHs and reduced their grazing upon some species of dinoflagellates.

INTRODUCTION

There are a large number of factors operating over a range of time and space scales that may determine the composition of phytoplankton communities. Across many coastal ecosystems an average of ~60% of daily primary production is grazed by microheterotrophs (MH) with a number of observations of grazing losses of ~100% per day (Calbet and Landry, 2004). Some grazing-dilution experiments have demonstrated that this grazing can be preferential (e.g. Gaul and Antia, 2001). Thus we know that grazing by MH is, potentially, a major determinant of phytoplankton community composition.

The formation of a phytoplankton bloom requires that favourable conditions are persistent for sufficient time such that net growth (μ_{net}) exceeds losses and biomass accumulates:

\[ \mu_{\text{net}} = \mu_{\text{gross}} - L + ai \]

where \( \mu_{\text{gross}} \) is the gross growth rate, \( L \) denotes losses (including respiration, grazing, sinking and advection) and \( ai \) are advective inputs. The forcing factors that have a high degree of control on \( \mu_{\text{gross}} \) are well known and include irradiance, temperature and nutrient availability. Any single factor will depend upon several other factors, e.g. irradiance is influenced by surface insolation, water column light attenuation, mixing depth and rate of vertical mixing. These factors are, in turn, influenced by a range of physical forcings, such as seasonal insolation, cloud cover, runoff and wind, which can all vary at different time scales. The environmental
heterogeneity arising from a multiplicity of interacting factors promotes species diversity (Ives and May, 1983) making phytoplankton ecology difficult to predict (Flynn, 2005) and may explain the paradox of the plankton (Hutchinson, 1961). Complexity does not, however, explain low-diversity blooms. Monospecific blooms require persistent conditions that are favourable to a particular taxon (Huisman et al., 1999).

Of the primary forcing factors influencing $\mu_{\text{max}}$ (irradiance, temperature and nutrients) temperature is normally considered to “set” $\mu_{\text{max}}$ while the photon flux and nutrient availability can act to reduce $\mu$ below $\mu_{\text{max}}$ (Baird et al., 2001). Simple models where growth is limited by one factor can predict phytoplankton community dominance (Tilman, 1977; Fasham et al., 1990). Spring blooms may be an example of limitation by a single factor (low irradiance during winter) leading to dominance by a limited community (Smetacek, 1985; Litchman, 1998; Butterwick et al., 2005). A competitive advantage under low light can be used in more complex models to predict community composition of blooms (Huisman et al., 1999; Jin et al., 2006; Litchman et al., 2006). Blooms of a range of taxa including small flagellates, dinoflagellates, cyanobacteria or diatoms (Tett et al., 1986; Mallin et al., 1991) during other times of the year are, arguably, less predictable. Similarly, the competitive advantages that allow a number of taxa with a relatively low $\mu_{\text{max}}$ (e.g. Emiliania, Aureococcus, Prymnesium, Gymnodinium) to form blooms are largely unknown. The possible mechanisms whereby taxa with a relatively low $\mu_{\text{max}}$ might dominate an ecosystem include:

(i) advective inputs that are positive, high and taxon-specific
(ii) losses (L) that are taxon-specific, e.g. sinking or differential grazing
(iii) taxa that overcome resource limitation(s) which adversely impact other taxa, e.g.:
   (a) intercepting more irradiance (e.g. are closer to the surface)
   (b) finding an alternative source of some limiting nutrient (e.g. using $N_2$ rather than dissolved inorganic nitrogen [DIN] or vertical migration for DIN)
   (c) having a different limiting nutrient

Given the time it takes for large blooms to develop, especially for taxa with low growth rates, the mechanism must be pervasive, considerable and sustained. It will be necessary to quantitatively investigate these mechanisms to make phytoplankton ecology a more predictable science.

In the case of the Huon Estuary, Tasmania, Australia, there are summer and autumn dinoflagellate blooms of *Ceratium* species and the chain forming *Gymnodinium catenatum*. Both taxa have maximum growth rates that are ~10% of the co-existing diatoms (at saturating irradiances and the prevailing temperatures). Blooms of *G. catenatum* have forced the closure of regional shellfish harvesting (Hallegraeff et al., 1995). Our investigations have focused on whether the phytoplankton community composition in the Huon Estuary is “top-down”-controlled by MH grazing or “bottom-up”-controlled by nutrient availability and local, or regional, water column physics. Top-down control, particularly in terms of its impact on species diversity was (Sherr and Sherr, 1994), and still is, poorly studied in phytoplankton ecology. The results from our grazing rate experiments are considered in the context of nine years of observations in the estuary and 60 years of regional water column sampling.

**METHODS**

**Background ecosystem characterization**

Several different sampling programmes running from 1944 through 2005 have provided data for analysis of the regional physics and the local phytoplankton dynamics. Sample locations (Fig. 1), sampling duration and intensity have varied; being monthly at one site (1944–2005), approximately weekly at five sites (1996–1998), monthly at a variable number of sites (2000–2005) or quarterly at ~20 sites (1996–1997). Sampling consisted of conductivity–temperature–depth (CTD) casts and either discrete bottle samples at fixed and variable depths, or integrated samples using a 12-m long tube. Samples for cell counts were preserved in acid Lugol’s solution (Parsons et al., 1984). Samples for pigments were filtered onto Whatman GF/F filters (nominal pore size ~0.7 μm) and held in the dark at ~176°C prior to analysis. Nutrient samples were stored at ~20°C prior to analysis. Analytical techniques for nitrate and/or nitrite (Wood et al., 1967), silicate (Murphy and Riley, 1962) and phosphate (Armstrong, 1951) were adapted and performed using Quick-Chem™ methods on a flow injection LACHAT® instrument as per the following protocols for nitrate and/or nitrite (Quick-Chem™ Method 31-107-04-1-A; detection limit ~0.03 μM), silicate (Quick-Chem™ Method 31-114-27-1-D; detection limit ~0.05 μM) and phosphate (Quick-Chem™ Method 31-115-01-1-G; detection limit ~0.02 μM). Ammonium was measured using the technique of Kerouel and Aminot.
Pigment analysis

Most pigment samples were analysed by high-performance liquid chromatography (HPLC) using methods developed by Wright et al. (1991) for extraction and gradient elution. The separated pigments were detected at 436 nm and identified against standard spectra using Waters Empower™ software. Concentrations of all pigments were determined from standards (Sigma™ or DHI Denmark). Primary pigments identified by HPLC included: Chl $c_1+c_2$, peridinin, 9'-cis-neoxanthin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, prasinoxanthin, violaxanthin, diadinoxanthin, alloxanthin, diatoxanthin, lutein, zeaxanthin, Chl $b$, Chl $a$, $\beta$, $\varepsilon$-carotene and $\beta,\beta$-carotene. Some samples were extracted and analysed for Chl $a$ spectrophotometrically using published methods (Parsons et al., 1984) and equations (Jeffrey and Humphrey, 1975).

CHEMTAX program

Pigment data were further analysed using CHEMTAX (Mackey et al., 1996) with an input matrix of pigment ratios derived from published literature (Mackey et al., 1996; Lewitus et al., 2005; Latasa, 2007). Samples collected weekly at five sites from 1996 to 1998 (Fig. 1) at three depths and using an integrating tube ($n = 1074$)
were analysed by HPLC (as above). Monthly averages of Chl \(a\), Chl \(b\), peridinin, 9’-cis-neoxanthin, 19’-butanoyloxyfucoxanthin, fucoxanthin, 19’-hexanoyloxyfucoxanthin, prasinoxanthin, violaxanthin, diadinoxanthin, alloxanthin, lutein, zeaxanthin, Chl \(b\) concentrations were used as input for CHEMTAX analysis using initial ratios from the Southern Ocean (Mackey et al., 1996). All final pigment ratios were consistent with literature values except Chl \(a/b\), which deviated up to 12% from initials if split across multiple Chl \(b\)-containing taxa. This problem was resolved by pooling all Chl \(b\)-containing taxa into one taxon, the Chlorophyta.

**Cell counts (including grazing experiments)**

The preserved samples were transferred to 1-L measuring cylinders, measured and allowed to settle for at least 24 h. After this time, approximately 90% of the volume was siphoned-off and the remaining sample was transferred to a 100-mL measuring cylinder, measured and again allowed to settle. After at least 24 h, approximately 90% of the volume was siphoned-off and the final volume recorded before the remaining sample was thoroughly mixed and an aliquot was taken for counting. The aliquot was put into a 1-mL Sedgwick Rafter counting chamber and examined using an inverted microscope. For most microplankton (cells generally larger than 20 \(\mu\)m diameter), at least 10% of a single slide was enumerated at 100× magnification (except when there were dense blooms of one or more microplankton species, when at least 20% was scanned). For nanoplankton (2–20 \(\mu\)m in diameter), the chamber was examined at 400× magnification until at least 200 cells of the dominant nanoplanlankton “taxon” had been counted. Small flagellates (<10 \(\mu\)m in diameter) in the nanoplankton were assigned to a single group (reported as “small flagellates”). From the grazing experiments, two entire chambers (2 mL) representing plankton concentrated from ~200 mL were enumerated at 100× magnification for MHs (20–200 \(\mu\)m in diameter) and larger phytoplankton species (>20 \(\mu\)m in diameter), such as G. catenatum. MHs, including non-photosynthetic dinoflagellates, were identified by reference to literature and corroborated by examining hundreds of live samples where pigmented versus non-pigmented cells were easily distinguished.

**Grazing experiments**

Analysis of 1996–1998 data (CSIRO Estuary Study Team, 2000) indicated that 27 sites in the lower Huon Estuary were spatially homogenous in terms of pigments. Six experiments were conducted at one location (Fig. 1) in the lower estuary during September 2003, November 2003, February 2004, July 2004, April 2005 and September 2005. A Seabird 19+ CTD was used to collect a vertical profile of temperature, conductivity, dissolved oxygen and fluorescence prior to sampling. For grazing experiments, samples were obtained from the Chl \(a\) maximum, which ranged from 0 to 7 m.

Grazing rates were measured using the technique developed by Landry and Hassett (1982), which requires a series of increasingly diluted seawater samples. We have modified this method using HPLC analysis of pigments and cell counts to achieve estimates of grazing on specific taxa. We used 2.4-L bottles and three replicates at 100%, 70%, 40% and 10% seawater (SW) diluted with 0.2 \(\mu\)m filtered site water obtained ~2 h prior to the collection of the samples and the commencement of the experiment. Water for the experiment was collected in 10-L Niskin bottles and gently mixed into a 50-L container. Three sub-samples at time zero \(T_0\) were immediately collected and preserved. These were used to describe the initial conditions. Sample water was added to bottles already containing appropriate amounts of 0.2 \(\mu\)m filtered water and then incubated for 24 h under *in situ* conditions. After incubation, samples were filtered and analysed for pigments (as above), and the concentrations used to calculate the apparent growth rate \(\mu_a\):
with 95% CI calculated as $SE \times t$ (with $df = 11$ and $t = 2.201$). An *a priori* decision was made to limit statistical comparisons to reduce the chance of a type I error. Statistical comparisons of growth rates, grazing rates and net growth rates for Chl $a$, fucoxanthin and peridinin were undertaken using general linear modelling followed by Bonferroni-corrected $t$-test to identify which temporal periods and pigments were significantly different. Within a single experiment, rates of grazing and growth were assessed by comparing intercepts and slopes from the least-squared lines of best-fit using conventional techniques (Armitage and Berry, 1994). The outcomes of statistical comparisons were considered significant if the probability ($P$) of the null hypothesis was $< 0.05$.

### Mesozooplankton

Zooplankton were collected from three sites in the Huon Estuary from October 2004 to October 2005 (Fig. 1). A Bongo net (mesh size 200 $\mu$m and mouth diameter 0.75 m) was towed at three knots for 3 min. The net was deployed to 20 m. After the tow, the nets were washed thoroughly with sea water and the catch collected in the closed cod-end. The catch was preserved with 10% formaldehyde and stored until sorted. In the laboratory, samples were split so that 400–1000 animals were counted per sample. All organisms were identified to some taxonomic level with species resolution for all copepods. Additional animals were collected on several sampling dates for the determination of carbon biomass. One to 50 freshly caught individuals (depending on size) of each common species were sorted, rinsed in filtered seawater followed by 300 $\mu$L distilled water, and dried at 60°C until constant mass was attained (Mettler microbalance; $\pm 1$ $\mu$g). Only animals that appeared intact when examined under a stereomicroscope were used. Specimens were analysed for CHN on a Thermo Finnigan EA 1112 Series Flash Elemental Analyser (Central Science Laboratories, University of Tasmania, Australia). Total carbon biomass (mg C m$^{-3}$) was determined by multiplying abundance by carbon mass for each taxon and summing the results. The empirical relationship demonstrated by Huntley and Boyd (1984) to explain 75% of the variation in growth rate for small zooplankton was used to estimate potential growth of the mesozooplankton:

$$G'_{\text{max}} = \left( \frac{1}{W} \right) \frac{dW}{dt} = 0.0542e^{0.1107T}$$

where $W$ is the carbon-specific growth rate of the mesozooplankton, $T$ the temperature and $G'_{\text{max}}$ is their
maximum mass-specific food-saturated growth rate. This was combined with the estimate of 70% assimilation efficiency (Conover, 1978) to estimate the assimilative capacity of the mesozooplankton:

\[
\text{Assimilative capacity} = 0.7G_{\text{max}}
\]

RESULTS

Environmental dynamics at the regional and local scale

Long-term (1944–2005) regional data from Maria Island were binned by month and showed a strong seasonal trend with average surface temperature rising from \(\sim 12^\circ\text{C}\) in August and September to highs of \(\sim 17^\circ\text{C}\) in mid-February (Fig. 2A). Subtracting the averaged monthly seasonal temperature from all observations indicated a weak \((r^2 = 0.12)\) but highly significant \((P < 0.001)\) positive linear relationship between time and deviation from average (Fig. 2B). The locally weighted regression indicates that the rate of temperature increase may not have been constant (Fig. 2B) and over the time period from 1996 to 2005, when there are local phytoplankton data, the regression is significant \((r^2 = 0.40, P = 0.002)\) and steeper than the long-term average (Fig. 2B). There were significant differences in the relationships between July and January temperatures over time (Fig. 2C). Winter temperatures have remained constant while summer temperatures are increasing. From this we conclude that the regional warming is seasonal and that the spring increase in temperature is faster now than 60 years ago.

Long-term average rainfall in the catchment as measured at Strathgordon (42.77°S, 146.04°E, period 1968–2004) and the Huon River flow were maximal in August and minimal in February (Fig. 3A and B). Mean monthly rainfall was highly correlated with the river flow averaged over the same month (Fig. 3C). During the period from 1996 to 1998, river flow and rainfall were somewhat different from the long-term means with a drier period from May to June (Fig. 3A and B). Representative vertical profiles for temperature and salinity from the centre of the estuary shows some vertical structure during every season (Fig. 3D and E). Stratification tended to be greatest in winter when a relatively thin \((\sim 1 \text{ m})\) layer of low-salinity water \((21\text{ practical salinity units})\) was present at the surface. Both spring and autumn had slightly deeper surface mixed layers of reduced salinity. Stratification was weakest in summer and then only sporadically present such that a relatively deep surface mixed layer \((15 \text{ m})\) developed.

Fig. 2. Time-series analysis of the water column temperature at Maria Island (see \(\Delta\) of Fig. 1) from 1945 to 2005. (A) Surface temperature data binned by month and fitted to a Gaussian curve, (B) Surface temperature—average monthly mean (from A) and fitted to a locally weighted (l.w.) regression (dashed line), a linear regression from 1944 to 2005 (solid line, \(P = 0.012\)) and a linear regression from 1996 to 2005 (thick grey line, \(P = 0.002\)). (C) Linear regressions fitted to surface temperatures from January (solid line, \(P = 0.008\)) or June (dotted line, \(P = 0.615\)) over the period 1944–2005.

Weekly samples from 1996 to 1998 at five sites and three depths (surface, subsurface and \(\sim 2 \text{ m}\) above bottom, where both the subsurface and near-bottom depths were variable across sites but overall mean
Fig. 3. Background environmental data on the Huon Estuary. (A) River run off (1986–1999) and during the period of intensive sampling (1996–1998). (B) Mean monthly rainfall (+) in catchment at Strathgordon (42.77°S, 146.04°E, period 1968–2004) and local daily rainfall at Geeveston (●) during 1996–1998, grey line is the running median. An unusual local rainfall event in January is circled. (C) Mean monthly rainfall from Strathgordon plotted versus mean monthly Huon River flow at Frying Pan Creek. Representative salinity (D) and temperature (E) profiles from (p) on Fig. 1. (F) Nutrient concentrations measured weekly 1996–1998 at five locations, three depths (surface, mid-depth and 2 m above bottom) and plotted as running medians, for NO₃+NO₂ the individual (five sites × three depths) values are also shown.
depths were 0, 2.5 and 15 m) were pooled by season and analysed for vertical and temporal trends in nutrient concentrations. Nitrate, phosphate and ammonium concentrations showed statistically significant effects of season and depth ($P < 0.001$, two-way ANOVA, data were not homogenous or normally distributed). Concentrations of all three increased significantly with depth while silicate concentrations were greatest at the surface (annual mean = 16.5 µM) and significantly (Kruskal–Wallis, $P < 0.001$) lower near the bottom (annual mean = 3.2 µM). Temporal trends were consistent across all depths and are presented as running medians of depth-averaged concentrations (Fig. 3F), with the exception of nitrate where all data are shown. There were no significant seasonal patterns in silicate concentrations. Depth-averaged nitrate and phosphate concentrations peaked in winter (Fig. 3F). Mean concentrations. Depth-averaged nitrate and phosphate concentrations were not homogenous or normally distributed). Nitrate, phosphate and ammonium concentrations showed statistically significant effects of season and depth ($P < 0.001$) lower near the bottom (annual mean = 3.2 µM). Temporal trends were consistent across all depths and are presented as running medians of depth-averaged concentrations (Fig. 3F), with the exception of nitrate where all data are shown. There were no significant seasonal patterns in silicate concentrations. Depth-averaged nitrate and phosphate concentrations peaked in winter (Fig. 3F). Mean summer surface concentrations of nitrate (0.15 µM) and phosphate (0.21 µM) were significantly less than other seasons (Kruskal–Wallis, $P < 0.001$ for both). Depth-averaged ammonium concentrations peaked strongly in autumn (Fig. 3F). On average, the DIN (DIN = NO$_3$+NO$_2$+NH$_4$) to dissolved inorganic phosphate (PO$_4$) ratio in surface waters was 1:1 and the DIN to dissolved silicon ratio was 1:33.

Light attenuation, measured as Secchi disk depth (SDD), is an exponential function of surface salinity ($s$):

$$SDD = 1.2 + 0.0249e^{-0.157}$$

where $r^2 = 0.89$, $P < 0.001$, $n = 495$ (all measurements with both SDD and surface salinity). The mean SDD was 3.7 m ($n = 871$); assuming that light attenuation ($k$) falls between 1.7/SDD (Poole and Atkins, 1929) and 1.13/SDD+0.10 (Holmes, 1970) and the 1% euphotic depth = 4.61/k, the mean euphotic depth would be ~11 m. Monthly mean SDD was greatest during January at 5.1 m ($n = 40$) falling to a median of 1.8 m ($n = 84$) during August. Mean SDD increased from 0.9 m at Huonville to 6.9 m over the ~35 km distance to the mouth of the Estuary or ~0.2 m km$^{-1}$ (linear regression, $P < 0.01$). Thus, the euphotic depth would be 0.4 m at Huonville and 20 m at Huon Island. At these locations, the water column was ~3 and 40 m (respectively), and thus the ratio of euphotic depth to overall depth was ~1:10 increasing to 1:2 down the estuary.

Characterization of the phytoplankton by light microscopy

Cell counts from the weekly samples including an integrated sample and three discrete depths at five sites, similar depths sampled quarterly from 20+ sites from 5 November 1996 to 5 October 1998 and integrated samples from 15 sites sampled monthly from 2002 to 2005 were pooled across depths and binned by month to examine seasonal trends. Sampling intensity varied such that the minimum number of samples in any one month was 41 (October) and the maximum was 143 (April). Numerically, the dominant “taxon” was a relatively constant background of small flagellates ($<10$ µm) with an average density of $451 \pm 99 \times 10^3$ L$^{-1}$. The seasonal dynamics, in terms of taxa identified to genera or species, were dominated by two diatoms, *Pseudonitzschia* and *Chaetoceros* (Fig. 4A). Densities of both rose sharply during a spring bloom to $>10^3$ L$^{-1}$ falling in late November and recovering to $>10^2$ L$^{-1}$ again between February and March. Cell densities of the two numerically dominant dinoflagellates, *Centratis* spp. and *G. catenatum* tended to be lowest in winter and early-spring reaching peaks in December and May (Fig. 4A).

There were also inter-annual differences in abundances. Data from the two stations sampled in 1997 ($n = 78$) and 1998 ($n = 51$), that were sampled again in 2004 and 2005 ($n = 21$), were compared. The data were pooled into seasons and then analysed for differences by three-way ANOVA (year, season and station) followed by Bonferroni $t$-tests for difference between years with 1996–1997 as the control year. *Centratis* spp., *Chaetoceros* spp. and small flagellates were all less abundant in 1996–1997 than 1997–1998. *Centratis* spp., small flagellates and *Skeletonema costatum* were more abundant in 2004–2005 than 1996–1997 and *Pseudonitzschia* spp. were less abundant. Mean annual cell density of *G. catenatum* was greatest in 1997–1998, but variability between stations was high and no statistically significant temporal pattern was observed (Table 1). Fifteen stations in D’Entrecasteaux Channel and the Huon Estuary (Fig. 1) were sampled monthly during 2002–2003 revealing a regional spring bloom of *S. costatum*, a summer bloom of *Centratis* spp. and an autumn bloom of *Pseudonitzschia* spp. (Fig. 4A). Calculating in situ growth rates of individual taxa on a regional basis (across all stations) indicated that all these blooms took $\geq$2 months to develop such that net in situ growth rates could be calculated across three or four data points. The in situ net growth rate for the spring bloom of *S. costatum* was 0.038 day$^{-1}$, the summer bloom of *Centratis* was 0.035 day$^{-1}$ and the autumn bloom of *Pseudonitzschia* was 0.045 day$^{-1}$.

Two offshore transects, in October and November 2004, collected surface phytoplankton samples from 20, 40, 60, 80, 100 and 150 km offshore (Fig. 1). All of the four dominant taxa were found offshore but at lower densities. During October–November 2004, the offshore
Fig. 4. Phytoplankton dynamics from D’Entrecasteaux Channel and the Huon Estuary. (A) Seasonal trends in the densities of the dominant diatoms and dinoflagellates (error bars +1 SE) from the Huon Estuary (five stations, three depths and integrated samples collected weekly and 20+ sites sampled quarterly from 1996 to 1998, data pooled by month). (B) Seasonal trends (dashed line) in monthly mean Chl a concentrations (+1 SE) and estimates of community composition derived from high-performance liquid chromatography pigments and CHEMTAX (Mackey et al., 1996) analysis, sampling as in (A). (C) Regressions of Chl a versus peridinin (n = 1074) and Chl a versus fucoxanthin (n = 1074), sampling as in (A). Note that latter regression line is indistinguishable from the x-axis. (D) Cell densities for dominant species or genera from 12 sites in Huon Estuary and D’Entrecasteaux Channel during July 2002 to March 2003, plotted versus month. Ceratium and G. catenatum densities on the right-hand axis, all others on left. Large open symbols indicate data used to calculate in situ growth rates of populations (see text), ○ denotes S. costatum, □ denotes Ceratium, ◊ denotes Pseudonitzschia.
Table I: A comparison of species densities at two stations in 1996–1997 (n = 78), 1997–1998 (n = 51) and the same two stations (Fig 1) again in 2004–2005 (n = 21) with sampling by integrating tube. Years were defined as August to July (e.g. 1996–1997 = August 1996 to July 1997), data were binned by season (winter, spring, summer and autumn) and data analysed by three-way ANOVA followed by Bonferroni t-tests to detect significant differences between stations, seasons and years. Underlined values are significantly different from 1996 to 1997 (P < 0.05), italics (P < 0.01), bold (P < 0.001).

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<td>Ceratium spp.</td>
<td>0.61</td>
<td>7.18</td>
<td>15.3</td>
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<tr>
<td>Pseudonitzschia spp.</td>
<td>68.2</td>
<td>107.7</td>
<td>107.7</td>
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<td>Chaetoceros spp.</td>
<td>25.0</td>
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<td>Gymnodinium ccatenatum</td>
<td>0.01</td>
<td>40.8</td>
<td>4.7</td>
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<tr>
<td>Small flagellates*</td>
<td>424</td>
<td>674</td>
<td>934</td>
</tr>
<tr>
<td>Skeletonema costatum*</td>
<td>0.12</td>
<td>0</td>
<td>21.8</td>
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*Failed Kolmogorov–Smirnov test for normality and passed the Levene median test for equal variance.

Characterization of the phytoplankton in the Huon Estuary by pigments

To assess the relative biomass of the various taxa, the marker pigments strongly associated with particular phyta or class of phytoplankton were used to assign a portion of the Chl a to a particular taxon by the matrix factorization program CHEMTAX (Mackey et al., 1996). Data from 1074 samples collected weekly at five sites from 1996 to 1998 were pooled by month and analysed to examine seasonal trends. CHEMTAX output was very consistent with literature-based ratios for all taxa except those containing Chl b. In Chl b-containing taxa, the Chl a allocations were distorted up to 12% outside initial values making the allocations to Euglenophyceae, Prasinophyceae and Chlorophyceae less reliable. To overcome this problem, all Chl b-containing taxa were summed and presented as chlorophytes (Fig 4B). The proportion of Chl a that was estimated as diatoms peaked at 53% during the spring bloom (October). Dinoflagellates reached monthly averages of 47, 57 and 54% during December, April and May. Based on CHEMTAX, the abundant <10 μm cells were largely cryptophytes (annual average ~11%), haptoptophytes (annual average ~15%), chlorophytes (annual average ~11%) and cyanobacteria (annual average ~2%). The haptoptophytes were most abundant in December when coccolithophorids blooms are regularly visible in regional SeaWiFS images (P Thompson, Hobart, personal observation).

The monthly mean Chl a concentrations ranged from 0.38 to 2.9 μg L^-1, but spatial and temporal variability (all samples from 1996–1998) were quite high with some 8% of samples having >3 μg L^-1. The dominance of dinoflagellates over diatoms in these high biomass samples can be assessed by plotting Chl a versus fucoxanthin and Chl a versus peridinin (Fig 4C). The slope of the Chl a versus peridinin relationship was 0.26:1 (r^2 = 0.90, P < 0.001, n = 1329) and if dinoflagellates have a weight to weight ratio of peridinin:Chl a ratio of 0.36:1 (Hallegraeff et al., 1991), then dinoflagellates averaged ~72% of the biomass in these dense patches. These dinoflagellates were not uniformly distributed throughout the water column with peridinin being significantly greater at intermediate depths relative to the surface. The weekly sampling from five locations during 1996–1998 indicates significant (Kruskal–Wallis one-way ANOVA on ranks, P < 0.001) differences in the overall median proportion of dinoflagellates at the surface, mid-depth and bottom of 0, 16 and 11% of total Chl a, respectively (assuming peridinin:Chl a = 0.36). Similarly, the peridinin:Chl a ratio peaked in mid-depth samples during October and in near-bottom samples in January. The zero slope of the Chl a versus fucoxanthin relationship suggests that diatoms and other fucoxanthin-rich taxa were not important components of the phytoplankton community in the highest density patches (Fig 4C).

Inter-annual comparisons were possible using three stations (Fig 1) sampled in 1996–1997 (n = 160), 1997–1998 (n = 159) and again in 2004–2005 (n = 41) with sampling by integrating tube and pigment analysis by HPLC. The data were binned by season (winter, spring, summer and autumn) and analysed by three-way ANOVA followed by Bonferroni t-tests to detect significant differences between stations, seasons and years (Table II). Mean annual concentrations of Chl a, peridinin, 19'-butanoyloxyfucoxanthin, fucoxanthin, 19'-hexanoyloxyfucoxanthin, prasinoxanthin, diadinoxanthin and allophycocyanin all increased significantly...
Table II: A comparison of pigments and pigment ratios at three stations in 1996–1997 (n = 160), 1997–1998 (n = 159) and the same three stations (Fig 1) again in 2004–2005 (n = 41) with sampling by integrating tube, pigment analysis by high-performance liquid chromatography. Years were defined as August to July (e.g. 1996–1997 = August 1996 to July 1997), but were pooled by season (winter, spring, summer and autumn) and data analysed by three-way ANOVA followed by Bonferroni t-tests to detect significant differences between stations, seasons and years. Underlined values are significantly different from 1996 to 1997 (P < 0.05), italics (P < 0.01), bold (P < 0.001).

<table>
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<td>0.07</td>
<td>5991</td>
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<td>0.000</td>
<td>0.000989</td>
<td>15.514</td>
<td>0.011</td>
<td>0.012</td>
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<tr>
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<td>0.290</td>
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<td>0.016</td>
<td>0.108</td>
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*Failed Kolmogorov–Smirnov test for normality and passed the Levene median test for equal variance.


Grazing experiments: gross growth rates, mortality and net growth rates

Samples collected for the grazing experiments had low concentrations of Chl a in spring and greater concentrations in autumn (Fig 5A). From September 2003 to February 2004, Chl a concentrations increased approximately four times from 0.36 to 1.32 μg L⁻¹, fucoxanthin by seven times and peridinin by approximately eight times, giving in situ pigment-specific net growth rates of 0.008, 0.013, 0.013 day⁻¹, respectively (note that these are lower than growth rates for specific taxa estimated from cell counts and monthly sampling). Dinoflagellates reached their greatest proportion of total Chl a (17%, from CHEMTAX) in February 2004. Phytoplankton diversity was significantly greater in summer (P < 0.001) than during other times of the year (Fig 5A) rising by a factor of 10 between early-spring and late-summer.

During the grazing experiments, dissolved inorganic nutrient (NO₃, NO₂, SiO₄, PO₄) concentrations were measured on the initial seawater at T₀ and T₂₄ and the diluent at T₀ and T₂₄. The T₀ and T₂₄ concentrations of nutrients were: phosphate (range = 0.14–0.89 μM), silicate (range = 0.81–18.3 μM), nitrate (range = 0.03–5.7 μM) and ammonium (range = 0.03–0.67 μM). The low nitrate concentrations during November and February in the T₀ and T₂₄ seawater samples raise some concerns about possible artefacts associated with nutrient limitation during the 24-h incubations. Growth rates during experiments conducted in November, February and March, however, were higher than those at other times of the year suggesting no negative effect of low nutrient concentrations on the experimental results.

Using the grazing-dilution technique (Landry and Hassett, 1982), the average annual gross Chl a-specific growth rate (μg) was estimated to be 0.87 day⁻¹ (Fig 5B), significantly greater (P = 0.012) than the peridinin-specific gross growth rate (0.31 day⁻¹), but not significantly (P = 0.929) different from the fucoxanthin-specific gross growth rate of (0.72 day⁻¹). For Chl a, peridinin and fucoxanthin, the average pigment-specific gross growth rate was 0.15 day⁻¹ in September 2003 rising significantly (P = 0.020) to 1.03 day⁻¹ in February 2004 and then falling significantly (P = 0.034) to 0.22 day⁻¹ by July 2004 (Fig 5B). The highest pigment-specific gross growth rate was 1.49 ± 0.04 day⁻¹ for fucoxanthin in February 2004.

Averaged over all sample periods, the pigment-specific mortality (m) rates were least for peridinin (~0.24 day⁻¹), greater for fucoxanthin (~0.33 day⁻¹) and greater again for Chl a (~0.61 day⁻¹) although quite variable and not significantly different (P = 0.067). The average pigment-specific mortality rates of Chl a, fucoxanthin and peridinin convert (after Stelfox-Widdicombe et al., 2000) into estimates of daily pigment-specific consumption rates of new production.
equalling 79, 56 and 79%, respectively. The average daily pigment-specific mortalities of “standing stock” were 39, 20 and 16% for Chl $\alpha$, fucoxanthin and peridinin, respectively (not significantly different, $P = 0.053$). Assuming a carbon:Chl $\alpha$ ratio of 40:1, the Chl $\alpha$-specific mortality rate indicates that the consumption by MHs of phytoplankton standing stock ranged from 0 to 44 mg carbon L$^{-1}$ day$^{-1}$. There were significant ($P = 0.023$) temporal differences in pigment-specific mortality rates but the Bonferroni $t$-tests did not detect which sample periods were different. Pigment-specific mortality rates tended to be lowest in early-spring (September) and greater in the remainder of the sample periods (data not shown). During late summer 2004, the peridinin-specific mortality was very low ($\leq 0.09$ day$^{-1}$) and significantly less ($P = 0.016$) than the rate of $-0.65$ day$^{-1}$ for fucoxanthin or $-0.81$ day$^{-1}$ for Chl $\alpha$. The results from this experiment are discussed in more detail below.

Over all sample periods, the mean Chl $\alpha$-specific net growth rate ($\mu_{g} - |m|$) was 0.26 day$^{-1}$, significantly ($P = 0.037$) greater than the

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Fig. 5. Data from 2003 to 2005 from a single experimental site used for grazing experiments (Fig. 1) and plotted by month. (A) Chl $\alpha$ concentrations and Shannon-Weiner diversity index, (B) gross growth rates ($\mu_{g}$) of pigments estimated by grazing-dilution technique, (C) net growth rates ($\mu - |m|$) for pigments and taxa from grazing-dilution experiments: \(P.\) pseudo denotes \(Pseudonitzschia\) pseudodelicatissima, \(N.\) clos denotes \(Nitzschia\) clusitrium, \(S.\) cos denotes \(Skeletonema\) costatum, $<10$ $\mu$m denotes small flagellates $<10$ $\mu$m, \(G.\) cat denotes \(G.\) catenatum, \(Cea\) denotes Centratiom, \(Chaet\) denotes \(Chartoceros\) spp., \(D.\) frag denotes \(Dactyliosolen\) fragilitissimus, \(R.\) set denotes \(Rhizosolenia\) setigera, \(P.\) sub denotes \(Pseudonitzschia\) subpacifica.
peridinin-specific net growth rate of 0.08 day\(^{-1}\), but not significantly different from the fucoxanthin-specific net growth rate of 0.39 day\(^{-1}\). The taxa with the most observations of positive net growth rates were microflagellates (cells < 10 \(\mu\)m, five of six), *Pseudonitzschia pseudodelicatissima* (four of six), *Nitzschia closterium* (three of six) and *Skeletonema costatum* (three of six). Growth rates estimated by cell counts and pigments in experiments were consistent with each other (Fig. 5C). The greatest net growth rates during spring were for diatoms and fucoxanthin. During spring, the *in vitro* net growth rates estimated from pigments and cell counts were consistent with the observations of *in situ* change. For example, in November 2003 the fucoxanthin-specific net growth rate was experimentally measured as 0.66 day\(^{-1}\) and the highest net growth rate measured experimentally for a taxon was for *S. costatum* at 2.1 day\(^{-1}\). These *in vitro* growth rates essentially mirror the changes observed from November to February, when *in situ* fucoxanthin concentrations showed net positive growth increasing from 0.66 to 0.85 \(\mu\)g L\(^{-1}\), while the diatom *S. costatum* jumped from \(-1600\) cells L\(^{-1}\) to \(-938 \times 10^3\) L\(^{-1}\). The other taxa observed to have positive *in situ* growth from September 2003 and February 2004 included small flagellates and the diatoms *N. closterium* and *P. pseudodelicatissima*.

Late summer (February 2004) was the only time when the peridinin-specific net growth rate was observed to be significantly greater than zero and the experiment conducted at that time yielded the best evidence of selective grazing upon specific taxa identified by marker pigments. For these reasons this experiment is discussed in some detail. Twelve diatom taxa were observed, only *N. closterium* and *P. pseudodelicatissima* had net growth rates significantly greater than zero at 1.29 \(\pm\) 0.21 day\(^{-1}\) and 1.32 \(\pm\) 0.23 day\(^{-1}\), respectively. These net growth rates were about two times the 0.85 day\(^{-1}\) net growth rate simultaneously observed for fucoxanthin (Fig. 5B). Of the four species of dinoflagellates observed, *G. catenatum*, *Dinophysis*, *Ceratium* and *Pomacentrum*, it was *G. catenatum* and *Ceratium* that had net growth rates of 0.73 and 0.63 day\(^{-1}\), which were significantly greater than zero and substantially greater than the net peridinin-specific growth rate of 0.18 day\(^{-1}\) (Fig. 5B). The greatest pigment-specific gross growth rates (\(\mu_g\)) were fucoxanthin (1.49 \(\pm\) 0.041 day\(^{-1}\)), Chl \(b\) (1.51 \(\pm\) 0.076 day\(^{-1}\)), Chl \(a\) (1.56 \(\pm\) 0.092 day\(^{-1}\)) and \(\beta\)-\(\beta\)-carotene (1.18 \(\pm\) 0.051 day\(^{-1}\)). Simultaneously, a range of pigment-specific gross growth rates were significantly less than Chl \(a\) including: peridinin (0.28 \(\pm\) 0.099 day\(^{-1}\), \(P = 7 \times 10^{-3}\)), zeaxanthin (0.87 \(\pm\) 0.052 day\(^{-1}\), \(P = 9 \times 10^{-5}\)), diadinoxanthin (0.17 \(\pm\) 0.096 day\(^{-1}\), \(P = 2 \times 10^{-9}\)), violaxanthin (0.31 \(\pm\) 0.22 day\(^{-1}\), \(P = 0.009\)), diadinoxanthin (0.57 \(\pm\) 0.06 day\(^{-1}\), \(P = 0.02\)) and alloxanthin (0.17 \(\pm\) 0.11 day\(^{-1}\), \(P = 2 \times 10^{-12}\)).

Predators and predation

Microheterotrophs

Overall, the dominant MHs were ciliates (47%), followed by heterotrophic dinoflagellates (28%) and tintinnids (17%). Small numbers (<100 L\(^{-1}\)) of most other grazers were observed with radiolarians, copepod nauplii, bivalve larvae, appendicularians (larval tunicates) and rotifers each averaging <1% of the total (Fig. 6A). Numbers of *Polykrikos schwartzii* peaked at 510 \(\pm\) 361 L\(^{-1}\) in February 2004. Temporal variation in MH abundance was high with coefficients of variation for mean abundance ranging from \(-80\%\) for ciliates to 226% for *P. schwartzii*. The abundance of MH was least in September 2003 at 749 L\(^{-1}\) and was significantly greater in late spring primarily because of an increase in ciliates. MH abundance peaked in autumn for an overall change in density by a factor of \(-8\). High densities were associated with lower community diversity, with the lowest diversity observed in late spring and autumn. Live samples were frequently observed using the inverted microscope and the heterotrophic dinoflagellate *P. schwartzii* was observed to attack chains of *G. catenatum* and physically tear-off individual cells before ingesting them. *P. schwartzii*’s temporal abundance was highly variable ranging from undetected in winter to a peak of \(-500\) cells L\(^{-1}\) in late February 2004 (Fig. 6B). The only other planktonic species that was observed to graze on *G. catenatum* was *Noctiluca scintillans*. *Noctiluca scintillans* was unreported from this region during the period 1996–1998 when weekly surveys of five sites and nine quarterly surveys of \(-20\) sites were conducted.
Sampling of 12 nearby sites in D’Entrecasteaux Channel and five sites in the Huon Estuary commenced in spring 2002 when *N. scintillans* was found throughout the region from spring to autumn with the greatest densities in summer (Fig. 6C).

**Mesozooplankton**

The total number of mesozooplanktons collected in the Huon Estuary during 2004 and 2005 increased from November to February when it reached a peak of 6800 individuals m$^{-3}$, thereafter it decreased during autumn and remained relatively stable at around 3500 individuals m$^{-3}$ throughout winter and spring (Fig. 7). The mesozooplankton assemblage was dominated by *N. scintillans*, small copepods (*Acartia tranteri*, *Paracalanus indicus*), cladocerans (*Pdon spp.*, *Eudone spp.*), and appendicularians (*Oikopleura spp.*). Other groups such as the ghost shrimp (*Lucifer hanseni*), larval decapods, salps and chaetognaths (*Sagitta spp.*) were seasonally abundant.

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Fig. 6. Microheterotrophs (MHs) from a single experimental site (Fig. 1) sampled from 2003 to 2005. (A) Mean abundance of grazers <200 μm. (B) Abundance and diversity of MH grazers in grazing dilution experiments as a function of month. (C) Abundance of *Noctiluca scintillans* at 12 or more sites in region over time (1996–2005).
The numerically abundant *N. scintillans* contributed an average of only 6% of the estimated carbon biomass of mesozooplankton. The greatest mean monthly mesozooplankton biomass was 38 mg carbon m\(^{-3}\) in February (Fig. 7). Assuming food-saturated, temperature-dependent growth (Huntley and Boyd, 1984), mesozooplankton could be expected to grow at 0.21 day\(^{-1}\) during October and November, rising to 0.32 day\(^{-1}\) in February and March. If the assimilation efficiency was 0.7 (Conover, 1978), the potential assimilative capacity (grazing rate) of mesozooplankton would rise sharply to a peak of \(\sim 7\) mg carbon m\(^{-3}\) day\(^{-1}\) (Fig. 7) or about 14% of the average standing stock of phytoplankton during February (Fig. 2B, assuming 40:1 carbon:Chl \(a\)). It is noted that the calculation of \(G_{\text{max}}\) assumes food-saturated growth (>3 \(\mu\)g Chl \(a\) L\(^{-1}\); Huntley and Boyd, 1984), a situation that occurred in only 1% of all depth-integrated samples and never during winter to early spring (June–October) in previous years in the Huon Estuary. In depth-specific samples, concentrations of >3 \(\mu\)g Chl \(a\) L\(^{-1}\) were found in 4% of all surface samples and in significantly more (Chi-squared, \(n = 1074\), \(P = 0.002\)) of all mid-depth (10.9%) and all bottom (11.1%) samples. The greatest proportion of observations of Chl \(a\) being >3 \(\mu\)g L\(^{-1}\) were 33% near the bottom during summer. The low densities of phytoplankton suggest that the assimilative capacity of mesozooplankton was less than estimated, especially during June–October.

**DISCUSSION**

We have demonstrated significant variation in the plankton biomass, community and diversity of the Huon Estuary over time scales from seasons to years. Here, we focus on the potential influence of irradiance, temperature, stratification, nutrient availability and grazing on the balance between diatoms and dinoflagellates in this ecosystem. We consider how these “bottom-up” and “top-down” factors may influence the phytoplankton dynamics over a range of time and space scales.

**Top-down factors**

Over all data from the Huon Estuary, the average phytoplankton losses because of grazing were \(\sim 79\%\) of daily new production and 38% of the standing stock. MH grazing of a similar magnitude has been observed around the world (Calbet and Landry, 2004) indicating the pivotal role of MH grazing in the control of phytoplankton dynamics. In the D’Entrecasteaux Channel and Huon Estuary, *in situ* net growth rates of spring, summer and autumn blooms of *S. costatum*, *Ceratium* and *Pseudonitzschia* were \(\sim 0.04\) day\(^{-1}\) or \(\sim 5\%\) of \(\mu_{\text{max}}\) for these two diatoms and \(\sim 20\%\) of \(\mu_{\text{max}}\) for the dinoflagellate, *Ceratium*. In contrast, the experimentally determined pigment-specific gross growth rates (estimated by extrapolation to zero predation) under ambient conditions of light, temperature and nutrients averaged

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**Fig. 7.** Mesozooplankton abundance, mean density \(\pm 1\) SE (\(n = 3\)) from November 2004 to November 2005 and assimilative capacity from measurements of total mesozooplankton weight (carbon) assuming that assimilation efficiency was 70% (Conover, 1978) and percent growth per day was 5.43\(^{0.112}\) (Huntley and Boyd, 1984) where \(T\) denotes temperature in degree Celsius.
The highest gross Chl \(a\) growth rates were observed in late summer, suggesting a significantly less grazing pressure, the experimentally strong selective grazing upon diatoms. Over periods of peridinin-specific loss rate was six times less than the late abundance was maximal (February 2004). In the grazing experiment where dinoflagellates and flagellates. In the grazing experiment where dinoflagellates and flagellates. It should be noted, however, that even with significantly less grazing pressure, the experimentally determined peridinin-specific net growth rate (0.18 day\(^{-1}\)) was lower than those of other taxa. Therefore, we suggest that some additional factor(s) must contribute to a relative increase in dinoflagellate net growth if they are to dominate the phytoplankton community.

The MH community observed in the Huon Estuary was similar in composition to those observed in coastal water bodies from Nova Scotia, Canada (Gifford, 1988) to South Africa (Froneman and McQuaid, 1997), with dominance by ciliates (aloricate) followed by tintinnids. Densities of both types of ciliates (aloricate + tintinnids) were \(\sim 1700 \text{ L}^{-1}\), very similar to the \(2100 \text{ L}^{-1}\) reported for waters around New Zealand (James and Hall, 1998; Hall et al., 2004). The major difference between MHs in the Huon Estuary and other coastal locations in the world seems to be the greater importance of heterotrophic dinoflagellates. Heterotrophic dinoflagellates are only rarely reported as the dominant grazers in coastal ecosystems, but when present they appear to be quite selective grazers (Johnson et al., 2003). Relatively large number of heterotrophic dinoflagellates are characteristic of the oceanic Australasian region north of the subtropical convergence (Wood, 1954) and the seas around Tasmania (Wood, 1964).

The abundance of the heterotrophic dinoflagellate \(P. schwartzii\) peaked in January and appears to contribute significantly to declines in the abundance of \(G. catenatum\). Polykrikos has been observed to be a major predator of other dinoflagellates in the Chesapeake (Johnson et al., 2003) and can grow faster (Jeong et al., 2001) than \(G. catenatum\). Under these circumstances, there would seem to be only a few mechanisms that would allow sufficient escape from MH predation for a \(G. catenatum\) bloom to develop. A simple predator–prey model (Holling, 1959) using realistic population densities and growth parameters for both species indicates that \(G. catenatum\) should not survive in the Huon Estuary without a spatial refuge or significant predation upon \(P. schwartzii\) itself.

In the Huon Estuary and D’Entrecasteaux Channel, the numerical abundance of mesozooplankton peaked in February as did their biomass. The increase in mesozooplankton combined with the assumed temperature-dependent grazing gives an approximately eight times rise in potential grazing rate between November and February. Integrated phytoplankton samples suggest that mesozooplankton would be unlikely \(\sim 1\%\) to encounter sufficient phytoplankton to saturate grazing during summer; near the bottom, 7 of 21 observations were \(>3 \text{ mg L}^{-1}\). If grazing on phytoplankton and food-saturated the mesozooplankton might consume an average of 7% of the phytoplankton standing stock per day (Huntley and Boyd, 1984); substantially less than the 38% consumed by microheterotrophs. Mesozooplankton are also likely to prey upon MHs and the peak in mesozooplankton grazing pressure in February coincides with a decline in MH abundance. These results suggest that the potential for a trophic cascade where mesozooplankton remove MHs and net phytoplankton growth increases (Hansen et al., 1993).

We speculate that such a trophic cascade might facilitate the observed late autumn blooms of \(G. catenatum\) by removing one of its main predators, \(P. schwartzii\).

**Bottom-up factors**

On a local scale, a number of strong seasonal cycles in bottom-up factors, such as temperature, light attenuation, nutrient concentrations, rainfall, river runoff and stratification may all influence phytoplankton community composition (Cloern, 1999; Adolf et al., 2006). In the Huon Estuary there are winter maxima in rainfall, river runoff, light attenuation, stratification and surface nutrient \(\text{NO}_3, \text{PO}_4\) concentrations. Coloured dissolved organic matter discharged from the Huon River (CSIRO Huon Estuary Study Team, 2000) and the annual pattern of insolation produce low-light conditions during winter. Light attenuation was most severe in the upper estuary where the ratio of photic depth \(z_p\) to the bottom \(z_m\) was 0.1 or about \(\frac{1}{2}\) the \(z_p/z_m\) of 0.2 generally required for net positive growth (Cloern, 1987). Thus, the upper reaches of the Huon Estuary are likely to be light-limited, whereas the majority of the estuary, especially towards the seaward end, had \(z_p/z_m\)
ratios of ≥0.5 and euphotic depths approaching 20 m. Low and fluctuating irradiance throughout winter are considered to provide an ecological niche that favours diatoms over other taxa (Smetacek and Passow, 1990; Litchman, 1998; Mitrovic et al., 2003) and are suggested as factors that establish diatoms as the seed species for spring blooms in the Huon Estuary and elsewhere.

Stratification of the water column is well known to favour dinoflagellates (Margalef, 1978; Smayda and Reynolds, 2001) but locally in the Huon Estuary stratification was maximal in winter and minimal in summer, a very different seasonal dynamic relative to many estuaries in the temperate northern hemisphere. During summer, the water column was periodically isohaline and isothermal (<0.1 psu and <0.1°C change from top to bottom) and easily mixed because of wind or overnight cooling. If the water column is rapidly mixed to the bottom there is no competitive advantage to vertical migration, at least in terms of exploiting higher concentrations of dissolved nitrogen at depth. During the summer and autumn seasons of 1996–1998 there were unusually heavy, episodic, rainfall and river high flows (this paper), increased estuary stratification, increased light attenuation (strong vertical separation of light and nutrients), decreased dissolved oxygen, and increased NH$_4^+$ in the bottom waters (CSIRO Huon Estuary Study Team, 2000), all potentially favouring dinoflagellates. Links between river flow and G. catenatum blooms have been previously demonstrated for the Huon Estuary (Hallegraeff et al., 1995). Gymnodinium catenatum tends to avoid the lower salinity surface layer (Doblin et al., 2006), a behaviour that has been shown by hydrodynamic and biogeochemical modelling to help retain it in this salt wedge estuary (Volkman et al., 2006).

Averaged over all measurements, the dissolved inorganic N:P and N:Si ratios were ~1:1 strongly suggesting the potential for nitrogen limitation of phytoplankton growth. This potential was most severe in summer when surface nitrate concentrations were at or near detection limits, while relatively high phosphate and silicate concentrations remained. The Huon River water has not been a significant source of DIN (CSIRO Huon Estuary Study Team, 2000), but it may supply some important micronutrients in its humic-laden waters (Doblin et al., 1999). Other significant inputs of nitrogen when ambient surface concentrations are low (approximate November–April) include 48% of the 778 tonnes annum$^{-1}$ (Volkman et al., 2006) from finfish farming in the Huon Estuary and D’Entrecasteaux Channel. Ammonium concentrations tended to be greater with depth and in areas of low dissolved oxygen (CSIRO Huon Estuary Study Team, 2000). Recent research showed that G. catenatum vertically migrated ~20 m in the Huon Estuary (Doblin et al., 2006) to scavenge nitrogen from depth (Armstrong et al., unpublished data); potentially a significant advantage in a stratified ecosystem with low DIN concentrations in the surface layer. These physical and chemical factors are likely to contribute to summer dinoflagellate blooms on a local scale, that is, in the Huon Estuary (Hallegraeff et al., 1995; Doblin et al., 2006).

The similarity of spring dinoflagellate communities in the Huon Estuary and a 100 km offshore suggests some processes that influence composition must be regional in scope. The two dominant autotrophic dinoflagellate genera, Ceratium and Gymnodinium, are considered sensitive to stratification (Fraga et al., 1990; Smayda and Reynolds, 2001). On a regional scale, stratification is mostly temperature-dependent and the long-term rise (1996–2005) in the local dominance by dinoflagellates may be related to the long-term regional rise in temperatures (Fig. 2) caused by the strengthening of the southward moving East Australia Current (EAC, Rintoul and Bullister, 1999). The fact that winter temperatures have not risen while summer temperatures have, indicates that the seasonal shallowing of the mixed layer depth is more rapid, a possible factor in the rising proportions of dinoflagellates throughout spring, summer and winter. We speculate that the strengthening EAC may also explain the recent arrival in southern Tasmania of the heterotrophic dinoflagellate, N. scintillans, a species that appears to have moved ~1700 km south from Moreton Bay over the last several decades (Wood, 1964; Dela-Cruz et al., 2002, 2003).

**CONCLUSIONS**

The phytoplankton, MH and mesozooplankton communities were seasonally variable in composition. Pigment-specific gross growth rates measured under *in situ* conditions of irradiance, temperature and nutrients but without grazing were ~20 times greater than net growth rates observed *in situ*. Therefore, grazing was the major factor reducing *in situ* growth rates. The abundance of MHs declined during summer, coincident with the peak in mesozooplankton abundance and their estimated grazing rate. Greater fucoxanthin-specific losses than peridinin-specific losses during summer suggest preferential grazing by MHs upon diatoms. A regional trend towards warmer summer surface waters may be a factor in the multi-year trend towards greater dinoflagellate dominance of the local phytoplankton community.
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