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Seasonal Variation of Antifouling Activities of Marine Algae from the Brittany Coast (France)

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Abstract: The antifouling activity of extracts (aqueous, ethanol, and dichloromethane) of 9 marine macroalgae against bacteria, fungi, diatoms, macroalgal spores, mussel phenoloxidase activity, and barnacle cypris larvae has been investigated in relation to season in bimonthly samples from the Bay of Concarneau (France). Of the extracts tested, 48.2% were active against at least one of the fouling organisms, and of these extracts, 31.2% were seasonally active with a peak of activity in summer corresponding to maximal values for water temperature, light intensity, and fouling pressure, and 17% were active throughout the year. This seasonal activity may be adaptive as it coincides with maximal fouling pressure in the Bay of Concarneau. Dichloromethane extracts of Rhodophyceae were the most active in the antifouling assays.

Keywords: antifouling, settlement, bacteria, barnacle, diatoms, fungi.

INTRODUCTION

All natural and man-made surfaces that are immersed in marine environments are potentially affected by the attachment of epibiotic and fouling organisms, respectively. For man-made surfaces, the process of fouling begins as soon as a substratum is immersed with the adsorption of macromolecules, predominantly proteins, lipolysaccharides, and polysaccharides (Baier, 1984; Wahl, 1989; Terlizzi et al., 2001). Subsequent microfouling and macrofouling by microbial slimes, algae, and invertebrates can result in major economic costs through, for example, corrosion (Little et al., 1999) and a reduction in the fuel efficiency of ships underway due to increased drag. Biofouling control is a worldwide problem in marine systems, which costs the U.S. Navy, for example, an estimated $1 billion per annum (Callow and Callow, 2002).

In the days of wooden sailing ships, antifouling methods included the use of arsenic, lime, and mercurial compounds to prevent shipworms and barnacles from destroying the hull. Although organotin compounds were synthesized almost 150 years ago, it was not until the 1960s that tributyltin (TBT) first appeared in marine paint for-

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mulations. By the 1970s most ships were coated with antifouling paint formulations based on release of toxic organotin or copper. The shipping industry still relies heavily on such coatings as they afford long-term protection and are comparatively cheap. Heavy metals are known, however, to be responsible for environmental damage to both terrestrial and marine life (Fent and Hunn, 1997). In the marine environment the continuous leaching of heavy metals has been responsible for shell thickening in oysters, sex changes in invertebrates, and possible genetic defects in other marine animals (Gibbs et al., 1987; Boyer, 1989; Ellis and Pattisinia, 1990; Axiak et al., 1995; Huet et al., 1996; Terlizzi et al., 1998, 2001; Granmo et al., 2002).

There have been a number of new developments in toxic antifouling coatings through innovations made by the paint industry. However, these tin-free coating formulations contain high concentrations of metals such as copper and zinc, in combination with booster biocides such as Irgarol and SeaNine 211 (Vouvoulis et al., 1999; Martinez et al., 2001), which may also pose environmental problems (Blanck, 2002; Ranke and Jastrorff, 2002; Omae, 2003). Moreover, these relatively new coatings are 2 to 3 times more expensive than TBT-based paints. It is increasingly evident that the future of fouling prevention lies in a nontoxic approach. Fouling release coatings based on silicone technology are a promising alternative to heavy-metal-based coatings, as is the use of secondary metabolites produced by marine organisms or synthetic compounds inspired by these natural products (Clare, 1996a; 1998; Holmström et al., 2002). Macroalgae are a rich source of natural bioactive products. In previous studies (Hellio et al., 2000a, 2000b, 2001a, 2001b, 2002), it was demonstrated that macroalgae from Brittany contain products with antifouling activity against marine bacteria, fungi, diatoms, seaweeds, and mussels. Little is known about the ecologic function of these compounds (de Nys et al., 1995; de Nys and Steinberg, 2002). Moreover, insufficient attention has been paid to evaluating the possible temporal variation in antifouling activity. Studies of chemical defenses in terrestrial organisms (Abarzua and Jakubowski, 1995) and marine organisms (Amade and Lemee, 1998; Steinberg and Vanaltena, 1992; Culioli et al., 2002) suggest that organisms vary widely in the production of chemical defenses associated with physical factors (temperature, light) and biological factors (e.g., grazing pressure), and season. The present study aimed to investigate seasonal variations in antifoulant activity of 9 macroalgal species collected from the Bay of Concarneau (France).

Materials and Methods

Collection and Preparation of Samples

Nine species of marine algae were collected every 2 months in 1999 (January, March, May, July, September, and November) from the West Coast of France (Concarneau Bay, Brittany, 47°52′ N, 3°55′ W). The algae studied included 3 Chlorophyta Ulvophyceae, *Enteromorpha intestinalis*, *Ulva lactuca*, and *Cladophora rupestris*; 3 Heterokontophyta, Phaeophyceae, *Ascophyllum nodosum*, *Sargassum muticum*, and *Ectocarpus siliculosus*; and 3 Rhodophyta, Florideophyceae, *Chondrus crispus*, *Laurencia pinnatifida*, and *Polysiphonia lanosa*.

After collection, the samples were rinsed with sterile seawater to remove associated debris. The cleaned material was then surface-dried in the shade at 30°C for 24 hours. The surface microflora were removed by washing the algal samples for 10 minutes with 30% ethanol (Hellio et al., 2000b).

Extracts A (aqueous), B (ethanol), and C (dichloromethane) were obtained as previously described by Hellio et al. (2000a). The extracts were stored at −40°C prior to their use.

Antifouling Tests

Screening for antifouling activity was performed using algal extracts at a concentration of 30 μg/ml. All assays were run in triplicate. Negative controls with the solvent carrier (5% DMSO v/v) were performed in every assay and showed no inhibition of the biological activities studied. In all our different assays (with the exception of the *Balanus amphitrite* test), we have studied the activities of bis (tributyltin) oxide (TBTO) and cupric sulfate ($CuSO_4$), at the concentration of 10 μg/ml as the controls (Hellio, 2000).

Antibacterial and Antifungal Tests

Six strains (B1 to B6) of marine bacteria were obtained from the Culture Collection of the University of Quimper (LUMAQ, France), which had been collected from the tidal zone in the Glénan Islands (Brittany, France) (47°43′95″ N, 4°01′85″ W). B1, B2, and B3 were gram-positive bacteria (*Bacillus*) isolated from the surface of *Enteromorpha* sp., *Gigartina* sp., and *Cladophora rupestris*. B4, B5, and B6 were gram-negative bacteria (*Bacillus*) isolated from the surface

of *Gelidium corneum*, *Enteromorpha* sp., and *Laminaria* sp. Three strains of marine fungi (F1 to F3), *Corollospora maritima*, *Lulworthia* sp., and *Dendryphiella salina*, were obtained from the Culture Collection of the School of Biological Sciences, University of Portsmouth, U.K.

Antibacterial and antifungal testing of the extracts was performed with agar-plated Petri dishes by a disk diffusion technique modified from Devi et al. (1997) as previously described by Hellio et al. (2001a). A sample consisting of 30 μg of product was loaded onto paper disks (6-mm diameter, Durieux, France). Microorganism cultures were grown in liquid DIFCO 2216 marine broth overnight, and 0.1-ml samples of the culture (10^6 cfu/ml) were spread over the agar. After incubation for 4 days at 20°C, the activity was evaluated by measuring the diameter (in millimeters) of the inhibition zones around the disks.

Inhibition of Diatom Growth

Diatomophyceae strains were obtained from Algobank, the Biological Resource Center of the University of Caen. These include *Amphora coffeaeformis* AC-2078 (D1), *Phaeodactylum tricornutum* AC-172 (D2), and *Cylindrotheca closterium* Ac-170 (D3). They were maintained in 100-ml Erlenmeyer flasks under continuous illumination 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ white fluorescent lamps at 18°C in Guillard's F/2 medium (Guillard and Ryther, 1972). The screening for bioactivity was performed as described by Sawant and Garg (1995). The effect of algal extracts (300 μg/ml) on the growth of microalgae (5×10^5 cells/ml) after 5 days of incubation was expressed as the percentage inhibition of growth (Hellio et al., 2002).

Inhibition of Attachment of Spores and Zygotes of Macroalgae

Spores of *Enteromorpha intestinalis* (M1) and *Ulva lactuca* (M2) were obtained by the osmotic shock method (Fletcher, 1989), and the protocol developed by Fletcher (1980) was used for the emission of zygotes from *Sargassum muticum* (M3). Antifouling tests were performed after Hattori and Shiruzi (1996). The effect of extracts (30 μg/ml) on macroalgal development was assessed by determining the percentage inhibition of attachment following incubation of spores or zygotes (600/ml) for 5 days at 20°C in plastic Petri dishes (35-mm diameter). The rate of inhibition of attachment was then calculated.

Inhibition of Phenoloxidase Activity of the Blue Mussel *Mytilus edulis*

Phenoloxidase activity was measured as a proxy for antifouling activity of extracts against *Mytilus edulis*, as previously described by Hellio et al. (2000a). Aliquots of pure enzyme were incubated at 25°C with 10 mM L-DOPA in 50 mM phosphate buffer (pH 6.8) and with the different algal extracts (30 μg/ml). Results are expressed as the percentage inhibition of the phenoloxidase activity compared to a control containing only buffer.

Inhibition of *Balanus amphitrite* Larval Settlement

Adult barnacles, *Balanus amphitrite*, were obtained from pier piling at the Duke University Marine Laboratory, Beaufort, North Carolina (courtesy of Dr. D. Rittschof). They were maintained at Newcastle University in aerated, filtered (10 μm) seawater, at 22°C, on a 14:10 light-dark cycle and fed daily on *Artemia* sp. The seawater was changed on alternate days. To obtain nauplii for cyprid culture, adults were left to dry overnight and then immersed in fresh seawater. Hatched nauplii were attracted to a point light source and collected by Pasteur pipette. They were reared to the cyprid stage on *Skeletonema costatum* as described in Billinghurst et al. (1998). After 4 to 5 days of culture, the cyprids were harvested using a 250-μm plankton mesh filter and aged for 3 days prior to use, in 0.45-μm-filtered seawater, at 6°C, in the dark (Rittschof et al., 1992; Clare, 1996b).

Settlement assays were conducted in 24-well microplates (Iwaki) with 15 to 20 cyprids per well in 2 ml of seawater. Six replicates of each algal extract were assayed at a concentration of 30 μg/ml. The plates were incubated in the dark at 28°C. Settlement was enumerated under a dissecting microscope after 12, 24, and 48 hours of incubation. The results were expressed as the percentage inhibition of the settlement compared to the controls.

Results

Antifouling Activity

Antifungal Activity

None of the Ulvophyceae tested (Table 1) showed antifungal activity. Of the 54 extracts of Phaeophyceae

Table 1. Effect of Positive Controls (TBTO and $CuSO_4$) and Ulvophyceae Extracts on Inhibition of Development of Fouling Organisms[a,b]

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
Control		+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	Nd
TBTO		+++	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	Nd
$CuSO_4$																		
1A	Jan.	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−
	March	−	−	−	−	−	−	−	−	−	+	−	−	++	++	++	−	−
	May	−	−	−	−	−	−	−	−	−	++	+	−	+++	++	+++	−	−
	July	−	−	−	−	−	−	−	−	−	++	+	−	+++	++	+++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	+	−	−	++	++	++	−	−
	Nov.	−	−	−	−	−	−	−	−	−	+	−	−	+	+	+	−	−
1B	Jan.	−	−	−	+	+	+	−	−	−	+	+	−	+	+	+	+	−
	March	−	−	−	+++	++	++	−	−	−	+	+	+	+	+	+	+	−
	May	−	−	−	+++	+++	+++	+	+	+	++	+	++	++	++	++	++	−
	July	−	−	−	++	+++	+++	+	+	+	++	+	++	++	++	++	++	−
	Sept.	−	−	−	++	++	++	−	−	−	+	+	+	++	+	++	++	−
	Nov.	−	−	−	+	+	+	−	−	−	+	+	−	+	+	+	+	−
1C	Jan.	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	++	−
	March	−	−	−	−	−	−	−	−	−	+	++	+	−	−	−	++	−
	May	−	−	−	−	−	−	−	−	−	++	++	++	+	+	−	+++	−
	July	−	−	−	−	−	−	−	−	−	+	++	+	+	+	−	+++	−
	Sept.	−	−	−	−	−	−	−	−	−	+	++	+	−	−	−	+++	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	++	−
2A	Jan.	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−
	March	−	−	−	−	−	−	−	−	−	+	+	+	++	++	++	−	−
	May	−	−	−	−	−	−	−	−	−	+	+	+	+++	+++	+++	−	−
	July	−	−	−	−	−	−	−	−	−	++	++	++	+++	++	+++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	++	+	+	−	−
	Nov.	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−
2B	Jan.	−	−	−	−	−	−	−	−	−	+	−	+	+	−	+	++	−
	March	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	++	−
	May	−	−	−	−	−	−	−	−	−	++	++	++	++	++	++	++	−
	July	−	−	−	−	−	−	−	−	−	++	++	++	++	+	++	++	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	++	−
	Nov.	−	−	−	−	−	−	−	−	−	+	−	+	+	−	+	++	−
2C	Jan.	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	++	−
	March	−	−	−	−	−	−	−	−	−	++	+	+	−	+	−	++	−
	May	−	−	−	−	−	−	−	−	−	++	++	++	++	++	+	+++	−
	July	−	−	−	−	−	−	−	−	−	++	++	++	+	++	+	++	−
	Sept.	−	−	−	−	−	−	−	−	−	++	+	++	+	+	−	++	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	++	−
3A	Jan.	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	++	−
	March	−	−	−	−	−	−	−	−	−	++	++	+	++	++	+	++	−
	May	−	−	−	−	−	−	−	−	−	++	++	++	+++	+++	+	++	−
	July	−	−	−	−	−	−	−	−	−	++	++	+	+++	+++	++	++	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	++	++	++	++	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	++	−
3B	Jan.	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−
	March	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++	+	−

(Continued)

Table 1. Continued

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
	May	−	−	−	−	−	−	−	−	−	++	++	++	+	++	++	+	−
	July	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++	+	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	+	++	++	+	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+	+	−
3C	Jan.	−	−	−	−	−	−	−	+	+	+	+	−	+	+	−	−	−
	March	−	−	−	−	−	−	+	++	++	+	+	−	++	+	+	−	−
	May	−	−	−	−	−	−	+++	+++	+++	++	++	−	+++	++	+	−	−
	July	−	−	−	−	−	−	++	+++	+++	+	+	−	+++	++	+	−	−
	Sept.	−	−	−	−	−	−	+	++	++	+	+	−	++	+	+	−	−
	Nov.	−	−	−	−	−	−	−	+	+	+	+	−	+	+	−	−	−

Nd, not determined.

[a] Number 1 indicates *E. intestinalis*; 2, *U. lactuca*; 3, *C. rupestris*.

[b] Results are presented as [−] for no inhibition, [+] for 1% to 30% inhibition, [++] for 31% to 59% inhibition, and [+++] for more than 60% inhibition; F1–F3, marine fungi; B1–B3, marine gram-positive bacteria; B4–B6, marine gram-negative bacteria; D1–D3, diatoms; M1–M3, macroalgae. A, aqueous fraction; B, ethanol fraction; C, dichloromethane fraction.

tested (Table 2), 8 extracts were active (4B and 5B). Extract 4B was active throughout the year toward F2 and F3, but a peak of activity against F1 was observed from March to July, although the level of activity was less during the rest of the year. Extract 5B showed constant inhibition of F1 and F2 throughout the year. Among the 54 extracts of Florideophyceae tested (Table 3), 18 showed antifungal activity (7B, 8B, and 8C). Fraction 7B showed inhibitory activity toward the 3 fungal strains tested throughout the year, but the highest level of activity was recorded between May and September. Although 8B was inactive toward F1, this extract showed activity toward F2 and F3, again peaking in May and July. Extract 8C was inactive against F1 but was consistently active toward F2 and F3.

Antibacterial Activity

Among the Ulvophyceae extracts tested (Table 1), only 2 showed activity (1B and 3C). Extract 1B was active throughout the year toward gram-positive bacteria, with a maximum level of inhibition in March and May for B1, and in May and July for B2 and B3. Regarding the inhibition of gram-negative bacteria, 1B was only weakly active in May and July toward B3, B4, and B5. Fraction 3C was active only on gram-negative bacteria. Seasonal variation was observed with a peak of activity in May for B4 and in May and July for B5 and B6.

Concerning the Phaeophyceae study (Table 2), 5 extracts (4B, 4C, 5B, 5C, and 6B) were active. Extract 4B was highly inhibitory throughout the year against B1, B2, B5, and B6. Seasonal variation in the activity of B3 and B4 was observed with a peak in May and July for B3 and from March to November for B4 (++). Extract 4C was active only on gram-positive strains. No seasonal variation was observed for the inhibition of B2. In contrast, extracts that had been harvested from March to September inhibited B1 growth, and extracts harvested from May to July inhibited B3 growth. Extract 5B was active toward all the bacterial strains tested and did not show any seasonal variation. Extract 5C was active only against gram-positive bacteria, without any seasonal variation in the inhibition of B1. Maximum activity against B2 and B3 was recorded in May and July. Extract 6B was active only on gram-positive bacteria. Unlike B1 and B3, there was no seasonal variation for B2.

Among the Florideophyceae extracts tested, 4 were found to be active: 8B, 8C, 9B, and 9C. Extract 8B was inactive against gram-negative bacteria and showed only weak activity against gram-positive bacteria. Extracts were only active from May to September on B1, B2, and B3. Extract 8C inhibited the growth of marine gram-positive bacteria without any seasonal variation. The highest levels of activity were for the inhibition of development of B1 and B2. The inhibition of B3 growth was intermediate throughout the year. Extract 9B inhibited the growth of marine gram-positive bacteria irrespective of season. Extract 9C was active against all the bacterial strains studied. No seasonal variation was observed for strains

Table 2. Effect of Phaeophyceae Extracts on Inhibition of Development of Fouling Organisms[a,b]

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
4A	Jan.	−	−	−	−	−	−	−	−	−	+	+	+	−	−	++	−	−
	March	−	−	−	−	−	−	−	−	−	+	+	+	−	−	++	−	−
	May	−	−	−	−	−	−	−	−	−	+	+	+	+	−	+++	−	−
	July	−	−	−	−	−	−	−	−	−	+	+	+	+	−	+++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	−	−	++	−	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	+	−	−	++	−	−
4B	Jan.	++	+++	+++	+++	+++	+	+	+++	+++	−	−	−	+	+	−	+++	++
	March	+++	+++	+++	+++	+++	+	++	+++	+++	−	−	−	+	+	−	+++	++
	May	+++	+++	+++	+++	+++	++	++	++	+++	+	−	−	++	++	−	+++	+++
	July	+++	+++	+++	+++	+++	++	++	+++	+++	+	−	−	++	++	−	+++	+++
	Sept.	++	+++	+++	+++	+++	+	++	+++	+++	−	−	−	+	+	−	+++	++
	Nov.	++	+++	+++	+++	+++	+	+	+++	+++	−	−	−	+	+	−	+++	++
4C	Jan.	−	−	−	++	++	+	−	−	−	+	++	−	−	++	+	+	−
	March	−	−	−	+++	++	+	−	−	−	+	++	−	−	++	+	+	−
	May	−	−	−	+++	++	++	−	−	−	+	++	−	−	+++	++	+	−
	July	−	−	−	+++	++	++	−	−	−	+	++	−	−	+++	++	+	−
	Sept.	−	−	−	+++	++	+	−	−	−	+	++	−	−	++	+	+	−
	Nov.	−	−	−	++	++	+	−	−	−	+	++	−	−	++	+	+	−
5A	Jan.	−	−	−	−	−	−	−	−	−	+	+	+	++	++	++	−	−
	March	−	−	−	−	−	−	−	−	−	+	++	+	++	++	++	−	−
	May	−	−	−	−	−	−	−	−	−	++	++	+	+++	+++	+++	−	−
	July	−	−	−	−	−	−	−	−	−	++	++	+	+++	+++	+++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	+	+	+	++	++	++	−	−
	Nov.	−	−	−	−	−	−	−	−	−	+	+	+	++	++	++	−	−
5B	Jan.	++	++	−	++	++	++	++	++	++	++	++	++	++	++	+	+++	+
	March	++	++	−	++	++	++	++	++	++	++	+++	++	+++	++	++	+++	+
	May	++	++	−	++	++	++	++	++	++	++	+++	++	+++	+++	+++	+++	++
	July	++	++	−	++	++	++	++	++	++	++	+++	++	+++	+++	+++	+++	++
	Sept.	++	++	−	++	++	++	++	++	++	++	+++	++	+++	++	+	+++	+
	Nov.	++	++	−	++	++	++	+	++	++	++	++	++	++	++	+	+++	+
5C	Jan.	−	−	−	++	++	+	−	−	−	+	++	+++	+++	+++	+++	+++	−
	March	−	−	−	++	++	+	−	−	−	++	+++	+++	+++	+++	+++	+++	−
	May	−	−	−	++	+++	++	−	−	−	++	+++	+++	+++	+++	+++	+++	−
	July	−	−	−	++	+++	++	−	−	−	++	+++	+++	+++	+++	+++	+++	−
	Sept.	−	−	−	++	++	+	−	−	−	++	+++	+++	+++	+++	+++	+++	−
	Nov.	−	−	−	++	++	+	−	−	−	+	++	+++	+++	+++	+++	+++	−
6A	Jan.	−	−	−	−	−	−	−	−	−	−	−	−	++	−	++	+++	−
	March	−	−	−	−	−	−	−	−	−	−	−	−	++	−	++	+++	−
	May	−	−	−	−	−	−	−	−	−	−	−	−	+++	−	+++	+++	−
	July	−	−	−	−	−	−	−	−	−	−	−	−	+++	−	+++	+++	−
	Sept.	−	−	−	−	−	−	−	−	−	−	−	−	++	−	++	+++	−
	Nov.	−	−	−	−	−	−	−	−	−	−	−	−	++	−	++	+++	−
6B	Jan.	−	−	−	++	++	++	−	−	−	+	−	+	+	−	−	+++	−
	March	−	−	−	+++	+++	++	−	−	−	+	−	+	+	−	−	+++	−
	May	−	−	−	+++	+++	++	−	−	−	+	−	+	++	−	+	+++	−
	July	−	−	−	+++	+++	++	−	−	−	+	−	+	++	−	+	+++	−
	Sept.	−	−	−	+++	+++	++	−	−	−	+	−	+	+	−	−	+++	−

(Continued)

Table 2. Continued

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
6C	Jan.	−	−	−	−	−	−	−	−	−	++	++	++	−	++	+	+++	+
	March	−	−	−	−	−	−	−	−	−	++	++	++	−	++	+	+++	+
	May	−	−	−	−	−	−	−	−	−	++	++	++	−	+++	++	+++	++
	July	−	−	−	−	−	−	−	−	−	++	++	++	−	+++	++	+++	+
	Sept.	−	−	−	−	−	−	−	−	−	++	++	++	−	++	+	+++	+
	Nov.	−	−	−	−	−	−	−	−	−	++	++	++	−	++	+	+++	+

[a] Number 4 indicates *A. nodosum*; 5, *S. muticum*; 6, *E. siliculosus*.
[b] Results are presented as [−] for no inhibition, [+] for 1% to 30% inhibition, [++] for 31% to 59% inhibition, and [+++] for more than 60% inhibition. F1–F3, marine fungi; B1–B3, marine gram-positive bacteria; B4–B6, marine gram-negative bacteria; D1–D3, diatoms; M1–M3, macroalgae. A, aqeuous fraction; B, ethanol fraction; C, dichloromethane fraction.

B1, B4, and B6. Seasonal variation in activity was observed against B2, with maximal inhibition in May and July. Maximal inhibition against B3 was from March to July, and high levels of inhibition against B5 occurred in May and July.

Antidiatom Activity

All of the extracts of Chlorophyceae tested (Table 1) showed antidiatom activity. Thus the extraction procedure and the period of collection were immaterial to activity. Extracts 1A, 3A, and 3C were active against the development of diatoms D1 and D2, with maximal activity recorded in May and July for 1A and 3A, and in May for 3C. Extracts 1B, 2A, 2B, 2C, 3A, and 3B inhibited the 3 strains tested. Extract 1B inhibited D2 with a low level of activity all year long. Extracts 1B and 2B exhibited maximal activity for D1, D2, and D3 in May and July (except for 1B with D2). Maximum activities were recorded in a single month for 1C with D1, 1C with D3, and 3B with D1/D2/D3 in May, and for 2A with D1/D2/D3 in July. In contrast, maxima of activity were recorded during several months for the combination 1C with D2 (March to September), 2C with D1/D2 (March to July), and 2C with D3 (May to September).

Eight extracts (4A, 4B, 4C, 5A, 5B, 5C, 6B, and 6C) from Phaeophyceae (Table 2) were active. Most of the active combinations showed no variation in activity during the year: 4A with D1/D2/D3, 4C with D1/D2, 5A with D3, 5B with D1/D3, 5C with D3, 6B with D1/D2, and 6C with D1/D2/D3. In contrast, seasonal variations activity was observed for some combinations with maxima of activity recorded from May to July for 4B with D1 and 5A with D1, from March to July for 5A with D2, from March to September for 5C with D1/D2, and from March to November for 5B with D2.

All of the extracts of Florideophyceae (Table 3) were active against at least one diatom strain. Seasonal variations of activity were measured for some combinations, with peaks of activity recorded in May (7C with D3), July (8A, 8B, and 8C with all strains), from May to July (9A with all strains), and from May to September (7C with D1). In contrast, no seasonal variation in activity was noted for the combinations 7A with D1/D2, and 7B, 3C and 9B with all strains.

Antimacroalgal Activity

Among the 54 extracts of Ulvophyceae tested (Table 1), 49 extracts were active toward at least one species of macroalga, and they all showed seasonal variation in activity. Maximum activity was recorded in May for 2A with M2, 2B with M2, and 2C with M1; from May to July for 1A with M1/M3, 1B with M2, 1C with M1/M2, 2A with M1/M3, 2B with M1/M3, 2C with M2/M3, 3A with M1/M2, and 3C with M1/M2; from May to September for 1B with M1/M3; from March to September for 1A with M2, 3B with M2/M3, and 3C with M3; and from July to September for 3A with M3.

Extracts of Phaeophyceae were all active against at least one macroalgal species (Table 2). Extract 5C was not seasonally active, being strongly inhibitory throughout the year against the 3 species of macroalgae. All the other active combinations (4A with M1/M3, 4B with M1/M2, 4C with M2/M3, 5A and 5B with all strains, 6A with M1/M3, 6B with M1/M3 and 6C with M2/M3) were subject to seasonal

Table 3. Effect of Florideophyceae Extracts on Inhibition of Development of Fouling Organisms[a,b]

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
7A	Jan.	−	−	−	−	−	−	−	−	−	+	++	−	++	++	++	−	−
	March	−	−	−	−	−	−	−	−	−	+	++	−	++	++	++	−	−
	May	−	−	−	−	−	−	−	−	−	+	++	−	+++	+++	+++	−	−
	July	−	−	−	−	−	−	−	−	−	+	++	−	+++	+++	+++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	+	++	−	++	++	++	−	−
	Nov.	−	−	−	−	−	−	−	−	−	+	++	−	++	++	++	−	−
7B	Jan.	+	+	++	−	−	−	−	−	−	++	++	+	−	++	+	+	−
	March	+	+	++	−	−	−	−	−	−	++	++	+	−	++	+	+	−
	May	++	++	+++	−	−	−	−	−	−	++	++	+	+	+++	++	+	−
	July	++	++	+++	−	−	−	−	−	−	++	++	+	+	+++	++	+	−
	Sept.	++	++	+++	−	−	−	−	−	−	++	++	+	+	++	+	+	−
	Nov.	+	+	++	−	−	−	−	−	−	++	++	+	−	++	+	+	−
7C	Jan.	−	−	−	−	−	−	−	−	−	−	−	+	++	−	−	+++	++
	March	−	−	−	−	−	−	−	−	−	−	−	+	++	−	−	+++	++
	May	−	−	−	−	−	−	−	−	−	+	−	++	+++	+	−	+++	++
	July	−	−	−	−	−	−	−	−	−	+	−	+	+++	+	−	+++	++
	Sept.	−	−	−	−	−	−	−	−	−	+	−	+	++	−	−	+++	++
	Nov.	−	−	−	−	−	−	−	−	−	−	−	+	++	−	−	+++	++
8A	Jan.	−	−	−	−	−	−	−	−	−	++	−	++	−	+	++	+	−
	March	−	−	−	−	−	−	−	−	−	++	−	++	−	+	++	+	−
	May	−	−	−	−	−	−	−	−	−	++	+	++	+	++	+++	+	−
	July	−	−	−	−	−	−	−	−	−	+++	+	+++	+	++	+++	+	−
	Sept.	−	−	−	−	−	−	−	−	−	++	+	++	−	+	++	+	−
	Nov.	−	−	−	−	−	−	−	−	−	++	−	++	−	+	++	+	−
8B	Jan.	−	+	++	−	−	−	−	−	−	++	+	+	+	−	−	−	−
	March	−	+	++	−	−	−	−	−	−	++	+	++	+	−	−	−	−
	May	−	++	+++	+	+	+	−	−	−	++	+	++	++	+	+	−	−
	July	−	++	+++	+	+	+	−	−	−	+++	++	+++	++	+	+	−	−
	Sept.	−	+	++	+	+	−	−	−	−	++	+	++	+	−	−	−	−
	Nov.	−	+	++	−	−	−	−	−	−	++	+	+	+	−	−	−	−
8C	Jan.	++	−	+++	+++	+++	++	−	−	−	+	−	++	+	++	+	−	−
	March	++	−	+++	+++	+++	++	−	−	−	+	+	++	+	++	+	−	−
	May	++	−	+++	+++	+++	++	−	−	−	++	+	++	++	+++	++	−	−
	July	++	−	+++	+++	+++	++	−	−	−	+++	++	+++	++	+++	++	−	−
	Sept.	++	−	+++	+++	+++	++	−	−	−	+	+	++	+	++	+	−	−
	Nov.	++	−	+++	+++	+++	++	−	−	−	+	+	++	+	++	+	−	−
9A	Jan.	−	−	−	−	−	−	−	−	−	−	−	+	−	++	++	−	−
	March	−	−	−	−	−	−	−	−	−	−	−	+	+	++	++	−	−
	May	−	−	−	−	−	−	−	−	−	+	+	++	+	+++	+++	−	−
	July	−	−	−	−	−	−	−	−	−	+	+	++	+	++	++	−	−
	Sept.	−	−	−	−	−	−	−	−	−	−	−	+	+	++	++	−	−
	Nov.	−	−	−	−	−	−	−	−	−	−	−	+	−	++	++	−	−
9B	Jan.	−	−	−	++	++	++	−	−	−	+++	+	++	+++	+++	+++	+++	+++
	March	−	−	−	++	++	++	−	−	−	+++	++	++	+++	+++	+++	+++	+++
	May	−	−	−	++	++	++	−	−	−	+++	++	++	+++	+++	+++	+++	+++
	July	−	−	−	++	++	++	−	−	−	+++	++	++	+++	+++	+++	+++	+++
	Sept.	−	−	−	++	++	++	−	−	−	+++	++	++	+++	+++	+++	+++	+++

(Continued)

Table 3. Continued

Extract	Month	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
	Nov.	−	−	−	++	++	++	−	−	−	+++	++	++	+++	+++	+++	+++	+++
9C	Jan.	−	−	−	++	++	+	++	++	++	++	++	+++	+++	+++	+++	+	−
	March	−	−	−	++	++	++	++	++	++	++	++	+++	+++	+++	+++	+	−
	May	−	−	−	++	+++	++	++	+++	++	++	++	+++	+++	+++	+++	+	−
	July	−	−	−	++	+++	++	++	+++	++	++	++	+++	+++	+++	+++	+	−
	Sept.	−	−	−	++	++	+	++	++	++	++	++	+++	+++	+++	+++	+	−
	Nov.	−	−	−	++	++	+	++	++	++	++	++	+++	+++	+++	+++	+	−

[a] Number 7 indicates *C. crispus*, 8, *L. pinnatifida*; 9, *P. lanosa.*

[b] Results are presented as [−] for no inhibition, [+] for 1% to 30% inhibition, [++] for 31% to 59% inhibition, and [+++] for more than 60% inhibition. F1–F3, marine fungi; B1–B3, marine gram-positive bacteria; B4–B6, marine gram-negative bacteria; D1–D3, diatoms; M1–M3, macroalgae. A, aqeuous fraction; B, ethanol fraction; C, dichloromethane fraction.

Table 4. Summary of Type of Activity (seasonal or yearly)[a,b]

Extract	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	Mussel	Barnacle
1a	−	−	−	−	−	−	−	−	−	S	S	−	S	S	S	−	−
1b	−	−	−	S	S	S	S	S	S	S	Y	S	S	S	S	S	−
1c	−	−	−	−	−	−	−	−	−	S	Y	S	S	S	−	S	−
2a	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	−	−
2b	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	Y	−
2c	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	S	−
3a	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	Y	−
3b	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	Y	−
3c	−	−	−	−	−	−	S	S	S	S	S	−	S	S	S	−	−
4a	−	−	−	−	−	−	−	−	−	Y	Y	Y	S	−	S	−	−
4b	S	Y	Y	Y	Y	S	−	−	−	Y	−	−	S	S	−	Y	S
4c	−	−	−	S	Y	S	−	−	−	Y	Y	−	−	S	S	Y	−
5a	−	−	−	−	−	−	−	−	−	S	S	Y	S	S	S	−	−
5b	Y	Y	−	Y	Y	Y	Y	Y	Y	Y	S	Y	S	S	S	Y	S
5c	−	−	−	Y	S	S	−	−	−	S	S	Y	Y	Y	Y	Y	−
6a	−	−	−	−	−	−	−	−	−	−	−	−	S	−	S	Y	−
6b	−	−	−	S	S	Y	−	−	−	Y	−	Y	S	−	S	Y	−
6c	−	−	−	−	−	−	−	−	−	Y	Y	Y	−	S	S	Y	−
7a	−	−	−	−	−	−	−	−	−	Y	Y	−	S	S	S	−	−
7b	S	S	S	−	−	−	−	−	−	Y	Y	Y	S	S	S	Y	−
7c	−	−	−	−	−	−	−	−	−	S	−	S	S	S	−	Y	−
8a	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	Y	−
8b	−	S	S	S	S	S	−	−	−	S	S	S	S	S	S	−	−
8c	Y	−	Y	Y	Y	Y	−	−	−	S	S	S	S	S	S	−	−
9a	−	−	−	−	−	−	−	−	−	S	S	S	S	S	S	Y	Y
9b	−	−	−	Y	Y	Y	−	−	−	Y	Y	Y	Y	Y	Y	Y	Y
9c	−	−	−	Y	S	S	Y	S	Y	Y	Y	Y	Y	Y	Y	Y	−

[a] F1–F3, marine fungi; B1–B3, marine gram-positive bacteria; B4–B6, marine gram-negative bacteria; D1–D3, diatoms; M1–M3, macroalgae. a, aqueous fraction; b, ethanol fraction; c, dichloromethane fraction.

[b] Results are presented as number 1 indicates *Enteromorpha intestinalis*; 2, *Ulva lactuca*; 3, *Cladophora runestris*; 4, *Ascophyllum nodosum*; 5, *Sargassum muticum*; 6, *Ectocarpus siliculosus*; 7, *Chrondus crispus*; 8, *Laurencia pinnatifida*; 9, *Polysiphonia lanosa*. [−] for nonactive; S, seasonal variation; Y, activity all year long.

Table 5. Period of Year Leading to Maximal Activity for Combination of Extract and Strain Subject to Seasonal Activities[a,b]

Extract	F1	F2	F3	B1	B2	B3	B4	B5	B6	D1	D2	D3	M1	M2	M3	*M. edulis*	*B. amphitrite*
1a	–	–	–	–	–	–	–	–	–	5-7	5-7	–	5-7	3-9	5.7	–	–
1b	–	–	–	3-5	5-7	5-7	5-7	5-7	5-7	5-7	–	5-9	5-9	5-7	5-9	5-9	–
1c	–	–	–	–	–	–	–	–	–	5	3-9	5	5-7	5-7	–	5-9	–
2a	–	–	–	–	–	–	–	–	–	7	7	7	5-7	5	5-7	–	–
2b	–	–	–	–	–	–	–	–	–	5-7	5-7	5-7	5-7	5	5-7	–	–
2c	–	–	–	–	–	–	–	–	–	3-9	5-7	5-9	5	5-7	5-7	5	–
3a	–	–	–	–	–	–	–	–	–	3-7	3-7	5	5-7	5-7	7-9	–	–
3b	–	–	–	–	–	–	–	–	–	5	5	5	3-9	3-9	3-9	–	–
3c	–	–	–	–	–	–	5	5-7	5-7	5	5	–	5-7	5-7	3-9	–	–
4a	–	–	–	–	–	–	–	–	–	–	–	–	5-7	–	5-7	–	–
4b	3-7	–	–	–	–	5-7	–	–	–	–	–	–	5-7	5-7	–	–	5-7
4c	–	–	–	3-9	–	5-7	–	–	–	–	–	–	–	5-7	5-7	–	–
5a	–	–	–	–	–	–	–	–	–	5-7	3-7	–	5-7	5-7	5-7	–	–
5b	–	–	–	–	–	–	–	–	–	–	3-9	–	3-9	5-7	5-7	–	5-7
5c	–	–	–	–	5-7	5-7	–	–	–	3-9	3-9	–	–	–	–	–	–
6a	–	–	–	–	–	–	–	–	–	–	–	–	5-7	–	5-7	–	–
6b	–	–	–	3-9	3-9	–	–	–	–	–	–	–	5-7	–	5-7	–	–
6c	–	–	–	–	–	–	–	–	–	–	–	–	–	5-7	5-7	–	–
7a	–	–	–	–	–	–	–	–	–	–	–	–	5-7	5-7	5-7	–	–
7b	5-9	5-9	5-9	–	–	–	–	–	–	–	–	–	5-9	5-7	5-7	–	–
7c	–	–	–	–	–	–	–	–	–	5-9	–	5	5-7	5-7	–	–	–
8a	–	–	–	–	–	–	–	–	–	7	5-9	7	5-7	5-7	5-7	–	–
8b	–	5-7	5-7	5-9	5-9	5-7	–	–	–	7	7	7	5-7	5-7	5-7	–	–
8c	–	–	–	–	–	–	–	–	–	7	7	7	5-7	5-7	5-7	–	–
9a	–	–	–	–	–	–	–	–	–	5-7	5-7	5-7	3-9	5	5	–	–
9b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9c	–	–	–	–	5-7	5-7	–	5-7	–	–	–	–	–	–	–	–	–

[a] F1–F3, marine fungi; B1–B3, marine gram-positive bacteria; B4–B6, marine gram-negative bacteria; D1–D3, diatoms; M1–M3, macroalgae. a, aqeuous fraction; b, ethanol fraction; c, dichloromethane fraction.

[b] Results are expressed by the month when the maximum antifouling activity was recorded (3, March; 5, May; 7, July; 9, September); Number 1 indicates *Enteromorpha intestinalis*; 2, *Ulva lactuca*; 3, *Cladophora rupestris*; 4, *Ascophyllum nodosum*; 5, *Sargassum muticum*; 6, *Ectocarpus siliculosus*; 7, *Chrondus*

variations, with a maximum intensity of activity in May and July.

All the extracts of Florideophyceae tested (Table 3) were active. Extracts 9B and 9C showed high inhibitory activity (+++) in all months tested. The activity of all the extracts varied seasonally, with the most active combinations (+++) being 7A with M1/M2/M3, 7B with M2, 7C with M1, 8A with M3, 8C with M2, and 9A with M2/M3.

Phenoloxidase Inhibition

Among the Ulvophyceae extracts tested (Table 1), extracts 1B, 1C, and 2C showed seasonal variation in anti-phenoloxidase activity, which was maximal from May to September. Extracts 2B, 3A, and 3B were active but did not exhibit any seasonal variation in their activity. The active fractions from Phaeophyceae (4B, 4C, 5B, 5C, 6A, 6B, and 6C; Table 2) and from Florideophyceae (7A, 8B, 8C, and 9A; Table 3) were highly inhibitory (+++), with the exception of 4C, 7C, and 8A, but none were seasonally active.

Inhibition of Barnacle Settlement

None of the 54 extracts of the Ulvophyceae (Table 1) were active. Among the Phaeophyceae extracts tested (Table 2), active ones showed seasonal variations, with the highest levels of inhibition recorded from May to July for 4B and 5B, and in May for 7B. Florideophyceaen extracts

7C and 9B were active without any seasonal variation (Table 3).

Global Analysis

Activity patterns of the algal extracts are summarized in Table 4. Attention is drawn to the following phenomena. Of all the extracts tested, 51.8% were inactive, 17% were active all year long, and 31.2% were seasonally active. Of the Chlorophyceae, 56.2% were inactive, 2% showed activity throughout the year, and 43.8% exhibited seasonal activity. Of the Phaeophyceae, 52.3% were inactive, 25.5% showed activity throughout the year, and 22.2% exhibited seasonal activity. Of the Rhodophyceae, 47% were inactive, 23.5% showed activity throughout the year, and 29.5% exhibited seasonal activity.

Among the extracts, 66.6% of the aqueous extracts were inactive, 6.5% showed activity throughout the year, and 26.9% exhibited seasonal activity. Of the ethanol extracts, 52.3% were inactive, 24.2% showed activity throughout the year, and 23.5% exhibited seasonal activity. Of the dichloromethane extracts, 47% were inactive, 19.6% showed activity throughout the year, and 33.4% exhibited seasonal activity.

The assays that were least susceptible to inhibition by the extracts were those that involved F1, F2, F3, B4, B5, B6, and *B. amphitrite* cyprids, with 85.2% of the combinations showing inactivity. The most susceptible assays were those that utilized D1, D2, D3, M1, M2, M3, and *M. edulis*, with more than 70% of the combinations leading to inhibition. Among this group, the strains that were sensitive to seasonal variation were M1, M2, and M3, with 84.2% of the combinations affected. *M. edulis* phenoloxidase activity was the least sensitive to seasonal variation of the extracts, since 84.2% gave the same level of activity all year round.

Table 5 shows clearly that maximal antifouling activity was never associated with the November or January extracts. Of the active fractions, 16.6% had a peak of activity for 1 month (group I) and 83.4% were maximally active for at least 2 months (group II). For group I, 56.5% of the combinations were active in May and 43.5% were active in July. Group II can be divided into 6 categories in terms of the timing of the peak period of activity: 68.7% from May to July, 13.4% from March to October, 12.5% from May to October, 3.6% from March to July, 0.9% from July to October, and 0.9% from March to May.

Discussion

Macroalgae produce a wide range of secondary metabolites, many of which exhibit a broad spectrum of bioactivity (Da Gama et al., 2002) and could potentially be used to develop new antifouling agents (de Nys and Steinberg, 2002). Some marine plants have evolved chemical and physical defenses against the settlement and growth of fouling organisms on their surfaces. A wide variety of marine natural products have been identified to have several activities against fouling organisms in bioassays (Clare, 1996a; Rittschof, 2000). Some pure compounds have been identified as natural antifoulants; however, the relationship between their ecologic role and their antifouling activities is not fully understood (de Nys and Steinberg, 2002). In previous studies (Hellio, 2000; Hellio et al., 2000a, 2000b, 2001a, 2001b, 2002), which were based on the screening of antifouling activities among 30 macroalgae collected in Brittany, we found that chemical defenses can be very specific, or very broad, depending on the method of extraction and the organisms in question. Similar observations were made by Da Gama et al. (2002).

Intraspecific chemical variation in the production of secondary metabolites has been demonstrated for a variety of marine algae (Hay, 1996). Several studies have examined variation in chemical defenses within individual thalli (Meyer and Paul, 1992, 1995; de Nys et al., 1996). Biogeographic variation in chemical defenses has also been studied for seaweed (Matlock et al., 1999; Hellio et al., 2001b). As marine fouling pressure varies during the year, being higher in spring and summer in temperate regions, the present study has focused on the possibility that antifouling defenses will also fluctuate seasonally. Concarneau (Brittany, France) was chosen as a sample site influenced by seasons. As the algae were always from the same place, geographical and spatial variation was eliminated. The available literature provides limited evidence of seasonal variation in secondary metabolite concentrations from marine macroalgae in relation to their antifouling activities. Indirect assessments were indicated by seasonal changes in epiphytic communities of chemically defended organisms (Harlin, 1996). Seasonal changes were highlighted for phenolic content of 9 species of algae (Steinberg and Vanaltena, 1992), for caulerpyne production in *Caulerpa taxifolia* (Amade and Lemee, 1998), and for the chemical composition of *Bifurcaria bifurcata* (Culioli et al., 2002).

The results of the screening assays presented here showed that 51.8% of the extracts were inactive, and of the remainder, 31.2% were seasonally active and 17% were active all year long. This is the first indication that macroalgae from the shores of Brittany possess different levels of antifouling activity during the year. That is not to say that the variable activity is adaptive, or that the biological activity that was detected has any ecologic significance. More work is needed to determine whether the compounds with antifoulant activity are released at the surface of the basibiont alga, where they can act on potential epibionts. Biological assays of conditioned water may provide some indication of potential polar metabolites. Nonpolar metabolites, which are likely to adsorb to the surface of the alga, could be assayed after extraction by the ''hexane dip'' (de Nys et al., 1998) or a comparable method. Nevertheless, inspection of the data for the algal species exhibiting seasonal activity shows that biological activity did not peak in any of the extracts collected in November or January. Rather, maximal activity was recorded from May to July, which corresponds to maximal values for water temperature, light intensity, and fouling pressure.

Although induction of chemical defenses by herbivores and space competition pressure may offer explanations for intraspecific differences in chemical defenses of benthic organisms, some physical characteristics of the environment can also be important (Amade and Lemee, 1988). For marine plants, nutrients, and allocation of resources to secondary metabolites are factors modifying seaweed susceptibility to herbivores and fouling (Renaud et al., 1990; Yates and Peckol, 1993; Cronin and Hay, 1996a, 1996b; Cronin et al., 1997), but these alterations could be due to some physical change as desiccation and light quality (Hay, 1996; Amade and Lemee, 1998).

In the present study temperature and light are presumably major physical factors influencing the seasonal production of metabolites with antifoulant action. From August to October, the activity of the extracts was at a higher level than from March to May. This difference can be explained by the temperature of the water, which, despite decreasing periods of sunshine, decreased less markedly because of the thermal inertia of the water. Consequently, we can hypothesize that the variation in antifouling activity was more directly dependent on light than on temperature variation. Similar results were obtained for caulerpyne production by *Caulerpa taxifolia* (Amade and Lemee, 1998). Light was also found to be a crucial factor for the production of phlorotannins by *A. nodosum* and *F. vesiculosus* (Pavia and Brock, 2000; Pavia and Toth, 2000). Production of antifouling compounds appears to rise from May to July, corresponding to the highest growth rate of the algae. This result is in accord with the findings of Dworjanyn et al. (1999), who reported that furanone levels on the surface of *Delisea pulchra* were highest near the actively growing apical tips and decreased toward the base of the frond.

Extracts of red algae from Brittany were prominent for their antifouling activity. Similar results were obtained in various screening programs performed in different seas (Moreau et al., 1984, 1988; Pesando and Caram, 1984; Steinberg and Vanaltena, 1992; Padmakumar and Ayyakkannu, 1997; Steinberg et al., 1997; Pawlik, 2000; Cho et al., 2001; Steinberg and de Nys, 2002) in which Rhodophyta species exhibited the best antifouling activities. As in a number of prior studies (Moreau et al., 1984, 1988; Pesando and Caram, 1984; de Nys et al., 1995; Cronin and Hay, 1996a; Crasta et al., 1997; Devi et al., 1997; Padmakumar and Ayyakkannu, 1997; Bhosale et al., 2002; Da Gama et al., 2002), nonpolar metabolites, which in the present case would have been extracted by dichloromethane, were clearly associated with antifouling activity.

It is assumed that there is a cost to the production of defensive compounds in terms of reduced growth rate, reproductive output, or competitive ability of the organisms producing them (Van Alstyne, 1988). The problems of allocation to defenses are further complicated by spatial or temporal variations in fouling rates. If fouling pressure is constant and predictable, then algae should maintain uniform levels of defense (Harvell, 1986). Ideally, defenses against fouling should vary with changes in fouling pressure (Van Alstyne, 1988). Our results highlight that the extracts were active, with little seasonal variation in activities toward marine bacteria and marine fungi. This result may reflect the ubiquity of microorganisms throughout the year. In contrast, diatoms and macroalgal spores, which colonize surfaces mainly in summer, were inhibited primarily by extracts of algae collected in spring and summer.

Diatoms, microalgae, and *M. edulis* phenoloxidase were particularly sensitive to inhibition by algal extracts. Previous studies (Cho et al., 2001; Hellio et al., 2000a, 2001b; Vannelle and Le Gal, 1995) have demonstrated the presence of antimussel (phenoloxidase inhibitory activity) compounds in seaweeds. These inhibitory activities are very important because mussels are among the most problematic of the so-called hard fouling species, for example, on offshore structures and seawater intakes (Jenner et al., 1998;

Rajogopal et al., 2003). Considering the high level of activity of our algal extracts toward other algal species, allelochemically mediated effects may be of importance. Allochemicals are known to regulate and control community structure and determine succession (Harlin, 1996). Interactions among marine algae, through the excretion of growth-inhibiting substances, have been suggested to play an important role in ecology (Harlin, 1996).

The organisms least susceptible to inhibition by the extracts tested were the cypris larvae of *B. amphitrite*, as well as the strains of marine fungi and gram-negative bacteria. The low level of activity toward *B. amphitrite* could relate to the fact that it is a tropical species and that the algae from Brittany do not have a targeted defense against it. It would be of interest in this regard to assay extracts against cyprids of a temperate fouling barnacle such as *Balanus improvisus* or *Elminius modestus*.

Marine fungi and gram-negative bacteria showed tolerance to the extracts tested. Some authors (Vaccaro et al., 1977; Duxbury and Bicknell, 1983; Barkay, 1987) have provided evidence that the response of heterotrophic bacteria to copper was effected by a simultaneous selection for bacterial species exhibiting an increased tolerance toward copper. The increasing number of observations of bacterial aquatic community resistance to heavy metals led to the conclusion that new antifouling products are urgently required. The results of the present study suggest that the selected algal species have little potential as a source of such natural products.

This study has highlighted the danger of relying on single collections of marine organisms for natural product studies. Clearly, the fact that bioassay results varied with the season of collection suggests that the content of secondary metabolites is variable. Whether the metabolites are synthesized, stored, and depleted, or produced continuously but at variable rates, is an important area for research. Pioneering research in this area has been conducted on furanone concentrations in *Delisea pulchra* (Dworjanyn et al., 1999; Wright et al., 2000), which demonstrated that furanones, which are stored in vesicles, are produced at different concentrations with respect to site of collection and season and between different life-history stages.

On the Brittany coast, a sampling program that focuses on the Rhodophyceae, collected in the months of May to July and utilizing a relatively polar solvent extraction protocol, offers the best chance of isolating secondary metabolites with antifouling potential. It could be argued that such studies should focus on products that are only released at the surface of the alga, that is, the site of potential epibiosis. Although this approach may identify compounds that function in antiepibiosis and target local epibionts (de Nys et al., 1998), it may overlook compounds that have antifoulant action against key fouling species that are not part of the natural epibiotic community of the basibionts under study.

Added in Proof: Since this paper was accepted, a publication has shown that *Enteromorpha* and *Ulva* are the same, not distint genera: Hayden, H.S., Blomster, J., Maggs C.A., Silva, P.C., Stanhope, M.J., and Waaland, R.J. (2003) "Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera." Eur. J. Phycol. 38: 277–294.

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