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Effect of nutrient availability on the uptake of PCB congener 2,2′,6,6′-tetrachlorobiphenyl by a diatom (*Stephanodiscus minutulus*) and transfer to a zooplankton (*Daphnia pulicaria*)

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Abstract

The objective of this study was to examine the importance of nutrient status of a diatom (*Stephanodiscus minutulus*) to the uptake of PCB congener #54 (2,2′,6,6′-tetrachlorobiphenyl) and the subsequent transfer of PCB to a pelagic grazing zooplankton (*Daphnia pulicaria*). The algae, which were grown under different nutrient treatments, were then fed to a zooplankton to examine the subsequent food chain transfer of PCB. Algal cultures were grown for at least 2 weeks in a steady state condition in (1) non-limiting, (2) low-Si, (3) low-N or (4) low-P media. Steady state algal cultures were dosed with 0.2 $\mu g\,L^{-1}$ PCB and were sampled for PCB uptake after 24 h. *D. pulicaria* were allowed to graze on these same cultures for 48 h before being analyzed for PCB body burdens. Low-Si (68% or 0.135 $\mu g\,L^{-1}$ of PCB) and low-P cultures (62%) had significantly higher percentage uptake of total PCB than the non-limiting (55%) or low-N (52%) treatments. When these values were divided by biochemical or elemental parameters, PCB per lipids ($\mu g\,\mu g^{-1}$) had one of the lowest coefficients of variation (CV) across the four treatments, indicating their importance in PCB uptake. When equal biovolumes of the four different treatment cultures were fed to zooplankton, both the low-N (13.9 ng PCB mg wet weight^{-1}) and the low-P (9.6 ng PCB mg wet weight^{-1}) grazing *D. pulicaria* had significantly higher PCB per wet weight than the low-Si (5.6 ng PCB mg wet weight^{-1}) and non-limited (2.6 ng PCB mg wet weight^{-1}) grazing *D. pulicaria*. There were no significant differences between algal nutrient treatments in PCB per wet weight of zooplankton grazing on clean algal food in PCB contaminated media. This study indicates that uptake of PCB by phytoplankton can be significantly altered by nutrient availability which subsequently affects transfer to zooplankton, potentially through such responses as grazing rate and lipid assimilation.
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Keywords: Algae; Nutrient limitation; Bioaccumulation; Lipid

1. Introduction

Polychlorinated biphenyls (PCBs) are a class of persistent chemicals that were manufactured from 1929 to 1975 for a variety of industrial uses including transformer oils, capacitor dielectrics and heat transfer fluids (Cairns et al., 1986). Most PCBs do not readily degrade in the environment and are lipophilic, and this chemical and physical stability is responsible for many of the environmental consequences (Erickson, 2001, 1997). PCBs have become a nearly ubiquitous environmental pollutant, being found in most human and animal adipose tissue and in nearly every climate or ecosystem on earth (Fiedler, 2001; Holoubek, 2001).

PCBs were produced and released in mixtures, the most popular of these being the Aroclors®. Each Aroclor® mixture (e.g. 1248, 1254 and 1260) was comprised of several different PCBs with differing levels of chlorination (1–10 chlorines). For congeners with $\log K_{ow} > 6$ or 6.5, slow contaminant uptake/elimination kinetics prevent algae from achieving equilibrium with water during high growth, while for congeners with $\log K_{ow} < 5.5$, high contaminant uptake/elimination kinetics allows algae to remain close to equilibrium with the water (Koelmans et al., 1995; Swackhamer and Skoglund, 1993). While the number of chlorines on a PCB molecule has a significant effect on the $\log K_{ow}$, and ultimately on the bioaccumulation factor (BAF), the position of these chlorines also has

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importance. PCBs which do not have chlorines in the 2,2′,6 or 6′ positions are classified as coplanar, while one or more chlorines in the above positions indicates *ortho*-substitution. Biomagnification factors (BMFs) for individual *ortho*-substituted PCBs are, on average, 25% higher than the BMFs of their coplanar PCB homologs (Willman et al., 1999), this difference ultimately means higher trophic levels could be more at risk for *ortho*-substituted PCB exposure. We chose to use a single congener to be better able to observe responses and congener #54 (2,2′,6,6′-tetrachlorobiphenyl) has a high *ortho*-substitution and a moderate log K_{ow} (5.21–6.05) (Jabusch and Swackhamer, 2005; Sabljiæ et al., 1993; Hawker and Connell, 1988).

For over 30 years the processes of bioaccumulation and biomagnification have been observed with persistent organic pollutants (POPs), such as PCBs. PCB entry into the aquatic food web is facilitated by bioaccumulation at the primary trophic level (Gerofke et al., 2005; Swackhamer and Skoglund, 1991). Phytoplankton represent a point of entry for such chemicals into the aquatic food web, where concentrations tend to magnify up the food chain, reaching levels of 25 million folds in top predators (Rasmussen et al., 1990; Oliver and Niimi, 1988; Norstrom et al., 1978). Phytoplankton play a critical role in controlling the fate of POPs in the water column because they are high in lipids and they serve as the base of both the pelagic and benthic food chains. Both modeling (Thomann and Connolly, 1984) and food web studies (Berglund et al., 1997) have shown that up to 99% of the PCBs in a top predator (such as lake trout) are accumulated by consumption rather than direct uptake from the water. PCBs are generally deposited in the lipid stores of organisms and Berglund et al. (2001) found that lipid content explained most of the variation of PCB concentration in phytoplankton in 19 southern Swedish lakes. They concluded that lipid content of phytoplankton increased with increased nutrient stress or lake trophy. It has been well established that nutrient limitation changes the morphological, biochemical and elemental characteristics of phytoplankton (Lynn et al., 2000; Weers and Gulati, 1997), however few studies (Datta, 2001; Halling-Sørensen et al., 2000; Kilham, 1998) have examined nutrient limitation induced changes in biochemistry of phytoplankton in regards to POP uptake. And even fewer studies (Kilham, 1998) have examined whether nutrient limitation induced changes in phytoplankton POP uptake can be transferred up the trophic ladder to zooplankton or beyond. Kilham (1998) performed an experiment using a *Nitzschia* sp. diatom, three nutrient regimes (low-Si, low-P and non-limited), polychlorinated dibenzofurans (PCDFs) and the zooplankton *Daphnia magna*. The results of Kilham (1998) showed a significantly higher PCDF transfer to zooplankton in response to increased algal lipids (low-Si > non-limited > low-P).

This study follows the Kilham (1998) study, but includes an N limited treatment and, with the data from Lynn et al. (2000), provides a more detailed examination of the algal biochemistry and elemental stoichiometry. In this study, we hypothesized that nutrient induced increases in lipid content of algae would lead to higher PCB uptake. Also, we further hypothesized that zooplankton grazing on algae with higher lipids and, subsequently, PCB would show higher PCB body burdens. Thus, the goals of this study were to examine the effects of nutrient limitation (Si, N and P) on the PCB (congener #54) uptake of an alga (*Stephanodiscus minutulus*, a freshwater diatom) and the subsequent effects on trophic transfer to a zooplankton (*Daphnia pulicaria*, a freshwater cladocera) via grazing.

2. Methods

2.1. Algae and zooplankton

S. minutulus clone was isolated by E.C. Theriot from Yellowstone Lake in Wyoming, USA and culture conditions are outlined in Lynn et al. (2000). Cultures of 600 mL were grown in 1 L flasks with normal or non-limiting (F) COMBO medium (Kilham et al., 1998) and in low-Si (S), low-N (N), or low-P (P) COMBO medium. Normal COMBO medium contained 100 μM Si, 1000 μM N, and 50 μM P, whereas low-Si medium contained 37.5 μM Si, low-N medium contained 50 μM N and 150 μM Si, and low-P medium contained 10 μM P. Nutrient levels in limited cultures were determined based on previous experience with elemental stoichiometry and necessary algae yields. Non-limited cultures were grown at μ_{max} (μ_{max} = 0.84 day^{-1} (Kilham et al., 1998)), the maximal growth rate, using semi-continuous dilutions (~300 mL day^{-1}) once each day (Kilham, 1978). Nutrient-limited cultures were maintained at 22% of μ_{max} using semi-continuous dilutions (100 mL day^{-1}) once each day, giving a 0.17 day^{-1} exchange rate. Aerated cultures were kept in an environmental chamber at 16 ± 1 °C with a 14:10 h L:D (light:dark) cycle and with 80 μmol m^{-2} s^{-1} of light. To ensure that the biochemical and elemental composition at a particular nutrient-limited growth rate was measured at a steady-state condition, culture fluoresence was monitored for 11 days before the beginning of the experiment.

The cladoceran *D. pulicaria* (clone isolated by C.E. Goulden from Lake Waynewood, PA) were maintained in 1-L flasks in full COMBO medium which was changed once each week. Zooplankton were maintained in a culture chamber at 20 ± 1 °C with a 14:10 h L:D cycle. These animals were fed a ration of >1.6 × 10^{7} μm^{3} mL^{-1} of non-limited *S. minutulus* three times each week. Gravid adult female *D. pulicaria* were separated evenly into 1 L beakers with 600 mL of COMBO medium. Adult *D. pulicaria* were removed from the beakers after 1 day and the beakers were stirred daily for four more days and fed as above. Juvenile *D. pulicaria* produced by these females (≤5 days old) were used in the zooplankton grazing experiment described below.

2.2. Experimental design

A 2 mL stock solution of PCB congener #54 (2,2′,6,6′-tetrachlorobiphenyl) [CAS No. 15968-05-5] at 100 μg mL^{-1} was purchased from AccuStandard Inc. (New Haven, CT). The stock solution was diluted in 1 L of acetone resulting in a 0.2 μg mL^{-1} working solution. Each algal culture flask (600 mL) was inoculated with 600 μL of working solution (0.2 μg mL^{-1}) resulting in a nominal PCB culture concentration of 0.2 μg L^{-1} or 120 ng PCB per flask. Contaminated zooplank-

ton medium was derived by adding 200 μL of working solution to 2 L of appropriate medium resulting in a PCB concentration of 0.02 $\mu g\,L^{-1}$.

Each algal treatment (F, S, N and P) had three replicate cultures which were dosed with a 2,2′,6,6′-tetrachlorobiphenyl working solution as described above. Algal cultures were dosed with PCBs just after the daily media change, maintaining the growth rates described above during the 24 h exposure period. After 24 h, duplicate 200 mL samples were taken from each culture for PCB analysis and the remaining algae for each treatment were pooled and used in the zooplankton feeding experiment. Algal PCB samples were centrifuged for 20 min at 5000 rcf, and the medium was decanted. The algae were then resuspended with 30 mL of hexane and stored in 40 mL pre-cleaned bottles with Teflon-lined lids at 4 °C until analysis for PCBs. Clean cultures (Lynn et al., 2000), exposed to the exact same experimental conditions, except addition of PCB, were used as clean food for zooplankton grazing experiments. Each algal nutrient treatment (F, S, N and P) had two zooplankton grazing treatments with three replicates per treatment. The first zooplankton treatment (#1) received PCB-dosed algae with nutrient appropriate PCB-contaminated media. The second zooplankton treatment (#2) received clean algae with nutrient appropriate PCB-contaminated media. Each zooplankton replicate contained 10 animals per beaker (≤5 days old) with 600 mL of appropriate media and algal food (Kilham et al., 1997a,b). Cell number (# mL^{-1}) and biovolume ($\mu m^3\,mL^{-1}$) of algal cultures was determined using a Coulter Counter (model ZB1) with Channelyser (model 256). The algal food ration for all treatments was $1.6 \times 10^7\,\mu m^3\,mL^{-1}$ or $9.6 \times 10^9\,\mu m^3\,beaker^{-1}$, which was not quantity limited for *D. pulicaria* fed the green alga *Ankistrodesmus falcatus* cultured under different growth rates and nutrient limitations (Kilham et al., 1997a,b). To prevent residual PCB contamination, algal food rations were centrifuged as described above and resuspended in appropriate zooplankton media before being added to the zooplankton experimental beakers. Media for zooplankton experiments were an order of magnitude less than the media used for algal cultures in regard to the limiting nutrient: Si 3.75 μM; N 5 μM; P 1 μM. Non-limiting media were the same for algal cultures and zooplankton experiments. The zooplankton grazing experiments lasted 48 h and were performed at 22 °C with low levels of light on a 16:8 h L:D. At the end of the zooplankton grazing experiment, *D. pulicaria* were removed from the beakers, pooled by replicate, blotted dry, weighed, placed in 30 mL of acetone:hexane (1:1, v:v) and stored at 4 °C in 40 mL pre-cleaned bottles with Teflon-lined lids until analysis for PCB.

2.3. PCB extraction and analysis

PCB were extracted and analyzed using previously described U.S. EPA recommended procedures (EPA, 1997; Erickson, 1997; Watts, 1980). All solvents used in PCB analysis were pesticide grade and were screened for organic contaminants prior to use. A 2 mL stock solution of PCB congener #65 (2,3,5,6-tetrachlorobiphenyl) [CAS No. 33284-54-7] at 100 $\mu g\,mL^{-1}$ was purchased from AccuStandard Inc. (New Haven, CT). Immediately prior to extraction, each algal and zooplankton sample received 0.5 mL of a 0.10 $\mu g\,mL^{-1}$ working solution of PCB congener #65 in acetone. Algal samples were then evaporated to ≤5 mL with ultrapure N_2 gas. Lipid clean-up was performed by eluting the sample through a micro-column of 2.0 g activated 100–200 mesh Florisil® (100 °C/24 h) with 10.0 mL hexane and evaporating back to the final volume (EPA, 1997; Erickson, 1997). Elemental sulfur was then removed by shaking 2-propanol (2 mL) and tetrabutylammonium sulfite (2 mL), adding ultra-pure water (8 mL) and reshaking. The organic extract was removed and mixed with 2.0 mL concentrated sulfuric acid (EPA, 1997; Jensen et al., 1977) and a 4 μL sub-sample was then analyzed by gas chromatography. Zooplankton samples were macerated in a ground-glass homogenizer with hexane. The samples were then transferred into 125 mL flasks with three rinses of hexane. The extracts were concentrated to near dryness in a Roto-evaporator (Buchi Model RE121). Reconstituted samples (1.0–2.0 mL in *iso*-octane) were then cleaned of interferences as described above and analyzed by gas chromatography.

Samples were analyzed for congeners 2,2′,6,6′-tetrachlorobiphenyl and 2,3,5,6-tetrachlorobiphenyl according to U.S. EPA SW-846 Method 8082 (EPA, 1997). Analysis was performed using a Hewlett-Packard (HP) Model 5890A gas chromatograph equipped with an electron capture detector and an HP Model 7673A Automatic Sampler. Samples were analyzed using a 60 m × 0.53 mm i.d. SPB-5 (0.5 μm film) fused silica megabore column (Supelco® Inc.) with ultra-high purity helium and nitrogen as carrier and makeup gases, respectively. The temperature program was set at 160 °C (6 min); 10 °C/min-235 °C (0 min); 0.9 °C/min-260 °C (10 min); Injector temperature, 280 °C; Detector temperature, 300 °C. PCB peak heights were quantified using an HP Model 3396A integrator. Calculations of congener concentrations were performed by multiple-peak linear regression analysis using Lotus-123® software. Five external standards for each congener were used for calibration curves. For every 10th sample, either a solvent blank or a standard was analyzed to determine instrument stability and confirmation of calibration curve, respectively.

2.4. Statistics

Sample PCB (2,2′,6,6′-tetrachlorobiphenyl) concentrations were corrected based on recoveries of congener #65 (2,3,5,6-tetrachlorobiphenyl), which were all within the acceptable range (75–125%) (EPA, 1997). PCB results for duplicate samples from a single algal culture flask were averaged before being used in any calculation or statistical test. A least square difference model with post hoc Bonferroni pair-wise correlation tests were performed with values from triplicate cultures for each treatment using SYSTAT Grad Pack v. 10.0 for Windows. Bioaccumulation, or bioconcentration, factors (BAFs) (Table 1) were based on either lipid (BAF_{lip} = [(ng PCB/mL algal culture)/(g lipid/mL of algal culture)]/[ng PCB/mL of media]) or dry weight (BAF_{dw} = [(ng PCB/mL algal culture)/(g dry weight/mL of algal culture)]/[ng PCB/mL of media]), using PCB data from this

Table 1
Bioaccumulation factors (BAF) and log BAF based on lipid (lip) and dry weight (dw) and biomagnification factors (BMF) for four algal treatments (F: non-limited, S: silica limited, N: nitrogen limited and P: phosphorus limited)

	Units	F	S	N	P
BAF_{lip}	$(ng\,g^{-1}/ng\,mL^{-1})\,10^4$	11.8	23.7	9.47	10.7
BAF_{dw}	$(ng\,g^{-1}/ng\,mL^{-1})\,10^4$	3.94	11.6	2.79	4.51
log BAF_{lip}	$ng\,g^{-1}/ng\,mL^{-1}$	5.07	5.37	4.98	5.03
log BAF_{dw}	$ng\,g^{-1}/ng\,mL^{-1}$	4.60	5.06	4.45	4.65
BMF	$ng\,flask^{-1}/ng\,flask^{-1}$	0.31	0.28	0.79	0.60

study and lipid or dry weight data taken from Lynn et al. (2000). PCB in media (ng PCB/mL of media) was calculated by subtracting the PCBs in the algae culture (ng PCB/mL of algal culture) from the nominal whole flask PCB mass (120 ng/flask). Biomagnification factors (BMFs) were calculated by dividing PCBs in zooplankton (ng/flask) by PCBs in the algae (ng/flask). Coefficients of variation (CV) were calculated by dividing the standard deviation (or square root of the variance) by the mean.

3. Results

Culture fluorescence steady-state observations and chlorophyll *a*, biochemical and elemental measurements for these algal cultures have been published in Lynn et al. (2000). There were minor differences in the pattern of PCB uptake between treatments when presented as: (1) percentage uptake of available PCB (Fig. 1A); (2) PCB in algal culture (Fig. 1B); (3) PCB per cell (Fig. 1C); (4) PCB per biovolume (Fig. 1D). However, all treatments were significantly different from each other at $p < 0.01$. The low-S and the low-P treatments had the highest percentage uptake of available PCB with 67.6% and 62.2%, respectively, and non-limited and low-N treatments were lower with 55.3% and 51.5%. The results are very similar when presented as PCB per mL of algal culture. The low-S and low-P treatments had the highest PCB uptake per mL with 13.5 and 12.5 $\mu g\,mL^{-1}\,10^5$, respectively, and low-N and non-limited treatments were lower with 10.3 and 11.1 $\mu g\,mL^{-1}\,10^5$. The differences between treatments become more pronounced and the low-N and non-limited treatments switch places when the results are expressed as PCB per cell (#) or biovolume (μm^3). The low-S treatment had significantly more PCB per cell (8.68 $\mu g\,cell^{-1}\,10^{-10}$) than the low-P (5.88 $\mu g\,cell^{-1}\,10^{-10}$), the low-N (3.78 $\mu g\,cell^{-1}\,10^{-10}$) and the non-limited (3.38 $\mu g\,cell^{-1}\,10^{-10}$) treatments. And the low-S treatment had significantly more PCB per biovolume (19.23 $\mu g\,\mu m^{-3}\,10^{-13}$) than the low-P (15.78 $\mu g\,\mu m^{-3}\,10^{-13}$), the low-N (13.69 $\mu g\,\mu m^{-3}\,10^{-13}$) and the non-limited (8.63 $\mu g\,\mu m^{-3}\,10^{-13}$) treatments.

There were significant differences ($p < 0.05$) between PCB per wet weight of zooplankton in PCB contaminated media grazing on PCB contaminated algal food, grown under differing nutrient-limited conditions during a 48 h experiment (Fig. 2). *D. pulicaria* fed low-N and low-P algae showed the greatest body burdens of PCB per wet weight with 12.1 and 10.1 $ng\,mg^{-1}$, respectively. *D. pulicaria* fed low-S and non-limited algae had lower body burdens of PCB per wet weight with 4.8 and

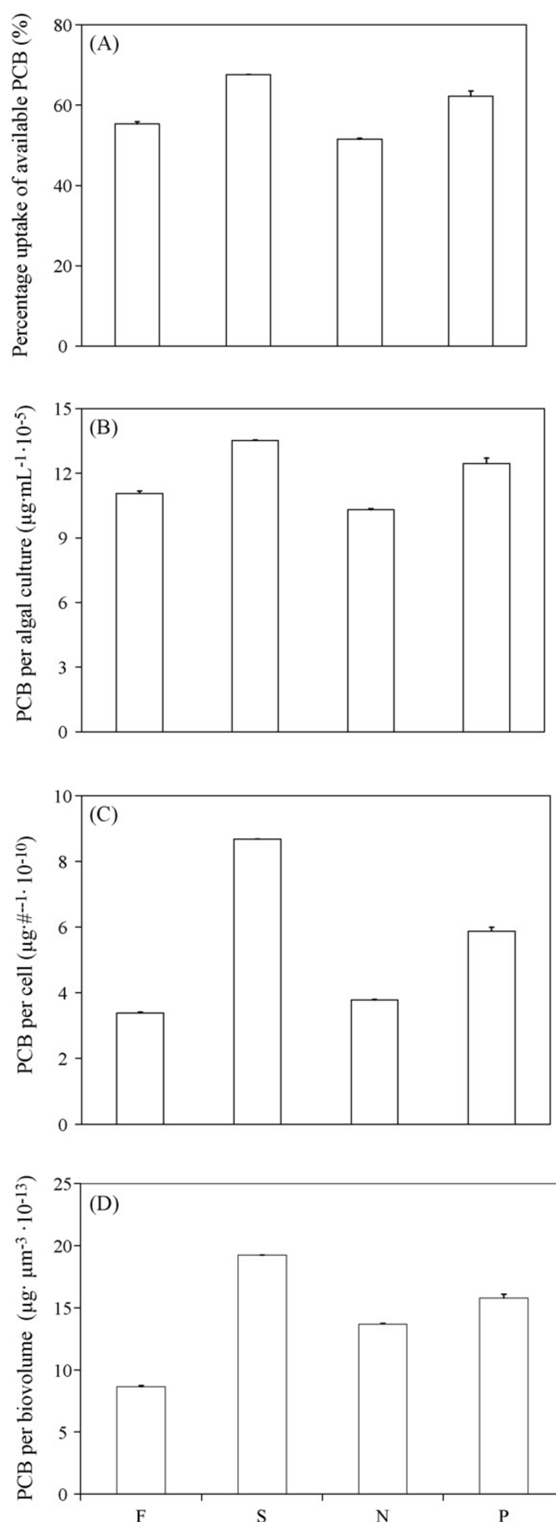

Fig. 1. Mean uptake for 24 h of PCBs (A) as a percentage; (B) per mL; (C) per cell; (D) per biovolume in *S. minutulus* algal cultures grown under differing nutrient treatments. (F: non-limited, S: silica limited, N: nitrogen limited and P: phosphorus limited). Bars indicate standard errors and $n = 3$ for each treatment. Within each panel (A–D), all treatments are significantly different from each other at $p < 0.01$.

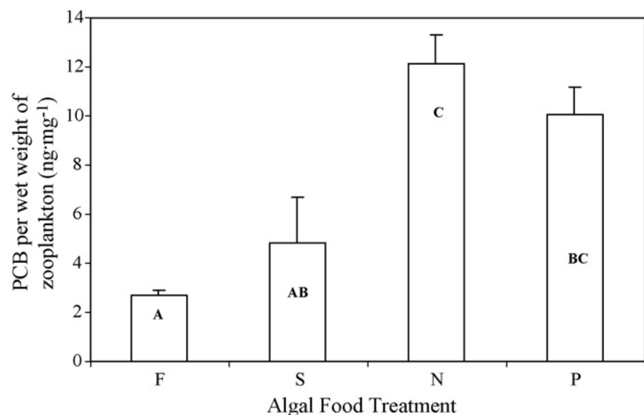

Fig. 2. Mean PCB per *D. pulicaria* wet weight after 48 h of grazing on equal biovolumes of *S. minutulus* grown under differing nutrient treatments and dosed with PCBs for 24 h (F: non-limited, S: silica limited, N: nitrogen limited and P: phosphorus limited). Bars indicate standard errors and $n = 3$ for each treatment with letters indicating significant differences at $p = 0.05$.

2.7 ng mg^{-1}, respectively. There were no significant differences from a 48 h grazing experiment ($p = 0.676$) in PCB body burden between zooplankton fed algae grown with differing nutrient levels where PCBs were added to the media only. Table 1 includes the calculated bioaccumulation factors (BAF) based on lipids (BAF_{lip} and log BAF_{lip}) and dry weight (BAF_{dw} and log BAF_{dw}) for the four algal treatments and the biomagnification factors (BMF) for algae to zooplankton transfer. The low-S BAFs (both BAF_{lip} and BAF_{dw}) were considerably higher than any of the other treatments and the low-N BAFs were lower than any of the other treatments. However, the BMFs show an inverse pattern to the BAFs with the low-N treatment having the highest BMF and the low-S treatment having the lowest BMF.

Although the algal cultures used in this experiment are the same ones used in Lynn et al. (2000) the biochemical/elemental data presented there and PCB data obtained in this study are inconsistent and direct correlations could not be made between PCB uptake and biochemical/elemental parameters. However, presuming that a specific biochemical/elemental parameter contributed heavily to PCB uptake (e.g. higher lipids leads to higher PCB), one would expect that expressing PCB per that biochemical/elemental parameter would result in a normalization between treatments. The stronger the relationship between PCB uptake and a biochemical/elemental parameter, the less variation one should see between nutrient treatments in PCB uptake expressed per that biochemical/elemental parameter and, therefore, the lower the coefficient of variation (CV) for that biochemical/elemental parameter across the four nutrient treatments. The average PCB per algal culture (μg PCB mL^{-1}) values, as shown in Fig. 1B, for each nutrient treatment were divided by several different biochemical or elemental parameters per algal culture (unit mL^{-1}) obtained from Lynn et al. (2000) producing treatment specific PCB/biochemical/elemental parameter (μg PCB unit^{-1}) values. The across treatment variance, mean and CV were then calculated for each of these values. Phospholipids and galactolipids + phospholipids had the lowest CV values with 0.25 followed by total lipids (0.29), triglyceride + phospholipids (0.30) and triglyceride + phospholipids + galactolipids (0.30). Other noteworthy CV values include particulate P (0.33), galactolipids (0.37), ash-free dry mass (0.44), protein (0.44), particulate N (0.44), particulate C (0.46), triglycerides (0.58), carbohydrate (0.60), chlorophyll *a* (0.61) and particulate Si (0.73).

4. Discussion

The results of this study support the hypothesis that a nutrient limited (i.e. low-Si) algae with a higher lipid content than a non-limited algae will, in turn, show a significantly increased PCB uptake. Further, the significant differences between all four nutrient treatments in regards to PCB uptake indicates that PCB uptake is very sensitive to biochemical and elemental changes in algae due to nutrient limitation. It is somewhat surprising that the results regarding algae to zooplankton transfer did not support the hypothesis of increased PCB transfer to zooplankton grazing on lipid-rich Si limited algae. However, it is impossible for any single study to accurately describe all possible situations of trophic POP transfer; limiting nutrients, algal food species, consumer species, consumer grazing rates and POP type all should play a role in determining the details of this interaction. Only after studying a number of different scenarios might it be possible to gain more understanding of the complex relationships involved with bioaccumulation and biomagnification.

4.1. Algal bioaccumulation of PCBs

It seems well founded that the amount of POP accumulation in algae is dependent on lipid quantity (Swackhamer, 1985) and several studies have shown that algal lipid levels vary in response to growth and nutrient regimes (Halling-Sørensen et al., 2000; Lynn et al., 2000; Kilham et al., 1997a,b). Datta (2001) grew the diatom *Cyclotella meneghiniana* in similar nutrient-limited conditions as those used here and observed very similar biochemical profiles as those in Lynn et al. (2000). Datta used a different PCB congener (2,2′,4,4′,5,5′-hexachlorobiphenyl) at a different concentration (7.5 μg L^{-1}) and ultimately showed a pattern of uptake (S > P > N > F) remarkably similar to these results (Fig. 1C). Kilham (1998) performed an experiment using a *Nitzschia* sp. diatom, three nutrient regimes (low-Si, low-P and non-limited) and tritiated polychlorinated dibenzofurans (PCDFs) and found a slightly different pattern of POP uptake in response to diatom nutrient treatment (S > F > P). Halling-Sørensen et al. (2000) found that BCFs for several POPs increased up to nine times as the total lipid content of the green algae, *Selenastrum carpricornutum*, increased as a consequence of nitrogen starvation. Regardless of the mechanism of lipid increase (low-Si in diatoms, low-N in green algae), the pattern of increased POP uptake in conjunction with increased lipids remains the same.

There is a body of literature which reports that lipids are a driving force to equilibrium at slow or low growth conditions, but under active growth conditions kinetic constraints prevent compounds from reaching equilibrium in the phyto-

plankton pool (Carlson and Swackhamer, 2006; Koelmans et al., 1995; Swackhamer and Skoglund, 1993). Swackhamer and Skoglund (1993) demonstrated that growth rates can exceed PCB uptake/elimination kinetics in algal cells resulting in growth dilution and depression of PCB concentrations under maximum growth conditions. However, their experiments were performed over 20+ days with static cultures and the growth rate was manipulated by temperature, not by nutrient limitation. While the non-limited algae (F) in this study was growing at maximum growth rate, unlike any of the other algal nutrient treatments, and the PCB per cell was significantly lower than any of the other treatments (Fig. 1C) the average cell densities of the four treatments after 24 h of PCB exposure were comparable ($F = 3.27 \times 10^5$, $S = 1.56 \times 10^5$, $N = 2.73 \times 10^5$, $P = 2.12 \times 10^5$). This indicates that growth dilution in this study is most probably not a confounding factor, however the differences in growth rates between the non-limited and limited treatments could possibly have consequences independent of altering the equilibrium kinetics.

The difference between the highest (S) and the lowest (N) BAF_{dw} in our experiments was only approximately three-fold. The BAF value (1.17×10^5) calculated by Lederman and Rhee (1982) for PCB uptake in the diatom *Fragilaria crotonensis* was higher than all the algal treatments in this study with the exception of the silica limited treatment (Table 1). Comparing our $BAFs_{dw}$ to those measured by Jabusch and Swackhamer (2004) in the green algae *Chlamydomonas reinhardtii*, our $BAFs_{dw}$ fall well below theirs. However, both those experiments used PCB congener #153 (2,2′,4,4′,5,5′-hexachlorobiphenyl), which has a significantly higher log K_{ow} (∼7.33) than any of the values associated with congener #54. Hawker and Connell (1988) list an empirical value for PCB #54 log K_{ow} as 5.48, while later giving a calculated value of 5.21. Sabljiæ et al. (1993) gave a measured log K_{ow} for 2,2′,6,6′-tetrachlorobiphenyl as 5.94, while more recently Jabusch and Swackhamer (2005) gave a value of 6.05. Thus it seems that the true log K_{ow} for this particular congener could range from 5.21 to 6.05.

Comparing our algal BAFs to those calculated by Berglund et al. (2000), based on 10–45 μm phytoplankton from 19 southern Swedish lakes, our BAFs fall dramatically below the field BAF correlation lines. Although, field measurements of PCB concentrations in phytoplankton from two Wisconsin lakes suggests that BAFs are lower when the community is dominated by diatoms than by nondiatoms (Swackhamer, 1985), which might explain our relatively low BAFs. Also there is a strong inverse relationship between the log BAF_{dw} and the number of *ortho*-chlorines (congener #54 has 4 *ortho*-chlorines (2,2′,6,6′); the maximum amount possible) (Swackhamer, 1985). Swackhamer and Skoglund (1994) indicate that the log BAF_{dw} for fourth degree *ortho*-substituted PCB after 1 day would be approximately 4.5, making only our silica limited BAF_{dw} high, perhaps in part due to the significantly increased lipid content. Swackhamer and Skoglund (1993) measured log BAF_{lip} for 40 representative PCB congeners in axenic cultures of *Scenedesmus* sp. After 24 h of exposure, they found log $BAFs_{lip}$ for low K_{ow} 5.2–6.0 to be between 5.0 and 5.5, corresponding with our calculations (Table 1). Taking all this into consideration, our BAFs seem to be consistent with other studies.

Conventional theory holds that PCBs and other lipid soluble POPs readily diffuse across the cell membrane and are sequestered into the subcellular lipid pools. Jabusch and Swackhamer (2005) measured the log K_{ow} of congener #54 as 6.05, they also measured the log K_{mw} (membrane/water partition constant) at 7.05. The method for measuring K_{mw} uses PCB-containing membrane vesicles from phospholipids, theoretically making K_{mw} a better predictor for PCB affinity of polar lipids and K_{ow} a better predictor for PCB affinity of neutral lipids. They found that within a homologue of chlorination (e.g. tetrachlorinated congeners 52, 54, 61, 77), the K_{mw}'s are very similar but the K_{ow}'s vary by more than an order of magnitude (Jabusch and Swackhamer, 2005). This suggests that *ortho*-substitution is important in PCB affinity of neutral lipids, but not so important in the affinity of polar lipids. One of the lowest coefficients of variation (CV) was with total lipids (0.29) and, in fact, PCBs per cell (Fig. 1C) show a striking resemblance to total lipids per cell in Lynn et al. (2000). However, triglycerides, the largest neutral lipid pool, had a relatively high CV (0.58) while phospholipids, charged lipids making up cell membranes, had the lowest CV (0.25). Galactolipids, lipids associated with the photosynthetic process, had a moderate CV of 0.37 and the remaining lipid classes quantified by Lynn et al. (2000) were too low to be considered significant. Recently Jabusch and Swackhamer (2004) investigated the contribution of surface absorption to the cell wall by the green alga *C. reinhardtii* in PCB uptake. Their research indicates that PCB uptake by algal cells is a kinetic mechanism with decreased uptake occurring as cell PCB levels increase and that surface absorption of PCBs to the cell wall accounted for less than 10% of the total bioaccumulation. Lederman and Rhee (1982), in an indirect evaluation of the physical adsorption to the diatom cell surface, found an order of magnitude less uptake in frustules than live or dead cells indicating that, at least in diatoms, cell surface adsorption is not a primary uptake mechanism. These results indicate that phospholipids, perhaps associated with internal membranes or organelles as opposed to cell walls, are an important factor in PCB uptake.

4.2. Transfer of PCBs from algae to zooplankton

There are quite a number of studies on transfer of PCBs from algae to blue mussels (*Mytilus edulis*), however there are few studies that have examined trophic transfer using pelagic zooplankton and diatoms. Kilham (1998) performed an experiment using a *Nitzschia* sp. diatom, three nutrient regimes (low-Si, low-P and non-limited), tritiated polychlorinated dibenzofurans (PCDFs) and the zooplankton *D. magna*. The results of Kilham (1998) showed a significantly higher PCDF transfer to zooplankton in response to increased algal lipids (low-Si > non-limited > low-P) which is a pattern that is in apparent contradiction with the results of this study. While Kilham (1998) used a different compound and a different diatom, it is still curious that these two very similar studies should yield different results. One other difference was that Kilham (1998) fed

algal rations based on cell number, while we used biovolume to determine algal rations. The food ration ($\mu m^3\ ml^{-1}$) chosen for this experiment was not quantity limited for *D. pulicaria* fed the green alga *A. falcatus* cultured under different growth rates and nutrient limitations (Kilham et al., 1997a,b). Therefore, the main difference between zooplankton treatments is the quality of the algae and it remains to be seen how differences in food quality can preferentially transfer different levels of PCB to zooplankton. We would expect a high lipid algae, with higher PCB accumulation, would produce higher PCB levels in grazing zooplankton, assuming constant grazing and assimilation rates by the *D. pulicaria*.

However, there is evidence that both grazing and assimilation in zooplankton varies in response to changes in food quality. Groeger et al. (1991) found that in short term feeding experiments, *Daphnia* accumulated more lipid when fed N-deficient algal cells than when fed green algae grown on N-sufficient media. The alga used was *Scenedesmus obliquus*, which increases lipid in response to N-limitation, however Sterner et al. (1992) found that *Daphnia* reared on low-P *Scenedesmus acutus* also accumulated large quantities of lipids. Studies have shown that green algae under N-limitation, such as *S. obliquus*, will alter cell wall morphology to become less digestible to predatory zooplankton. This raises the question of whether the high lipid *S. obliquus* was indeed a lipid rich food source or, if because of decreased digestibility, it was a low lipid food source. Many studies indicate that nutrient limitation, particularly N, in green algae (most notably *S. acutus*) decreases filtering or clearance rates in zooplankton (Lürling and van Donk, 1997; Sterner and Smith, 1993; van Donk and Hessen, 1993). However, Lürling and van Donk (1997) found there were no differences between clearance rates of *Daphnia pulex* on non-limited and N-limited diatoms (*Synedra tenuissima*) indicating a possible difference between algal types. There is a prevalent theory that nutrient-limited green algae undergo morphological changes in their cell walls and this leads to a reduced grazing pressure (van Donk et al., 1997). However, the siliceous cell walls of diatoms appear to be resistant to morphological changes in cell walls which affect digestibility.

Wagner and Kamjunke (2001) found evidence that extracellular products released by *Asterionella formosa* during photosynthesis triggered the reduction of the filtration rate in *Daphnia galeata*. They found a significant correlation between primary production measured *in situ* and the reduction of filtration rate in the filtrate of reservoir water. This indicated that in diatoms, an increase in photosynthesis and primary production can release dissolved chemicals into the water that inhibit or reduce the filtering rate in *D. galeata*. One might suggest the avoidance of grazing losses by algae should be most important in conditions of limiting nutrients or low primary production rates, but perhaps the algae are unable to produce these appetite quelling compounds while under extreme nutrient stress. This theory is supported by Liu and Dagg (2003), who found that mesozooplankton grazing rates were substantially higher in a nutrient limited area near the Mississippi River discharge point than in more nutrient rich areas.

Our low-N algae were chlorophyll *a* depleted (Lynn et al., 2000) and most likely had a reduced photosynthetic rate compared to the other algal treatments (Pedersen, 1995). Following the above theory, this could result in a higher filtration rate of *D. pulicaria* grazing on low-N food and consequentially a higher PCB uptake. In support of this, our data indicate a distinct negative trend between algal chl *a* content (Lynn et al., 2000) ($N < P < S < F$) and PCB uptake by *D. pulicaria*. The low-Si treatment of Kilham (1998) had the highest lipids and the highest PCDF accumulation, but this treatment was described as having almost “no color”. While Kilham (1998) reported little or no difference in calculated *Daphnia* filtering rates, the color, and presumably the chlorophyll content, of the respective algal treatments, was correlated with zooplankton PCDF uptake. This raises the possibility that the chlorophyll content of the zooplankton food may not only affect the filtration rate, but could also affect the rate of lipid assimilation. Cauchie et al. (1999) measured lipid content and biomass of *D. magna* and chlorophyll *a* in ponds over a 10-month period and found that *Daphnia* lipid levels, as a function of weight (mg g dry mass^{-1}), increased with decreased chlorophyll *a* levels. This inverse correlation raises the possibility that when the available food exhibited low chlorophyll *a* levels, the *Daphnia* increased their deposition of lipids. This lends support to the theory that zooplankton lipid assimilation can be different with differing algal food qualities, thus leading to preferential uptake of lipid associated PCB.

This study indicates that the trophic transfer of a PCB can be significantly modulated by the nutrient status of the phytoplankton and the subsequent grazing and assimilation rates of zooplankton. These factors imply that some ecosystems may be at higher risk for biomagnification as different nutrients can be limiting not only in different lakes or surface waters, but also on a seasonal level (Kreeger et al., 1997). Diatoms commonly play a key role in the spring algal blooms which occur in temperate lakes during turnover and this mixing process is very likely to release sediment bound PCBs into the water column increasing exposure to phytoplankton. While this study illuminates some new possibilities in algal PCB uptake and transfer to zooplankton, there is an indication that this process is significantly dependent on algal species and compound type. Therefore there is a need to perform more experiments examining growth and nutrient-limiting effects on bioaccumulation in algae and transfer to consumers.

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References

Berglund, O., Larsson, P., Brönmark, C., Greenberg, L., Eklöv, A., Okla, L., 1997. Factors influencing organochlorine uptake in age-0 brown trout (*Salmo trutta*) in lotic environments. Can. J. Fish. Aquat. Sci. 54, 2767–2774.

Berglund, O., Larsson, P., Ewald, G., Okla, L., 2000. Bioaccumulation and differential partitioning of polychlorinated biphenyls in freshwater, planktonic food webs. Can. J. Fish. Aquat. Sci. 57, 1160–1168.

Berglund, O., Larsson, P., Ewald, G., Okla, L., 2001. The effect of lake trophy on lipid content and PCB concentrations in planktonic food webs. Ecology 82, 1078–1088.

Cairns, T., Doose, G.M., Froberg, J.E., Jacobson, R.A., Siegmund, E.G., 1986. Analytical chemistry of PCBs. In: Waid, J.S. (Ed.), PCBs and the Environment, vols. 1–3. CRC Press Inc., Boca Raton, FL, USA, pp. 1–46.

Carlson, D.L., Swackhamer, D.L., 2006. Results from the US Great Lakes fish monitoring program and effects of lake processes on bioaccumulative contaminant concentrations. J. Great Lakes Res. 32, 370–385.

Cauchie, H.-M., Jaspar-Versali, M.-F., Hoffmann, L., Thomé, J.-P., 1999. Analysis of the seasonal variation in biochemical composition of *Daphnia magna* Straus (Crustacea:Branchiopoda:Anomopoda) from an aerated wastewater stabilisation pond. Ann. Limnol.-Int. J. Limnol. 35, 223–231.

Datta, S., 2001. Relationship Between PCB Uptake and Nutrient Limitation in Three Algal Species. Ph.D. Thesis. Drexel University, Philadelphia, PA, USA, pp. 114.

EPA, U.S., 1997. Test methods for evaluating solid wastes. US Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC.

Erickson, M.D., 1997. Analytical Chemistry of PCBs, 2nd ed. CRC Press Inc., Boca Raton, FL, USA, p. 667.

Erickson, M.D., 2001. In: Robertson, L.W., Hansen, L.G. (Eds.), Introduction: PCB Properties, Uses, Occurrence, and Regulatory History. PCBs: Recent Advances in Environmental Toxicology and Health Effects. University of Kentucky Press, Lexington, KY, xi–xxx.

Fiedler, H., 2001. In: Needham, L., Fiedler, H. (Eds.), Global and Local Disposition of PCBs. Origin of PCBs and Characterization of Exposures, Section I. University of Kentucky Press, Lexington, KY, USA, pp. 11–15.

Gerofke, A., Komp, P., McLachlan, M., 2005. Bioconcentration of persistent organic pollutants in four species of marine phytoplankton. Environ. Toxicol. Chem. 24, 2908–2917.

Groeger, A.W., Schram, M.D., Marzole, G.R., 1991. Influence of food quality on growth and reproduction in *Daphnia*. Freshwater Biol. 26, 11–19.

Halling-Sørensen, B., Nyholm, N., Kusk, K.O., Jacobsson, E., 2000. Influence of nitrogen status on the bioconcentration of hydrophobic organic compounds to *Selenastrum capricornutum*. Ecotox. Environ. Saf. 45, 33–42.

Hawker, D.W., Connell, D.W., 1988. Octanol–water partition coefficients of polychlorinated biphenyl congeners. Environ. Sci. Technol. 22, 382–387.

Holoubek, I., 2001. In: Needham, L., Fiedler, H. (Eds.), Polychlorinated Biphenyl (PCB) Contaminated Sites Worldwide. Origin of PCBs and Characterization of Exposures, Section I. University of Kentucky Press, Lexington, KY, USA, pp. 17–26.

Jabusch, T.W., Swackhamer, D.L., 2004. Subcellular accumulation of polychlorinated biphenyls in the green alga *Chlamydomonas reinhardtii*. Environ. Toxicol. Chem. 23, 2823–2830.

Jabusch, T.W., Swackhamer, D.L., 2005. Partitioning of polychlorinated biphenyls in octanol/water, triolein/water, and membrane/water systems. Chemosphere 60, 1270–1278.

Jensen, S., Renberg, L., Reutergardh, L., 1977. Residue analysis of sediment and sewage sludge for organochlorines in presence of elemental sulfur. Anal. Chem. 49, 316–318.

Kilham, S.S., 1978. Nutrient kinetics of freshwater planktonic algae using batch and semi-continuous methods. Mitt. Int. Ver. Theor. Angew. Limnol. 21, 147–157.

Kilham, S.S., 1998. Effects of physiological state on the bioaccumulation of toxic chemicals in algae and their transfer to zooplankton. Verh. Int. Ver. Theor. Angew. Limnol. 26, 1734–1736.

Kilham, S.S., Kreeger, D.A., Goulden, C.E., Lynn, S.G., 1997a. Effects of algal food quality on fecundity and population growth rates of *Daphnia*. Freshwater Biol. 38, 639–647.

Kilham, S.S., Kreeger, D.A., Goulden, C.E., Lynn, S.G., 1997b. Effects of nutrient limitation on biochemical constituents of *Ankistrodesmus falcatus*. Freshwater Biol. 38, 591–596.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., Herrera, L., 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377, 147–159.

Koelmans, A.A., Anzion, S.F.M., Lijklema, L., 1995. Dynamics of organic micropollutant biosorption to cyanobacteria and detritus. Environ. Sci. Technol. 29, 933–940.

Kreeger, D.A., Goulden, C.E., Kilham, S.S., Lynn, S.G., Datta, S., Interlandi, S.J., 1997. Seasonal changes in the biochemistry of lake seston. Freshwater Biol. 38, 539–554.

Lederman, T.C., Rhee, G.Y., 1982. Bioconcentration of a hexachlorobiphenyl in Great Lakes planktonic algae. Can. J. Fish. Aquat. Sci. 39, 380–387.

Liu, H.B., Dagg, M., 2003. Interactions between nutrients, phytoplankton growth, and micro- and mesozooplankton grazing in the plume of the Mississippi River. Mar. Ecol. Prog. Ser. 258, 31–42.

Lürling, M., van Donk, E., 1997. Life history consequences for *Daphnia pulex* feeding on nutrient-limited phytoplankton. Freshwater Biol. 38, 693–709.

Lynn, S.G., Kilham, S.S., Kreeger, D.A., Interlandi, S.J., 2000. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (Bacillariophyceae). J. Phycol. 36, 510–522.

Norstrom, R.J., Hallett, D.J., Sonstegard, R.A., 1978. Coho salmon (*Oncorhynchus kisutch*) and herring gulls (*Larus argentatus*) as indicators of organochlorine contamination in Lake Ontario. J. Fish. Res. Board Can. 35, 1401–1409.

Oliver, B., Niimi, A., 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ. Sci. Technol. 22, 388–397.

Pedersen, M.F., 1995. Nitrogen limitation of photosynthesis and growth—comparison across aquatic plant communities in a Danish estuary (Roskilde Fjord). Ophelia 41, 261–272.

Rasmussen, J.B., Rowan, D.J., Lean, D.R.S., Carey, J.H., 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. 47, 2030–2038.

Sabljiæ, A., Güsten, H., Hermens, J., Opperhuizen, A., 1993. Modeling octanol/water partition coefficients by molecular topology: chlorinated benzenes and biphenyls. Environ. Sci. Technol. 27, 1394–1402.

Sterner, R.W., Hagemeier, D.D., Smith, R.F., Smith, W.L., 1992. Lipid ovary indexes in food-limited *Daphnia*. J. Plankton Res. 14, 1449–1460.

Sterner, R.W., Smith, R.F., 1993. Clearance, ingestion and release of N and P by *Daphnia obtusa* feeding on *Scenedesmus acutus* of varying quality. Bull. Mar. Sci. 53, 228–239.

Swackhamer, D.L., 1985. The Role of Water Particle Partitioning and Sedimentation in Controlling the Fate and Transport of PCBs in Lakes. Ph.D. Thesis. University of Wisconsin, Madison, WI, USA, 259 pp.

Swackhamer, D.L., Skoglund, R.S., 1991. In: Baker, R.A. (Ed.), The Role of Phytoplankton in the Partitioning of Hydrophobic Organic Contaminants in Water. Organic Substances and Sediments in Water: Processes and Analytical, vols. 2/3. CRC Press LLC, Boca Raton, FL, pp. 91–105.

Swackhamer, D.L., Skoglund, R.S., 1993. Bioaccumulation of PCBs by algae—kinetics versus equilibrium. Environ. Toxicol. Chem. 12, 831–838.

Swackhamer, D.L., Skoglund, R.S., 1994. Kinetic-Model for Organic Contaminant Bioaccumulation in Phytoplankton. Abstracts of Papers of the American Chemical Society. 207:136-ENVR.

Thomann, R.V., Connolly, J.P., 1984. Model of PCB in Lake Michigan lake trout food chain. Environ. Sci. Technol. 18, 65–72.

van Donk, E., Hessen, D.O., 1993. Grazing resistance in nutrient-stressed phytoplankton. Oecologia 93, 508–511.

van Donk, E., Lurling, M., Hessen, D.O., Lokhorst, G.M., 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. Limnol. Oceanogr. 42, 357–364.

Wagner, A., Kamjunke, N., 2001. Reduction of the filtration rate of *Daphnia galeata* by dissolved photosynthetic products of edible phytoplankton. Hydrobiologia 442, 165–176.

Watts, R., 1980. Analysis of pesticide residues in human and environmental samples. A compilation of methods selected for use in pesticide monitoring programs. Research Triangle Park, NC, US Environmental Protection Agency Section 5, A, 1.

Weers, P.M.M., Gulati, R.D., 1997. Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnol. Oceanogr. 42, 1584–1589.

Willman, E.J., Manchester-Neesvig, J.B., Agrell, C., Armstrong, D.E., 1999. Influence of ortho-substitution homolog group on polychlorobiphenyl bioaccumulation factors and fugacity ratios in plankton and zebra mussels (*Dreissena polymorpha*). Environ. Toxicol. Chem. 18, 1380–1389.