

Preliminary Studies on the Growth of Selected 'Green Tide' Algae in Laboratory Culture: Effects of Irradiance, Temperature, Salinity and Nutrients on Growth Rate

R. Taylor^{a, b*} R. L. Fletcher^b and J. A. Raven^a

^a Department of Biological Sciences, University of Dundee, Dundee DD1 4HN, U. K. (present address of RT)

^b Institute of Marine Sciences, University of Portsmouth, Ferry Road, Eastney, Portsmouth, Hampshire, PO4 9LY, U. K.

* Corresponding author

During the summer months, Langstone Harbour, a eutrophic inlet on the south coast of England, is subject to blooms of green macroalgae commonly referred to as 'green tides'. In a series of laboratory experiments, the vegetative growth of eight 'green tide' algae collected from the harbour was examined under a range of irradiances, temperatures, salinities and N and P concentrations. Test algae were *Chaetomorpha linum*, *Cladophora dalmatica*, *Enteromorpha compressa*, *Enteromorpha linza*, *Percursaria percursa*, *Rhizoclonium tortuosum*, *Ulva curvata* and *Ulva rigida*. All algae showed a broad tolerance to the environmental conditions of irradiance, temperature and salinity. The most rapid growth rates were recorded in irradiances ranging from 18 to 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$, whilst the temperature promoting the most rapid algal growth ranged from 10 to 20 °C. With respect to salinity, the highest growth rates were recorded for algae cultured in the range 6.8 to 27.2 PSU. Several species were tolerant of salinities as low as 3.4 and 0 PSU. All algae showed a broad tolerance to a wide range of N and P concentrations. Maximum growth rates were recorded in nutrient concentrations ranging from 10 to 100 $\text{mmol m}^{-3} \text{PO}_4$, 100 to 1000 $\text{mmol m}^{-3} \text{NO}_3$ and 60 to 100 $\text{mmol m}^{-3} \text{NH}_4$. At these concentrations of N and P, considerable biomass was attained by the algae, with growth rates up to 21% per day. This, coupled with their broad environmental tolerance, is likely to promote their ecological success in the shallow, nutrient-enriched waters of Langstone Harbour and, indeed, in eutrophic waters worldwide.

Introduction

Langstone Harbour, on the south coast of England, is a sheltered, relatively enclosed tidal inlet. It has a total area of 19 km² of which approximately 15 km² are intertidal. The harbour is shallow, with a mean depth of 3.9 m, and is fully marine.

The waters of the harbour are subject both to agricultural run-off and light industrial pollution. The input of sewage, however, is more substantial. Biologically treated effluent is discharged from the local sewage works and enters the watercourse in the north of the harbour at a rate of $45 \times 10^3 \text{ m}^3 \text{ d}^{-1}$. The input of nutrients, coupled with the shallow, sheltered and enclosed characteristics of the harbour has greatly influenced the flora of the waters, promoting the rapid growth of nuisance, r-selected species (Taylor *et al.* 1998, Taylor 1999).

For example, the excessive growth of green algae (predominantly of *Ulva* and *Enteromorpha* species) has been reported in the harbour since the 1970s and the site has more recently been recognised as a 'possible eutrophication problem area' by the Oslo and Paris commissions (OSPAR convention).

Numerous investigations have been conducted with regard to the presence of 'green tide' algae in Langstone Harbour (e. g. Southgate 1972, Montgomery and Soulsby 1980, Soulsby *et al.* 1978, Lowthion *et al.* 1985, Montgomery *et al.* 1985), the most extensive reports being based on ecological studies. To date, however, very little is known about the ecophysiology of these nuisance algae. Algal growth rates may be regulated by environmental parameters such as irradiance and temperature (see Lüning 1990, Lobban and Harrison 1994 for reviews), whilst salinity may be one of the most important environmental factors to influence algal distribution (Kim *et al.* 1990). The availability of nutrients may also be one of the primary factors regulating the growth and development of macroalgae (DeBoer 1981), with nitrogen (e. g. Topinka and Robbins 1976, DeBoer and Ryther 1977) and phosphorus (e. g. Lapointe 1986, 1987, Harrison *et al.* 1990, Lapointe *et al.* 1992) being the elements most frequently limiting the growth of macroalgae in marine waters. At eutrophic sites it is thought that the role of N and P in the regulation of algal growth is of particular importance (Fletcher 1996).

In the current investigation, a number of 'green tide' algae were collected from the exposed intertidal mudflats of Langstone Harbour. Laboratory studies were undertaken to determine the response of these algae to a range of irradiances, temperatures and salinities. The N and P requirements of the algae were also recorded in terms of minimum growth requirements and those concentrations promoting the most rapid growth.

The aims of the investigation were two-fold:

(1) To investigate the ecophysiology of the 'green tide' algae from Langstone Harbour, using a monofactorial approach, in an attempt to elucidate why such algae are so successful in Langstone Harbour and other nutrient-enriched sites;

(2) To provide extensive data on the environmental tolerances and optima of these algae. This information may be of considerable use to those wishing

to culture these or similar 'green tide' algae in the laboratory.

Materials and Methods

Preparation of plant material

Samples of eight 'green tide' algae were collected from the intertidal mudflats of Langstone Harbour during low tide. These were *Ulva rigida* C. Agardh; *Ulva curvata* (Kützing) De Toni; *Enteromorpha compressa* (Linnaeus) Greville; *Enteromorpha linza* (Linnaeus) J. Agardh; *Cladophora dalmatica* Kützing; *Chaetomorpha linum* (O. F. Müller) Kützing; *Rhizoclonium tortuosum* (Dillwyn) Kützing; *Percursaria percursa* (C. Agardh) Rosenvinge. Collections were made from Langstone Harbour during the spring-summer period at the early stages in 'green tide' development. The ecological range for the harbour is shown in Table I.

All algae were returned to the laboratory and washed and cleaned to remove any surface contaminants. *Ulva rigida*, *U. curvata*, *Enteromorpha compressa* and *E. linza* were present in sufficient abundance for the material to be used immediately. Samples of *Cladophora dalmatica*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Percursaria percursa* were isolated and grown up in unialgal culture until sufficient material for the experimental work was obtained. Batch culture was conducted at 20 °C and 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16:8, L:D photoperiod. Algae were grown in modified Von Stosch culture medium (Von Stosch 1964).

Table I. Physico-chemical parameters recorded for Langstone Harbour (1994–1996 survey, Pye 2000).

Environmental parameter	Range
Salinity (PSU)	32.3–34.7
Secchi depth (m)	1.5–3.2
Temperature (°C) sediment	3.5–21.7
seawater	4.5–19.8
air	4.7–19.0
Nitrate (mg m^{-3})	< 5–1610
Ammonia (mg m^{-3})	< 6–938
Phosphate (mg m^{-3})	< 5–872

Table II. Culture regimes used to investigate the influence of irradiance, temperature, salinity, N and P on the growth of selected 'green tide' algae.

	Study 1: Temperature variable	Study 2: Irradiance variable	Study 3: Salinity variable	Study 4: [PO ₄ ³⁻] variable	Study 5: [NO ₃ ⁻] variable	Study 6: [NH ₄ ⁺] variable
Temperature (°C)	10, 15, 20, 25, 30	15	15	15	15	15
Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	72	0, 9, 18, 44, 88, 175	72	72	72	72
Salinity (PSU)	34.0	34.0	0, 3.4, 6.8, 10.2, 13.6, 17.0, 20.4, 23.8, 27.2, 30.6, 34.0	34.0	34.0	34.0
[PO ₄ ³⁻] (mmol m^{-3})	30	30	30	0, 10, 20, 30, 50, 100, 200	30	30
[NO ₃ ⁻] (mmol m^{-3})	500	500	500	500	0, 10, 50, 200, 400, 800, 1000	0
[NH ₄ ⁺] (mmol m^{-3})	0	0	0	0	0	0, 10, 20, 40, 60, 80, 100

Irradiance, temperature and salinity experiments (culture studies 1–3)

Algae were cultured in a modified Von Stosch medium under a wide range of environmental conditions (Table II).

Irradiance was from cool-white fluorescent tubes with a 16:8, L:D photoperiod, whilst culture rooms provided a constant temperature. To obtain the range of salinity values investigated, seawater at 34 practical salinity units (PSU) was diluted using distilled water enriched in the same manner as the modified Von Stosch medium.

N and P experiments (culture studies 4–6)

The effects of nutrients on algal growth were investigated using clean, 'nutrient low' seawater collected offshore in the open waters of the Solent (analysis showed this to contain $< 9 \text{ mmol m}^{-3} \text{ NO}_3^-$, $< 1 \text{ mmol m}^{-3} \text{ NH}_4^+$ and $< 0.8 \text{ mmol m}^{-3} \text{ PO}_4^{3-}$). This water was used to produce a basic culture medium that consisted of Von Stosch solution with no added N or P. Enrichments of PO_4^{3-} and NO_3^- or NH_4^+ were then added (as NaH_2PO_4 , $2\text{H}_2\text{O}$, NaNO_3 and NH_4Cl , respectively), to the culture medium in various concentrations (Table II).

Culture techniques and growth measurements

Vegetative material of each of the eight 'green tide' algae was cultured in standard plastic Petri dishes containing 30 mL of the appropriate medium. Dishes were then placed in culture rooms, under the appropriate environmental conditions. Petri dishes were regularly repositioned to ensure uniform irradiance was received by all culture vessels. With the exception of the two *Ulva* species and *Enteromorpha linza*, algal growth was measured as change in fresh weight with time. The wet weight of the initial inocula in each Petri dish was in the range 0.050 to 0.100 g.

For the foliose algae, *Ulva rigida*, *U. curvata* and *Enteromorpha linza*, discs (7 mm diameter) were cut from thalli using a stainless steel borer. Growth was measured as the increase in disc area with time. All procedures were conducted in triplicate.

Growth measurements were taken and culture media replaced in all cultures every 3 days for a total experimental period of 15 days. Growth rates were determined as relative growth rate $\times 100$ (Evans 1972, adapted by Lüning 1990) using the equation:

$$\% \text{ increase per day} = \frac{100 \ln(N_2/N_1)}{T_2 - T_1}$$

where N_1 and N_2 are wet weight or disc area at times T_1 and T_2 , respectively.

Data from all experiments were tested for normality and homogeneity prior to statistical treatment using analysis of variance (ANOVA).

Results

Figures 1 to 3 show the effect of environmental parameters on algal growth. Figures 4 to 6 show the effect of the different nutrients. Data are summarised

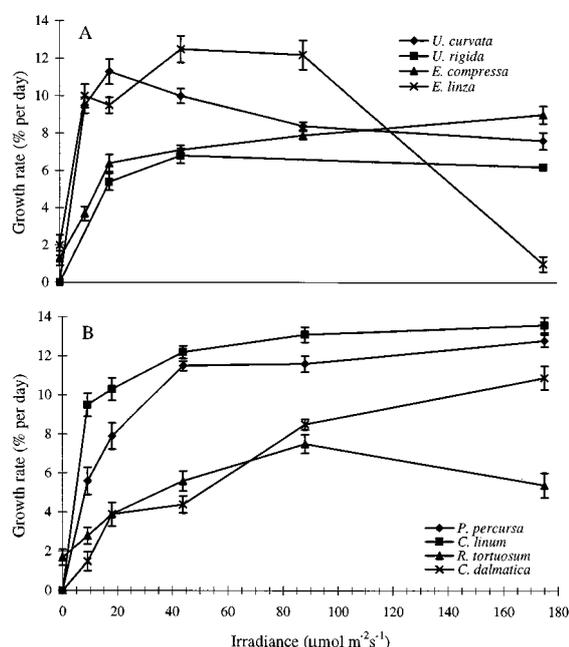


Fig. 1. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) under a range of irradiance levels. Error bars represent SE ($n = 3$).

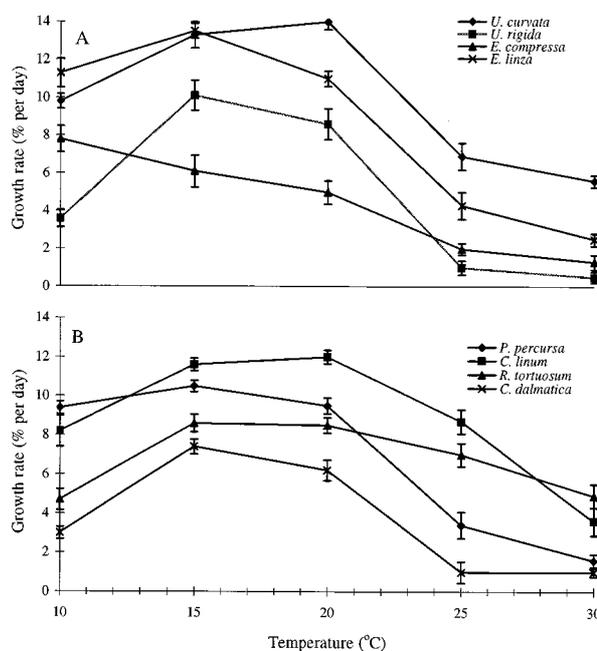


Fig. 2. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) under a range of temperature conditions. Error bars represent SE ($n = 3$).

in Table III, which lists the conditions promoting the highest growth rates.

The effect of irradiance on algal growth

Growth was significantly affected by irradiance

($p < 0.001$), with all algae showing a broad tolerance. With the exception of *Enteromorpha linza*, which showed a dramatic reduction in growth when the irradiance level increased from 88 to 175 $\mu\text{mol m}^{-2} \text{s}^{-1}$, all species were relatively tolerant of the highest irradiance level investigated. Indeed, for *Percursaria per-*

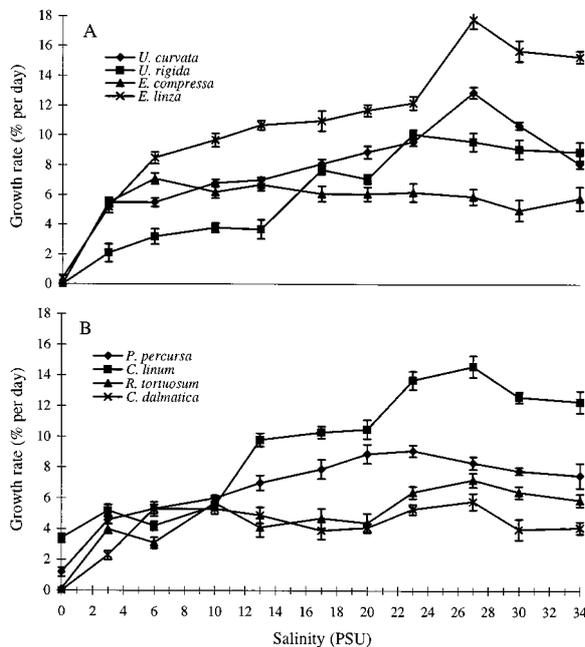


Fig. 3. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) in a range of salinity levels. Error bars represent SE (n = 3).

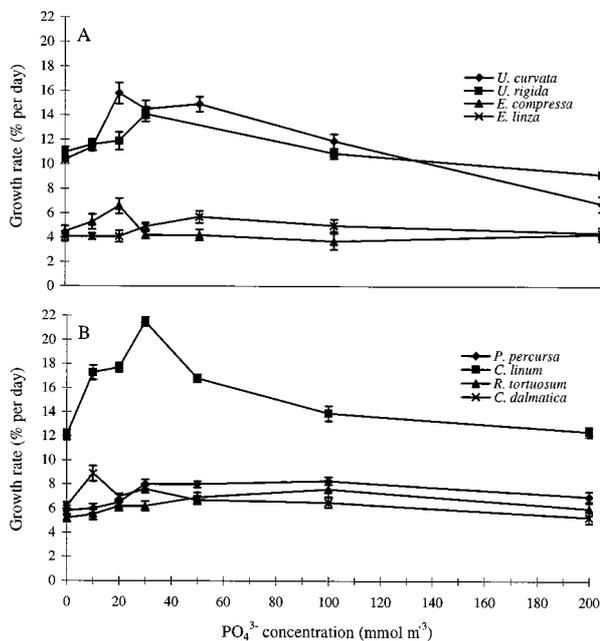


Fig. 4. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) in a range of PO_4^{3-} concentrations. Error bars represent SE (n = 3).

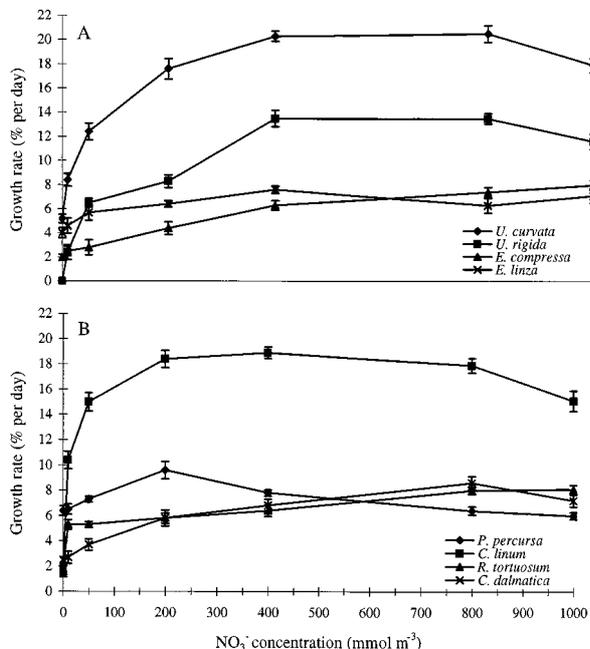


Fig. 5. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) in a range of NO_3^- concentrations. Error bars represent SE (n = 3).

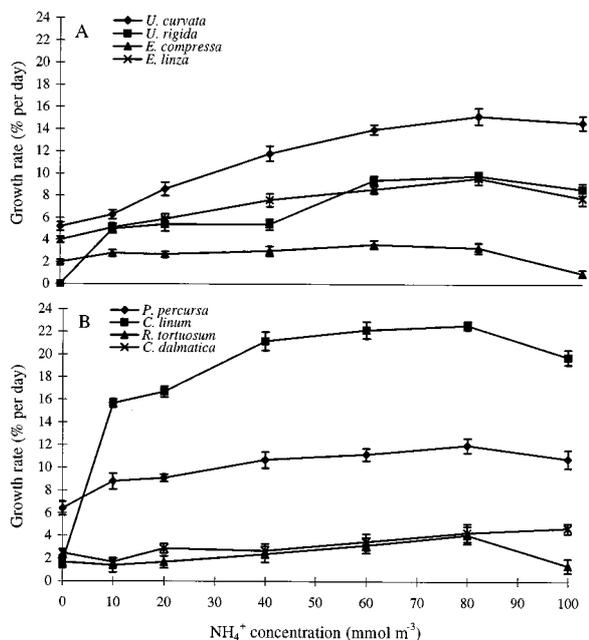


Fig. 6. Growth of *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *E. linza* (A) *Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Cladophora dalmatica* (B) in a range of NH_4^+ concentrations. Error bars represent SE (n = 3).

Table III. Growth conditions found to promote the highest growth rates of selected 'green tide' algae in laboratory culture.

	Temperature (°C)	Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Salinity (PSU)	[PO_4^{3-}] (mmol m^{-3})	[NO_3^-] (mmol m^{-3})	[NH_4^+] (mmol m^{-3})
<i>P. percursa</i>	15	175	23.8	100	100	80
<i>U. curvata</i>	20	18	27.2	20	800	80
<i>U. rigida</i>	15	54	23.8	30	400–800	80
<i>C. linum</i>	20	175	27.2	30	400	80
<i>R. tortuosum</i>	15–20	88	27.2	100	1000	80
<i>E. compressa</i>	10	175	6.8	20	1000	60
<i>E. linza</i>	15	72	23.8	50	400	80
<i>C. dalmatica</i>	15	175	27.2	10	800	100

percursa, *Chaetomorpha linum*, *Enteromorpha compressa* and *Cladophora dalmatica*, highest growth rates were at this irradiance. This suggests that these species, in particular, may be tolerant of even higher light levels.

The irradiance levels promoting the most rapid growth rates were generally high, apart from *Ulva curvata* and *U. rigida* which grew most rapidly at 18 and 44 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. All species grew well at the lower irradiances, with *Rhizoclonium tortuosum*, *Enteromorpha compressa* and *E. linza* apparently growing in complete darkness – this probably resulted from short bursts of light during the growth measurements. Growth re-occurred in all species originally cultured at 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$ following illumination (data not shown).

The effect of temperature on algal growth

Growth of all algae was significantly affected by temperature over the range investigated ($p < 0.05$). All species grew at the lowest temperature of 10 °C. The most rapid growth of *Enteromorpha compressa* was at this temperature, but for all other species growth increased at the higher temperature of 15 °C. The highest growth rates of all species except *E. compressa* were generally recorded at either 15 or 20 °C. Towards the end of the culture period, the growth of all species declined at 25 and 30 °C. These temperatures promoted rapid growth over short time periods (data not shown), but examination of material under the light microscope showed that prolonged culture caused plant tissue damage.

The effect of salinity on algal growth

Growth of all algae was significantly affected by salinity ($p < 0.005$), and in general, growth increased with increasing salinity up to 23.8 to 27.2 PSU (70–80% full seawater). The exception to this was *E. compressa* which, while showing a broad tolerance to all salinities tested, exhibited the most rapid growth at 6.8 PSU (20% full seawater).

Four of the species investigated (*Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *Cladophora dalmatica*) exhibited no growth at 0 PSU, though

growth resumed upon placing the material in full seawater (data not shown). The remaining algae (*Percursaria percursa*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Enteromorpha linza*) demonstrated some growth in freshwater, though growth rates were low. All algae tested showed a wide tolerance to salinity, exhibiting growth in 3.4 to 34 PSU (10 to 100% full seawater).

The effect of phosphorus (as PO_4^{3-}) on algal growth

All algae showed a broad tolerance with growth occurring at concentrations up to 200 mmol m^{-3} . In *Percursaria percursa*, *Ulva curvata*, *U. rigida* and *Chaetomorpha linum*, growth rates were significantly affected by PO_4^{3-} concentration ($p < 0.05$), with growth increasing with increasing PO_4^{3-} concentration up to an optimum level. In the remaining four algae, growth was not significantly affected by external PO_4^{3-} concentration.

The highest growth rate for *Cladophora dalmatica* was recorded at 10 $\text{mmol m}^{-3} \text{PO}_4^{3-}$, whilst *Ulva curvata*, *U. rigida*, *Enteromorpha compressa* and *Chaetomorpha linum* grew most rapidly at either 20 or 30 $\text{mmol m}^{-3} \text{PO}_4^{3-}$. The most rapid growth rates observed for the remaining species were recorded at the higher PO_4^{3-} concentrations, the most rapid growth of *Enteromorpha linza* recorded at 50 mmol m^{-3} and of *Percursaria percursa* and *Rhizoclonium tortuosum* at 100 mmol m^{-3} . All species grew at low rates in seawater unenriched with PO_4^{3-} .

The effect of nitrogen (as NO_3^- and NH_4^+) on algal growth

The growth of all algal material was significantly affected by the addition of both NO_3^- ($p < 0.05$) and NH_4^+ ($p < 0.05$) to the culture medium.

Percursaria percursa showed a significantly different response to the two N-sources ($p < 0.01$), and at concentrations up to 100 mmol m^{-3} , highest growth rates were recorded when NH_4^+ was used as the N-source. For *Ulva curvata*, highest growth rates were recorded when NO_3^- was the N-source, but analysis showed that, overall, differences in growth

due to the two N-sources were not significant. This was also the case for *U. rigida*, in which NH_4^+ promoted the highest growth rates. Of all the algae tested, *U. rigida* was the only species to show zero growth in a seawater medium with no N-enrichment.

In *Chaetomorpha linum*, NH_4^+ promoted significantly higher growth rates than NO_3^- ($p < 0.05$). This alga had a broad tolerance to NO_3^- with rapid growth from 0 to 1000 mmol m^{-3} .

The response of *Rhizoclonium tortuosum* was significantly different for the two N-sources ($p < 0.05$), with highest growth rates when NO_3^- was the N-source. When NH_4^+ was used, growth was reduced above 80 mmol m^{-3} . Overall, the two N-sources did not have a significantly different effect on the growth of *Enteromorpha compressa*, but $80 \text{ mmol m}^{-3} \text{ NO}_3^-$ promoted the highest growth. The growth rate of *E. linza* was highest when NH_4^+ was the N-source, there being a significant difference in growth between NH_4^+ and NO_3^- ($p < 0.001$).

In *Cladophora dalmatica*, growth occurred at a similar rate at the lower concentrations of both N-sources. At the higher concentrations, growth rates were highest in NO_3^- , but overall, there was no significant difference in algal growth in either of the N-sources.

The concentration of N promoting the highest growth rates varied widely with both N-source and algal species. Most rapid growth of most species occurred in the lower to mid-range of NO_3^- concentrations, though all algae showed a broad tolerance to higher concentrations. Indeed, highest growth rates were recorded at $1000 \text{ mmol m}^{-3} \text{ NO}_3^-$ in both *Enteromorpha compressa* and *Rhizoclonium tortuosum*. When NH_4^+ was used as the N-source, all algae showed maximum growth at the mid- to high range of concentrations. In *Enteromorpha compressa*, highest growth rates were at $60 \text{ mmol m}^{-3} \text{ NH}_4^+$. The most rapid growth of all other species was at 80 mmol m^{-3} with the exception of *Cladophora dalmatica* in which the highest growth rate was at $100 \text{ mmol m}^{-3} \text{ NH}_4^+$, suggesting that *C. dalmatica* in particular, may be tolerant of concentrations of NH_4^+ higher than those tested.

Discussion

Irradiance

The experimental data reveal all the investigated 'green tide' algae were broadly tolerant of a wide range of irradiance levels, from 9 to $175 \mu\text{mol m}^{-2} \text{ s}^{-1}$. All species exhibited rapid growth rates, even at lower irradiances; rapid growth under these conditions being explained by the general morphology of these species. All algae are either thin and sheet-like or tubular, or are filamentous providing a large surface area:volume quotient and allowing all constituent cells to be potentially photosynthetically active.

The tolerance of these algae to low irradiances finds support in the literature, with laboratory studies on the growth rate of intertidal *Enteromorpha clathrata* (Roth) J. Agardh showing the alga to be intolerant of high irradiances, with optimum growth at just $36 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (Fitzgerald 1978). Indeed, Sand-Jensen (1988) suggests that light saturation for growth of *Ulva lactuca* Linnaeus occurs at $55 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

With respect to high irradiances, most species investigated here responded favourably to irradiances up to $175 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Although we were unable to test higher light levels in the laboratory, the highest growth rates of *Percursaria percursa*, *Chaetomorpha linum*, *Enteromorpha compressa* and *Cladophora dalmatica* were recorded at $175 \mu\text{mol m}^{-2} \text{ s}^{-1}$, suggesting that these species, in particular, are probably tolerant of higher irradiance levels. Fortes and Lüning (1980), for example, suggest that the growth of *Ulva lactuca* may not be inhibited at irradiances up to $225 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Gordon *et al.* (1980) have similarly demonstrated that in *Cladophora albida* (Hudson) Kützing growth inhibition does not occur until $200\text{--}300 \mu\text{mol m}^{-2} \text{ s}^{-1}$, whilst in the field, there are numerous reports of 'green tide' algae appearing in abundance in shallow lagoons and estuaries where there is comparatively little attenuation of incident radiation (see Fletcher 1996 for a review).

Temperature

All algae investigated had a broad tolerance to the temperatures investigated. At the lowest temperature (10°C) growth rates were high, and in general the most rapid growth was at $15\text{--}20^\circ\text{C}$. At the lowest temperature (10°C), growth rates were very high, suggesting that all species will continue to thrive at temperatures lower than this. Indeed, *Enteromorpha intestinalis* (Linnaeus) Link from the harbour has been shown to tolerate temperatures below freezing (Taylor and Fletcher 1999).

With respect to the higher temperatures tested in the growth studies, data show all 'green tide' species from Langstone Harbour to be tolerant of temperatures up to 25 and 30°C , though plant tissue damage became evident towards the end of the culture period. Gessner (1970) suggests that for most marine algae, tolerance to high temperatures largely relates to temperatures experienced by these plants in their natural habitat; in this respect, there are reports of *Cladophora albida* from Australia, for example, surviving up to 40°C (Gordon *et al.* 1980). Innes (1988) shows that an alga's position on the shoreline may also influence its temperature tolerance, with high- and low-intertidal plants of *Enteromorpha linza* showing different temperature responses.

Salinity

All algae tested had a broad tolerance to salinity. The highest growth rates were recorded in culture media

of < 100% full seawater, with the most rapid growth generally between 23.8 and 27.2 PSU (70 and 80% full seawater).

All species were tolerant of the lower salinities. Indeed, *Enteromorpha compressa* grew most rapidly at just 6.8 PSU (20% full seawater). That 'green tide' algae are generally tolerant of low salinities has been shown in laboratory studies (McComb *et al.* 1981) and is illustrated in the field by the appearance of a variety of green species in areas like the brackish Baltic Sea (Wallentinus 1981, 1991, Schramm 1991) and the Danish fjords (Frederiksen 1987). In the current investigation, four of the species (*Percursaria percursora*, *Chaetomorpha linum*, *Rhizoclonium tortuosum* and *Enteromorpha linza*) demonstrated tolerance of low salinities by actively growing at 0 PSU. Lobban and Harrison (1994) suggest that *Cladophora* is also able to cross the so-called salinity barrier, but in the current investigation *C. dalmatica* showed no growth at 0 PSU. All species that did not grow at 0 PSU were able to tolerate 15 days in the non-saline medium and resumed normal growth when transferred to full seawater. A similar report has been made for *Enteromorpha clathrata* (Roth) Greville (Biebl 1956).

Algal growth rates in the current investigation remained high at 34 PSU, suggesting tolerance to even higher salinities than this. Support for this is provided by reports of *Cladophora* sp. (Birch *et al.* 1981, 1983), *Chaetomorpha* sp. and *Ulva rigida* (Lavery and McComb 1991) in Peel Inlet, Western Australia, where salinity in the summer rises to 50 PSU (Gordon *et al.* 1980). Pérez-Ruzafa *et al.* (1989) similarly document the growth of several 'green tide' species in the hyper-saline Mar Menor Lagoon, S. E. Spain.

Many articles in the current literature suggest that *Enteromorpha* species, in particular, have a broad salinity tolerance. Reed and Russell (1979) and Young *et al.* (1987) suggest that samples of *Enteromorpha intestinalis* from the high intertidal tolerate salinities from 0 to 51 PSU, whilst Kim *et al.* (1990) have demonstrated growth of *E. multiramosa* Bliding in the range 8 to 48 PSU. Optimum growth of *E. clathrata* was between 25 and 35 PSU (Fitzgerald 1978), whilst *E. linza* grew best at 30 to 35 PSU (Kjeldsen and Phinney 1971). The latter authors suggest that *Enteromorpha* species grow best in near-to-normal salinity, but in the current investigation the most rapid growth of *E. linza* was recorded at 23.8 PSU (70% full seawater), whilst *E. compressa* had highest growth rates at 6.8 PSU (just 20% full seawater).

Phosphorus (as PO_4^{3-})

Four species were significantly affected by the addition of P to the culture medium with growth being greatly enhanced. In the literature, very few reports document the growth of macroalgae in response to PO_4^{3-} when applied singly. Gordon *et al.* (1981) working with *Cladophora albida* showed optimum

growth of this alga to occur at a PO_4^{3-} concentration of just 2.63 mmol m^{-3} and similarly, in the current investigation, the highest growth rate of *C. dalmatica* was at just 10 mmol m^{-3} PO_4^{3-} . For the remainder of the species tested, highest growth was at 20 to 30 mmol m^{-3} PO_4^{3-} .

The ability of all the algae tested to grow well in a wide range of PO_4^{3-} concentrations concurs with their excessive growth in Langstone Harbour. In the northern region of the harbour, in particular, phosphates derived from detergents and the partial mineralisation of organic matter in sewage, are present in large amounts, enriching the waters and fuelling plant growth (Montgomery *et al.* 1985).

Nitrogen

In the current investigation, *Percursaria percursora*, *Chaetomorpha linum* and *Enteromorpha linza* grew most rapidly in culture media enriched with NH_4^+ , whilst highest growth rates of *Rhizoclonium tortuosum* were in media enriched with NO_3^- . For the remaining species there was no significant difference in the growth of the algae in either N-source.

Similar findings (i. e. the assimilation of NH_4^+ and NO_3^- to promote equally high growth rates) have been reported for *Chondrus crispus* Stackhouse (Neish and Fox 1971, Neish and Shacklock 1971, Prince 1974) and in *Fucus* spp. (Topinka and Robbins 1976), whilst work by Hanisak (1979) on *Codium fragile* (Suringar) Hariot subsp. *tomentosoides* (Van Goor) Silva shows the alga to grow equally well with NO_3^- , NO_2^- or NH_4^+ .

Lapointe and Ryther (1979) found that the growth of *Gracilaria foliifera* (Forskål) Børgesen was highest when NH_4^+ was used as the N source at N loadings just adequate to support exponential growth. At higher loadings NO_3^- promoted optimum growth. A preference for NO_3^- has been documented for several algal species (see Iwasaki 1967, Nasr *et al.* 1968, Rao and Mehta 1973), whilst Koutropoulos *et al.* (1991) found *Ulva* spp. to grow better when NH_4^+ was used as the N-source rather than NO_3^- . The authors report almost 30% more growth in NH_4^+ , but in the current investigation there was no significant difference in the growth of *Ulva curvata* or *U. rigida* when grown in NO_3^- or NH_4^+ .

The highest growth rates of most species occurred in the lower to mid range of NO_3^- concentrations tested here, though all algae had a broad tolerance to the higher concentrations. In tests with NH_4^+ , all algae grew most rapidly in the mid- to high range of concentrations.

Many algal species are similarly tolerant of high N concentrations, with previous studies reporting the optimum N concentration for growth to be in the range of 120 to 2140 mmol m^{-3} (DeBoer *et al.* 1978). The literature suggests that in macroalgae tolerances to N are generally broad. Morgan *et al.* (1980) report

the growth of *Palmaria palmata* (Linnaeus) Kuntze for some 5 weeks in seawater enriched with 0.5 to 2 mol m⁻³ NO₃⁻ or NH₄⁺. Wu *et al.* (1984) similarly recorded optimum growth of *Porphyra yezoensis* Ueda and *Gracilaria verrucosa* (Hudson) Papenfuss at 0.5 and 5 mol m⁻³ NH₄⁺, respectively. Laycock *et al.* (1981) found *Chondrus crispus* to be tolerant of additions of 0.5 mol m⁻³ NH₄⁺ four times weekly, whilst Iwasaki (1967) observed that the growth of the *Conchocelis* phase of *Porphyra tenera* Kjellman was only inhibited by concentrations greater than 7.0 mol m⁻³.

Concentrations of inorganic N between approximately 35 and 357 mmol m⁻³ have been shown to promote optimum growth in *Cladophora albida* (Gordon *et al.* 1981), although the authors also reported the alga to grow well in a nutrient-free medium. In the current investigation all species, with the exception of *Ulva rigida*, were similarly able to maintain growth in a seawater medium unenriched with N, suggesting that, for a limited time period at least, the algae are able to rely on internal nutrient reserves for growth. The utilisation of accumulated internal nutrient reserves has been shown in both *Enteromorpha* and *Ulva* by Fujita (1985) and in *Ulva* by Rosenberg and Ramus (1982).

At both optimal and sub-optimal conditions, considerable biomass was attained by all algae. All algae were able to withstand periods of low irradiance and darkness, reflecting their ability to survive the winter months in northern latitudes where daytime irradiance may be low. Similarly, it suggests that the algae are well adapted to the shaded conditions associated with the thick mats of algae commonly found during the summer months on the Langstone Harbour mudflats. Tolerance to high light intensities was also demonstrated, the algae being subjected to high irradiance on a 16:8 L:D photoperiod. This reflects the ability of 'green tide' species to tolerate such conditions in the field during the summer months. At low tide the upper-most layers of algae on the harbour's mudflats are exposed for lengthy periods to the effects of strong solar radiation.

The tolerance of the algal species to high temperatures (25 and 30 °C) may similarly be explained by

the exposure of the algal mats to such conditions during low tide in the summer months. At 10 °C, the lowest temperature investigated, algal growth rates were high. This suggests that all species will continue to thrive at temperatures below this. Indeed, they are tolerant of winter temperatures in the harbour of below 5 °C.

With respect to salinity, all algae had a broad tolerance range. Tolerance to higher salinities is demonstrated by the occurrence of significant field populations in the fully marine Langstone Harbour, whilst the euryhaline nature of 'green tide' species is reflected in the wide range of habitats they occupy, these being of both high and low salinity.

In addition to exhibiting rapid growth under a wide range of environmental conditions, all 'green tide' algae in the current investigation showed a broad tolerance to a wide range of N and P concentrations. The ability to grow well in high PO₄³⁻ concentrations is reflected by the excessive growth of several 'green tide' species in the north of the harbour where sewage effluent is released. Levels of N in Langstone Harbour are similarly elevated by the presence of sewage, and in the current investigation the growth rate of all algae was greatly enhanced by the addition of high concentrations of N to the culture medium.

Considerable biomass was attained by algae cultured at elevated N and P concentrations, whilst all species were broadly tolerant of light, temperature and salinity. It is suggested, therefore, that when combined, all these factors undoubtedly contribute towards the success of 'green tide' algae in Langstone Harbour and, indeed, in eutrophic waters worldwide.

Acknowledgements

This work was carried out with financial assistance from the European Commission, under the auspices of the EUMAC project, within the framework of the Environmental Programme (Contract no. EV5V-CT93-0290).

Accepted 26 February 2001.

References

- Biebl, R. 1956. Lichtresistenz von Meeresalgen. *Protoplasma* 46: 63–89.
- Birch, P. B., D. M. Gordon and A. J. McComb. 1981. Nitrogen and phosphorous nutrition of *Cladophora* in the Peel-Harvey system. *Bot. Mar.* 24: 281–387.
- Birch, P. B., J. O. Gabrielson and K. S. Hamel. 1983. Decomposition of *Cladophora*. 1. Field studies in the Peel-Harvey estuarine system, Western Australia. *Bot. Mar.* 26: 165–171.
- DeBoer, J. A. 1981. Nutrients. In: (C. S. Lobban and M. J. Wynne, eds) *The Biology of Seaweeds*, Botanical Monographs Vol 17. Blackwell Scientific Publications, Oxford. pp. 356–392.
- DeBoer, J. A. and J. H. Ryther. 1977. Potential yields from a waste-recycling algal mariculture system. In: (R. Krauss, ed.) *The Marine Plant Biomass of the Pacific Northwest Coast*. Oregon State University Press, Corvallis, Oregon. pp. 231–249.
- DeBoer, J. A., H. J. Guigli, T. L. Israel and C. F. D'Elia. 1978. Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. *J. Phycol.* 14: 261–266.

- Evans, G. C. 1972. *The Quantitative Analysis of Plant Growth*. Blackwell Scientific Publications, Oxford. pp. 734.
- Fitzgerald, W. J. 1978. Environmental parameters influencing the growth of *Enteromorpha clathrata* (Roth) J. Ag. in the intertidal zone in Guam. *Bot. Mar.* 21: 207–220.
- Fletcher, R. L. 1996. The occurrence of 'Green Tides' – a review. In: (W. Schramm and P. H. Nienhuis, eds) *Ecological Studies, Vol. 123. Marine Benthic Vegetation*. Springer-Verlag, Berlin/Heidelberg. pp. 7–43.
- Fortes, M. D. and K. Lüning. 1980. Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgoländer wiss. Meeresunters.* 34: 15–29.
- Frederiksen, O. T. 1987. The fight against eutrophication in the inlet of "Odense fjord" by reaping of sea lettuce (*Ulva lactuca*). *Wat. Sci. Tech.* 19: 81–87.
- Fujita, R. M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. *J. Exp. Mar. Biol. Ecol.* 92: 283–301.
- Gessner, F. 1970. Temperature: Plants. In: (O. Kinne, ed.) *Marine Ecology, Vol. 1 (1)*. Wiley-Interscience, New York. pp. 363–406.
- Gordon, D. M., P. B. Birch, and A. J. McComb. 1980. The effect of light, temperature and salinity on photosynthetic rates of an estuarine *Cladophora*. *Bot. Mar.* 23: 749–755.
- Gordon, D. M., P. B. Birch and A. J. McComb. 1981. Effects of inorganic phosphorus and nitrogen on the growth of an estuarine *Cladophora* in culture. *Bot. Mar.* 24: 93–106.
- Hanisak, M. D. 1979. Nitrogen limitation of *Codium fragile* spp. as determined by tissue analysis. *Mar. Biol.* 50: 333–337.
- Harrison, P. J., M. H. Hu, Y. P. Yang and X. Lu. 1990. Phosphate limitation in estuarine and coastal waters of China. *J. Exp. Mar. Biol. Ecol.* 140: 79–87.
- Innes, D. J. 1988. Genetic differentiation in the intertidal zone in populations of the alga *Enteromorpha linza* (Ulvales: Chlorophyta). *Mar. Biol.* 97: 9–16.
- Iwasaki, H. 1967. Nutritional studies of the edible seaweed *Porphyra tenera*. II. Nutrition of *Conchocelis*. *J. Phycol.* 3: 30–34.
- Kim, K. Y., I. K. Lee and I. C. Chung. 1990. Effects of temperature and salinity on germination and vegetative growth of *Enteromorpha multiramosa* Bliding (Chlorophyceae, Ulvales). *Korean J. Bot.* 33: 141–146.
- Kjeldsen, C. K. and H. K. Phinney. 1971. Effects of variations in salinity and temperature on some estuarine macro-algae. *Proc. Int. Seaweed Symp.* 7: 301–308.
- Koutropoulos, D., G. Nikolaidis and S. Haritonidis. 1991. Biomass response of the macrophyte *Ulva* spp. to nitrogen enriched seawater. *Oebalia* 17: 65–72.
- Lapointe, B. E. 1986. Phosphorus-limited photosynthesis and growth of *Sargassum natans* and *Sargassum fluitans* (Phaeophyceae) in the western North Atlantic. *Deep Sea Res.* 33: 391–399.
- Lapointe, B. E. 1987. Phosphorus- and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Mar. Biol.* 95: 561–568.
- Lapointe, B. E. and J. H. Ryther. 1979. The effects of nitrogen and seawater flow rate on the growth and biochemical composition of *Gracilaria foliifera* var. *angustissima* in mass outdoor cultures. *Bot. Mar.* 22: 529–538.
- Lapointe, B. E., M. M. Littler and D. S. Littler. 1992. Nutrient availability to marine macroalgae in siliciclastic versus carbonate-rich coastal waters. *Estuaries* 15: 75–82.
- Lavery, P. S. and A. J. McComb. 1991. Macroalgal-sediment nutrient interactions and their importance to macroalgal nutrition in a eutrophic estuary. *Estuar. Coast. Shelf Sci.* 32: 281–295.
- Laycock, M. V., K. C. Morgan and J. S. Craigie. 1981. Physiological factors affecting the accumulation of L-citrullinyl-L-arginine in *Chondrus crispus*. *Can. J. Bot.* 59: 522–527.
- Lobban, C. S. and P. J. Harrison. 1994. *Seaweed Ecology and Physiology*. Cambridge University Press. pp. 366.
- Lowthion, D., P. G. Soulsby and M. C. M. Houston. 1985. Investigation of a eutrophic tidal basin. I. Factors affecting the distribution and biomass of macroalgae. *Mar. Environ. Res.* 15: 263–284.
- Lüning, K. 1990. *Seaweeds: Their Environment, Biogeography and Ecophysiology*. Wiley-Interscience, New York. pp. 527.
- McComb, A. J., R. P. Atkins, P. B. Birch, D. M. Gordon and R. J. Lukatelich. 1981. Eutrophication in the Peel-Harvey Estuarine System, Western Australia. In: (B. J. Neilson and L. E. Cronin, eds) *Estuaries and Nutrients*. Humana Press, New Jersey. pp. 323–342.
- Montgomery, H. A. C. and P. G. Soulsby. 1980. Effects of eutrophication on the intertidal ecology of Langstone Harbour, U. K., and proposed control measures. *Prog. Wat. Tech.* 13: 287–294.
- Montgomery, H. A. C., P. G. Soulsby, I. C. Hart and S. L. Wright. 1985. Investigation of a eutrophic tidal basin: Part 2: Nutrients and environmental aspects. *Mar. Environ. Res.* 15: 285–302.
- Morgan, K. C., P. F. Shacklock and F. J. Simpson. 1980. Some aspects of the culture of *Palmaria palmata* in greenhouse tanks. *Bot. Mar.* 23: 765–770.
- Nasr, A. H., I. A. Bekheet and R. K. Ibrahim. 1968. The effects of different nitrogen and carbon sources on amino acid synthesis in *Ulva*, *Dictyota* and *Pterocladia*. *Hydrobiologia* 31: 7–16.
- Neish, A. C. and C. H. Fox. 1971. Greenhouse experiments on the vegetative propagation of *Chondrus crispus* (Irish Moss). *Tech. Rept. No. 12, Halifax, NS Atlantic Regional Laboratory*. National Research Council of Canada. pp. 35.
- Neish, A. C. and P. F. Shacklock. 1971. Greenhouse experiments (1971) on the propagation of strain T4 of Irish Moss. *Tech. Rept. No. 14, Halifax, NS Atlantic Regional Laboratory*. National Research Council of Canada. pp. 35.
- Pérez-Ruzafa, A., J. D. Ros, C. Marcos, R. Ballester and I. M. Pérez-Ruzafa. 1989. Distribution of the macrophyte beds in a hypersaline coastal lagoon (the Mar Menor, SE Spain) and its recent evolution related to changes in salinity. In: (C.-F. Boudouresque, A. Meinesz, E. Fresi and V. Gravez, eds) *Proceedings of the 2nd International Workshop in Posidonia Beds*. GIS Posidonie Publications, Marseille. pp. 49–62.

- Prince, J. G. 1974. Nutrient assimilation and growth of some seaweeds in mixtures of seawater and secondary sewage treatment effluents. *Aquaculture* 4: 69–79.
- Pye, K. E. 2000. The effects of eutrophication on the marine benthic flora of Langstone Harbour, South Coast of England. PhD Thesis, University of Portsmouth, U. K. pp. 260.
- Rao, P. S. and V. B. Mehta. 1973. Physiological ecology of *Gelidiella acerosa* Forskål Feldman et Hamel. *J. Phycol.* 9: 33–335.
- Reed, R. H. and G. Russell. 1979. Adaptation to salinity stress in populations of *Enteromorpha intestinalis* (L.) Link. *Estuar. Coast. Mar. Sci.* 8: 251–258.
- Rosenberg, G. and J. Ramus. 1982. Ecological growth strategies in the seaweed *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): soluble nitrogen and reserve carbohydrates. *Mar. Biol.* 66: 251–259.
- Sand-Jensen, K. 1988. Photosynthetic responses of *Ulva lactuca* at very low light. *Mar. Ecol. Prog. Ser.* 50: 195–201.
- Schramm, W. 1991. Eutrophication and recent changes in macrophyte vegetation in the western Baltic (Kiel Bay). In: (R. Delepine, ed.) *Workshop Proceedings of COST 48, subgroup III, St. Malo, France, 10–13th March 1991*. Cost 48, Commission of the European Communities, Brussels. pp. 69–78.
- Soulsby, P. G., D. Lowthion, and M. C. M. Houston. 1978. Observations on the effects of sewage discharged into a tidal harbour. *Mar. Pollut. Bull.* 9: 242–245.
- Southgate, B. A. 1972. Langstone Harbour study. *Report to Hampshire River Authority and the Hampshire County Council*. pp. 20.
- Taylor, R. 1999. The 'green tide' threat in the UK – a brief overview with particular reference to Langstone Harbour, south coast of England and the Ythan Estuary, east coast of Scotland. *Bot. J. Scotl.* 51: 195–203.
- Taylor, R. and R. L. Fletcher. 1999. A simple method for the freeze-preservation of zoospores of the green macroalga *Enteromorpha intestinalis*. *J. appl. Phycol.* 11: 257–262.
- Taylor, R., R. L. Fletcher and K. Pye. 1998. The 'green tide' phenomenon in the North Sea and English Channel: case studies of the Ythan estuary and Langstone Harbour. In: (G. W. Scott and I. Tittley, eds) *Changes in the Marine Flora of the North Sea*. CERC Publications, Scarborough. pp. 166
- Topinka, J. A. and J. V. Robbins. 1976. Effects of nitrate and ammonium enrichment on growth and nitrogen physiology in *Fucus spiralis*. *Limnol. Oceanogr.* 21: 659–664.
- Von Stosch, H. A. 1964. Wirkung von Jod und Arsenit auf Meeresalgen in Kultur. *Proc. Int. Seaweed Symp.* 4: 142–150.
- Wallentinus, I. 1981. Chemical constituents of some Baltic macroalgae in relation to environmental conditions. *Proc. Int. Seaweed Symp.* 10: 363–370.
- Wallentinus, I. 1991. The Baltic Sea gradient. In: (A. C. Mathieson and P. H. Nienhuis, eds) *Ecosystems of the World – 24 Intertidal and Littoral Ecosystems*. Elsevier, Amsterdam. pp. 83–108.
- Wu, C., Y. Zhang, R. Li, Z. Peng, J. Zhang and X. Fan. 1984. On the utilization of the ammonium-nitrogen by two red algae, *Porphyra yezoensis* and *Gracilaria verrucosa*. *Hydrobiologia* 116/117: 475–477.
- Young, A. J., J. C. Collins and G. Russell. 1987. Ecotypic variation in the osmotic responses of *Enteromorpha intestinalis* (L.) Link. *J. Exp. Bot.* 38: 1309–1324.