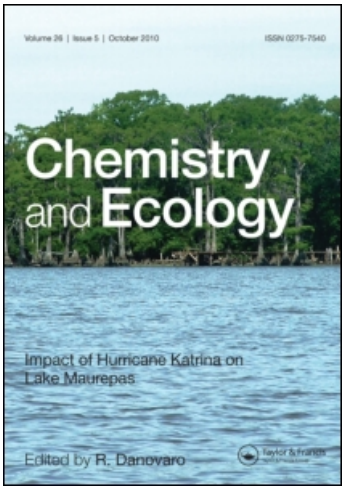

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THE INFLUENCE OF VARYING PLANKTONIC AND PERIPHYTIC ALGAL BIOMASS ON EXPOSURE TO POLYCHLORINATED BIPHENYLS IN MESOCOSMS

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Algal populations, either suspended in the water column (planktonic) or present on the walls of an enclosure (periphytic), develop differently depending upon the physical scale of the system. This study determined whether these variations altered the speciation and therefore exposure of polychlorinated biphenyls (PCBs) in estuarine mesocosms. Exposure was defined as the fraction of applied contaminant taken up by plankton after two hours. Using a three phase equilibrium model, the partitioning of a suite of PCBs within variously-sized mesocosms was predicted using laboratory derived distribution coefficients and measured levels of planktonic and periphytic algal biomass. In mesocosms having large wall surface area to volume ratios, sorption of hydrophobic PCBs to periphyton significantly decreased contaminant exposure. However, within the range of planktonic algal biomasses observed in this study, the regulation of PCB exposure was relatively invariant between variously-sized mesocosms. To minimize sorption of hydrophobic organic contaminants (HOCs) to periphyton and reduce artefacts inherent with this partitioning, we suggest using mesocosms with low wall surface area to volume ratios (less than or equal to 1). In addition, periphytic biomass should be quantified regularly and a three-phase equilibrium approach used to predict the actual exposure concentrations.

Keywords: PCBs; mesocosms; periphyton; exposure; ecotoxicology

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INTRODUCTION

Numerous studies have focused on the exposure of hydrophobic organic contaminants (HOCs) to either single species of plankton at a bench-top scale (Ko, 1994; Sijm *et al.*, 1995; Stange and Swackhamer, 1994) or to field populations (Biggs *et al.*, 1980; Harding and Phillips, 1978a; Ko and Baker, 1995; Lenderman and Rhee, 1982). Bridging the gap between these widely varying spatial scales, mesocosms (enclosures ranging in volume from $0.1-100\,m^3$) provide a means to study chemical effects on larger scale systems consisting of a myriad of organisms and their associated biological feedbacks (*e.g.*, Fairchild *et al.*, 1992; Giddings *et al.*, 1996; Howick *et al.*, 1991; Rodgers and Peters, 1983; Swartzman *et al.*, 1990; Webber *et al.*, 1992). By employing mesocosms, ecotoxicologists may easily control parameters such as light, temperature, nutrients, and number of species, while evaluating the speciation and subsequent exposure of toxicants to the system. However, extrapolation of results from these scaled-down versions of natural systems has been problematic due to inherent factors within these enclosures which act to decrease the applied dose of contaminant. Therefore, before mesocosms can be used reliably as surrogates for natural systems in contaminant exposure studies, an understanding of the effects on exposure by these factors must be developed.

The scale-dependent production of planktonic algal biomass may exert a significant control on HOC exposure. Biological communities, especially algal populations, vary in abundance as the size of the enclosure is varied (Perez *et al.*, 1991 and 1997). For instance, Perez *et al.* (1991) investigated the fate and ecological effects on systems dosed with the pesticide Kepone using three different sizes of marine mesocosms. Even in the absence of the contaminant perturbation, phytoplankton abundance, as well as the time to achieve maximum algal biomass, was directly related to container size. Due to these spatial and temporal scale variances in algal biomass, contaminant exposure also varied with mesocosm size. Biological feedbacks, *i.e.*, the increased production of algal exudates with increased algal biomasses may, in turn, also lead to decreased exposure of contaminants to organisms due to sequestration (Swartzman *et al.*, 1990). Thus, for particle-sorptive contaminants such as polychlorinated biphenyls

(PCBs), the actual dose which an organism encounters is rarely equal to applied dose.

Planktonic populations and biological feedbacks are governed, to some extent, by the spatial scale of the enclosure; yet how does this scale dependency ultimately influence the exposure of the contaminants to the organisms themselves? Because HOCs have a high sorptive affinity for both colloidal and particulate organic carbon (POC), mesocosms with higher dissolved organic carbon (DOC) and POC likely sequester more HOCs, thus reducing exposure (*via* passive uptake) to higher trophic levels. In pure algal cultures, researchers have found a negative correlation between bioconcentration factors (BCFs) of PCBs and density of algae (Ko, 1994; Sijm *et al.*, 1995; Richer and Peters, 1993). Sijm *et al.* suggested that the apparent decrease of algal BCFs was a result of reduced bioavailability of the contaminants in the aqueous phase due to binding to exudates.

The above studies were conducted using simple media containing a single algal species, often at densities greatly exceeding those found in natural systems. In mesocosms, where more realistic yet complex mixtures of protozoan, bacterial and planktonic algal populations exist in the water column, exposure of HOCs may be more difficult to assess. However, generally it has been observed that productive systems sequester more contaminants and subsequently decrease exposure to organisms (Gunnarsson and Rosenberg, 1997; Larrson *et al.*, 1992; McCarthy and Bartell, 1988). For example, McCarthy and Bartell supplemented their modelling study with laboratory experiments investigating the role of trophic status in modifying the bioavailability of contaminants and subsequent decreases in dose to biota. For phytoplankton, zooplankton, benthic invertebrates and omnivorous fish, the 15–30% reduction in toxic effects observed corresponded to increases in dissolved organic matter in the system.

Changes in mesocosm spatial scale may not only influence contaminant exposure through differences arising in the abundance, diversity and timing of water column constituents, but may also be influenced by periphyton (algal wall growth). Periphyton, common in natural communities as well as in physical enclosures such as mesocosms, may represent a significant phase on to which hydrophobic pollutants may sorb (Chen *et al.*, 1997; Dudzik *et al.*, 1979 and

Howell *et al.*, 1972). Sorption to periphyton, depending upon the wall surface area to volume ratio of the enclosure, is hypothesized to decrease the exposure concentration significantly below the applied dose. An understanding of the degree to which exposure concentrations are reduced within enclosed systems will aid in extrapolating results from mesocosm research to natural aquatic systems.

The objective of this study was to investigate the exposure of HOCs (using PCBs as representative contaminants) in mesocosms of varying algal biomass. In comparison to Turner's (1996) method for producing variations in algal biomass within mesocosms (varying light levels), the biomass of planktonic populations, as well as periphytic communities, were varied by employing enclosures of various dimensions, periodically scraping periphyton from the enclosure's walls and nutrient addition. A suite of PCBs having a wide range of octanol-water partition coefficients (K_{ow}) was spiked into subsamples collected from each of the various mesocosms. Determination of distribution coefficients for PCBs in the water columns from these mesocosms throughout the development of planktonic communities provided insight into partitioning behaviour of contaminants over a realistic range of particulate and dissolved organic carbon values. Because direct contaminant addition to the mesocosms was not possible, sorption of PCBs to periphyton was modelled using an equilibrium partitioning approach employing empirical data gathered from the mesocosm experiment.

MATERIALS AND METHODS

Mesocosm Facilities

The Multiscale Experimental Ecosystem Research Center (MEERC) at the University of Maryland Center for Environmental Science was established as one of U.S. Environmental Protection Agency's Exploratory Research Centers. The underlying hypothesis of MEERC is that responses of ecological systems to external perturbations differ as the system's spatial and complexity scales change. The far-reaching goal of the MEERC project is to develop a new generation of scale dependent, ecosystem models which can be used to extrapolate

experimental results from controlled mesocosms to real management problems.

The four indoor cylindrical MEERC mesocosms of various dimensions were fabricated from opaque fibreglass (Tab. I). The sizes and shapes were chosen to provide a range in mesocosm dimensions. All mesocosms were subjected to identical environmental conditions, including light temperature, mixing regimen, and water column replacement rates (Chen *et al.*, 1997). Each mesocosm was intermittently stirred by rotating paddles, creating similar turbulence throughout all mesocosms, regardless of size (Petersen *et al.*, 1997; Sanford, 1997).

Mesocosm Experiment

Water column samples were collected weekly from four mesocosms (Fig. 1) during a four week MEERC mesocosm experiment from July 25 to August 22, 1995. Natural estuarine sediment collected from the Choptank River, Maryland, USA, a sub-estuary of the Chesapeake Bay, was used. Sediments were heated to eliminate live benthic infauna and were placed in each mesocosm to a depth of 10 cm. The mesocosms were filled with filtered Choptank River (0.5 μm) water with salinity of ca. 11%. On the initiation of the mesocosm experiment, 10‰ of the volume of each mesocosm was filled with unfiltered water to seed the mesocosms with a natural community of plankton and copepod nauplii, as well as ambient nutrients existing in the estuary water at the time of the experiment. Organisms larger than copepod nauplii (*e.g.*, copepidites, gelatinous zooplankton, fish larvae) were screened out from the fill water.

Once filled, the benthic and pelagic biological communities within the mesocosms were allowed to develop. To simulate natural flushing,

TABLE I Dimensions and volumes of employed mesocosms of the Multiscale Experiment Ecosystem Research Center (MEERC). Periphyton was scraped from the walls of mesocosm B1 but left on the walls in A, B2 and C

Mesocosm	*Volume*, m^3	*Depth*, *m*	*Diameter*, *m*	*Wall surface area*: *Volume*
A	0.10	1.0	0.35	11
B1	0.10	0.50	0.50	7.5
B2	0.10	0.50	0.50	7.5
C	10.0	1.0	3.5	1.1

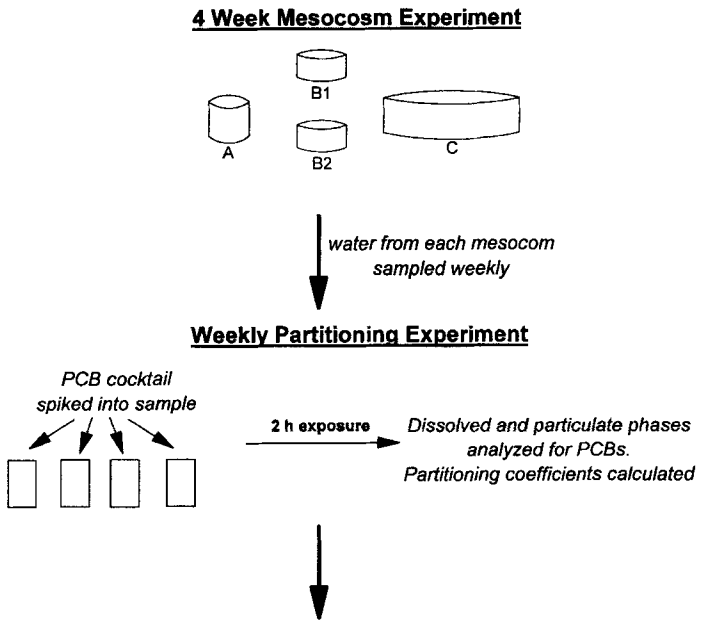

FIGURE 1 Flow chart outlining the generation of data required for formulation of the exposure model for variously-sized mesocosms containing both planktonic and periphytic algae.

10% of the volume of each mesocosm was removed and replaced with filtered (0.5 μm) Choptank River water daily. Two weeks after the initiation of the experiment, the mesocosms were inoculated with ammonium (NH_4^+), phosphate (PO_4^{3-}) and dissolved silica to achieve concentrations of 50, 3.1 and 50 μM, respectively. Chlorophyll *a* (Chl *a*) concentrations were measured every second or third day.

Periphyton was allowed to accumulate on the walls and surface of the sediments in mesocosms A, B2, and C, and was periodically scraped from the walls of mesocosm B1 and allowed to settle or suspend in the water column. Periphytic biomass was measured by placing replicate strips (2 cm wide) of mesocosm wall material on the interior of the mesocosm walls (Chen *et al.*, 1997). As benthic and planktonic communities developed, periphytic communities colonized these strips. Strips were harvested periodically and chlorophyll *a* (units of μg Chl *a* per wall surface area) was measured using standard

acetone extraction methods (Chen *et al.*, 1997). Dry weight of periphyton was not measured directly. In order to convert periphytic biomass from units of μg Chl *a* per wall surface area to mg dry weight per wall surface area (or volume), water column algal Chl *a* was related to total suspended particles (TSP) and extrapolated to periphyton.

Sample Collection

The partitioning of PCBs between the dissolved and particulate phases was tracked in water column samples collected from each of the four mesocosms (A, B1, B2 and C) on a weekly basis (Fig. 1). Samples for the partitioning experiments were collected in stainless steel, air tight tanks. Additional water was collected and analyzed for TSP, dissolved organic carbon (DOC), and particulate organic carbon (POC), from which the fraction organic carbon (foc) was calculated. All samples were transported within four hours of collection to the Chesapeake Biological Laboratory (University of Maryland System, Solomons, MD) for processing and experimentation. For TSP determination, a volume of the sample was passed through a tared 0.4 μm Nucleopore filter, dried at 50°C, and reweighed. Dissolved organic carbon (DOC) was measured by an automated persulphate digestion at 100°C using a Model 700 Carbon Analyzer. For POC determination, a known volume of water was filtered through at 25 mm glass fibre filter (Whatman GF/F) and total carbon was analyzed by high temperature (950°C) combustion using a Model #240-XA elemental analyzer (Leemen Labs, Inc.).

HOC Partitioning

A cocktail containing nineteen polychlorinated biphenyl (PCB) congeners (AccuStandard Inc.) was prepared in acetone from individual neat standards (Tab. II). The congeners encompassed a wide range of log K_{ow} values (4.55 to 8.59). The volume of acetone carrier (100 μl) which was spiked into the individual water samples was kept low to minimize the amount of DOC introduced into the systems. The resulting PCB congener concentrations in the water ranged from 3 to 276 $ng\,l^{-1}$ (Tab. II), well below their aqueous solubilities.

TABLE II Properties and total concentrations of the polychlorinated biphenyls used in the laboratory partioning experiment. Total concentrations refer to the total concentration in the spiked tanks

IUPAC no.	*Substitution pattern*	*log K_{ow}*	*Total concentration (ng/L)*
1	2-	4.55	5.94
10	2,6-	5.09	5.64
15	4,4′	5.33	6.27
19	2,2′,6-	5.34	11.89
34	2′,3,5-	5.73	10.89
52	2,2′,5,5′-	6.18	11.15
77	3,3′,4,4′-	6.40	29.31
97	2,2′,3′,4,5-	6.65	27.88
100	2,2′,4,4′,6-	6.23	5.56
101	2,2′,4,5,5′-	6.94	31.51
104	2,2′,4,6,6′-	6.64	3.01
105	2,3,3′,4,4′-	6.91	29.75
118	2,3′4,4′,5-	6.89	25.29
126	3,3′,4,4′,5-	6.89	29.01
156	2,3,3′,4,4′,5-	7.65	30.43
170	2,2′,3,3′,4,4′,5	7.86	159.58
180	2,2′,3,4,4′,5,5′-	7.76	275.76
194	2,2′,3,3′,4,4′,5,5′-	8.11	25.15
206	2,2′,3,3′,4,4′,5,5′,6-	8.59	88.79

Immediately after spiking a sample, the tank was sealed and gently agitated for several minutes. The exposure tank was allowed to equilibrate for two hours in a temperature-controlled room of 20 ± 0.1°C. Periodic inversion and agitation of the tanks ensured complete mixing. After the 120 minute exposure time, a 1 litre subsample of the water from the tank was filtered using a precombusted 47 mm glass fibre filter (GF/F) with a nominal pore size of 0.7 μm. The receiver flask contained 5 ml of dichloromethane (DCM). The filtrates were stored in a refrigerator until extraction. The filters were folded, wrapped in aluminum foil and frozen for later extraction. Using an equilibrium partitioning model, data from both the mesocosm and weekly partitioning experiments, the exposure of PCBs within the mesocosm experiment was predicted (Fig. 1).

HOC Extraction and Analyses

Prior to extraction, two internal standards (IUPAC 30: 2,4,6-trichlorobiphenyl and IUPAC 204: 2,2′,3,4,4′,5,6,6′-octachloro-biphenyl) were

added. Filtrate samples were extracted by liquid–liquid separation using DCM. Solvent was reduced by evaporation under pressure and replaced with hexane. Samples were further concentrated to 1 ml. Particulate PCB concentrations were evaluated by extracting the filters three times with DCM using sonication. Extracts were combined, concentrated by evaporation under pressure, and replaced with hexane. The sample was further concentrated to 1 ml under nitrogen. The concentrated extract was purified by liquid–solid chromatography (4 g to 6% deactivated aluminium trioxide) using petroleum ether as the eluant. The eluant was collected, concentrated, replaced with hexane and further concentrated.

PCBs were quantified using a Hewlett Packard 5890 gas chromatograph with a ^{63}Ni electron capture detector (GC-ECD). The column was a 60 m × 0.023 mm i.d. DB-5 capillary column (J & W Scientific) with hydrogen as the carrier gas (flow of 1 ml min^{-1}) and nitrogen as the make-up gas (flow of 30 ml min^{-1}). An autosampler (HP 7673) injected a 2 µl sample in the splitless injection mode. Identification of PCB congeners was based on comparison of retention times to those found for the cocktail. All spiked congeners were well above analytical detection limits.

RESULTS AND DISCUSSION

Mesocosm Experiment

Despite the fact that all mesocosms were filled with water from the same source and were subjected to identical physical forcing functions, algal populations in the mesocosms soon diverged after the initiation of the experiment. Both TSP concentrations, measured only on those days when exposure experiments were conducted, and Chl *a* concentrations between mesocosms varied widely in magnitude and in timing (Figs. 2a–b). The largest TSP and Chl *a* values were measured in mesocosm B1. This is a direct result of the scraping of periphyton which consequently suspended biomass in the water column of that mesocosm. In comparison, TSP and Chl *a* values in mesocosm B2 did not change very much. In mesocosm C, a distinct planktonic bloom occurred prior to nutrient addition as evident by a

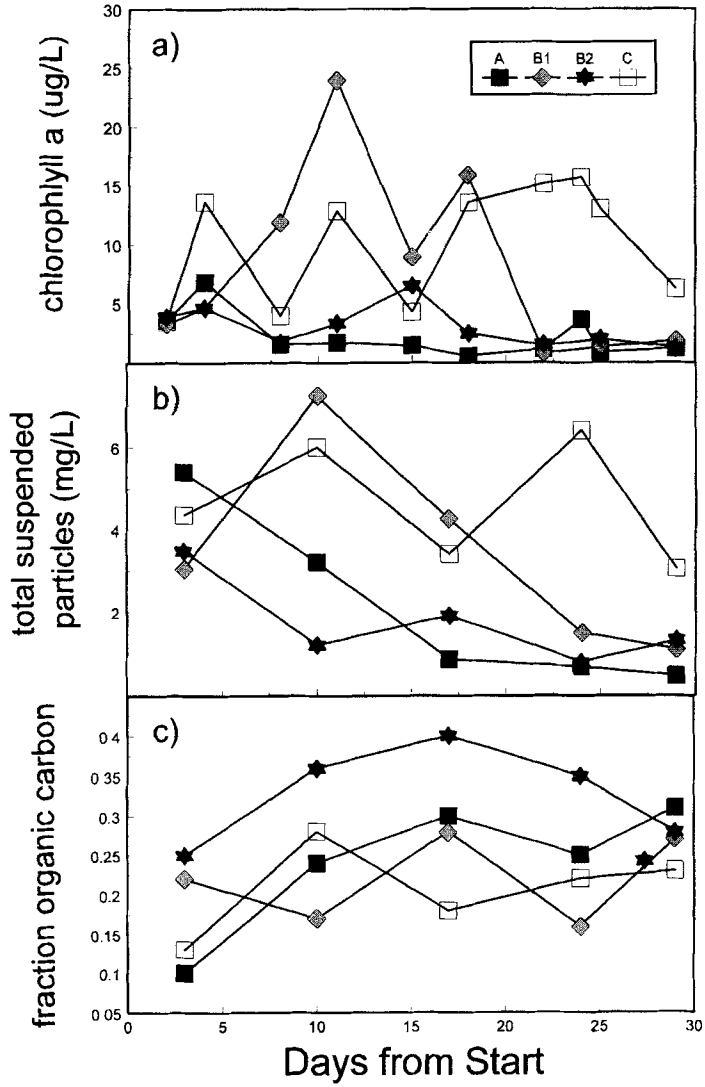

FIGURE 2 (a) Chlorophyll *a* concentrations (Chl *a*); (b) total suspended particles (TSP) and (c) fraction organic carbon (foc) *versus* time in the four mesocosms. A nutrient pulse occurred on day 15 and the water column was sampled on the days 3, 10, 17, 23 and 29 to evaluate partitioning of PCBs.

large TSP and Chl *a* concentrations. In the same mesocosm, the planktonic algae responded to the nutrient addition (day 15) to a relatively large degree, shown by a broad peak in Chl *a* concentrations

(Fig. 2a). Mesocosm A did not contain any noticeable phytoplankton bloom either before or after the addition of nutrients. This may have been due to competition for nutrients between planktonic and periphytic algae in an enclosure having higher wall surface area to volume ratios. Linear regression between TSP and Chl *a* concentrations within the four mesocosms was highly significant ($R^2 = 0.80$) suggesting that the majority of the suspended particles were intact phytoplankton. There was little correlation between TSP and DOC ($R^2 = 0.05$) suggesting that even during bloom conditions, algal exudates did not contribute significantly to the DOC pool. In fact, although not shown, dissolved organic carbon concentrations throughout the duration of planktonic development remained relatively invariant (ranging from 3.2 to 3.9 $mg\,l^{-1}$). Values for fraction organic carbon ranged from 0.1% to 0.4% over the course of the summer experiment. In the three mesocosms in which periphyton was left to accumulate on the walls of the enclosure, the foc of suspended matter increased during the first week, denoting a shift from more abiotic to more biotic particles with time, possibly due to resuspension of sediments during the early days of the experiment. After the first week, mesocosm B2 consistently had the highest foc values, except for the last day of sampling. The values of all ancillary measurements mentioned are within ranges found in the Chesapeake Bay during the summer.

Periphytic biomass was measured throughout the experiment (Chen *et al.*, 1997). In mesocosms A and B2, periphytic biomass dominated the total biomass (planktonic + periphytic) in the enclosures after the first week (Fig. 3). The total biomass within mesocosm C, that enclosure of smallest wall surface area to volume ratio, was almost entirely composed by planktonic algae, except for day 17. The effects of wall scraping were evident by substantially reduced periphytic biomasses in mesocosm B1 compared to B2. Also, periphyton that was scraped from the wall became suspended in the water column, increasing values of planktonic algal biomasses compared to the untouched counterpart (mesocosm B2).

As in this study, Perez *et al.* (1991) observed differences in periphytic and planktonic communities when they investigated the influence of spatial scales between three differently sized mesocosms. In both studies, varying mesocosm size and shape, changes intensity and, for some, timing of phytoplanktonic blooms. However, their study

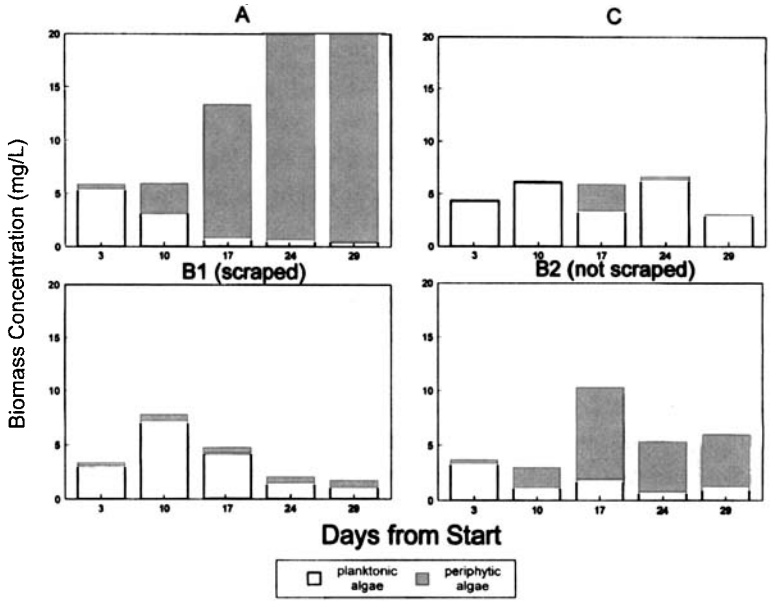

FIGURE 3 Comparison of the fraction of periphytic and planktonic biomass in mesocosms A, B1, B2 and C during the four week mesocosm experiment.

showed that in the absence of an external perturbation, smaller mesocosms had lower but more immediate increases in algal density than large-size systems. Perez *et al.* (1991) attributed their size dependent responses to either variations in benthic species or container surface area, or some combination of the two. They speculated that variations in the latter would control the timing of the phytoplankton bloom. Smaller mesocosms having a higher wall surface area to volume ratio would support higher wall growth populations that would in turn be more effective at releasing nutrients to support water column blooms.

In a concurrent project to this one, the differences in both phytoplankton and periphyton abundances from sets of three (triplicate) mesocosms of each dimension were evaluated (Chen *et al.*, 1997). For planktonic and periphytic biomass, coefficient of variations (C.V.; s^2/mean) ranged from 0% to 30% and 0% to 20%, respectively. Throughout the mesocosm experiment, C.V. values were low (5%) except for those days following nutrient addition when values

increased (up to 30%). The relatively low C.V. values indicated that the three mesocosms of each differently sized mesocosm behaved similarly, and that the phytoplanktonic and periphytic biomasses observed in the mesocosms we sampled from (A, B1, B2 and C) were primarily a result of the dimension of the enclosure, rather than random variation within treatments.

Partitioning Experiment

Through the use of mesocosms of varying spatial scale, variable wall cleaning techniques (scraped *versus* not scraped) and a nutrient addition, varying algal biomass both within the water column and on the walls of the mesocosms were produced. Yet, how to do these differences influence how the water column constituents sequester PCBs, and subsequently determine the exposure of these contaminants? To answer this, we conducted partitioning experiments to quantify differences, if any, in the partitioning of HOCs to the organic phases (dissolved and particulate) contained in the water column of the mesocosms and to elucidate the factors important in controlling contaminant exposure.

Because the purpose of this research was to investigate differences in HOC partitioning between mesocosms of varying size and shape and subsequent varying algal biomasses each produced, the exposure time was kept to a minimum (2 h) to preserve sample integrity. That is, minimizing the handling time after sample collection ensured that the communities that were present in the mesocosms did not alter significantly once removed (*i.e.*, senescent). Numerous studies have found that the uptake of HOCs by algae is rapid, often reaching a pseudo- or true steady-state within two hours (Ko, 1994; Harding and Phillips, 1987a, b; Mailhot, 1987; Richer and Peters, 1993 and Sijm *et al.*, 1995). In one of the first studies of PCB uptake by phytoplankton, Harding and Phillips (1978b) determined that greater than 90% of the ultimate (48 hour) uptake of 2,2′,4,5,5′-pentachlorobiphenyl occurred within the first 30 minutes of exposure, followed by a small amount of additional uptake during the next two days. Mailhot (1987) and Richer and Peters (1993) observed similar time courses, which they empirically modelled using a square hyperbola.

Ko (1994) exposed *Isochrysis galbana* to a continuous flow of PCB-laced air and found a rapid uptake of the contaminants in the early hours followed by a slower uptake over the days to follow. Rapid (*i.e.*, time scale of minutes) initial uptake accounted for between 10% and 90% of the total PCB uptake by the estuarine chrysophyte.

It has been generally assumed that uptake of PCBs into plankton is rapid, but recent work has challenged this assumption. Stange and Swackhamer (1994) and Swackhamer and Skoglund (1993) have suggested that kinetically-limited uptake of PCBs by rapidly-growing phytoplankton maintains subsaturated conditions in natural waters. Further, Ko (1994) found that an initial rapid uptake of PCBs was followed by a second sorption step in which the contaminants reached sorptive equilibrium at longer time scales. However, in this research, exposure of PCBs was defined as the amount of contaminant taken up by algae after two hours (rapid first step uptake) and not as the ultimate uptake of contaminant into plankton occurring on the order of days to weeks.

The partitioning between the dissolved and particulate phases after a two hour exposure period was evaluated by calculating distribution coefficients (K_d) for each PCB congener. The distribution coefficient describes the observed partitioning of organic chemicals in the field and is defined as the ratio of the observed concentration of PCB in the particulate phase (C_p) to the observed concentration of PCB in the dissolved phase (C_d). The measured dissolved concentration (C_d) includes not only the freely dissolved phase PCBs but also incorporates the colloidally-bound PCB fraction which passes through a glass fibre filter. Log K_d *vs* log K_{ow} plots were constructed for the four mesocosms over the four weeks (day 3, 10, 17, 23 and 29) of the experiment (Fig. 4). If K_d is expressed in units of 1 kg^{-1}, a 1 : 1 relation between these two quantities based on partition theory is expected Karickoff *et al.* (1979), but this is rarely observed in practice (*e.g.*, Ko and Baker, 1995 and Swackhamer and Skoglund, 1993). More pertinent to this study is the observation that for a given PCB at a given time, the values for K_d appear to be different between mesocosms. On day 3 of the experiment, minimal differences in distribution coefficients were observed (Fig. 4a). During this early portion of the experiment, spatial scaling influences on the production of POC and DOC were not as pronounced as in later sampling days.

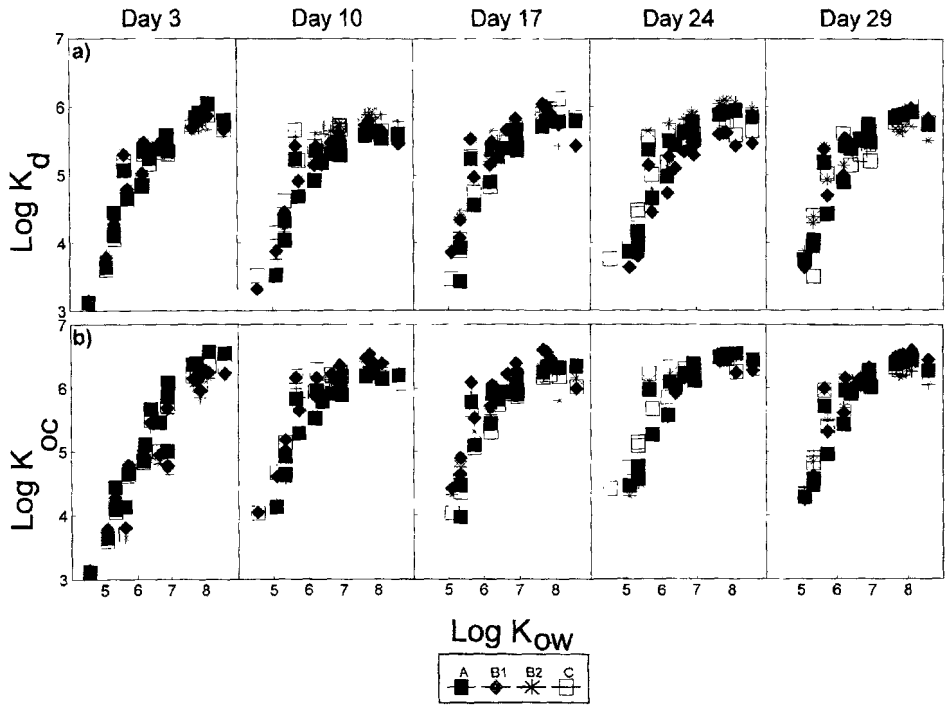

FIGURE 4 Plots of (a) log distribution coefficient (log K_d) and (b) log organic carbon normalized distribution coefficient (log K_{oc}) *versus* log octanol-water partition coefficient (log K_{ow}) for days 3, 10, 17, 23 and 29 for mesocosms A (filled square), B1 (filled diamond), and B2 (star), and C (open square).

Essentially all four mesocosms had very similar water column constituents at this early stage of the experiment, therefore only slight differences in partitioning between mesocosms were observed. In the weeks following (days 10, 17, 23 and 29), differences in partitioning between the four mesocosms were suggested by diverging log K_d *versus* log K_{ow} plots. Values for K_d were normalized to the total suspended particulate concentrations, therefore differences in partitioning, although small, arose from different sorption affinities of the particles and not simply a consequence of particle concentration. According to stepwise multiple regression analysis, approximately 75% of the variation in distribution coefficients over the duration of the experiment was attributed to the variation in the octanol–water partition coefficient and to a much lesser degree, the foc of the particles. Because of the influence of foc on partitioning, organic carbon normalized distribution coefficients (K_{oc}) were calculated and plotted (Fig. 4b). For days 10, 17, 23 and 29, the differences in partitioning may be explained by normalizing to the organic carbon fraction of the particulate phase, further reducing the differences in partitioning behaviour between mesocosms. Although normalizing to organic carbon reduces some of the residual differences in partitioning among the mesocosms, unnormalized distribution coefficients were relatively uniform in the first place, suggesting HOC partitioning was similar even though the abundance and composition of POC and DOC pools may have been different. In fact, the ranges of K_{oc} values for a given K_{ow} from this laboratory investigation were much less than those found in field studies (*e.g.*, Ko and Baker, 1995).

Despite the fact that the production of sequestering moieties in the water column is dependent on the physical scale of mesocosm, contaminant exposure as regulated by water column organic matter did not vary much between the variously-sized mesocosms. This is likely due to the narrow range of particulate and colloidal organic matter concentrations in our experiments and also the short time scales (weeks). The narrow range in the abundance of the sequestering moieties translates to an equally small range in partitioning behaviours. Perhaps more eutrophic mesocosms may have produced differences in partitioning akin to those seen by Sijm *et al.* (1995) using algal cultures. However, the realistic situations the MEERC mesocosms afforded, indicate that ranges in particulate and colloidal

organic matter in the water columns of varying mesocosm and natural systems do not have a large effect on the partitioning behaviour of PCBs over these time scales.

Modelling the Wall Effect

Periphyton has the ability to sequester HOCs (Perez *et al.*, 1991). Past MEERC experiments have shown that periphytic biomass was inversely related to mesocosm diameter and was greatest in summer experiments when periphyton dominated autotroph biomass and organic production within the mesocosms (Chen *et al.*, 1997). It is intuitive to suggest that periphyton has a potentially large influence on the dynamics of organic matter production in mesocosms, especially in those enclosures of high wall surface area to volume (WSA:V) ratios. When comparing biomass values for planktonic *versus* periphytic populations (Fig. 3), periphytic biomass dominated the total POC in some mesocosms (A and B2) suggesting that periphytic growth would be a confounding factor when evaluating exposure in enclosures.

An equilibrium partition model was used to semi-empirically model HOC sorption to particulate organic matter in the water column, sediments and periphyton:

$$F_d = \frac{1}{1 + (TSP * K_d) + (PB_{wall} * K_{wall}) + (PB_{sed} * K_{sed})}$$

$$F_p = (K_d * TSP * C_d)$$

$$F_{sed} = (K_{sed} * SA_{sed} * C_d)$$

$$F_{wall} = (K_{wall} * SA_{wall} * C_d)$$

where

C_d : concentration of PCB in dissolved phase ($kg\,l^{-1}$)
F_d : fraction of PCB in dissolved phase
F_p : fraction of PCB in particulate phase
F_{wall} : fraction of PCB in wall periphyton
F_{sed} : fraction of PCB in surficial periphyton (algal mats on sediment)
K_d : distribution coefficient ($l\,kg^{-1}$)
K_{wall} : wall periphyton–water partition coefficient ($l\,kg^{-1}$)
K_{sed} : sediment-water partition coefficient ($l\,kg^{-1}$)

PB_{wall} : periphytic biomass on wall normalized to volume of mesocosm ($kg\,l^{-1}$)
PB_{sed} : periphytic biomass on sediment normalized to volume of mesocosm ($kg\,l^{-1}$)
TSP : total suspended particles ($kg\,l^{-1}$).

The observed distribution coefficients calculated from our laboratory experiments (K_d) were used in the above equations to describe the partitioning between the dissolved and particulate phases. Partitioning to the wall periphyton and the sediments, which were commonly covered with periphyton, were described by the same empirically-derived distribution coefficients (K_d). This was justified because this represented the partitioning of PCBs between the dissolved phase and a particulate phase composed primarily of algae. Although other researchers have found varying distribution coefficients or bioconcentration factors between pelagic algal species (*e.g.*, Harding and Philips, 1978b) our assumption that $K_d = K_{wall} = K_{sed}$ is a valid first approximation because there are no known published studies which have evaluated HOC partitioning to periphyton. In addition, in past and subsequent MEERC mesocosm experiments, periphyton and planktonic algae were characterized as being primarily composed of diatoms (Chen *et al.*, 1997). Moreover, carbon content to Chl *a* ratios for planktonic and periphyton algae were similar (100 : 1 *vs* 140 : 1 respectively), further supporting our approximation that the partition coefficients found for PCBs to planktonic algae are equal to those for periphytic algae.

Using data collected from both the mesocosm experiment (PB_{wall} and TSP) and the partitioning experiment (K_d), an equilibrium model was used to calculate the distribution of PCB congener 156 (2,3,3′,4,4′,5-hexachlorobiphenyl) in the four mesocosms if the enclosures were dosed directly with contaminants (Fig. 5). According to the model, on the third day of the experiment, congener 156 would have partitioned primarily to the suspended particulate matter in the water column of each mesocosm. On this day, both TSP and periphytic biomass were similar in all mesocosms. By the second week (day 10), differences in partitioning to the four phases (dissolved, particulate, periphyton, sediment) among the four mesocosms evolved. Most notable would be the large extent of HOC partitioning to the

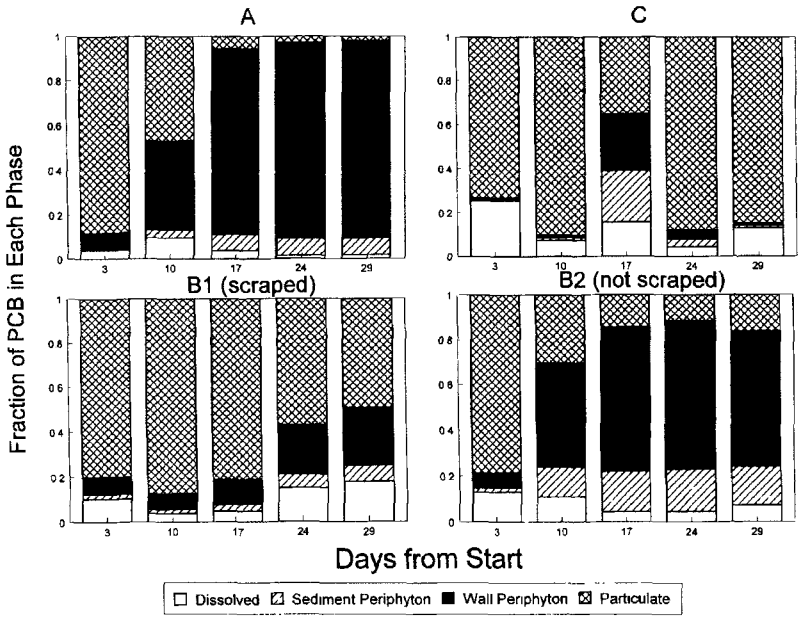

FIGURE 5 Distributions of PCB congener 156 (2,3,3′,4,4′,5-hexachlorobiphenyl) in mesocosms A, B1, B2 and C for the four week mesocosm experiment based on the three-phase equilibrium partitioning model.

periphytic phase in mesocosms A and B2 (40% and 45%, respectively), those enclosures having relatively high WSA:V ratios compared to mesocosm C, and those in which periphyton was not scraped from the walls. By day 17, the model estimates that PCB congener 156 would be primarily bound to periphyton in the three smaller mesocosms but fractionated almost equally to the four phases in mesocosm C. As the experiment progresses and periphytic populations develop in all mesocosms, the fraction of contaminant that partitioned to periphyton in mesocosms A, B1 and B2 increases. In mesocosm A, for example, periphytic growth is responsible for sequestering the vast majority of the available contaminant (up to 95%). Conversely, for mesocosm C, by the end of the experiment (day 29), 90% of the PCB is bound to suspended particulate matter, with less than 4% associated with the periphyton. On day 17 in mesocosm C, periphytic growth in the sediment would have been successfully competing with pelagic particulates for contaminants due to relatively

low *TSP* values and large sediment surface area to volume ratio. However, mesocosm C eventually responded to the nutrient pulse of day 15 by producing a relatively large planktonic bloom which dominated the total particulate organic carbon pool, and subsequently sequestration of the majority of the PCB by this phase would have occurred.

From this equilibrium-based approach, the influence of wall periphyton on the partitioning of contaminants, especially in those mesocosms of relatively large WSA:V ratios, was evident. Within the times scales of this experiment, the exposure of HOCs was largely controlled by the abundance (biomass) of either periphytic or planktonic algae. The sheer abundance of periphyton most likely swamped any differences in partitioning arising from differing sorptive capacities of the surfaces of the two algal forms.

The above model may be modified in order to act as predictive tool regarding the exposure of HOCs in mesocosms of any dimension

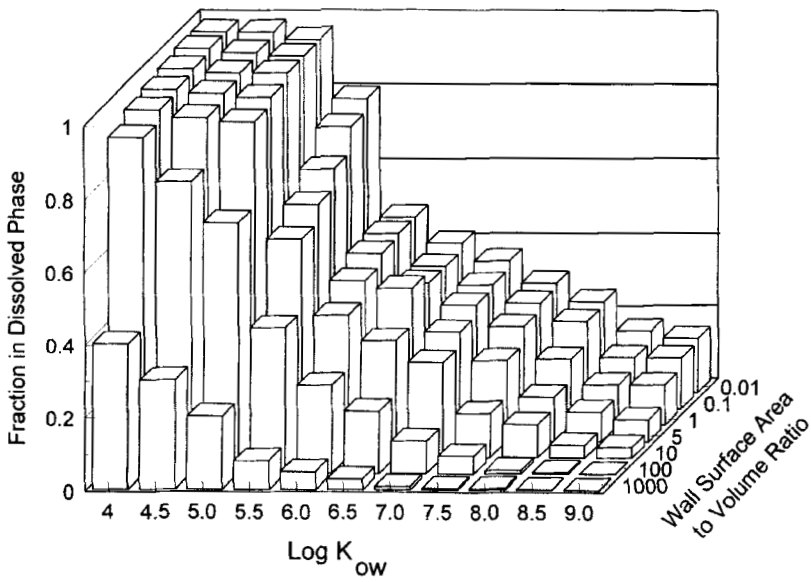

FIGURE 6 Fraction of dissolved phase PCB as a function of hydrophobicity of contaminant (log K_{ow}) and wall surface area to volume ratio based on the three-phase equilibrium partitioning model assuming periphytic and planktonic biomasses of 1 g wet wgt m^{-2} and 2 mg l^{-1}, respectively.

containing both planktonic and periphytic algae. Keeping both suspended and wall algae biomass constant (1 g wet/m^2 and 2 mg l^{-1}, respectively), the dissolved or "exposure" fraction of an HOC having a particular K_{ow} and WSA:V may be predicted (Fig. 6). As expected, with decreasing WSA:V ratios and K_{ow}values (decreasing hydrophobicity), more of the contaminant would be found in the exposure fraction. For enclosures having a WSA:V of 1 or less, differences in exposure concentrations are not large, suggesting that for most HOCs (with the exception of highly hydrophobic compounds), a mesocosm C dimension is sufficient to dampen the effects of sorption to periphyton.

CONCLUSIONS

The popularity of using mesocosms as surrogates for natural aquatic systems in ecotoxicological research will undoubtedly continue. However, for exposure studies utilizing mesocosms having WSA:V ratios greater than 1 and containing periphyton, the actual dose of hydrophobic organic contaminants that organisms will be exposed will be at times significantly less than of the applied dose. Periphyton may be scraped and removed from the enclosures to minimize this artefact but removal of periphyton may potentially remove HOCs as well. Moreover, HOCs may sorb to the surfaces of the enclosure walls regardless of degree of algal covering. A more viable alternative when conducting these types of exposure studies is to quantify periphytic biomass regularly and to use a three-phase equilibrium approach to predict the actual exposure concentrations or to use larger enclosures.

Periphytic biomass is not the only scale-dependent effect that may influence partitioning of HOCs. For example, for relatively volatile HOCs, water surface area to volume ratio would be of importance when considering volatization of the applied dose. In addition, mesocosm dimension may influence the extent of photolysis for those photosensitive HOCs. In mesocosm studies investigating the exposure of HOCs to higher trophic levels, such as zooplankton and planktivorous fishes, spatial variations may also effect the ultimate accumulation of contaminants. Depending on the dimension of the enclosure, higher organisms may preferentially graze on periphytic algae or algal mats on the sediment surface rather than planktonic

algae, thus, shifting their exposure to the contaminants. Further research entailing these aspects will supplement this research and expand our understanding regarding the use and misuse of mesocosms as surrogate systems for exposure studies.

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