EVALUATING THE USE OF ERGOSTEROL AS A BIOINDICATOR FOR ASSESSING FUNGAL RESPONSE TO WATER QUALITY[*]

D. E. PADGETT[1*], M. A. MALLIN[2] and L. B. CAHOON[1]

[1] *Department of Biological Sciences; and* [2] *Center for Marine Sciences Research, University of North Carolina at Wilmington, NC, U.S.A.*
(corresponding author)*

Abstract. This study investigated the *in situ* production of fungal biomass on presterilized woody substrates submerged in natural estuarine and freshwater bodies enriched with nitrogen and phosphorus-containing chemical compounds. Results suggest that the membrane-bound sterol ergosterol may hold significant promise as an quantitative indicator of fungal response to water quality. We suggest that realistic assessment of the environmental health of natural waters should include a suite of bioassays that could measure the response of living organisms to pollution much more directly than could be predicted by current, indirect monitoring techniques.

Keywords: bioindicators, ergosterol, water quality

1. Introduction

Most published studies dealing with environmental monitoring of natural water bodies report concentrations of specific chemical moieties at monitored sites but do not directly evaluate their individual or collective effects on living organisms (e.g. Lebo and Sharp, 1993; Chui, 1997). Derived data then are used to infer the impacts of such perturbations on aquatic organisms and to predict the consequences for food webs and human economy (USEPA, 1992; NOAA, 1996).

Although direct bioassays would be more informative for assessing the true impact of pollutants on life processes, they are more difficult to do thus are not routinely undertaken (APHA, 1995). We would argue, however, that the complexity of natural ecosystems might make organisms respond differently to pollution than one might predict based on indirect monitoring techniques alone. This could be caused by unanticipated interactions (e.g. synergisms or even overriding effects) between physical and chemical factors at the site and would not be detectable by quantifying the individual factors separately. Newell and Wall (1998), for instance, showed that ascomycetous fungi grew more profusely on salt marsh cordgrass established in soils heavily polluted with mercury and polychlorinated biphenyls when supplemental nitrogen was available. We, therefore, think it timely that science should begin to develop realistic bioassays both to assess the environmental

[*] Contribution No. 228 UNCW Center for Marine Sciences Research

health of natural waters more accurately and to aid in reevaluating the predictive significance of currently used water quality indicators.

The purpose of the present study was to explore the utility of a unique fungal sterol as a bioindicator of fungal response to water quality. Our investigation focused on quantifying the effects of septic and pulp mill effluent on fungal mass accumulation on a submerged cellulosic substrate at selected freshwater and estuarine sampling stations.

We do not propose that all aquatic organisms will respond to environmental insults as do fungi, but view the present experiments as an initial step toward developing a bioindicator of the environmental health of a fundamentally important group of eukaryotic decomposers.

2. Materials and Methods

Two separate experiments (herein called the 'paired sampling study' and the 'Brunswick County study') that employed similar methodologies were conducted to address this question. Both utilized a 'bait line' design (described below) whereby autoclaved birch-wood tongue depressors were incubated *in situ* as a substrate (bait) for aquatic, cellulolytic fungi. Additionally, each experiment evaluated fungal growth on the baits through time by quantifying a membrane sterol (ergosterol) known to be a unique indicator of living thalli of important cellulolytic basidiomycetous, ascomycetous, and deuteromycetous species (Newell, 1992).

Monthly monitoring of physical and chemical parameters of the respective water columns had been ongoing at all sampling stations for several years (Table I) and continued through the present experiments.

2.1. 'Paired sampling study' bait deployment

This experiment used paired sampling sites (in New Hanover and Columbus County, North Carolina, USA) whose physical and chemical profiles were very similar relative to monitored parameters (Table I) except that one member of each pair was impacted by some form of point-source anthropogenic effluent and the other was unimpacted (control). New Hanover is a coastal county in southeastern North Carolina bounded by the Atlantic Intracoastal Waterway on the east and by the Cape Fear River on the west. The river is sufficiently wide at its mouth that tidal influence is significant as far as twenty miles upstream rendering most county streams mesohaline. Although the river extends into adjacent Columbus County, its tidal salt wedge dissipates before reaching our sampling pair at Riegelwood.

Three sampling pairs were in estuarine water and one was in a non-saline region of the Cape Fear River upstream and downstream of the effluent outfall at the International Paper Corp. pulp mill at Riegelwood. A fifth pair also was in non-saline water and served as our methodological control in that neither member had

TABLE I

Water column physical and chemical parameters monitored monthly for paired sampling (PS) and Brunswick County (BC) experiments. Some parameters for the paired sampling experiment differed at estuarine (E) sites compared to non-saline, riverine (R) sites

| Water column | PS | | |
parameter	E	R	BC
BOD (5 da and 20 da)	X	X	
Chlorophyll a	X	X	X
Conductivity		X	
Fecal coliform	X	X	X
Nitrogen as:			
Ammonia	X	X	
Inorganic N	X		
Kjeldahl N		X	
Nitrate + Nitrite	X	X	
Total N		X	X
Dissolved oxygen	X	X	X
Phosphorus as:			
Orthophosphate	X	X	
Total P	X	X	X
pH		X	X
Salinity	X	X	X
Suspended solids		X	X
Temperature	X	X	X
Turbidity		X	X

an effluent input. Paired sites were assigned the same station number, but distinguished from each other by the lower-case letters 'p' (polluted) and 'c' (control). All estuarine pairs exhibited similar salinity fluxes (ranging from about 10 to 30 parts per thousand at low and high tides respectively) and are herein distinguished from the riverine pairs by the parenthetical designations (E) and (R). Sterile tongue depressors were attached to 20 lb. test-strength monofilament fishing line in such a way that they could be submerged completely, but would float in the water column throughout the incubation period. Triplicate lines each securing three substrates were tied to bricks and submerged at each site in May 1997. This 'bait line' arrangement allowed triplicate substrates to be harvested for fungal biomass quantification after one, two, and three months of submergence from each sampling site. Whereas station 4 (the International pulp mill) was the only non-saline test pair, we

repeated our experiment there from 1 September through 30 October 1997 to verify initial results.

2.2. BRUNSWICK COUNTY STUDY BAIT DEPLOYMENT

This study was conducted in selected freshwater sites in Brunswick County, North Carolina which has very similar topography and vegetation to and is located immediately to the southwest of New Hanover County. Sampling was done from May through September 1998 but did not involve a paired site design. The methodology further differed from that of the paired study in that all baits were preweighed before submergence to permit determination of mass loss during submergence, sampling was at three week intervals rather than monthly, and single rather than triplicate substrates were harvested at each sampling interval.

2.3. HARVEST AND ANALYSES

Harvested baits were returned to the laboratory in an ice chest and cleaned of adherent sediment and epibiotic growth. The paired sampling study baits were then transferred to 15 mL of absolute methanol and stored at 4C. Brunswick County samples were immediately frozen in liquid nitrogen, lyophilized, and weighed to determine mass loss then stored in methanol.

All ergosterol extractions were done within 7 days of methanol storage using the method of Newell et $al.$ (1988) as modified by Padgett and Posey (1993, wherein extracted tissue was left in the reflux flask through the alcoholic base step). Detection and quantification of ergosterol in all samples was done with an ICI Corp. High Performance Liquid Chromatograph (HPLC) equipped with a 5μm C-18 reverse-phase column, an absorbance detector wavelength of 282 nm, and a 100% methanol mobile phase flowing at 2 mL min^{-1}. Mean ergosterol concentrations for triplicate baits from the paired sampling study were compared within pairs for each sampling interval using Student's t-test ($p < 0.05$).

Water quality analyses were done monthly at all stations for both experiments (Table I) using standard methods as required by the state of North Carolina (Mallin et $al.$, 1997). Mean values of monitored parameters from the paired sampling study were compared within pairs using Student's t-test ($p < 0.05$) to identify effluent components that could explain differences in fungal mass for the sampling sites.

Preliminary analysis of Brunswick County water quality data indicated that stations differed principally in total nitrogen and phosphate concentrations. Therefore, mean values for the sampling period were calculated for these parameters at each station and used to rank stations from most to least nutrient enriched. N and P values were compared to week 9 ergosterol and substrate biomass loss data for the same station using Pearson correlation analysis to detect trends that could explain relative fungal growth.

TABLE II

Mean nitrogen and phosphate concentration (ppb) in water columns at paired sampling stations during sampling period. Values differing significantly within pairs (Student's t-test, P < 0.05) are bold

Station pair	N + N[a]	Ammonia	Phosphate
1c (E)	**136.4**	30.3	48.2
1p (E)	**454.1**	33.3	72.0
2c (E)	**23.6**	**26.3**	4.9
2p (E)	**42.3**	**42.7**	7.4
3c (E)	**6.7**	23.7	30.3
3p (E)	**43.7**	64.3	54.0
4c (R)	730.0	**96.7**	71.1
4p (R)	823.3	**166.6**	79.3
5c (R)[b]	320.0	76.7	38.3
5c'(R)[b]	383.3	60.0	32.7

[a] Nitrite + Nitrate

[b] Methodological control pair

3. Results

Statistical comparisons of monitored water column parameters revealed no significant differences within pairs for the paired sampling study except for nitrogen and/or phosphate (Table II). Similarly, nitrogen and phosphate concentration data from Brunswick County stations are summarized in Table III.

For both studies, ergosterol contents of baits increased progressively with increasing submergence duration in the great majority of cases except as noted in Table IV for the paired sampling experiment and in Table V for the Brunswick County study. We further noted that our methodological control sampling pair (station 5c,c') exhibited statistically similar ergosterol accumulations at each sampling interval.

We noted a highly significant statistical correlation of both total N and total P concentrations with ergosterol content of baits for the week nine harvest for the Brunswick County study when stations B4 (whose bait could not be found) and B31 (where there was high ergosterol even in relatively low N, P water) were excluded from the data set. The ergosterol to total N correlation coefficient (r) was 0.947 (n = 8, p < 0.0001) while that of total P with ergosterol was 0.905 (n = 8, p < 0.002).

The most unexpected finding from the paired sampling experiment was that mean ergosterol content of baits from nitrogen and/or phosphate-enriched estuarine sites was significantly less than from their respective controls (Student's t-test, p < 0.05). The reverse was true in the single freshwater test pair [station 4(R)] where

TABLE III

Mean (ppb) of total nitrogen and total phosphorus content of surface water at Brunswick County sampling stations for study period

Station	Mean (ppb)		Composite rank[c]	Molar N:P
	Total N[a]	Total P[b]		
B3	2981 (2)	1314 (2)	2	5.0
B4	3253 (1)	1477 (1)	1	4.9
B8	390 (9)	7 (10)	9	123.0
B9	609 (6)	80 (3)	4	16.7
B14	627 (5)	22 (7)	5	63.1
B18	369 (10)	18 (8)	8	45.4
B22	761 (3)	42 (5)	3	40.3
B26	669 (4)	58 (4)	3	25.6
B28	573 (7)	16 (9)	7	79.3
B31	415 (8)	34 (6)	6	26.9

[a] Number in parentheses represents station rank in total N hierarchy
[b] Number in parentheses represents station rank in total P hierarchy
[c] Composite rank of stations based on total N + total P

ammonia-rich effluent from the International Paper Corp. pulp mill was associated with fungal biomass more than twice that of the control after three months of bait submergence. Repeat sampling (Sept – Oct) at this same pair showed similar results. All Brunswick County sampling sites were in fresh water and, like pair 4(R), generally yielded ergosterol concentrations that were proportional to nutrient enrichment (Table V: compare ranked data for N + P with that for ergosterol). We found that station B31 was a notable exception to this generality (Table V) in that its baits yielded substantial ergosterol (station ranked third out of 10) despite relatively low water column N + P concentrations (station ranked sixth out of 10).

Also included in Table V are mass loss data for the preweighed baits deployed in the Brunswick County study. Although rates of mass decrease varied from station to station, all substrates showed progressively greater loss through time. The magnitude of substrate mass reductions largely was proportional to ergosterol concentration except for stations B18, where the bait lost 16% of its original mass over nine weeks despite containing no detectable ergosterol, and station B31, whose bait lost only 11% of its mass over nine weeks yet yielded the third highest ergosterol concentration found. Despite these unexpected result, Pearson correlation comparisons of week nine ergosterol to percent mass loss for all stations yielded a correlation coefficient of 0.6736 ($p = 0.047$, n = 9).

TABLE IV

Mean ergosterol content (μg g^{-1} of extracted substrate) of tongue depressors after *in situ* incubation at paired sampling stations (n=3). Standard error shown in parentheses after each value. Values suffixed by asterisk are significantly different from paired control (Student's t-test, p < 0.05) for same harvest. Values suffixed by same letter are not significantly different from other values in same row (i.e. no difference through time)

| Station | Months of bait submergence | | |
	1	2	3
1p (E)	34.9 (4.9)A	57.1 (6.9)*A	69.7 (10.0)*B
1c (E)	51.7 (5.1)A	141.1 (18.9)B	66.6 (8.6)B
2p (E)	14.2 (0.1)*A	67.0 (19.1)B	111.5 (5.7)*B
2c (E)	61.8 (9.1)A	lost	143.6 (8.2)B
3p (E)	7.4 (1.5)*A	17.0 (7.8)A	39.2 (13.8)B
3c (E)	bt[a]	41.4 (7.5)A	54.0 (10.6)A
4p (R)[b]	257.0 (19.8)A	477.9 (74.0)*B	486.3 (76.2)*B
4c (R)[b]	nh[c]	271.6 (17.7)A	208.6 (21.2)A
4p (R)[d]	232.8 (15.2)A	411.4 (5.8)*B	
4c (R)[d]	205.9 (7.8)A	319.8 (18.9)B	
5c (R)	176.0 (25.6)	276.7 (8.4)	271.3 (26.4)
5c' (R)	161.1 (21.9)	242.4 (21.9)	248.8 (23.4)

[a] Ergosterol present but below chromatographic integration threshold.
[b] Values for June-August harvests.
[c] Baits not harvested due to flooding.
[d] Values for September and October replicate experiment.

4. Discussion

Experimental results clearly show that water-borne propagules of cellulolytic fungi invade woody substrates very soon after their submergence and grow thereupon at rates influenced by chemical conditions in the surrounding water columns. In our freshwater sites for both studies increased nitrogen and/or phosphate content largely had a positive effect on fungal growth that generally was proportional to the concentration of N and P (e.g. compare ranked values for nutrient content with ergosterol in Table V). This finding is in agreement with Suberkropp's (1995) observation that freshwater hyphomycetes growing on dead leaves in Alabama streams can obtain inorganic nutrients from the water column.

TABLE V

Ergosterol content (μg g^{-1} lyophilized substrate) and % dry mass lost by substrate at each harvest in Brunswick County experiment

Station	% Mass Lost			Ergosterol (μg g^{-1})			Station Rank[a]		
	wk 3	wk 6	wk 9	wk 3	wk 6	wk 9	Erg.	Mass	N + P
B3	13	19	23	90.8	123.6	145.8	2	1	2
B4	12	18	NR[b]	132.4	153.0	NR	1	2	1
B8	2	3	4	0	0	TR[c]	9	10	9
B9	2	3	5	TR	8.4	13.9	8	9	4
B14	4	8	15	0	20.7	40.3	5	6	5
B18	3	9	16	0	0	0	10	4	8
B22	3	18	18	33.1	53.9	62.0	4	3	3
B26	3	12	13	0	12.6	29.5	7	4	3
B28	2	6	10	TR	23.8	7.6	6	8	7
B31	4	9	11	10.7	62.0	92.5	3	7	6

[a] Numbers in field indicate relative station rank in mean ergosterol (Erg.),% mass loss (Mass), or nutrient (N + P) hierarchies.
[b] Substrate not recovered at harvest.
[c] Ergosterol present but below chromatographic integration threshold.

Fungal growth at freshwater station pair 4 in Columbus County also was greater where there was nutrient enrichment. In this case, ergosterol content of baits at the polluted site just below the International Paper mill effluent outfall was 2.3 times more than at the upstream paired site after three months submergence. This presumably was due to the significantly greater N content (as ammonia) of the effluent water.

Although these results would argue in favor of accurately predicting fungal activity based on N and P water quality assays, this conclusion is confounded by results from Brunswick county station B31. This site ranked third in terms of mean ergosterol production despite being sixth in the composite N + P hierarchy (Table V). During the course of this study we discovered that B31 was near a leaking residential septic system. The unexpectedly higher fungal mass, therefore, could have been stimulated more by organic loading of the water column than by N or P. If fungal production at this station resulted from utilization of dissolved organic carbon in the water column more than from hydrolysis of lignocellulose of the bait, this could explain the relatively low bait mass loss.

We noted that some unknown factor(s) at stations B8 and B18 prevented detectable fungal growth (Table V). This was associated with very low substrate mass loss at B8 but not at B18 where loss ranked fourth. We cannot explain this difference particularly in light of the 123:1 and 45:1 N:P ratios at the two stations respectively since both should have promoted considerable fungal production. We

simply cite this as evidence that fungal production cannot always be predicted on the basis of N and P concentrations alone.

Nutrient enrichment in estuarine water surprisingly was associated with statistically significant decreases ($p < 0.05$) in mean fungal growth on baits at two of the three sampling pairs (1E and 2E) compared to their controls. The mean ergosterol value for the third saline pair (3E) also was significantly lower at the polluted site after one month, but this difference decreased with longer incubation and was not statistically significant by the second month. This finding is contrasted with the enhanced fungal growth noted by Newell *et al.* (1996b) on salt marsh cordgrass standing-moribund tissue from a tidal wetland plot fertilized with ammonium nitrate. We cannot explain what factors combined to cause salt tolerant fungi to respond to nutrient enrichment in exactly the opposite way than did those in the Newell *et al.* study or to the present freshwater species. We would point out, however, that this phenomenon would not have been predictable based on water quality data alone.

Numerous studies have documented the fact that the growth form of filamentous fungi enables them to penetrate and decompose solid cellulolytic substrates more quickly than is common for single-celled bacteria (Padgett *et al.*, 1985; Newell *et al.*, 1996a; Dickinson and Pugh, 1974; Eaton and Hale, 1993; Jennings, 1995; Dix and Webster, 1995). Even though many bacteria secrete similar suites of hydrolytic enzymes as do plant-decomposing fungi, the substrate-penetrating ability of hyphal growth enables filamentous fungi to reach deep recesses of a solid substrate more quickly. As a consequence, fungi are more important than bacteria at least in the initial stages of wood degradation. Progressive substrate softening, however, alters the surface to volume ratio in favor of bacterial decomposers (Benner *et al.*, 1984). This together with the generally proportional relationships between substrate mass loss and ergosterol content in the present study suggests that fungi were the principal decomposers of our woody substrates at least during the initial weeks of submergence.

Numerous studies (e.g. Newell *et al.*, 1989; Padgett and Posey, 1993; Gessner *et al.*, 1991) have shown that lignocellulolytic fungi contain ergosterol in their cell membranes. Newell *et al.* (1987), furthermore, has shown that its concentration can be affected by the nutritional state of the fungus while Padgett and Posey (1993) reported that ergosterol content can vary both inter and intraspecifically. Nonetheless, we agree with Newell (1996) that ergosterol can be a significant indicator of fungal participation in plant litter decomposition, but feel that ergosterol data alone impart a somewhat simplistic view of fungal-mediated decomposition (reasoning discussed below).

In reality, members of natural decomposer communities (principally bacteria and fungi) compete with each other as they attempt to colonize substrates (Dickinson and Pugh, 1974). Dynamics of this competition are reflected in ongoing changes in microbial community composition on a given substrate through time (Dix and Webster, 1995). As this relates to the present study, the ergosterol extracted from a

given substrate was derived from the sum total of the (unidentified) fungal species present thus can be quantified only as μg ergosterol g^{-1} of substrate. This fact would appear to reduce the utility of ergosterol as a useful water quality bioassay, but rather argues for the need to combine such data with identifications of fungal species present on the substrate (Cooke, 1969).

The underlying significance of the ergosterol assay in the present context is rooted not only in the importance of fungal communities as decomposers but also in their documented value as a food source in the detritus-based food web (e.g. Kaushik and Hynes, 1968; Barlocher and Kendrick, 1973a; Barlocher and Porter, 1986; Barlocher and Newell, 1994)). These studies have shown that aquatic detritivores most often feed on emergent as well as submerged plant litter not because of its nutritional value *per se*, but rather to access high quality microbial protein contained therein. Microbial energy, in turn, is passed to higher trophic levels as larger animals feed on the detritivores then are preyed upon themselves.

One of the environmental implications of pollutants that alter fungal activity is the resulting impact that such alterations might have on higher trophic levels via this detritus-based food web. If pollutants shift competitive balances of fungal communities in favor of species that are less palatable to detritivores (Barlocher and Kendrick, 1973b; Barlocher, 1985), there could be substantial consequences at higher trophic levels. It is possible that some currently unexplained biotic consequences of pollution could be caused by such a subtle yet profound shift in trophic dynamics or by conditions that favor bacterial or unidentified mixotrophic decomposers over fungi.

In our opinion the preceding discussion argues for inclusion of the ergosterol assay in a suite of bioassays that should be administered wherever realistic evaluations of environmental health of aquatic systems are sought. We suggest that ergosterol analyses reflect the relative health of the fungal decomposer community and should be interpreted by comparison to baseline values (e.g. prior to onset of the particular environmental insult) or to data from similar but unimpacted areas (Newell and Wall, 1998).

References

APHA: 1995, 'Standard methods for the examination of water and wastewater', 19th ed. *American Public Health Association*, Washington, D. C.

Barlocher, F.: 1985, 'The role of fungi in the nutrition of stream invertebrates', *Bot. J. Linnean Soc.* **91**, 83–94.

Barlocher, F. and Kendrick, B.: 1973a, 'Fungi in the diet of *Gammarus pseudolimnaeus*', *Oikos* **24**, 295–300.

Barlocher, F. and Kendrick, B.: 1973b, 'Fungi and food preferences of *Gammarus pseudolimnaeus*', *Arch. Hydrobiol.* **72**, 501–516.

Barlocher, F. and S. Y. Newell.: 1994. 'Phenolics and proteins affect palatability of *Spartina* leaves to the gastropod *Littoraria irrorata*', *Mar. Ecol.* **15**, 65–75.

Barlocher, F. and C. W. Porter.: 1986. 'Digestive enzymes and feeding strategies of three stream invertebrates', *J. N. Am. Benthol. Soc.* **5**, 58–66.

Benner, R., Newell, S. Y., Mac Cubbin, A. E. C. and Hodson, R. E.: 1984. 'Relative contributions of bacteria and fungi to rates of degradation of lignocellulosic detritus in salt-marsh sediments', *Appl. Environ. Microbiol.* **48**, 36–40.

Chui, P. C.: 1977, 'Characteristics of stormwater quality from two urban watersheds in Singapore', *Environ. Monitoring and Assessment* **44**, 173–181.

Cooke, W. B.: 1969, 'Our mouldy earth: a study in the fungi of our environment with emphasis on water', *U. S. Dept. Of Interior Water Pollution Control Admin.* Cincinnati, OH.

Dickinson, C. H. and Pugh, G. J. F.: 1974, *Biology of Plant Litter Decomposition*, vols. 1 and 2, Academic Press, London. 775 pp.

Dix, N. J. and Webster, J.: 1995, *Fungal Ecology*, Chapman and Hall, London. 549 pp.

Eaton, R. A. and Hale, M. D. C.: 1993, *Wood: Decay, Pests, and Protection*, Chapman and Hall, London, 546 pp.

El-Sharouny, H. M. M.: 1989, 'Pollution effects on fungi inhabiting organic debris in the Nile water', *Egypt. J. Microbiol.* **24**, 405–413.

Gessner, M. O., Bauchrowitz, M. A. and Escautier, M.: 1991, 'Extraction and quantification of ergosterol as a measure of fungal biomass in leaf litter' *Microbial Ecol.* **22**, 285–291.

Jennings, D. H.: 1995, *The Physiology of Fungal Nutrition*, Cambridge Univ. Press, Cambridge, 622 pp.

Kaushik, N. K. And Hynes, H. B. N.: 1968, 'Experimental study on the role of autumn-shed leaves in aquatic ecosystems', *J. Ecol.* **56**, 229–243.

Lebo, M. H. And Sharp, J. H.: 1993, 'Distribution of phosphorus along the Delaware, an urbanized coastal plain estuary', *Estuaries* **16**, 290–301.

Mallin, M. A., Posey, M. H., Moser, M. L., Shank, G. C., McIver, M. R., Alphin, T. D., Ensign, S. H. and Merritt, J. F.: 1997, 'Environmental assessment of the lower Cape Fear River system', *UNC Wilmington CMSR report* **97–01**, 150 pp.

Newell, S. Y.: 1992, 'Estimating fungal biomass and productivity in decomposing litter', in: G. C. Carroll and D. T. Wicklow (eds.), *The fungal community: its organization and role in the ecosystem, 2nd ed*, Marcel Dekker Inc., New York. 521–561.

Newell, S. Y.: 1996, 'Establishment and potential impacts of eukaryotic mycelial decomposers in marine terrestrial ecotones', *J. Exp. Mar. Bio. Ecol.* **200**, 187–206.

Newell, S.Y., Miller, J. D. and Fallon, R. D.: 1987, 'Ergosterol content of salt-marsh fungi: effect of growth conditions and mycelial age', *Mycologia* **79**, 688–695.

Newell, S. Y., Arsuffi, T. L. and Fallon, R. D.: 1988, 'Fundamental procedures for determining ergosterol content of decaying plant litter by liquid chromatography', *Appl. Environ. Microbiol.* **54**, 1876–1879.

Newell, S. Y., Fallon, R. D. and Miller, J. D.: 1989, 'Decomposition and microbial dynamics for

standing, naturally positioned leaves of the salt marsh grass *Spartina alterniflora*', *Mar. Bio.* **101**, 471–481.

Newell, S. Y., Porter, D. and Lingle, W. L.: 1996a, 'Lignocellulosis by ascomycetes (fungi) of a saltmarsh grass (smooth cordgrass)', *Microsc. Res. Technol.* **33**, 32–46.

Newell, S. Y., Arsuffi, T. L. and Palm, L. A.: 1996b, 'Misting and nitrogen fertilization of shoots of a saltmarsh grass: effects upon fungal decay of leaf blades', *Oecologia* **108**, 495–502.

Newell, S. Y. And Wall, V. D.: 1998, 'Response of salt marsh fungi to the presence of mercury and polychlorinated biphenyls at a Superfund site', *Mycologia* **90**, 777–784.

NOAA: 1996, 'NOAA's estuarine eutrophication survey, volume 1: South Atlantic region', *Office of Ocean Resources and Assessment*, National Oceanic and Atmospheric Administration, Silver Spring, MD.

Padgett, D. E. And Posey, M. H.: 1993, 'An evaluation of the efficiencies of several ergosterol extraction techniques', *Mycol. Res.* **97**, 1476–1480.

Padgett, D. E., Hackney, C. T. and Sizemore, R. K.: 1985, 'A technique for distinguishing between bacterial and non-bacterial respiration in decomposing *Spartina alterniflora', Hydrobiologia* **122**, 113–119.

Suberkropp, K.: 1995, 'The influence of nutrients on fungal growth, productivity, and sporulation during leaf breakdown in streams', *Can. J. Bot. (Suppl. 1)* **S1361–S1369**.

USEPA: 1992, 'The national estuary program after four years - a report to congress', *EPA report no. 503/9-92/007*, United States Environmental Protection Agency, Office of Water, Washington, D.C.