

Video Article

Laboratory Estimation of Net Trophic Transfer Efficiencies of PCB Congeners to Lake Trout (*Salvelinus namaycush*) from Its Prey

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Abstract

A technique for laboratory estimation of net trophic transfer efficiency (γ) of polychlorinated biphenyl (PCB) congeners to piscivorous fish from their prey is described herein. During a 135-day laboratory experiment, we fed bloater (*Coregonus hoyi*) that had been caught in Lake Michigan to lake trout (*Salvelinus namaycush*) kept in eight laboratory tanks. Bloater is a natural prey for lake trout. In four of the tanks, a relatively high flow rate was used to ensure relatively high activity by the lake trout, whereas a low flow rate was used in the other four tanks, allowing for low lake trout activity. On a tank-by-tank basis, the amount of food eaten by the lake trout on each day of the experiment was recorded. Each lake trout was weighed at the start and end of the experiment. Four to nine lake trout from each of the eight tanks were sacrificed at the start of the experiment, and all 10 lake trout remaining in each of the tanks were euthanized at the end of the experiment. We determined concentrations of 75 PCB congeners in the lake trout at the start of the experiment, in the lake trout at the end of the experiment, and in bloaters fed to the lake trout during the experiment. Based on these measurements, γ was calculated for each of 75 PCB congeners in each of the eight tanks. Mean γ was calculated for each of the 75 PCB congeners for both active and inactive lake trout. Because the experiment was replicated in eight tanks, the standard error about mean γ could be estimated. Results from this type of experiment are useful in risk assessment models to predict future risk to humans and wildlife eating contaminated fish under various scenarios of environmental contamination.

Video Link

The video component of this article can be found at <https://www.jove.com/video/51496/>

Introduction

Of all of the factors affecting the rate at which fish accumulate contaminants, the efficiency with which fish retain contaminants from the food that they eat is one of the most important¹⁻³. Risk assessment models have been developed to predict future risks to both people and wildlife eating contaminated fish under various scenarios of environmental contamination, and the reliability of these predictions critically depends on the accuracy of the estimates of the efficiency at which fish retain contaminants from their food⁴.

The efficiency with which the contaminant in the food ingested by the predator is transported through the gut wall is known as gross trophic transfer efficiency⁵. A portion of the quantity of the contaminant transported through the gut wall of the predator may eventually be lost through depuration and/or metabolic transformation. The efficiency with which the contaminant in the food ingested by the predator is retained by the predator, including any losses due to elimination and metabolic transformation, is known as net trophic transfer efficiency⁶.

Gross trophic transfer efficiency of organic contaminants to fish from their prey appears to vary with the contaminant's chemical properties, including lipid affiliation as measured by the octanol-water partition coefficient, K_{ow} ^{3,7}. According to an empirical relationship developed by Thomann³, gross trophic transfer efficiency is relatively high when $\log K_{ow}$ is equal to a value between 5 and 6. Gross trophic transfer efficiency declines exponentially at a rate of 50% per unit of $\log K_{ow}$ as $\log K_{ow}$ increases from 6 to 10, according to the Thomann³ relationship.

Nevertheless, the gross and net trophic transfer efficiencies of polychlorinated biphenyl (PCB) congeners to fish from their prey do not appear to follow the Thomann³ relationship in most cases. Although the trophic transfer efficiencies of PCB congeners to lake whitefish (*Coregonus clupeaformis*) from its food followed the relationship proposed by Thomann⁸, trophic transfer efficiencies of PCB congeners were either just weakly related or not related at all to $\log K_{ow}$ for Atlantic salmon (*Salmo salar*)⁹, rainbow trout (*Oncorhynchus mykiss*)¹⁰, coho salmon (*Oncorhynchus kisutch*)¹¹, and northern pike (*Esox lucius*)¹¹.

The overall goal of this study was to present a laboratory technique for estimating the net trophic transfer efficiencies of PCB congeners to a piscivorous fish from its prey. Lake trout (*Salvelinus namaycush*) was chosen as the piscivorous fish for our experiment because lake trout are relatively easy to maintain in laboratory tanks. Bloater (*Coregonus hoyi*) was selected as the prey fish to be fed to the lake trout because bloater

is eaten by lake trout in its natural setting¹². In addition, we determined whether the net trophic transfer efficiencies for lake trout estimated from our laboratory experiment followed the Thomann³ relationship. We also determined whether the degree of activity by the lake trout had a significant effect on net trophic transfer efficiency (γ) of the PCB congeners. Activity by lake trout in the Laurentian Great Lakes is believed to have recently increased because changes in the food webs have caused lake trout to allocate more energy toward searching for food¹³. Lake trout were forced to exercise in one set of tanks by subjecting these lake trout to relatively high flow rates, whereas the other lake trout were permitted to remain relatively inactive by subjecting them to relatively low flow rates. Finally, the specific details of our laboratory procedure that need to be carefully followed to ensure the highest degree of accuracy in the γ estimates and to make the laboratory results applicable to the field are discussed, as well as future directions for research building on our laboratory technique. Net trophic transfer efficiency can be estimated both in the laboratory and in the field, and advantages and disadvantages are associated with both approaches. Accuracy in the estimate of γ depends on the accuracy of the estimate of food consumption. The amount of food eaten by fish in the laboratory can be accurately determined when proper protocols are followed, whereas the amount of food eaten by fish in the field is typically estimated via bioenergetics modeling. Use of bioenergetics modeling to derive the amount of food eaten has the potential to introduce a substantial amount of uncertainty into the estimates of food consumption. Fish bioenergetics models have been shown to estimate food consumption with no detectable bias for the case of lake trout^{14,15}, but considerable bias in bioenergetics model estimates of food consumption has been detected for the case of lake whitefish^{15,16}. On the other hand, estimates of net trophic transfer efficiency estimated in the laboratory may not be applicable to the field due to a difference in feeding rates between the laboratory and the field¹⁷. Evidence from both the laboratory and the field suggest that feeding rate can influence γ ^{14,17}.

The methodology used in the present study for estimating γ in the laboratory is applicable to situations where the predator fish is fed prey fish, and the amount of prey fish eaten by the predator can be accurately tracked. To accomplish this, the experimenter must weigh all of the food before placement in the tank; and the experimenter must be able to remove all of the uneaten food from the tank, and then weigh the uneaten food. In addition, an adequate suite of mixers and blenders should be available to obtain a sufficient degree of homogenization of the samples of both predator and prey fish. Finally, the gas chromatography – mass spectrometry instrumentation used to determine the PCB congener concentrations must be capable of detecting and quantifying individual PCB congeners at relatively low concentrations.

Protocol

1. Laboratory Experiment

1. Obtain the prey fish to be fed to the predator fish during the experiment. Preferably these prey fish should be captured in the field, frozen, and stored at about -30 °C. Consider commercial fishing operations as a potential source for the prey fish.
2. Introduce the predator fish into the laboratory tanks to be used for the experiment. Up to 15 predator fish have been introduced into each of 870-L tanks, and up to 30 predator fish have been introduced into each of 2,380-L tanks in previous studies^{16,18}.
3. Acclimate the predator fish to a diet of the selected prey fish. Once acclimated, the predator fish should remain on this diet for 1-3 months before beginning the experiment.
4. Set aside samples of prey fish by randomly selecting 10 to 20 composite samples from the batch of prey fish. Number of prey fish in a composite sample could range from 3 to 100, depending on the size of the prey fish. Each composite sample should be double bagged, frozen, and stored at about -30 °C.
5. Initiate the experiment by sacrificing 30 to 50% of the fish in each of the tanks.
 1. To euthanize the fish, mix 8 g of Finquel with 45 L of water in a large plastic container and then place the fish in the container with the Finquel solution.
 2. Once euthanized, place all of the sacrificed fish from one tank into a bag, then double bag, and store at about -30 °C until time of the processing.
 3. Weigh each of the fish remaining in each of the tanks, and record the weights; an anesthetic will likely be needed to conduct the weighing.
 4. To anesthetize the fish, mix 4.6 g of Finquel with 45 L of water in a large plastic container, and then place the fish in the container with the Finquel solution.
 5. Wait a few minutes for the anesthetic to take effect before weighing the fish.
6. On each day of the experiment, thaw an appropriate amount of prey fish, and cut the prey fish into pieces weighing roughly 1 to 5 g. Weigh the quantity of prey fish to be placed in each of the tanks, then drop the prey fish pieces into each tank and allow the predator fish about 1 hr to feed. Then remove all uneaten food, allow the food to air dry for about 20 min, and then weigh the uneaten food for each of the tanks. Record the amount of food placed in the tank and the amount of uneaten food for each of the tanks each day.
NOTE: For the representative experiment, lake trout were fed as much food as they would consume during one feeding period each day¹⁸. However, the predator fish could also be placed on fixed rations^{16,19}.
7. Terminate the experiment by sacrificing all of the remaining predator fish in each of the tanks. To euthanize the fish, mix 8 g of Finquel with 45 L of water in a large plastic container and then place the fish in the container with the Finquel solution. Record the weight of each of the sacrificed fish. For reliable results, the experiment must run for at least 100 days, preferably for at least 130 days. Place all of the fish from a tank into one bag, then double bag, and store at about -30 °C until time of processing.

2. Fish Homogenization

1. Select a set of predator fish and/or prey fish composites for thawing. Allow the composites to partially thaw. Each composite may require 0.5 to 1 hr to homogenize.
2. Using the appropriate-sized blenders, homogenize each of the composites. For each composite, place a sample (from 50 to 100 g) of the homogenate into a cleaned, acetone-rinsed, and labeled jar. Then cap the jar and store the jar at about -30 °C until the time of processing.
3. Wash all equipment used to homogenize the fish, and then properly rinse with distilled water and methanol, between samples.

3. Extraction

1. Weigh 20.0 g of thawed homogenized fish tissue in a 200-ml beaker.
2. Add approximately 40 g of sodium sulfate and mix well with spatula.
3. Add surrogate spike solution containing congeners 30, 61, 161, and 166. Spike at a concentration that yields a final concentration of 20 ng/ml in the extract.
4. Allow the sample to dry at RT while mixing every 20 min.
5. Allow the sample to reach a consistency of dry sand, at which point the sample is ready for extraction.
6. Set up the Soxhlet extraction apparatus with a 500-ml flask containing Teflon boil chips, Soxhlet, and condenser.
7. Add the dried fish mixture to a glass thimble with a coarse-fritted disc bottom or paper thimble.
8. Add 150 ml of 50% hexane and 50% dichloromethane to the beaker used for the sample and stir while scraping the walls of the beaker with a spatula.
9. Transfer the solvent to the top of the Soxhlet with the flask attached and allow it to cycle through the Soxhlet and into the flask.
10. Repeat a second time with 150 ml again.
11. Place the Soxhlet with the attached flask onto the heating element and attach the condenser.
12. Turn on the heating element and bring the solvent to a gentle boil, then extract for a minimum of 16 hr making sure that cold water is supplied to the condensers.
13. After allowing the solvent to cool, check to see if any of the sample flasks contain water. For those flasks containing water, add sodium sulfate and swirl until the water is absorbed by the sodium sulfate.
14. Concentrate the sample using a nitrogen sample concentrator or a Kaderna Danish (KD) glassware setup with a hot water bath.
15. Allow the sample to evaporate to a volume of less than 2 ml, and then bring to a final volume of 5 ml by using small washings of hexane to transfer the sample from the glassware used to a 5-ml volumetric flask.
16. Transfer to a 10-ml vial and label with sample information.

4. Extract Clean-up

1. Prepare acidified silica gel by adding 44 g of concentrated sulfuric acid to 100 g of activated silica gel.
2. Add 10 g of acidified silica gel into a small chromatography column containing a small plug of glass wool at the bottom.
3. Add 1 ml of sample extract to the column after pre-cleaning the column with 10 ml of hexane.
4. Elute the column with 20 ml of hexane and collect in tapered 20-ml glass tube.
5. Place the glass tube on the nitrogen evaporator (N-Vap) apparatus under a stream of nitrogen and immersed in hot water.
6. Evaporate to less than 1 ml but not to dryness.
7. Remove from the N-Vap apparatus and transfer to 1-ml volumetric flask with small washings of hexane.
8. Transfer to a 1.8 ml autosampler vial labeled with sample information.
9. Spike 4 μ l of internal standard into the vial. The sample is now ready for analysis.

5. Analysis by Gas Chromatography – Mass Spectrometry Using Negative Chemical Ionization

1. Use standards to calibrate the instrument: The standards are available in mixtures consisting of groups of well separated congeners. Mixes 1-5 consist of nearly all of the congeners found in Arochlors 1016, 1221, 1232, 1242, 1248, 1254, and 1260. Mix 1 is used as a multi-level calibration mix, and system linearity is confirmed by preparing at least five calibration levels at concentrations between 2 and 100 ng/ml. Mixes 2-5 are used as single point calibrations for each congener.
2. Set up the chromatography – mass spectrometry system in the negative chemical ionization mode with hydrogen as the carrier gas (1 ml/min) and methane as the reagent gas.
3. Use a fused-silica, capillary column (60 m $\times$ 0.25 mm inner diameter) coated with DB-XLB at 0.25- μ m film thickness for separation. Program oven temperature from 60 to 212 $^{\circ}$ C at 25 $^{\circ}$ C/min, then to 260 $^{\circ}$ C at 1 $^{\circ}$ C/min, and then to 280 $^{\circ}$ C at 4 $^{\circ}$ C/min, with a final hold time of 4 min. Injector and transfer line temperatures should be set at 280 $^{\circ}$ C. Inject 1 to 2 μ l of the sample using the splitless injection mode.
4. Analyze all standards and samples by the internal standard method using 13 C-labeled decachlorobiphenyl.
5. Perform a check on the initial calibration by running a second source standard and Arochlors 1242 and 1260, and then compare predicted values for the Aroclor congeners with the observed amounts from this check procedure.
6. Once the initial calibration procedure has been successfully accomplished, complete analysis of all samples. Run a calibration check every ten samples, using any of the calibration mixtures from the initial calibration.

6. Calculation of Net Trophic Transfer Efficiency

1. Calculate net trophic transfer efficiency, γ , for each combination of tank and PCB congener using the following equation:

$$\gamma = \frac{([PCB_f]W_f) - ([PCB_i]W_i)}{\text{amount of the PCB congener ingested}}$$
 where $[PCB_f]$ is the average PCB congener concentration of the predator fish in the tank at the end of the experiment, W_f is the average weight of the predator fish in the tank at the end of the experiment, $[PCB_i]$ is the average PCB congener concentration of the predator fish in the tank at the start of the experiment, W_i is the average weight of the predator fish at the start of the experiment, and the amount of PCB congener ingested refers to the weight of the PCB congener ingested, on average, by each lake trout in the tank during the course of the experiment.
2. Calculate the denominator in the above equation by multiplying the average concentration of the PCB congener in the prey fish composites by the average amount (weight) of the prey fish eaten per predator fish in the tank during the entire course of the experiment.

Representative Results

Lake trout showed a substantial amount of growth during the experiment, as the initial lake trout mean weights ranged from 694 to 907 g while the final lake trout mean weights ranged from 853 to 1,566 g (**Table 1**). The average amount of food consumed by a lake trout during the course of the 135-day experiment ranged from 641 to 2,649 g. Mean PCB congener concentrations in the lake trout increased during the experiment, as mean PCB congener concentrations ranged from 0.01 to 7.14 ng/g (wet-weight basis) at the start of the experiment while mean PCB congener concentrations ranged from 0.03 to 29.31 by the conclusion of the experiment (**Table 2**). Averaging across the 10 composite samples of September-caught bloater, PCB congener concentrations ranged from 0.03 to 26.56 ng/g. Averaging across the 10 composite samples of May-caught bloater, PCB congener concentrations ranged from 0.03 to 23.52 ng/g (**Table 2**). Refer to Madenjian *et al.*²¹ for more details on the bloater used in the experiment.

Mean estimates of γ ranged from 0.309 to 0.988, based on averaging across all eight tanks (**Table 3**). Standard errors for these mean estimates ranged from 0.029 to 0.227. For all 75 of the PCB congeners, mean γ for the active lake trout did not significantly differ from mean γ for the inactive lake trout. Thus, active lake trout retained the PCB congeners from the food that they consumed with nearly the same efficiency as inactive lake trout.

As the degree of chlorination increased from 5 to 10 chlorine atoms per molecule, estimates of γ showed a slight decrease (**Figure 1**). However, γ did not vary significantly with degree of chlorination of the PCB congeners (one-way ANOVA: $F = 2.16$; degrees of freedom [df] = 6, 67; $p = 0.0579$). Averaging γ across all 75 congeners, the mean value was 0.664.

As $\log K_{ow}$ increased from 6.0 to 8.2, γ declined exponentially (**Figure 2**). This rate of decline was significantly different from zero (t test: $t = -4.09$; $df = 64$; $p = 0.0001$), but was equal to just 7% per unit of $\log K_{ow}$. Based on the fitted curve, γ was equal to 0.70 at $K_{ow} = 6$, and γ was equal to 0.61 at $K_{ow} = 8$ (**Figure 2**).

For 66 of the 75 PCB congeners, the standard error about the mean estimate of γ was small (≤ 0.05) (**Table 3**). For six of the nine other PCB congeners, the standard errors about the mean estimate of γ were fairly low (≤ 0.10). Higher standard errors were associated with a lower degree of chlorination (three to five chlorine atoms per molecule).

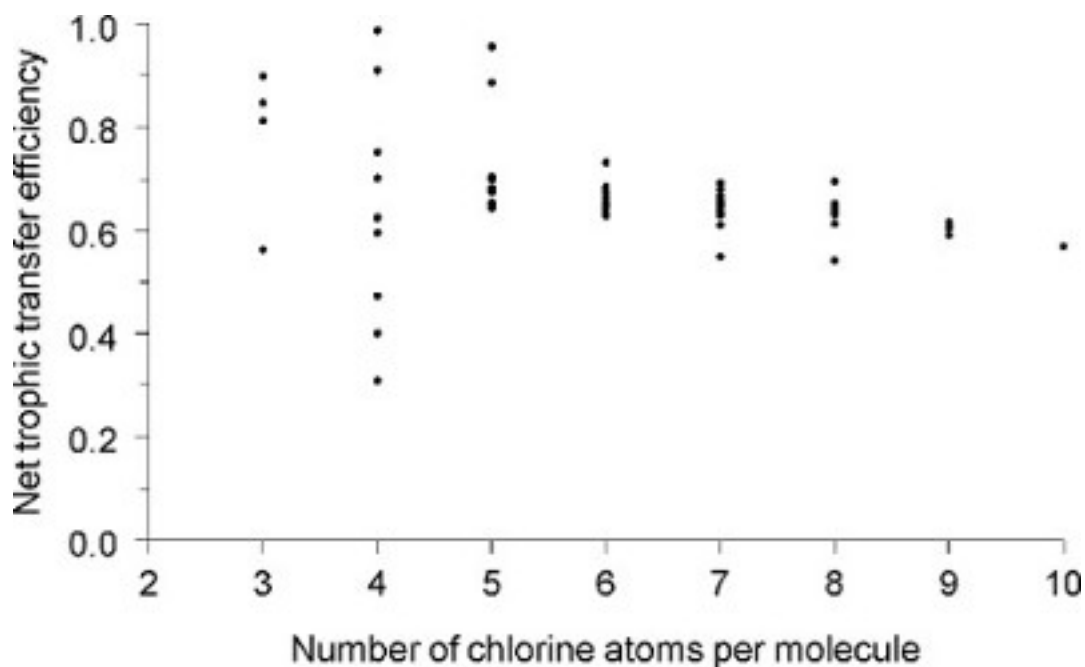


Figure 1. Estimates of net trophic transfer efficiency (γ) of PCB congeners to lake trout from its prey depicted as a function of the number of chlorine atoms per molecule of the PCB congener. Estimates were based on a laboratory experiment, during which bloaters were fed to the lake trout. Figure reproduced with permission from Madenjian *et al.*¹⁸.

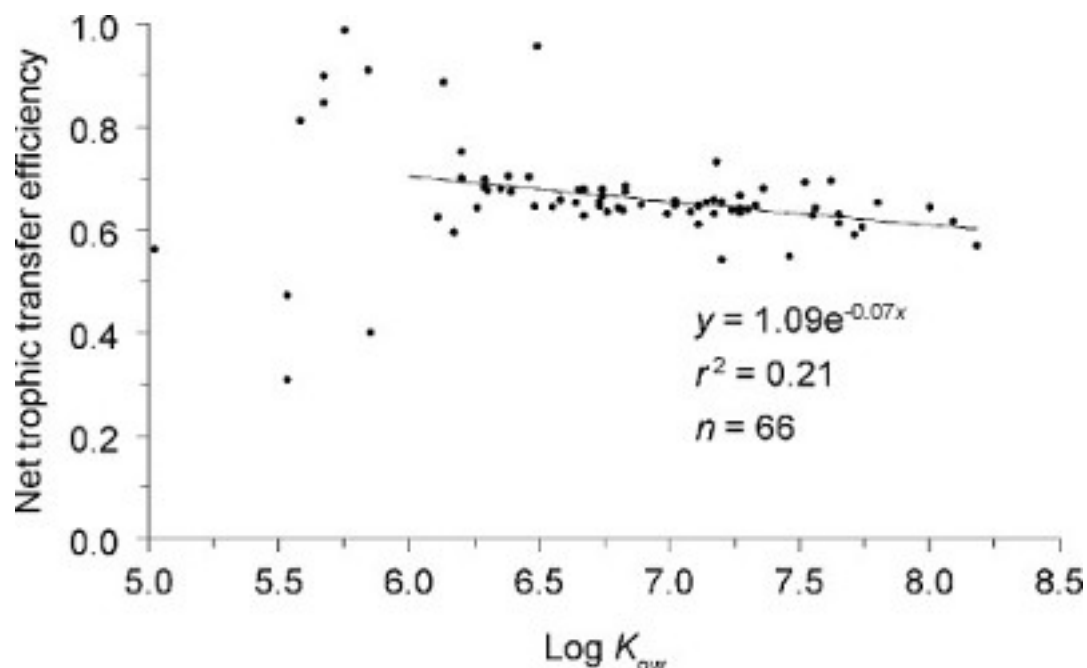


Figure 2. Estimates of net trophic transfer efficiency (γ) of PCB congeners to lake trout from its prey depicted as a function of the log K_{ow} of the PCB congener. Estimates were based on a laboratory experiment, during which bloaters were fed to lake trout. The fitted regression line for congeners with log K_{ow} greater than 6 is also displayed. The r^2 value for the fitted regression line represents the amount of variation in log γ explained by log K_{ow} . Figure reproduced with permission from Madenjian *et al.*¹⁸.

Table 1. Initial average weights and final average weights of lake trout used in the 135-day laboratory experiment. Bloaters were fed to the lake trout. Also included is the average amount of food eaten by a lake trout during the entire course of the experiment. Table reproduced with permission from Madenjian *et al.*¹⁸.

Tank number	Initial mean weight of lake trout (g)	Final mean weight of lake trout (g)	Consumption (g)
1	907	1,345	1,734
2	860	1,339	1,999
3	890	1,518	2,344
4	817	1,566	2,649
5	694	1,242	1,870
6	729	853	641
7	754	1,050	1,203
8	729	1,092	1,336

Table 2. Initial and final PCB congener concentrations in lake trout, averaged across the eight tanks used during the 135-day laboratory experiment. Average PCB congener concentrations in the September-caught and May-caught bloaters fed to the lake trout during the experiment are also shown. Table reproduced with permission from Madenjian *et al.*¹⁸. PCB congeners were numbered according to Ballschmiter *et al.*²⁰.

PCB congener	Initial lake trout PCB congener mean concentration (ng/g)	Final lake trout PCB congener mean concentration (ng/g)	September-caught bloater PCB congener mean concentration (ng/g)	May-caught bloater PCB congener mean concentration (ng/g)
19	1.62	3.41	3.27	2.01
22	0.41	0.66	0.36	0.32
28	1.22	2.24	1.27	0.82
31	1.19	1.97	1.13	0.67
44	1.10	2.08	1.09	0.84
45	0.66	1.74	2.25	1.71

46	0.81	2.51	5.23	3.73
47	1.88	5.72	9.10	5.81
52	2.11	3.76	2.05	1.66
60	0.59	2.04	2.10	1.50
63	0.19	0.68	0.74	0.52
70	3.05	10.25	9.43	6.62
74	0.76	2.76	2.35	1.79
82	0.26	0.91	0.80	0.75
83	0.45	1.60	1.62	1.28
85	1.70	6.63	6.38	5.15
87	1.12	3.47	3.09	2.46
92	1.17	4.16	3.91	3.06
95	2.22	5.06	3.09	2.59
97	1.04	3.37	3.08	2.45
99	3.19	12.38	11.95	9.59
101	3.33	10.25	8.90	7.37
105	2.88	11.35	10.80	9.28
110	4.53	15.78	15.55	12.31
115	0.20	1.03	0.69	0.54
117	0.25	1.24	1.19	0.98
118	6.20	24.17	22.94	19.35
124	0.22	0.79	0.77	0.63
128	1.58	6.26	6.03	5.37
130	0.85	3.26	3.24	2.85
131	0.77	2.97	2.89	2.52
134	0.14	0.44	0.42	0.36
135	0.84	3.19	3.16	2.62
137	0.46	1.77	1.67	1.49
138	7.14	28.31	26.56	23.52
141	0.71	2.50	2.45	2.17
144	0.08	0.22	0.19	0.18
146	2.34	9.10	8.96	7.86
149	2.38	8.18	8.25	6.72
151	0.47	1.53	1.43	1.27
156	0.68	2.65	2.31	1.96
158	0.64	2.42	2.36	1.99
163	2.92	10.24	10.07	8.94
164	0.47	1.81	1.79	1.58
167	0.43	1.65	1.64	1.43
170	1.03	3.94	3.71	3.47
171	0.39	1.46	1.43	1.26
172	0.38	1.45	1.41	1.30
174	0.48	1.83	1.84	1.67
175	0.11	0.42	0.42	0.37
176	0.03	0.09	0.09	0.09
177	0.72	2.67	2.65	2.45

178	0.61	2.33	2.26	2.03
179	0.17	0.60	0.58	0.55
180	3.35	12.84	11.97	10.73
183	1.18	4.44	4.32	3.79
185	0.04	0.14	0.14	0.14
187	3.12	12.07	11.65	10.67
190	0.27	1.02	1.18	1.02
191	0.05	0.20	0.20	0.17
193	0.27	1.03	0.94	0.87
194	0.46	1.73	1.66	1.55
195	0.14	0.54	0.53	0.49
196	0.30	1.12	1.15	1.03
197	0.06	0.23	0.23	0.20
199	0.67	2.44	2.17	2.12
200	0.01	0.03	0.03	0.03
201	0.14	0.53	0.52	0.48
202	0.31	1.14	1.12	1.02
203	0.48	1.83	1.83	1.61
205	0.02	0.09	0.09	0.08
206	0.19	0.70	0.70	0.65
207	0.07	0.25	0.26	0.24
208	0.11	0.41	0.43	0.40
209	0.11	0.36	0.38	0.36

Table 3. Mean estimates of net trophic transfer efficiency (γ) of PCB congeners to lake trout from its prey. Estimates were based on a 135-day laboratory experiment, during which lake trout were fed bloaters. For each congener, γ estimates from all eight tanks were averaged to yield the mean estimate. Standard error of the mean is enclosed in parentheses. Table reproduced with permission from Madenjian *et al.*¹⁸. PCB congeners were numbered according to Ballschmiter *et al.*²⁰.

PCB congener	Mean γ	Standard error of mean
19	0.563	0.046
22	0.813	0.127
28	0.900	0.086
31	0.848	0.065
44	0.988	0.058
45	0.474	0.058
46	0.309	0.035
47	0.401	0.029
52	0.911	0.059
60	0.625	0.034
63	0.596	0.036
70	0.702	0.039
74	0.753	0.050
82	0.700	0.038
83	0.644	0.039
85	0.677	0.037
87	0.699	0.038

92	0.681	0.032
95	0.887	0.102
97	0.683	0.032
99	0.675	0.035
101	0.705	0.035
105	0.678	0.035
110	0.647	0.037
115	0.957	0.227
117	0.704	0.050
118	0.680	0.035
124	0.655	0.037
128	0.666	0.035
130	0.644	0.034
131	0.659	0.037
134	0.646	0.032
135	0.653	0.034
137	0.675	0.035
138	0.686	0.033
141	0.639	0.037
144	0.680	0.050
146	0.650	0.034
149	0.628	0.036
151	0.653	0.034
156	0.733	0.051
158	0.657	0.032
163	0.632	0.042
164	0.648	0.035
167	0.642	0.033
170	0.668	0.039
171	0.649	0.038
172	0.649	0.035
174	0.646	0.037
175	0.632	0.038
176	0.636	0.046
177	0.636	0.031
178	0.654	0.040
179	0.647	0.034
180	0.681	0.036
183	0.654	0.038
185	0.611	0.036
187	0.659	0.036
190	0.549	0.031
191	0.629	0.032
193	0.693	0.037
194	0.654	0.035
195	0.643	0.039

196	0.614	0.037
197	0.640	0.040
199	0.696	0.036
200	0.543	0.042
201	0.634	0.040
202	0.639	0.036
203	0.631	0.036
205	0.645	0.038
206	0.617	0.036
207	0.606	0.039
208	0.592	0.038
209	0.570	0.037

Discussion

For the most accurate estimates of γ , the experimenter must be able to accurately track both the amount of food placed in each of the tanks and the amount of uneaten food in each of the tanks during the course of the experiment. To accomplish this, the experimenter must be able to remove all of the uneaten food from the tanks and accurately determine its weight. In addition to accurate tracking of the food actually eaten by the predator fish, accurate estimation of γ may also depend on sufficient duration of the experiment. Given that widely cited laboratory studies specifically designed to estimate trophic transfer efficiency of PCBs to fish from their food ranged from 105 to 224 days in duration^{22,23}, a duration of at least 100 days, and preferably at least 130 days, is recommended. Further, bias may be introduced into the estimation of γ by an insufficient number of predator fish sampled for PCB determinations at the start of the experiment¹⁴. The probability of obtaining a sample of predator fish with PCB concentrations not representative of the average PCB concentration for all of the predator fish in the tank increases with decreasing sample size. Ideally, half of the fish in the tank should be sacrificed for PCB determinations at the start of the experiment.

To maximize relevancy and applicability of the laboratory experiment results to the field, a prey fish that is typically eaten by the predator fish in the field should be fed to the predator fish during the laboratory experiment. Net trophic transfer efficiency may depend on the nature of the food matrix containing the PCB congeners^{11,24}. Evidence from previous studies has suggested that estimates of γ based on a commercial pellet diet may be substantially less than γ estimates based on predator fish feeding on actual prey fish¹⁷. Hence, a diet of prey fish rather than a processed or synthesized diet is recommended.

To minimize uncertainty in the estimates of γ , both the predator fish and prey fish composites should be well homogenized. The degree of homogenization depends, in part, on the available set of blenders and mixers. For large predator fish, a large mixer may be needed to initiate the homogenization process. A subsample of the homogenate from the large mixer may then be transferred to a smaller mixer, where a higher degree of homogenization can be achieved.

Accurate determination of the PCB congener concentrations in the homogenized fish tissue samples is a key component of the process of accurately estimating γ for the various PCB congeners. The samples must be properly cleaned during the follow-up to the extraction process to remove matrix interferences and to achieve a low level of detection for the PCB congeners. Use of a gas chromatography – mass spectrometry system with a negative chemical ionization source operated in the single ion mode can lead to detection levels as low as 0.02 ng/ml in the extract for the more highly chlorinated PCB congeners, although the detection limit for the lower chlorinated PCB congeners would be considerably higher than this value²⁵. An electron capture detector can be substituted for the negative chemical ionization instrument and this approach will provide low level detection, but will also be more susceptible to matrix interferences. Depending on the PCB congener concentrations in the homogenized fish tissue samples, the researcher will need to decide as to which approach (negative chemical ionization or electron capture) is more appropriate. For very low PCB congener concentrations, the electron capture approach may have to be used. It should be pointed out that measurements near the detection limit often have relatively low precision and accuracy due to analytical error²⁶.

The methodology detailed in this study could be easily adapted to address new research questions in the field of PCB accumulation in fish. For example, as mentioned above, γ may be influenced by feeding rate. Previous work has suggested that γ decreases with increasing rate of food consumption^{14,17}. Exactly how does γ change with increasing feeding rate? Do the relationships between γ and degree of chlorination or between γ and $\log K_{ow}$, which have been elucidated in this study for fish fed ad libitum, remain consistent at lower feeding rates? Which of the following two factors has a greater influence on γ : the amount of food consumed each day or the frequency of feeding (*i.e.*, feeding once every day versus feeding once every two or three days)? Which of the following two factors has a greater influence on γ : the weight of food consumed each day or the amount of energy in the food consumed each day? The methodology detailed in this study is well suited to answer these questions, because both feeding rate and food type can be controlled in the laboratory.

Disclosures

None.

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