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Pigment signatures reveal temporal and regional differences in taxonomic phytoplankton composition off the west coast of Ireland

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The composition of phytoplankton assemblages in April, 1998 in Galway Bay and during a summer phytoplankton bloom occurring southwest of Ireland in August, 1998, was characterized by pigments measured by high-performance liquid chromatography. Pigment data reflecting phytoplankton assemblages dominated by diatoms in Galway Bay and dinoflagellates in the southwest of Ireland were compared to phytoplankton cell counts. Significant relationships were found between the fucoxanthin concentrations and the diatom cell numbers ($P < 0.0002$ r^2 0.63) during April, and between fucoxanthin ($P < 0.0001$ r^2 0.79), 19′hexanoyloxyfucoxanthin ($P < 0.0001$ r^2 0.77) concentrations and Gyrodinium aureolum cell numbers during the summer bloom.

The accurate description of phytoplankton communities usually involves time-consuming microscopic examination and a high level of taxonomic skill. Alternatively phytoplankton groups may be characterized by the presence or absence of pigments. Chlorophyll and carotenoid pigments, either singly or in combination, have been used successfully for chemosystematic identification of phytoplankton in oceanic waters (Jeffrey *et al.*, 1975; Gieskes and Kraay, 1983; Wright and Jeffrey, 1987; Barlow *et al.*, 1993). Although pigments may vary among cells within a taxon or between taxa, the abundance of the diagnostic pigments generally reflects the major distributions of phytoplankton to the division or class level (Millie *et al.*, 1993).

The aim of this research was to evaluate the utility of high-performance liquid chromatography (HPLC)-based pigment analyses for detecting temporal and regional changes in the phytoplankton composition off the west coast of Ireland. A specific objective was to establish whether pigment ratios can be used to characterize particular phytoplankton populations. Observations were made during 1998 between April 1 and April 7 in Galway Bay and between August 9 and August 23 off the southwest coast of Ireland (Figure 1A and B).

Water samples were taken from the surface mixed layer (1 m), and sub-surface (10–15 m) from which 55 ml subsamples were taken and preserved with Lugol's iodine for microscopic analysis. Between 0.75 and 1 l samples were filtered through 25 mm Whatman GF/F filters and immediately frozen by storage in liquid nitrogen until HPLC analysis could be carried out after the cruise. In the laboratory, the frozen filters were extracted in 5 ml 90% HPLC-grade acetone, ultrasonicated for 30 s and centrifuged to remove cellular debris. The method chosen in this study (Barlow *et al.*, 1993) is a modification of the reverse-phase method described previously (Mantoura and Llewelyn, 1983). The HPLC system was calibrated for each pigment with commercial standards. Pigments were identified by injecting samples of phytoplankton reference cultures whose pigment composition has been documented in the literature (Jeffrey *et al.*, 1997). To confirm peak identity a set of samples was analysed with on-line photo diode array spectroscopy (UV 6000 LP). Phytoplankton was enumerated by a modified Utermohl's

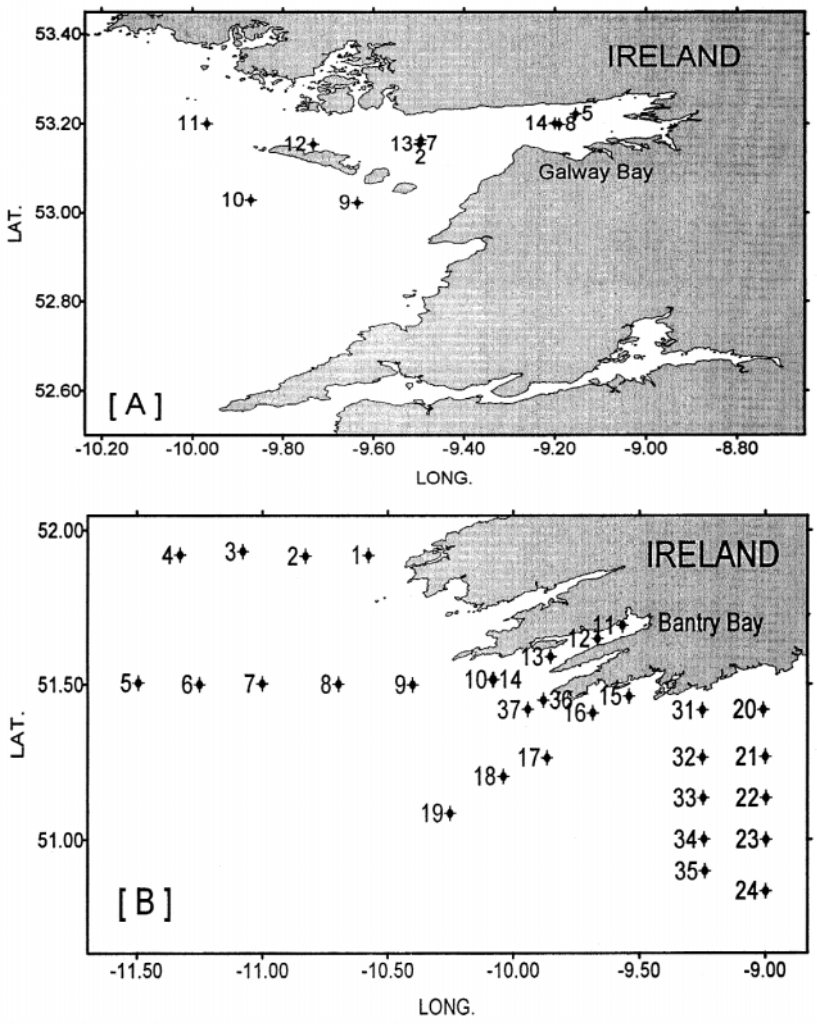

Fig. 1. (**A**) Location of sampling stations in Galway Bay (April 1–7, 1998). (**B**) Location of sampling stations in southwest Ireland (August 9–23, 1998).

technique, counting samples after sedimentation using a phase-contrast inverted microscope (Hasle, 1978). Satellite images of ocean colour (SeaWIFS) were received from the Remote Sensing Data Analysis Service (RSDAS) at Plymouth Marine Laboratory.

Chlorophyll *a* concentration varied from 2.4 to 1.2 μg l^{-1} in a westward to seaward direction along the transect (Figure 2A).The maximum surface concentration of 2.4 μg l^{-1} was observed at the inner station 14 at the end of the cruise. Stations 13 and 14 were sampled three times, during the cruise so Figure 2A and B show the average values and standard deviation for these stations. Fucoxanthin and chlorophyll *c1* + *c2* were the main accessory pigments in all samples. Variations in the surface concentrations of these pigments showed similar spatial trends to those of chlorophyll *a* (Figure 2B). The lowest surface concentrations of pigments were observed at stations 9 and 10 outside the bay. Data from these stations are shown as isolated points on Figures 2A and B. Only at stations 11 and 12 was there a noticeable sub-surface pigment maximum (lower panel in Figure 2A and B). In most stations diatoms were generally dominant (Figure 3). Fucoxanthin is the dominant biomarker for diatoms. It is not surprising, therefore that in the spring of 1998 samples from Galway Bay had fucoxanthin as the dominant carotenoid. Their concentrations were slightly lower at stations 9 and 10, similar to the chlorophyll distribution. *Thalassiosira* spp. were the dominant diatom species in Galway Bay. The second most dominant forms were species from the genera *Chaetoceros* and *Rhizosolenia*. Surface distributions of diatoms and microflagellates were generally very similar among stations along the transect.

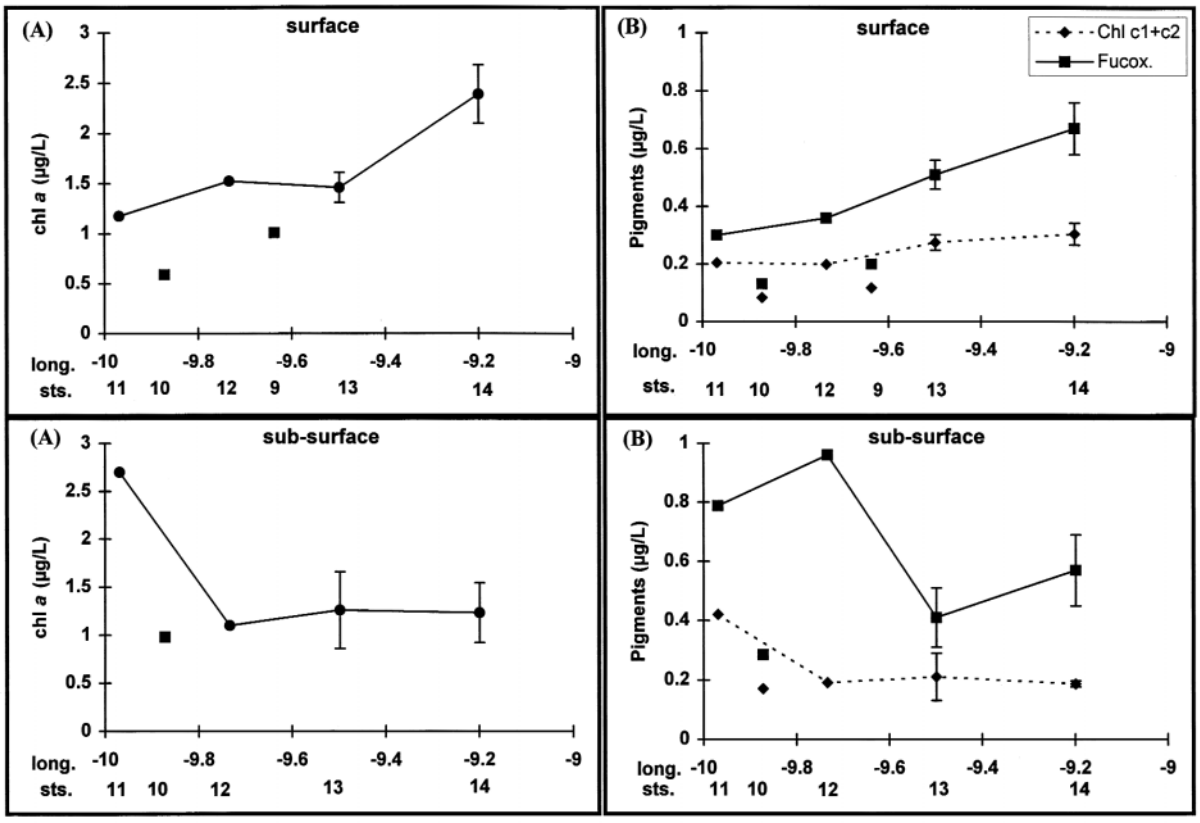

Fig. 2. Distribution of surface and sub-surface pigments in Galway Bay during April, 1998. (**A**) Chlorophyll *a* (Chl *a*); (**B**) chlorophyll *c1* + *c2* (Chl *c1* + *c2*) and fucoxanthin (Fucox.).

The contribution of microflagellates, which primarily consisted of small cells (<5 µm), to the total biomass was high in the most offshore stations (stations 9, 10 and 11) of Galway Bay (Figure 3). Results of HPLC analysis did not, however, suggest any changes in phytoplankton dominance even though high microflagellate concentrations (56–78% cell volume) were observed in these stations (Figures 2B and 3). This suggests that these microflagellates are heterotrophic organisms. The dominance of diatoms and microflagellates in the sub-surface layer was highly variable.

Significant linear relationships were found between chlorophyll *a* and both fucoxanthin and chlorophyll *c1* + *c2* (Figure 4A and B). Ratios of 1.8 for the chlorophyll *a* to fucoxanthin ratio and 6.8 for the chlorophyll *a* to chlorophyll *c1* + *c2* were obtained. The chlorophyll *a* to fucoxanthin ratio observed (1.8) was within the range of 1.08–2.3 reported for diatoms *in situ* in comparable areas (Wright and Jeffrey, 1987; Tester *et al.*, 1995). The high correlation (r^2 0.63) between diatom cell count and fucoxanthin concentrations also confirms this (Table I). Diadinoxanthin and β-carotene were also observed in very low concentrations during the April 1998 cruise. The ratios of these pigments to chlorophyll *a* are summarized in Table I.

There was considerable variability in the concentration and composition of phytoplankton pigments around the southwest of Ireland during the August 1998 cruise. Chlorophyll *a* concentrations in excess of 20 µg l^{-1} were measured in surface waters (Figure 5). These high surface chlorophyll *a* concentrations declined significantly offshore in the southwestern part of Ireland. SeaWIFS satellite data taken just before (August 4) and during (August 15) the cruise show an area of enhanced chlorophyll extending from the southwest of Ireland in a southeastwards direction (Figure 6). This can be compared with the contours of chlorophyll *a* derived from measurements between August 9 and August 23 1998. There was good agreement between the *in situ* and satellite data in both magnitude and spatial distribution (Figures 5 and 6).

The results of the pigment analyses (Figure 7A) showed that surface and sub-surface chlorophyll *a* values were very

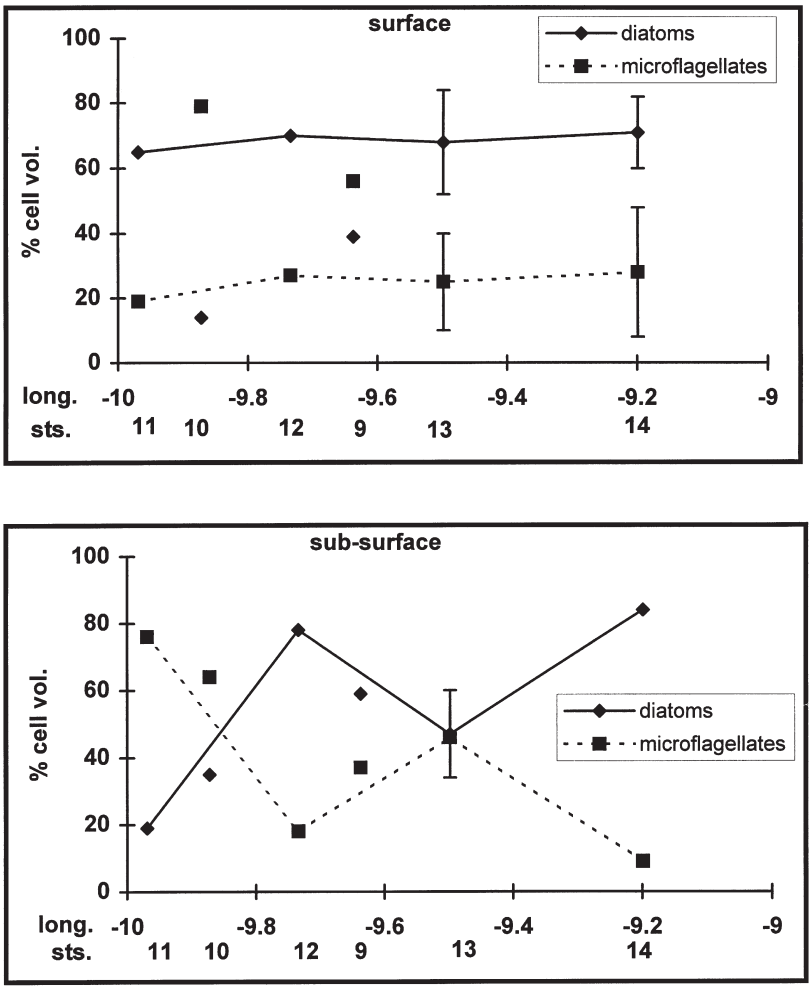

Fig. 3. Surface and sub-surface phytoplankton abundance (% cell volume) in Galway Bay during April, 1998. The lines join stations along the transect, other stations are plotted as isolated points.

similar between stations 5–8 but sharply increased from station 9, and reached a maximum at stations 13 (surface) and 14 (sub-surface) just inside Bantry Bay. The distributions of the carotenoid fucoxanthin, 19′hexanoyloxyfucoxanthin, chlorophyll *c1* + *c2* and *c3* were very similar to that of chlorophyll *a* (Figure 7B). Most pigments were present at all stations at the sub-surface but their concentrations varied. Maximum sub-surface concentrations were observed between stations 9–13 (Figure 7B). At stations 9, 13 and 14 the dominant phytoplankter was the dinoflagellate *Gyrodinium aureolum* (Figure 8A). Maximum sub-surface cell numbers were observed at station 14. The second most dominant dinoflagellate species was *Gymnodinium* spp. Diatom cells were observed at stations 9, 14, 13, 12 and 11 along the transect and *Rhizosolenia setigera* and *Leptocylindrus danicus* species were dominant among the diatoms.

Gyrodinium aureolum was also present in large concentrations off the southwest coast, forming a surface or near-surface bloom (Figure 8B). The distribution of this organism was again similar to that of chlorophyll *a* and certain carotenoids along the north to south transect (stations 20–24) (Figures 8B and 9A and B). Maximum surface concentrations were recorded at station 24 at 1 m depth with values of 23.0 μg chlorophyll *a* l^{-1}, 5.2 μg fucoxanthin l^{-1}, 1.9 μg 19′hexanoyloxyfucoxanthin l^{-1}, 1.4 μg chlorophyll *c1* + *c2* l^{-1}, 0.9 μg chlorophyll *c3* l^{-1} and 940 cells ml^{-1} of *Gyrodinium aureolum* (Figures 8B and 9A and B). Maximum sub-surface concentrations were observed at station 24. Note that in both transects the trend in pigment distribution is similar to that of phytoplankton (Figures 7A and B, 8A and B and 9A and B). Diadinoxanthin, diatoxanthin and β-carotene were also

Table I: Chlorophyll a and phytoplankton abundance to accessory pigment ratios

			19′hexanoyl	Chlorophyll	Chlorophyll			
		Fucoxanthin	oxyfucoxanthin	*c1* + *c2*	*c3*	Diadinoxanthin	Diatoxanthin	β-carotene
April	Chlorophyll *a*	$y = 1.8x + 0.67$		$y = 6.8x - 0.04$				
		$r^2 = 0.43$, $n = 21$		$r^2 = 0.88$, $n = 21$				
	Diatoms (cells ml^{-1})	$y = 0.01x + 0.18$		$y = 0.005x + 0.13$				$y = 23.5x + 0.24$
		$r^2 = 0.63$, $n = 16$		$r^2 = 0.50$, $n = 16$				$r^2 = 0.76$, $n = 19$
August	Chlorophyll *a*	$y = 4.3x$	$y = 11.5x + 0.23$	$y = 10x + 0.7$	$y = 24x + 0.73$	$y = 11.9x + 0.04$	$y = 43.5x + 0.76$	$y = 38.5x - 0.005$
		$r^2 = 0.98$, $n = 51$	$r^2 = 0.95$, $n = 51$	$r^2 = 0.72$, $n = 51$	$r^2 = 0.73$, $n = 51$	$r^2 = 0.95$, $n = 31$	$r^2 = 0.79$, $n = 21$	$r^2 = 0.96$, $n = 26$
	G. aureolum	$y = 0.005x + 0.6$	$y = 0.002x + 0.2$	$y = 0.002x + 0.17$	$y = 0.001x + 0.06$			
	(cells ml^{-1})	$r^2 = 0.79$, $n = 51$	$r^2 = 0.77$, $n = 51$	$r^2 = 0.61$, $n = 51$	$r^2 = 0.68$, $n = 51$			

P values were between $P < 0.0001$ and $P < 0.003$ for all analyses.

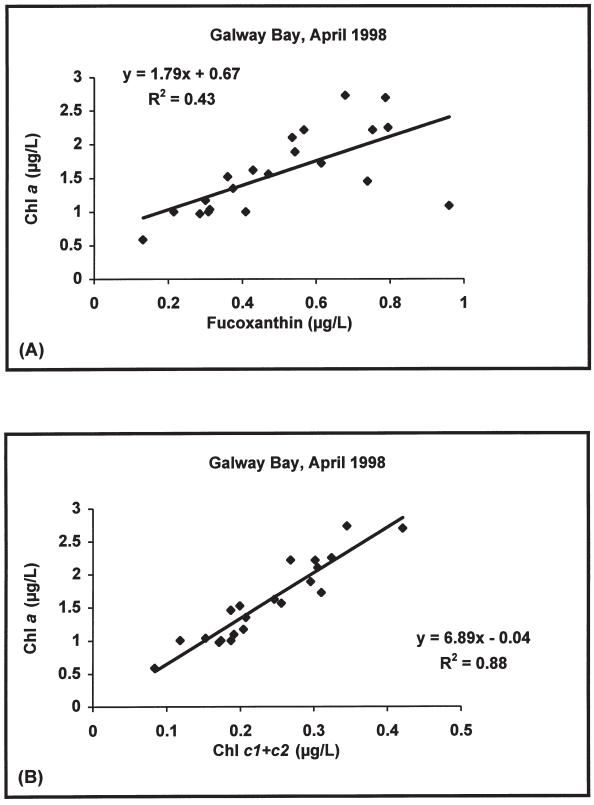

Fig. 4. Linear relationship between: (**A**) chlorophyll *a* (Chl *a*) and fucoxanthin concentrations; (**B**) Chl *a* and chlorophyll *c1* + *c2* (Chl *c1* + *c2*) concentrations during April, 1998.

observed in the working area. The ratios of these pigments to chlorophyll *a* are summarized in Table I.

The characteristic carotenoid of photosynthetic dinoflagellates is peridinin (Jeffrey *et al.*, 1975), in some species, including *Gyrodinium aureolum* and *Gymnodinium galatheanum*, peridinin is replaced by fucoxanthin and/or its derivatives (Bjornland and Tangen, 1979; Tangen and Bjornland, 1981). For example, *Gyrodinium aureolum* is a common red tide dinoflagellate in North European waters (Johnsen and Sakshaug, 1993; Raine and McMahon, 1998). The complementary biomarker chlorophyll *c* (*c1* + *c2* and *c3*) is also present in these species (Johnsen and Sakshaug, 1993). The results in this study showed that fucoxanthin, 19′hexanoyloxyfucoxanthin and chlorophyll *c1* + *c2* and *c3* were present in significant concentrations at stations containing *Gyrodinium aureolum* on the southwest coast of Ireland (August, 1998) and fucoxanthin and chlorophyll *c1* + *c2* were present in Galway Bay (April, 1998), when diatom species were dominant.

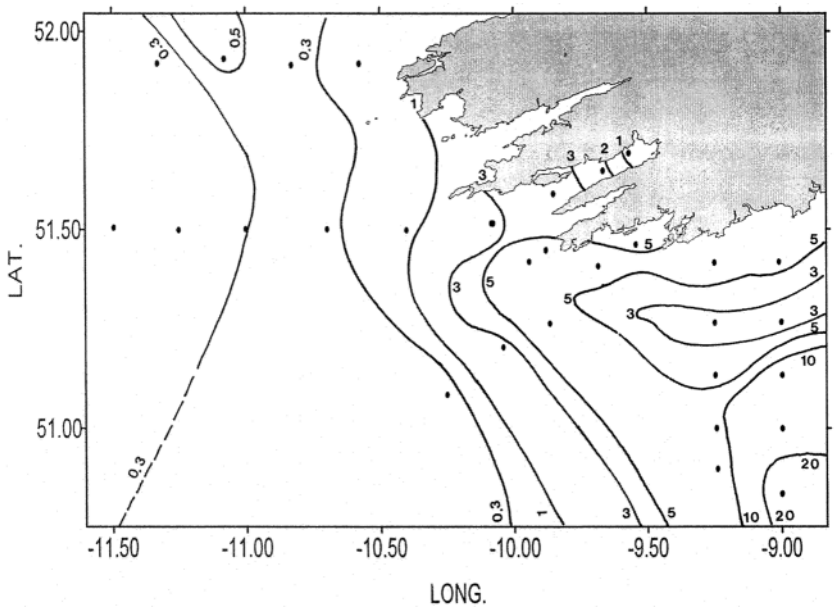

Fig. 5. Surface (1 m) distribution of chlorophyll *a* (μg l^{-1}) during August, 1998.

The chlorophyll *a* to fucoxanthin ratio of 4.3 observed in August was much higher than either the 1.8 observed in the spring during the present study (Table I), when diatoms were dominant, or other values reported for diatoms (Wright and Jeffrey, 1987; Tester *et al.*, 1995). The observed chlorophyll *a* to fucoxanthin ratio of 4.3 and the chlorophyll *a* to 19′hexanoyloxyfucoxanthin ratio of 11.5 (Figure 10) are close to the values reported for *Gyrodinium aureolum* in culture (Johnsen and Sakshaug, 1993) of ~3 and 8 respectively. A significant relationship was also observed between the chlorophyll *a* and chlorophyll *c* (*c1* + *c2* and *c3*) (Table I) and calculated ratios are comparable to the ratio of *Gyrodinium aureolum* in culture. These are between 5 and 7 compared with 10 for chlorophyll *c1* + *c2* and between 8 and 20 compared to 24 for chlorophyll *c3* (Johnsen and Sakshaug, 1993).

Ratios of chlorophyll *a* to fucoxanthin in nanocrypsophyceae are also as high as, or higher than, those of diatoms (Vesk and Jeffrey, 1987). However, microscopic analyses indicated the absence of the former group. The chlorophyll *a* to accessory pigment ratios from this study are within the range of ratios of other field samples for spring 1998 in Galway Bay and are close to culture ratios for August 1998 in southwest Ireland. As far as the authors are aware, this is the first record for these pigment ratios for this organism *in situ*.

Significant linear relationships were found not only between chlorophyll *a* and fucoxanthin, 19′hexanoyloxyfucoxanthin, chlorophyll *c1* + *c2* and chlorophyll *c3* (Table I) but also between cell abundance and these pigments (r^2 0.79, r^2 0.77, r^2 0.61 and r^2 0.68, respectively). This would confirm that *Gyrodinium aureolum* was the most important carrier of fucoxanthin, 19′hexanoyloxyfucoxanthin and chlorophyll *c* (*c1* + *c2* and *c3)* in the samples from the fieldwork on August 1998. However, any coexistence of diatoms and *Gyrodinium aureolum* in samples might complicate the estimation of the contribution of diatoms to the flora using pigment data alone. The high fucoxanthin concentrations and chlorophyll *c1* + *c2* were due largely to *Gyrodinium aureolum*. There were no significant relationships between diatom cell counts and fucoxanthin and chlorophyll *c1* + *c2* concentrations during August 1998 even in these samples where diatoms were more numerous. However, there was a significant relationship between *Gyrodinium aureolum* cell counts and fucoxanthin and chlorophyll *c1* + *c2* concentrations during August 1998. A similarly significant relationship was observed between diatom cell counts and fucoxanthin and chlorophyll *c1* + *c2* concentrations during April 1998 (Table I).

In conclusion, this study indicates that HPLC is a powerful tool for describing phytoplankton populations,

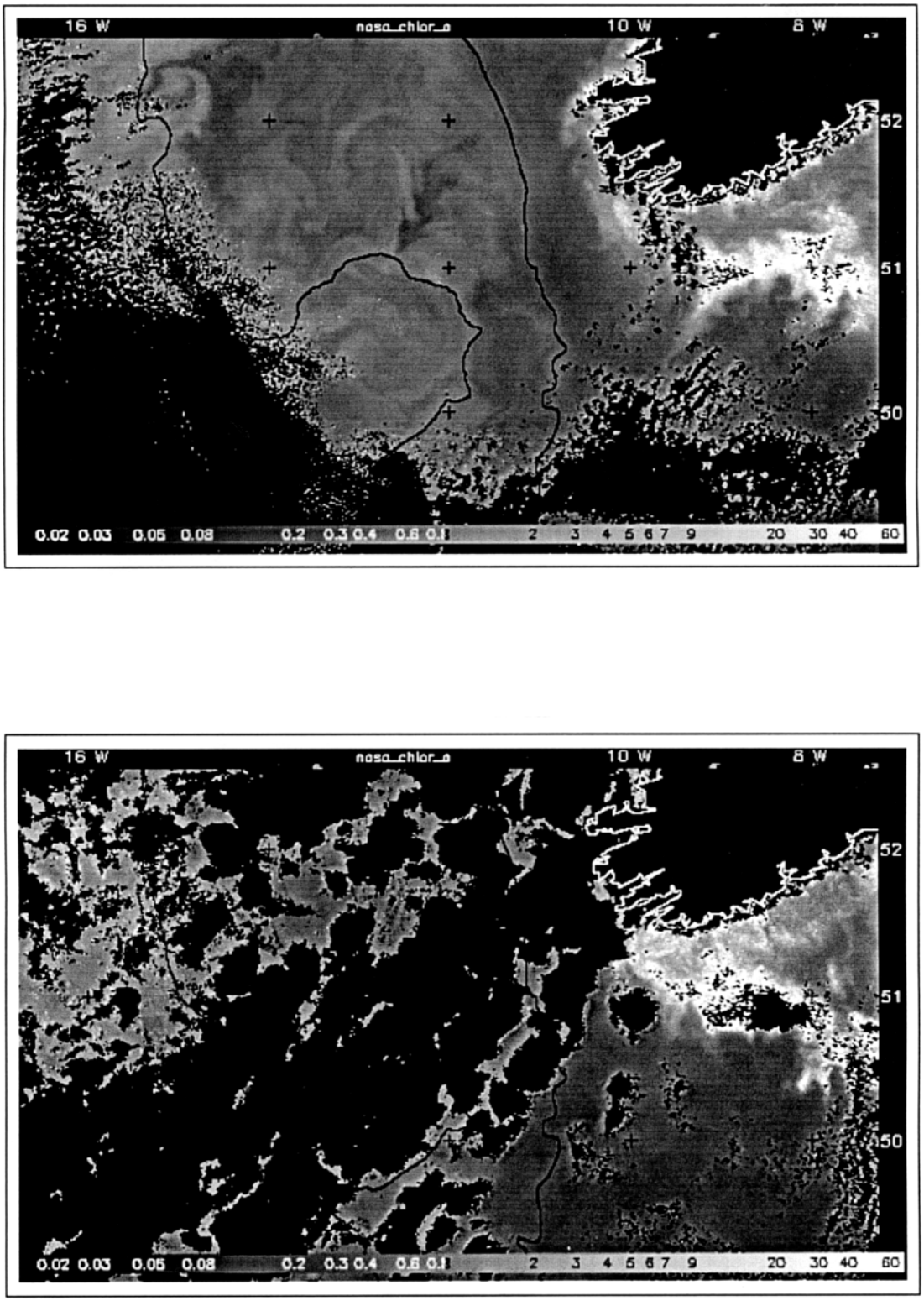

Fig. 6. SeaWIFS chlorophyll *a* (μg l^{-1}) images on August 4 and 15, 1998.

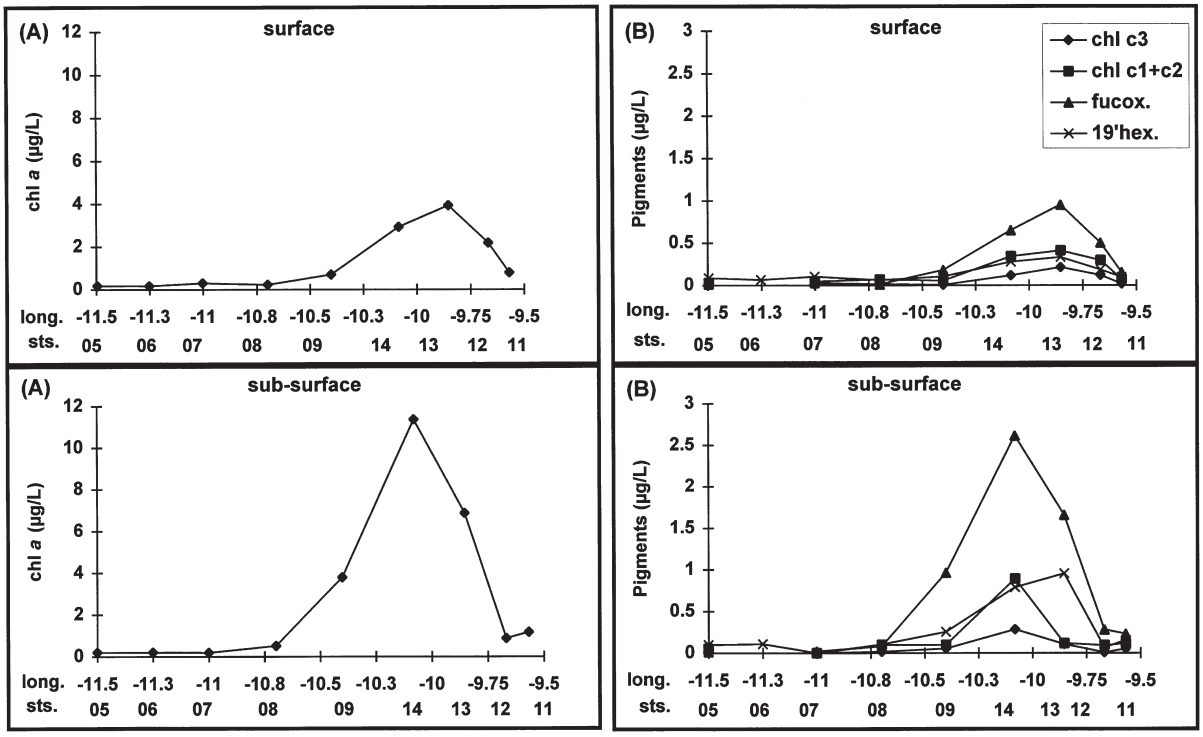

Fig. 7. Distribution of surface and sub-surface pigment concentrations along an east to west transect (stations 5 to 14). (**A**) Chlorophyll *a* (Chl *a*), (**B**) fucoxanthin (Fucox.), 19′hexanoyloxyfucoxanthin (19′hex), chlorophyll *c1* + *c2* (Chl *c1* + *c2*) and chlorophyll *c3* (Chl *c3*).

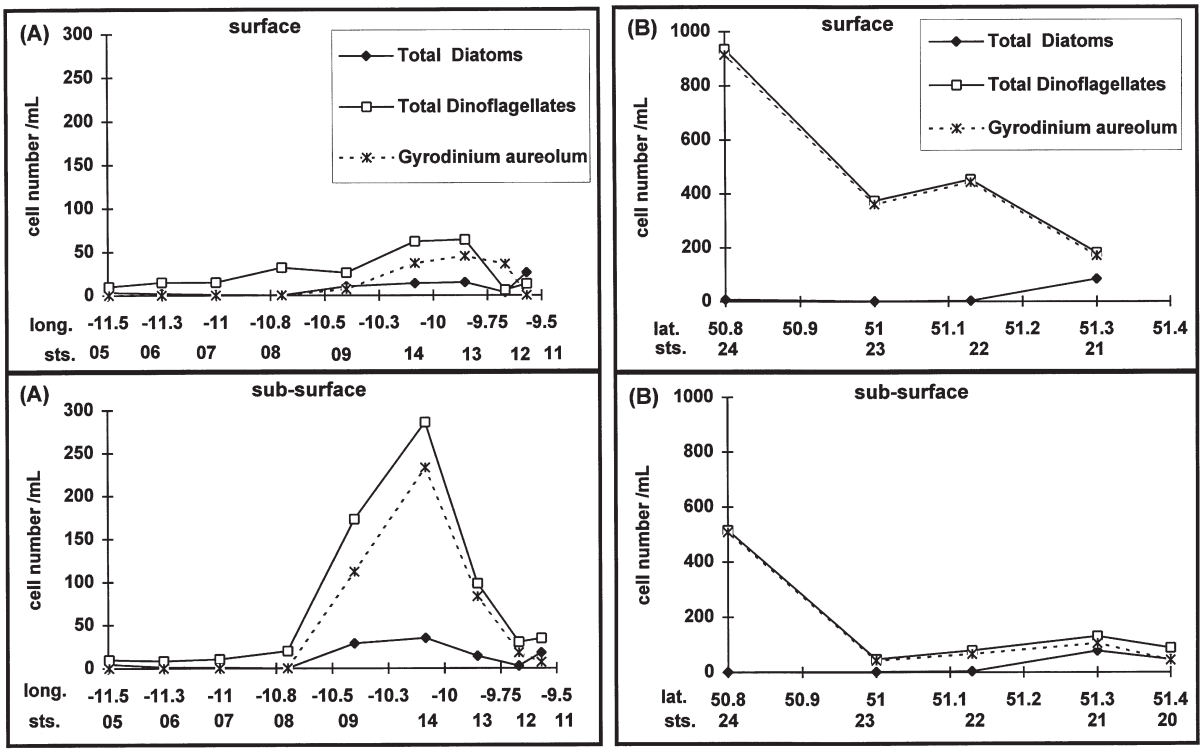

Fig. 8. Phytoplankton abundance in surface and sub-surface layers during August, 1998. (**A**) East to west transect (stations 5 to 14), (**B**) north to south transect (stations 20 to 24).

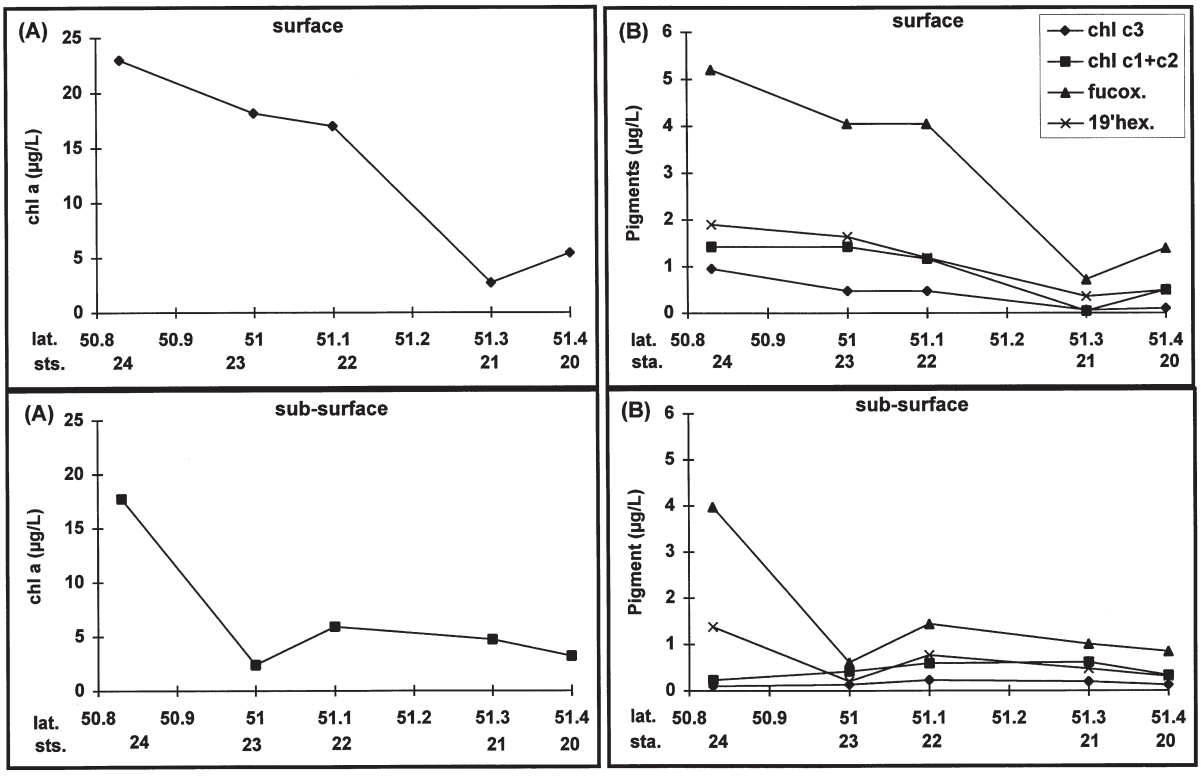

Fig. 9. Distribution of surface and sub-surface pigment concentrations along a north to south transect (stations 20 to 24). (**A**) Chlorophyll *a* (Chl *a*), (**B**) fucoxanthin (Fucox.), 19′hexanoyloxyfucoxanthin (19′hex), chlorophyll *c1 + c2* (Chl *c1 + c2*) and chlorophyll *c3* (Chl *c3*).

even when two differing assemblages have the same dominant accessory pigments. Results presented here have shown that a diatom and *Gyrodinium aureolum* community can be distinguished through simple pigment ratios, even though both contain the same biomarker (fucoxanthin). HPLC ratios could also be used to differentiate *Gyrodinium aureolum* blooms from other dinoflagellate populations comprised of species containing peridinin, which might be expected during summer around the Irish coast and elsewhere. Although no single technique is ideal for resolving all information about phytoplankton community structure, the importance of the ability of HPLC to distinguish a potentially harmful dinoflagellate population such as *Gyrodinium aureolum* from others cannot be understated, given the occurrence of this organism and other toxic chloroplast-containing dinoflagellates around southwestern Ireland, such as *Dinophysis acuminata* and *D. acuta* which have had adverse economic effects on the aquaculture industry (McMahon *et al.*, 1998).

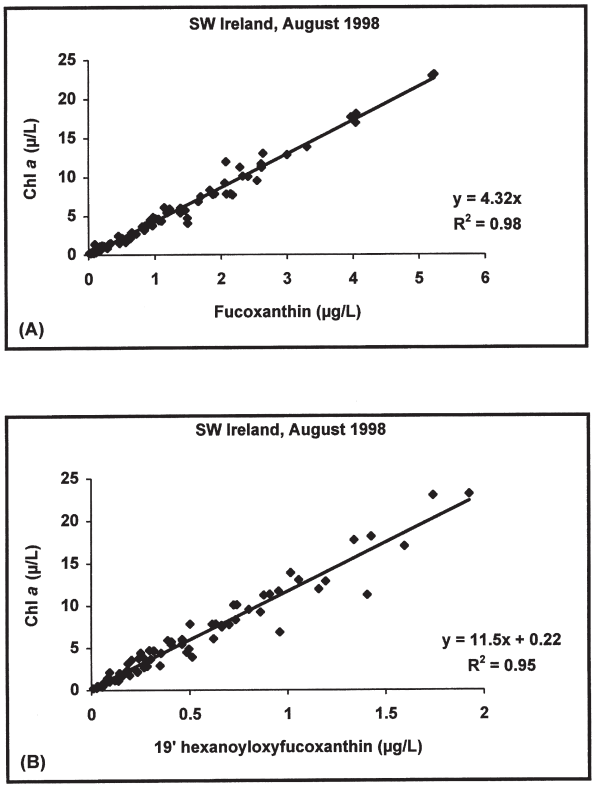

Fig. 10. Linear relationship between (**A**) chlorophyll *a* (Chl *a*) and fucoxanthin concentrations, and (**B**) Chl *a* and 19′hexanoyloxyfucoxanthin concentrations during August, 1998.

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