Euro-limpacs

Project no. **GOCE-CT-2003-505540**

Project acronym: **Euro-limpacs**

Project full name: **Integrated Project to evaluate the Impacts of Global Change on European Freshwater Ecosystems**

Instrument type: **Integrated Project**

Priority name: **Sustainable Development**

Deliverable No. 24
Report – Modelling the distribution of atmospherically transported organochlorinecompounds in the food-web of a high mountain lake (Redo, Pyrenees)

Due date of deliverable: **Month 12**
Actual submission date: **Month 12**

Start date of project: **1 February 2002** Duration: **5 Years**

Organisation name of lead contractor for this deliverable: **CSIC**

Revision: Final

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level (tick appropriate box)		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	**X**
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

This deliverable comprises a paper submitted Environmental Toxicology and Chemistry and a paper published in Environmental Science and Technology.

1. Distribution of Polycyclic Aromatic Hydrocarbons in the Food Web of a High Mountain Lake (Pyrenees)
Environmental Toxicology and Chemistry, in press
Ingrid Vives, Joan O. Grimalt, Marc Ventura and Jordi Catalan

2. The roles of food and water in the bioaccumulation of organochlorine compounds in high mountain lake fish.

Environmental Science and Technology 38, 4269-4275 (2004)
J. Catalan, M. Ventura, I. Vives and J.O. Grimalt

Running head: **Polycyclic aromatic hydrocarbons in a mountain lake food web**

Corresponding author:

Joan O. Grimalt

Department of Environmental Chemistry (IIQAB-CSIC)

Jordi Girona, 18

08034-Barcelona

Catalonia

Spain

Telephone: +34934006122

Fax: +34932045904

e-mail: jgoqam@cid.csic.es

Total words: 6458

Total references: 48

Total tables: 2

Total figures: 5

24

25

26 **DISTRIBUTION OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE FOOD**

27 **WEB OF A HIGH MOUNTAIN LAKE (PYRENEES)**

28

29

30 **INGRID VIVES[†], JOAN O. GRIMALT*[†], MARC VENTURA[‡] and JORDI CATALAN[‡]**

31

32

33 [†]Department of Environmental Chemistry, Institute of Chemical and Environmental Research

34 (CSIC), Jordi Girona 18, 08034-Barcelona, Catalonia, Spain.

35

36 [‡]Limnology Group (CSIC-UB). Center for Advanced Studies of Blanes (CEAB-CSIC). Accés

37 Cala St. Francesc, 14. Blanes 17300. Catalonia. Spain.

38

39 ---

40

*To whom correspondence may be addressed (jgoqam@cid.csic.es)

Disclaimer: The research work reported in this manuscript is new and original. It is not under evaluation for publication in any other journal.

Abstract

The contents of polycyclic aromatic hydrocarbons (PAHs) in the food web organisms included in the diet of brown trout from a remote mountain lake have been investigated. The preferential habitat and trophic level of the component species has been assessed from the signature of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$). Subsequently, the patterns of accumulation and transformation of these hydrocarbons in the food chain have been elucidated. Most of the food web organisms exhibit PAH distributions largely dominated by phenanthrene, which agrees with its predominance in atmospheric deposition, water and suspended particles. Total PAH levels are higher in the organisms from the littoral habitat than from the deep sediments or the pelagic water column. However, organisms from deep sediments exhibit higher proportions of higher molecular weight PAH than those in other lake areas. Distinct organisms exhibit specific features in their relative PAH composition that points to different capacities for uptake and metabolic degradation. Brown trout shows an elevated capacity for metabolic degradation since they have lower PAH concentrations than food and they are strongly enriched in lower molecular weight compounds. The PAH levels in trout highly depend on organisms living in the littoral areas. Fish exposure to PAH may therefore vary from lake to lake according to the relative contribution of littoral organisms to their diet.

Keywords: Polycyclic Aromatic Hydrocarbons, Food-web, Trout, Phenanthrene, Invertebrates

INTRODUCTION

The overall environmental concentrations of polycyclic aromatic hydrocarbons (PAHs) have increased extensively in the 20th century as consequence of the general enhancement of combustion processes [1, 2]. These compounds are released directly to the atmosphere, both in the form of gas and in association to particles, where they are transported over long distances and become global contaminants [3]. In this respect, recent studies on sediments, water and air have demonstrated that these compounds are significant pollutants in remote areas such as high mountain lake ecosystems [1, 4-6].

These hydrocarbons and their metabolites have been widely studied because of their carcinogenic and mutagenic properties [7-10]. Thus, some PAH are included in the EU and US lists of priority pollutants [11]. In fish, toxico-hepatic lesions have been related to PAH exposure [12].

One important aspect that needs to be assessed is whether the overall increase of long-range transported of PAH may have an effect on remote ecosystems or in some of their organisms. In this respect, little information is available on the mechanisms of incorporation of these compounds from atmosphere to food web and to high predators such as fish. Whereas fish possess mixed-function oxygenase systems that rapidly metabolize PAH [12-14], these enzymes are poorly developed in some invertebrates that have a lower rate of metabolic degradation [15, 16]. As a consequence, PAHs can be found in benthic and water column organisms even in areas of low pollution [17, 18]. Invertebrates are the main components of the intermediate trophic levels of aquatic food webs [19] and thus their accumulated PAHs are transferred to higher trophic levels, such as fish [13]. Thus, low PAH concentrations in fish do not necessarily imply that they are not receiving significant pollutant fluxes and that they are free of stress. More research is needed to understand to what extent the low degradation

capacity of invertebrates is general and whether PAH mixtures could undergo some degree of transformation. Integrated studies on the PAH pathways throughout the trophic webs up to fish are also required to improve our estimates of fish exposure to those pollutants.

High altitude mountain lakes offer unique environments for the assessment of the transfer mechanisms of atmospherically transported organic pollutants into biota. These lakes do not contain organic contaminant sources in their watersheds. The pollutants found in the organisms originate from atmospheric inputs and are distributed through the complexities of the food web structure and its dynamics. In addition, food webs tend to be simpler than in low land lakes which facilitates sampling and description of the relationships between organisms.

In the present study Lake Redon (Pyrenees) is chosen as model case for these ecosystems. The portion of the food web part related to the diet of the unique top predator in the lake, brown trout (*Salmo trutta*), has been investigated. The study of PAH body contents illustrates how these compounds distribute among the different types of organisms according to their feeding modes and habitats.

MATERIALS AND METHODS

Study area.

Lake Redon (formerly Redó) (42º 38’ N, 0º 46’ E) is a high mountain lake located in the central Pyrenees (Catalonia, Spain). This lake is situated at 2240 m above sea level, above the regional tree line and far from local pollution sources. It has a surface area of 24 ha, a maximum depth of 73 m and a volume of 7.7 hm^3. Water residence time is ca. 4 yr [19] and there is only one outflow. The ice-free period is from June to December [19]. The relative small watershed (155 ha) is scarcely vegetated; alpine meadows alternate with large areas of

bare granodioritic rock. Lake water composition is very low in phosphorus (total P ca. 0.1 µmol/L) and acid neutralizing capacity (ca. 40 µeq/L) [19].

Sample Collection and Handling.

Fish sampling followed standard test fishing procedures with multifilament gillnets. All fish were measured and dissected and their sex was determined on site. Liver was wrapped in a pre-cleaned aluminium foil and kept frozen (-20ºC) until analysis. Fifteen liver samples were analyzed. The length and weight of the collected specimens were respectively 286 ± 26 mm and 230 ± 58 g (mean ± standard deviation). Average condition factor and age of the trouts analyzed were 0.97 ± 0.09 $g \cdot cm^{-3}$ and 11 ± 4 year old, respectively. There were not significant differences between the analyzed males and females (6 and 7, respectively) on those two factors (Analysis of variance, ANOVA, $p < 0.05$).

Distinct sampling methods were used for collecting organisms in the three main lake habitats. Kick sampling was chosen for littoral organisms, Ekman drags for deep sediment species and plankton nets for pelagic zooplankton. Samples were kept cold during transport and were later identified and separated in the lab into distinct taxa for stable isotope and PAH analysis. For PAH analyses a minimum wet weight of 0.5 g was obtained pooling individuals of a common taxa. Replicates were analyzed when enough material was available. We tried to include as many organisms as possible from the estimated brown trout diet **(Table 1)** in the lake. The components finally analyzed were belonging to the littoral habitat, e.g., plecoptera (*Arcynopterix compacta, Siphonoperla torrentium),* megaloptera *(Sialis lutaria),* coleoptera *(Platambus maculatus),* gastropoda *(Radix ovata) and* cyanobacteria *(Nostoc)*, to the deep sediment habitat, e.g., diptera (chironomidae), crustacea (*Eurycercus lamellatus*) and bivalvia (*Pisidium*), and to the pelagic habitat, e.g., crustacea (*Daphnia pulicaria*).

PAH analysis.

Fish liver was extracted and analyzed for PAHs as described elsewhere [20]. Briefly, liver was ground with activated sodium sulfate spiked with perdeuterated anthracene, pyrene and benzo[*ghi*]perylene, and introduced in pre-cleaned cellulose cartridges. The tissue was Soxhlet extracted (n-hexane:dichloromethane, 4:1, v/v) for 20 hours and purified through an aluminium oxide chromatographic column. Elution with hexane:dichloromethane (1:2; v/v; 30 mL) contained all the studied PAHs. Further on, extracts were concentrated to 2 mL by vacuum rotary evaporation (20 ºC, 20 Torr), then to near dryness under gentle nitrogen flow and redissolved to 50 µl with iso-octane.

Average water content in brown trout tissue (74.2±1.8 %, n=8) was calculated by drying in a vacuum sealed-dissecator at 20ºC to constant weight. This value was used to convert fish wet weight based PAH concentrations in fish to dry weight values for subsequent comparison with invertebrate and algal levels.

These hydrocarbons were analyzed in invertebrates and algae following a method that was slightly modified from the one described above [21]. Briefly, all samples were dried in a vacuum sealed-dissecator at 20ºC to constant weight to determine dry weight. Tissues were Soxhlet extracted with n-hexane-dichloromethane (4:1, v/v) for 20 hours. Then, perdeuterated PAHs were added and clean up was continued as described above.

Before chromatographic analysis, an internal standard of perdeuterated perylene was added to all sample vials as reference to improve injection precision. Samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS, Trace, Thermo, Bremen, Germany). This instrument was equipped with a 50 m x 0.25 mm i.d. HP-5MS capillary column coated with 5% phenyl 95% methylpolysiloxane (film thickness 0.25 µm). Samples were injected in splitless mode. The oven temperature program started at 90ºC (held for 1 min) to 120ºC at 10ºC·min^{-1}, and then to 310ºC at 4ºC·min^{-1} (holding time 15 min). Injector, transfer line and ion source temperatures were 280ºC, 280ºC and 200ºC, respectively.

166 Stringent precautions were kept for maintenance of the injector under clean conditions
167 avoiding adsorptions that could deviate the system from linearity and increase the limits of
168 detection and quantification. Helium (1.1 $mL \cdot min^{-1}$) was used as carrier gas. Data acquisition
169 was in electron impact (70 eV) and selected ion monitoring (40 ms dwell time). The ion mass
170 program is reported elsewhere [4, 22].

171 All major PAHs were analyzed, including fluorene, phenanthrene, anthracene,
172 fluoranthene, pyrene, benz[*a*]anthracene, chrysene+triphenylene, benzo[*b*]fluoranthene,
173 benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*ah*]anthracene, indeno[*1,2,3-cd*]pyrene and
174 benzo[*ghi*]perylene. Many of them are described as mutagenic, carcinogenic and teratogenic
175 by the International Agency for Research on Cancer [23]. Evidence of oxidation stress in fish
176 from high mountain lakes upon exposure to these compounds has been reported [24].

177 Procedural blanks were analyzed for every set of six samples. The recoveries of the
178 surrogate standards were calculated for each sample. Average values were 77%, 80% and
179 120% for perdeuterated anthracene, pyrene and benzo[*ghi*]perylene, respectively.
180 Identification and quantification of all studied compounds were performed by an external
181 standard method. Relative response to perylene-d_{10} was calculated and this value was also
182 corrected by the recovery of the surrogate standards.

183 Residue analysis n-hexane, dichloromethane, iso-octane, methanol, acetone, and
184 analysis grade anhydrous sodium sulfate were from Merck (Darmstadt, Germany).
185 Aluminium foil was rinsed with acetone and let dry at ambient temperature prior to use.
186 Neutral aluminium oxide type 507C was from Fluka AG (Buchs, Switzerland). Cellulose
187 extraction cartridges (20 mm i.d. x 80 mm long) were from Whatman (Maidstone, England).
188 PAHs mix9 and perdeuterated PAHs were purchased from Dr. Ehrenstorfer (Augsburg,
189 Germany).

Aluminium oxide, sodium sulfate and the cellulose cartridges were pre-cleaned by Soxhlet extraction with dichloromethane:methanol (2:1, v/v) for 24 h before use. Sodium sulfate and aluminium oxide were activated overnight at 400ºC and 120ºC, respectively.

Stable Isotope Analysis

Samples for stable isotope analysis were previously dried during 24 h at 60 ºC. In order to assure complete combustion, a small amount of vanadium pentaoxide was added before packaging the dried samples in tin capsules. Samples were analyzed in a Delta C Finnigan MAT mass spectrometer (Bremen, Germany) coupled online with a Carlo Erba CHNS (Milan, Italy) elemental analyzer, via a Finnigan conflo 2 interface. Specific standards provided by the International Agency of Atomic Energy (IAEA) were used for calibrating the isotopic signal. Sucrose (IAEA CH6), polyethilene (IAEA CH7) and graphite (IAEA-USGS 24) were used for carbon, and ammonium sulfate (IAEA-USGS 25, IAEA-N1 and IAEA-N2) and potassium nitrate (IAEA-NO3) were used for nitrogen [25]. A complete batch of standards was run at the beginning and at the end of each analytical session, and IAEA CH6 and CH7, and IAEA-N1 and IAEA-NO3 were run every twelve samples for linearity control. Special care was taken in weighting the samples and the standards in order that both had similar amplitudes. Results are reported using atmospheric nitrogen and PeeDee belemnite (PDB) carbonate as references. Reproducibility was better than 0.1 ‰ and 0.3 ‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Lipid Content Determination

Lipid content in fish liver was determined gravimetrically. For the rest of organisms we used a different approach because of the low amount of dry mass available. The lipid content was estimated from measured elemental carbon and nitrogen assuming that the main

body constituents were lipids, proteins, ashes and chitin [20, 26, 27]. Lipid percentage was calculated by difference, after estimating proteins from nitrogen content and multiplying by 6.25 [26], and using literature values for ash [20, 27] and chitin [28] content. This method was not applicable to *Nostoc*. Cyanobacteria use carbohydrates as energy reserve, their lipid content is low and rather constant because it corresponds exclusively to structural compounds. Therefore, a literature value is used for this organism [29].

RESULTS AND DISCUSSION

Food web structure

The $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of the samples collected in Estany Redon is shown in **Figure 1**. The lowest level of consumers shows a broad differentiation on the $\delta^{13}C$ signature, responding to the $\delta^{13}C$ of the food. Values ranged between -29.8‰ and –13.4‰, the end-members corresponding to small pelagic cladocerans (*D. pulicaria*) and littoral invertebrates (*R. ovata, P. maculatus*), respectively. The organisms living in the deep sediment habitat showed intermediate $\delta^{13}C$ values between -26‰ and -18‰. This range of values is common when littoral and, particularly, herbivores scraping on benthic algae are included in the food web studies [25, 31]. Phytoplankton tend to present an elevated isotopic discrimination respect to the signature of the available water CO_2, generally showing $\delta^{13}C$ values between –30 to –20‰ [30] but epilithic benthic algae have a lower discrimination, with values in the range of –20‰ to -8‰ [30] because of the boundary layer effect on diffusive processes around benthic algae [32]. Sediment matter has intermediate $\delta^{13}C$ values between pelagic and littoral organisms. The characteristic carbon signature in the primary consumers of the three different habitats is progressively lost at higher trophic levels (Fig. 1), as predators use food sources from different habitats. Since snails (*Radix*) present $\delta^{13}C$ values of ca. –13‰

240 we should expect values for epilithic algae (mainly diatoms) of about -12‰ and, in fact, these
241 are the values found in lake Redon in other studies [33].

242 The lower isotopic values of *Nostoc*, a colony forming cyanobacteria living
243 epilithically, can be interpreted according to two main processes. First, isotopic discrimination
244 is lower in cyanobacterial rubisco than in eukaryotic enzymes. Second, cyanobacteria possess
245 a CO_2 concentrating mechanism that includes active HCO_3^- uptake. The bicarbonate
246 contribution to the cyanobacterial $\delta^{13}C$ signature is higher as lower is the concentration of
247 CO_2 in water [34]. Accordingly, in soft and oligotrophic waters, such are those of lake Redon,
248 low growth rates and scarce free CO_2 result in higher $\delta^{13}C$ values [35].

249 The observed $\delta^{15}N$ values ranged between –4.6‰ (*Nostoc* sp.) and 3.6 ‰ (*S. trutta*).
250 Negative $\delta^{15}N$ values for primary producers, which shift the remaining food web values
251 downwards, are found in natural environments or experimental conditions in which nitrogen is
252 in excess with respect to other nutrients required by the algae [36]. This situation is relatively
253 rare in marine systems and terrestrial vegetation but it is common in the present alpine
254 freshwater systems because of the elevated atmospheric deposition of nitrogen pollution [37].
255 In addition the deposited compounds may have an isotopic signature that is negatively shifted
256 from molecular nitrogen in air [38]. In lake Redon, nitrogen available for algae (NO_3^-, NH_4^+)
257 is several orders of magnitude higher than phosphorus [19]. Nitrogen discrimination during
258 algal uptake is therefore expected to be high. Similar or even lower $\delta^{15}N$ values have been
259 found in food webs of alpine streams [39].

260 Assuming an enrichment factor of 3.5‰ $\delta^{15}N$ per trophic level change [40], the total
261 $\delta^{15}N$ span of Lake Redon involves a short food chain from primary producers to top predators
262 (a mean of 2.2 trophic transferences). Brown trout located at the higher position of the food
263 web do not contain piscivorous specimens. *S. torrentium* and *S. lutaria* belongs to an
264 intermediate level of predators between fish and herbivorous or detritivorous organisms.

265

266 *Polycyclic aromatic hydrocarbons in the food web*

267 The distributions of PAH in the food web organisms are dominated by phenanthrene
268 (ranging between 22-70 % of the total analyzed PAHs), followed by fluorene, fluoranthene
269 and pyrene (Fig. 2). Anthracene is present in small proportions (between 1-5 % of the total
270 analyzed PAH). The sum of the thirteen PAH considered for study range between 18 (*D.*
271 *pulicaria*) and 900 ng g^{-1} dw (*S. torrentium*), a 50-fold difference **(Table 2)**. No clear
272 qualitative differences are observed between distinct trophic levels.

273 Examination of the accumulated amounts exhibits a correspondence with the living
274 habitat of the organisms **(Fig. 3)**. The lowest total value is observed in *D. pulicaria,* the
275 pelagic species, and the highest values are found in the littoral insect larvae such as *P.*
276 *maculatus, S. lutaria*, *A. compacta* and *S. torrentium*. In the littoral habitat *Nostoc* and *Radix*
277 are the only organisms deviating from these high values. However, they have very low lipid
278 content. After normalization by lipids both organisms appear among those with highest PAH
279 levels (Table 2). The deep sediment organisms, e.g., *E. lamellatus*, *Pisidium* and
280 Chironomidae, exhibit intermediate values between littoral and pelagic species.

281 The higher concentrations in the organisms living in the littoral habitat are in
282 agreement with previous studies in the same lake showing more efficient PAH transfer to
283 underlying sediments at shallower water column. Thus, sediments situated at 72 and 30 m had
284 PAH concentrations of 630 and 2200 ng/g, respectively [41]. These differences suggest that
285 PAH undergo significant degradation upon transport through the water column.

286 The PAH distributions found in the organisms are predominated by low molecular
287 weight (LMW) compounds, between fluorene and pyrene (Table 2, Fig. 3). Higher relative
288 proportion of high molecular weight (HMW) PAHs are found in the organisms of the deep
289 sediment which constitutes a differential feature from pelagic and littoral species (Fig. 2).

290

291 *Polycyclic aromatic hydrocarbons in the water column and D. pulicaria*

292 Previous studies in Estany Redon have shown that phenanthrene is the dominant PAH
293 in high mountain lake waters, both in the dissolved and the particulate fractions (Fig. 2; [6]).
294 This is also the case in *D. pulicaria*. However, there are a number of compounds that are
295 found in higher proportion in both water phases than in *D. pulicaria,* e.g., fluoranthene,
296 pyrene, chrysene, benzofluoranthenes, indeno[1,2,3-*cd*]pyrene and benzo[ghi]perylene,
297 indicating an apparent selective bioaccumulation of phenanthrene in *Daphnia*. Comparison of
298 the PAH concentrations found in water (dissolved phase) [6] and in *D. pulicaria* (lipid
299 normalized) with the expected octanol-water partitioning (K_{ow}) [*42*] show that phenanthrene
300 and anthracene are higher in the cladoceran than theoretically expected **(Fig. 4)**, which
301 suggest a biomagnification process through particle ingestion. The lack of biomagnification
302 for the other PAH may be due either to selective metabolic degradation, which seems
303 unlikely, or to selective intake during food digestion.

304

305 *Polycyclic aromatic hydrocarbons in sediments and benthic organisms*

306 The sedimentary PAH composition is dominated by fluoranthene, chrysene,
307 benzofluoranthenes, indeno[1,2,3-*cd*]pyrene and benzo[ghi]perylene and differs significantly
308 from the PAH distributions in the benthic organisms, either those from the littoral zone or in
309 the deep areas. As shown in Figure 2, HMW PAH constitute about 67% of the total
310 sedimentary mixtures of these hydrocarbons whereas the organisms exhibit a higher LMW
311 content with a high predominance of phenanthrene. This later hydrocarbon is the dominant
312 PAH in atmospheric deposition [43], water, suspended particles and most of the organisms.
313 Therefore its decrease in the sedimentary distributions should reflect a preferential
314 degradation with respect to other PAHs.

315 *E. lamellatus* is the organism exhibiting the highest proportion of HMW PAH and
316 therefore the one with a PAH composition more similar to the one in the sediments (Fig. 2).
317 This cladoceran has a small size that allows it swimming between the sediment debris and
318 eating organic detritus and their associated bacterial flora. The other benthic organisms,
319 including those living partially buried in the sediments such as *Pisidium* and some
320 chironomidae larvae, exhibit a substantial lower amount of HMW PAH than *E. lamellatus.*
321 In the case of chironomidae, the larvae exhibit the high predominance of phenanthrene
322 that is common to most organisms. However, the pupae are dominated by fluoranthene (Fig.
323 2). This change in composition occurs during major metabolic changes in the organism
324 because the pupal stage does not involve any feeding. Comparison of the PAH concentrations
325 between larval and pupal stages shows a general PAH decrease in the latter (Table 2). The
326 decrease in fluoranthene is smaller than for the other PAHs, which suggest a lower metabolic
327 degradation rate for this compound in invertebrates. Accordingly, a higher concentration of
328 fluoranthene could be expected as longer is the life time of an invertebrate. This is generally
329 the case of the insects included in the present study (Fig. 2) whose insect larvae are larger and
330 tend to have longer life cycles (*Sialis*, *Platambus*, plecoptera).
331
332 *Polycyclic aromatic hydrocarbons in brown trout*
333 Among the distinct fish organs PAH are found in liver and in much lower
334 concentrations in other tissues [8, 44, 45]. This uneven distribution constitutes a major
335 difficulty for food web studies. However, in a first approach, the concentrations in liver can be
336 considered for comparison. The clear predominance of phenanthrene in the trout livers
337 examined in the present study (Fig. 2) is consistent with the PAH profiles found in fish livers
338 of other freshwater [46] and marine systems [22, 47, 48].

For most hydrocarbons, between phenanthrene and benzo[*a*]pyrene, comparison of the PAH concentrations in liver of *S. trutta* and water with K_{ow} shows higher ratios than the expected octanol-water partitioning (Fig. 4), suggesting that food and not diffusive exchange through the gills is the likely source of PAH into fish liver. The lower abundance of indeno[*1,2,3-cd*]pyrene, benzo[*ghi*]perylene and dibenzo[*ah*]anthracene may reflect either their higher degree of metabolism degradation or lower fish bioavailability due to their larger size [16].

Comparison between lipid normalized PAHs in the main components of the fish-diet [22] with the PAH content in fish liver **(Fig. 5)** shows that most of the food organisms accumulate significantly lower concentrations of LMW PAH than *S. trutta.* In contrast, chironomid larvae and *E. lamellatus* accumulate higher concentrations of the HMW PAH than trout. *D. pulicaria* is the only main food trout component whose concentrations do not exceed in any case the amounts found in trout liver.

Calculation of the ratios between lipid normalized PAH concentrations in fish liver and food (Table 1) provides an estimation of the proportion of the PAH intake that is effectively retained in fish (Fig. 5). The PAH values for the pooled fish diet are higher than in fish liver. Having in mind that liver exhibits substantially larger concentrations than the other fish organs, the differences observed indicate that there is a metabolic transformation of PAH in fish which eliminate a substantial part of the ingested hydrocarbons. The selective enrichment of LMW PAH in fish liver when compared to the composition in food intake gives further ground to this observation.

Chironomidae and *D. pulicaria* constitute about 65% of the fish food (Table 1) [33]. The concentrations in these two organisms are lower than in trout liver. Therefore the secondary fish diet components, the organisms from the littoral zones, seem to be very important to explain the PAH levels found in the livers of fish examined in the present study.

This particular habitat may play a key role in the transfer of air transported PAH into high organisms in mountain lakes. Lake Redon is large and deep in comparison to average high mountain lakes. Thus, in this lacustrine ecosystem the difference between central and lateral environments is more significant. The relevance of the organism living in littoral zones for the incorporation of PAH into fish is therefore more apparent. In shallower high mountain lakes that difference may not be so remarkable due to the lower extend of PAH degradation during settling through short water columns.

CONCLUSIONS

Most of the food web organisms exhibit PAH distributions largely dominated by phenanthrene, which contrasts with the PAH composition in sediments, but agrees with its predominance in deposition, water and suspended particles in the water column. PAHs content increase from pelagic to littoral organisms, with intermediate values in organisms inhabiting deep sediments.

In addition to this main pattern, some specific features are related to the distinct organisms or living stages. In the pelagic habitat, *D. pulicaria* shows a relative decrease in HMW compounds with respect to the suspended particles, largely phytoplankton, that constitute its food. *E. lamellatus* shows a PAH distribution that is close to that in sediment whereas other organisms such as *Pisidium* or chironomidae larvae that also live in close contact with the sediment exhibit much higher differences. A significant change in PAH composition is observed between chironomidae larvae and pupae, indicating that metamorphism during the latter stage also involves high metabolic PAH degradation in which fluoranthene is the less affected hydrocarbon. In the littoral habitat, the organisms living

longer also show a higher proportion of fluoranthene. Bioavailabity and degradation capacities seem to vary significantly among organisms with different living ways and life cycles.

In fish, the selective enrichment of LMW PAH and the lower lipid normalized PAH concentrations in liver relative to food intake evidences a significant metabolic transformation of these hydrocarbons. Although average fish food shows higher PAH concentration than fish liver, the two main diet components, chironomidae and *Daphnia,* have lower content. Therefore, the secondary diet components, mainly the organisms from the littoral zone, play a critical role for PAH supply into fish in those mountain lakes. These secondary fish diet components may accumulate higher PAH levels in shallower lakes than the one studied therefore involving higher fish exposure to PAHs.

Acknowledgement. Financial support from the EU projects EMERGE (EVK1-CT-1999-00032) and EUROLIMPACS (GOCE-CT-2003-505540) is acknowledged. One of us, IV, thanks Generalitat de Catalunya (Catalan Autonomous Government) for a Ph.D. fellowship. Partial funding for research on the Pyrenean lakes is acknowledged to Generalitat de Catalunya. We are thankful to B.L. van Drooge (ICER-CSIC), N. García-Reyero (IBMB, CSIC), G. de Mendoza (University of Barcelona), J.C. Massabuau (University of Bordeaux 1), R. Lackner (University of Innsbruck), R. Chaler and D. Fanjul.

414 **REFERENCES**

415

416 [1] Fernández P, Vilanova R, Martínez C, Appleby P, Grimalt J. 2000. The historical record
417 of atmosferic pyrolitic pollution over Europe registered in the sedimentary PAH from
418 remote mountain lakes. *Environ Sci Technol* 24:1906-1913.
419 [2] Howsam M, Jones K. 1998. Sources of PAHs in the environment. In Neilson AH, eds, *The*
420 *Handbook of Environmental Chemistry*, Vol 3, Part I-PAHs and related compounds.
421 Springer, Berlin, Germany.
422 [3] Wania F, Mackay D. 1996. Tracking the distribution of persistent organic pollutants.
423 *Environ Sci Technol* 30:390A-396A.
424 [4] Fernández P, Vilanova R, Grimalt J. 1999. Sediment fluxes of polycyclic aromatic
425 hydrocarbons in European high altitude mountain lakes. *Environ Sci Technol* 33:3716-
426 3722.
427 [5] Fernández P, Grimalt J, Vilanova R. 2002. Atmospheric gas-particle partitioning of
428 polycyclic aromatic hydrocarbons in high mountain regions of Europe. *Environ Sci*
429 *Technol* 36:1162-1168.
430 [6] Vilanova R, Fernández P, Martínez C, Grimalt J. 2001. Polycyclic aromatic hydrocarbons
431 in remote mountain lake waters. *Water Res* 35:3916-3926.
432 [7] Lehr RE, Jerina DM. 1997. Metabolic activations of polycyclic hydrocarbons: structure
433 activity relationship. *Arch Toxicol* 39:1-6.
434 [8] Varanasi U, Stein J, Nishimoto M, Reichert W, Collier T. 1987. Chemical carcinogenesis
435 in feral fish: uptake, activation, and detoxication of organic xenobiotics. *Environ Health*
436 *Perspect* 71:155-170.
437 [9] Black J, Maccubbin A, Johnston C. 1988. Carcinogenicity of benzo(a)pyrene in rainbow
438 trout resulting from embryo microinjection. *Aquat Toxicol* 13:297-308.

439 [10] Aas E, Baussant T, Balk L, Liewenborg B. Andersen O. 2000. PAH metabolites in bile,
440 cytochrome P4501A and DNA adducts as environmental risk parameters for chronic oil
441 exposure: a laboratory experiment with Atlantic cod. *Aquat Toxicol* 51:241-258.
442 [11] http://oaspub.epa.gov/srs/Dynamic-Build.setup
443 [12] D'Adamo R, Pelosi S, Trotta P, Sansone G. 1997. Bioaccumulation and biomagnification
444 of polycyclic aromatic hydrocarbons in aquatic organisms. *Mar Chem* 56:45-49.
445 [13] Varanasi U. 1989. *Metabolism of Polyclyclic Aromatic Hydrocarbons in the Aquatic*
446 *Environment*. CRC Press, Boca Raton, FL, US.
447 [14] Collier T, Varanasi U. 1991. Hepatic activities of xenobiotic metabolizing enzyms and
448 biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental
449 contaminants. *Arch Environ Contam Toxicol* 20:462-473.
450 [15] Livingstone DR. 1998. The fate of organic xenobiotics in aquatic ecosystems:
451 quantitative and qualitative differences in biotransformation by invertebrates and fish.
452 *Comp Biochem Psysiol* 120: 43-49.
453 [16] Varanasi U, Reichert W, Stein J, Brown D, Sanborn H. 1985. Bioavailability and
454 biotransformation of aromatic hydrocarbons in benthic organisms exposed to sediment
455 from an urban estuary. *Environ Sci Technol* 19: 836-841.
456 [17] Eadie B, Faust W, Gardner W, Nalepa T. 1982. Polycyclic aromatic hydrocarbons in
457 sediments and associated benthos in Lake Erie. *Chemosphere* 11:185-191.
458 [18] Gewurtz S, Lazar R, Haffner G. 2000. Comparison of polycyclic aromatic hydrocarbon
459 and polychlorinated biphenyl dynamics in benthic invertebrates of Lake Erie, USA.
460 *Environ Toxicol Chem* 19:2943-2950.
461 [19] Ventura M, Camarero L, Buchaca T, Bartumeus F, Livingstone D, Catalan J. 2000. The
462 main features of seasonal variability in the external forcing and dynamics of a deep
463 mountain lake (Redó, Pyrenees). *J Limnol* 59:97-108.

464 [20] Hedges JI, Baldock JA, Gelinas Y, Lee C, Peterson ML, Wakeham SG. 2002. The
465 biochemical and elemental compositions of marine plankton: A NMR perspective. *Mar*
466 *Chem* 78:47-63.
467 [21] Vives I, Grimalt JO. 2002. Method for integrated analysis of polycyclic aromatic
468 hydrocarbons and organochlorine compounds in fish liver. *J Chromatogr B* 768:247-254.
469 [22] Carrera G, Fernandez P, Vilanova R, Grimalt JO. 1998. Analysis of trace polycyclic
470 aromatic hydrocarbons and organochlorine compounds in atmospheric residues by solid-
471 phase disk extraction. *J Chromatogr A* 823:189-196.
472 [23] International Agency for Research on Cancer. 1983. *Monographs on the Evaluation of*
473 *the Carcinogenic Risk of Chemicals to Humans: Polynuclear Aromatic Hydrocarbons.*
474 Vol. 32. WHO, Lyons, France.
475 [24] Escartin E, Porte C. 1999. Biomonitoring of PAH pollution in high-altitude mountain
476 lakes through the analysis of fish bile. *Environ Sci Technol* 33: 406-409.
477 [25] Gonfiantini R. 1978. Standards for stable isotope measurements in natural compounds.
478 *Nature* 271: 534-536.
479 [26] Gnaiger E, Bitterlich G. 1984. Proximate biochemical-composition and caloric content
480 calculated from elemental CHN analysis - A stoichiometric concept. *Oecologia* 62:289-
481 298.
482 [27] Vollenweider RA. 2000. Concerning calculation methods and limitations of proxy-
483 estimates of proteins, carbohydrates and lipids in crustacean zooplankton from CHN
484 analyses. *J Limnol* 59:170-178.
485 [28] Cauchie H. 2002. Chitin production by arthropods in the hydrosphere. *Hydrobiologia*
486 470: 63-96.
487 [29] Ahlgren G, Gustafsson I, Boberg M. 1992. Fatty acid content and chemical composition
488 of freshwater microalgae. *J Physio* 28:37-50.

489 [30] Fry B, Sherr EB. 1989. $\delta^{13}C$ Measurements as indicators of carbon flow in marine and
490 freshwater ecosystems. In Rundel PW, Ehleringer JR, Nagy KA, eds, *Stable Isotopes in*
491 *Ecological Research,* Springer-Verlag, New York, US, pp 196-229.
492 [31] Hecky RE, Hesslein RH. 1995. Contribution of benthic algae to lake food webs as
493 revealed by stable isotope analysis. *J N Am Benthol Soc* 14:631-653.
494 [32] France RL. 1995. Differentiation between littoral and pelagic food webs in lakes using
495 stable carbon isotopes. *Limnol Oceanogr* 40:1310-1313.
496 [33] Catalan J, Ventura M, Vives I, Grimalt JO. 2004. The roles of food and water in the
497 bioaccumulation of organochlorine compounds in high mountain lake fish. *Environ Sci*
498 *Technol* 38:4269-4275.
499 [34] Goericke R, Montoya JP, Fry B. 1994. Physiology of isotopic fractionation in algae and
500 cyanobacteria. In Lajtha K, Michener RH, eds, *Stable Isotopes in Ecology and*
501 *Environmental Science*, Blackwell Scientific Publications, Oxford, UK, pp 187-221.
502 [35] Erez J, Bouevitch A, Kaplan A. 1998. Carbon isotope fractionation by photosynthetic
503 aquatic microorganisms: experiments with *Synechococcus* PCC7942, and a simple carbon
504 flux model. *Can J Bot* 76:1109-1118.
505 [36] Waser NAD, Harrison PJ, Nielsen B, Carvert SE, Turpin DH. 1998. Nitrogen isotope
506 fractionation during the uptake and assimilation of nitrate, nitrite, ammonium, and urea by
507 a marine diatom. *Limnol Oceanogr* 43:215-224.
508 [37] Camarero L, Catalan J. 1993. Chemistry of bulk precipitation in the central and eastern
509 Pyrenees (northeast Spain). *Atmos Environ* 27:83-94.
510 [38] Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Ann Rev Ecol Syst*
511 18:293-320.

512 [39] Zah R, Burgherr P, Bernasconi SM, Uehlinger U. 2001. Stable isotope analysis of
513 macroinvertebrates and their food sources in a glacier stream. *Freshwater Biol* 46:871-
514 882.
515 [40] Vander Zanden MJ, Rasmussen JB. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic
516 fractionation: Implications for aquatic food web studies. *Limnol Oceangr* 46:2061-2066.
517 [41] Vilanova R. 2000. Study of environmental change from the analysis of persistent organic
518 pollutants in European high mountain lakes. Ph.D. thesis. University of Barcelona.
519 Barcelona. Catalonia. Spain.
520 [42] Mackay D, Ying Shiu W, Ching Ma K. 1992. *Illustrated Handbook of Physical-*
521 *Chemical Properties and Environmental Fate for Organic Chemicals*, 1rst edition edition.
522 Lewis Publishers. Chelsea, MI, USA.
523 [43] Fernández P, Carrera G, Grimalt JO, Ventura M, Camarero L, Catalan J, Nickus U, Thies
524 H, Psenner R. 2003. Factors governing the atmospheric deposition of polycyclic aromatic
525 hydrocarbons to remote areas. *Environ Sci Technol* 37:3261-3267.
526 [44] Vanarasi V, Stein JE. 1991. Disposition of xenobiotic chemicals and metabolites in
527 marine organisms. *Environ Health Perspect* 90:93-100.
528 [45] Hellou J, Warren WG. 1997. Polycyclic aromatic hydrocarbons and saturated
529 hydrocarbons in tissues of flatfish: Insight on environmental exposure. *Mar Environ Res*
530 43:11-25.
531 [46] Pointet K, Milliet A. 2000. PAHs analysis of fish whole gall bladders and livers from the
532 Natural Reserve of Camargue by GC/MS. *Chemosphere* 40: 293-299.
533 [47] Baumard P, Budzinski H, Garrigues P, Sorbe J, Burgeot, T, Bellocq J. 1998.
534 Concentrations of PAHs (Polycyclic Aromatic Hydrocarbons) in various marine
535 organisms in relation to those in sediments and to trophic level. *Mar Pollut Bull* 36:951-
536 960.

537 [48] van der Oost R, Beyer J, Vermeulen N. 2003. Fish bioaccumulation and biomarkers in
538 environmental risk assessment: a review. *Environ Toxicol Pharmacol* 13**:**57-179.
539

FIGURE CAPTIONS

FIGURE 1. $\delta^{13}C$ and $\delta^{15}N$ composition of the organism analyzed for polycyclic aromatic hydrocarbons in this study (Lake Redon, Pyrenees).

FIGURE 2. Relative composition of polycyclic aromatic hydrocarbons (%) in the main food web components of Lake Redon (Pyrenees). 1, fluorene; 2, phenanthrene; 3, anthracene; 4, fluoranthene; 5, pyrene; 6, benz[*a*]anthracene; 7, chrysene+triphenylene; 8, benzo[*b*]fluoranthene; 9, benzo[*k*]fluoranthene; 10, benzo[*a*]pyrene; 11, indeno[*1,2,3-cd*]pyrene; 12, benzo[*ghi*]perylene; 13, dibenzo[*ah*]anthracene.

FIGURE 3. Principal component analysis of the content of polycyclic aromatic hydrocarbons (PAH) of the food web organisms from Lake Redon (Pyrenees). The concentrations of PAH (Table 2) were root-squared transformed to decrease the influence of the most abundant compounds in the ordination. The first two principal components accounted for 97.6% of the total variance. Fl, fluorene; Phe, phenanthrene; A, anthracene; Fla, fluoranthene; Py, pyrene; BaA, benz[*a*]anthracene; Chr, chrysene; BFlas, benzo[*b*+*k*]fluoranthenes; BaPy, benzo[*a*]pyrene; IndPy, indeno[*cd*-1,2,3]pyrene; BPer, benzo[*ghi*]perylene; DibahA, dibenz[*ah*]anthracene; LMW, PAH between fluorene and pyrene; HMW, PAH between benz[*a*]anthracene and dibenz[*ah*]anthracene. Littoral habitat organisms (squares): Arc, *Arcynopterix compacta;* Sip*, Siphonoperla torrentium,* Sia, *Sialis lutaria,* Pla, *Platambus maculatus,* Rad, *Radix ovata* and Nos, *Nostoc* sp. Deep sediment organisms (diamonds), Chl, Chp, chironomidae larvae and pupae; Eur, *Eurycercus lamellatus* and Pis, *Pisidium.* Pelagic organism (circle): Dap, *Daphnia pulicaria.* Top predator (star): Sal, *Salmo trutta*.

563 **FIGURE 4.** Comparison between the octanol-water partition coefficients [43] and the ratios
564 between lipid normalized polycyclic aromatic hydrocarbons in *S. trutta* (liver), *D.*
565 *pulicaria* and water (dissolved phase) [6].
566 **FIGURE 5.** Representation of the lipid normalized concentrations of polycyclic aromatic
567 hydrocarbons in fish diet, c*hironomidae* (larva and pupa), *D. pulicaria* and *E. lamellatus*
568 by reference to the levels in fish liver. The horizontal line gives the reference for
569 compounds in higher or lower lipid normalized concentrations than in fish liver. Numbers
570 in abscissas refer to the PAH list indicated in the caption of Figure 2.

Table 1. Estimated annual averaged brown trout diet in Lake Redon (Pyrenees)

Diet component	Contribution (% volume)
Chironomidae (pupae)	33.7
Daphnia pulicaria	20.1
Chironomidae (larvae)	12.6
Sialis lutaria	4.6
Terrestrial insects	4.3
Eurycercus lamellatus	5.6
Radix ovata	4.7
Platambus maculatus	2.5
Pisidium sp.	1.0
Siphonoperla torrentium	0.3
Nostoc sp.	0.2
Other organisms	6.8
Unidentifiable material	0.7

Table 2. Lipid content (%) and concentrations of individual polycyclic aromatic hydrocarbons (PAH) in each species analyzed (ng/g dw). Fl, fluorene; Phe, phenanthrene; A, anthracene; Fla, fluoranthene; Py, pyrene; BaA, benz[*a*]anthracene; Chr, chrysene; BFlas, benzo[*b*+*k*]fluoranthenes; BaPy, benzo[*a*]pyrene; IndPy, indeno[*cd*-1,2,3]pyrene; BPer, benzo[*ghi*]perylene; DibahA, dibenz[*ah*]anthracene; Low molecular weight (LMW) PAH encompass between fluorene and pyrene; High molecular weight (HMW) PAH encompass between benz[*a*]anthracene and dibenz[*ah*]anthracene.

Species	Lipid content	Fl	Phe	A	Fla	Py	BaA	Chr	BFlas	BaPy	IndPy	BPer	DibahA	total PAH	LMW	HMV	total PAH per lipid
Littoral habitat																	
Nostoc sp.	1.7	3.6	31	1.8	3.5	2.9	0.08	0.36	0.52	0.23	0.15	0.18	0.15	44	43	1.7	2616
Radix ovata	0.6	6.7	46	3.4	5.6	4.4	0.13	0.54	1.1	0.31	0.55	0.63	<LOD[a]	69	66	3.3	11514
Platambus maculatus	9.9	93	310	31	140	80	14	43	23	12	25	18	<LOD	790	650	140	7976
Sialis lutaria	21.1	41	114	16	87	3.3	2.5	7.9	2.9	2.0	3.7	7.4	<LOD	400	370	26	1885
Arcynopterix compacta	26.5	92	268	26	117	7.1	4.2	35	14	11	20	14	<LOD	610	510	98	2299
Siphonoperla torrentium	37.7	117	340	34	162	97	13	74	<LOD	<LOD	37	28	<LOD	900	750	150	2393
Deep sediment habitat																	
Eurycercus lamellatus	37.9	23	92	9.3	65	45	18	55	42	24	18	14	11	420	230	180	1099
Pisidum sp.	13.2	14	45	4.9	22	13	2.2	12	9.0	1.9	4.6	3.8	<LOD	130	100	34	1012
Chironomidae larvae	30.4	19±6.7	110±21	11±3.2	39±38	24±26	4.3±15	19±41	25±11	7.9±7.8	9.6±16	9.8±9.1	13±10	290±70	200±50	89±21	956
Chironomidae pupae	39.9	10±10	12±7.5	3.0±2.9	36±45	3.9±1.4	1.2±3.2	4.8±9.6	3.5±3.1	2.2±4.0	2.6±4.8	2.5±3.9	0.24±0.14	85±35	68±35	17±8	205
Pelagic habitat																	
Daphnia pulicaria	54.4	1.4	10	0.74	2.2	1.1	0.22	0.54	0.72	0.31	0.21	0.28	<LOD	18	16	2.3	33
Top predator																	
Salmo trutta	4.6[b]	9.8±3.4	47±16	3.1±1.9	8.7±5.2	7.5±3.7	0.6±0.6	2.4±1.6	1.5±0.65	0.55±0.60	0.15±0.10	0.20±0.20	0.20±0.15	82±20	76±16	5.6±1.2	1776

[a] <LOD. Below limit of detection (0.1-0.14 ng/g dw). [b]Lipid content in liver (in muscle 3%)

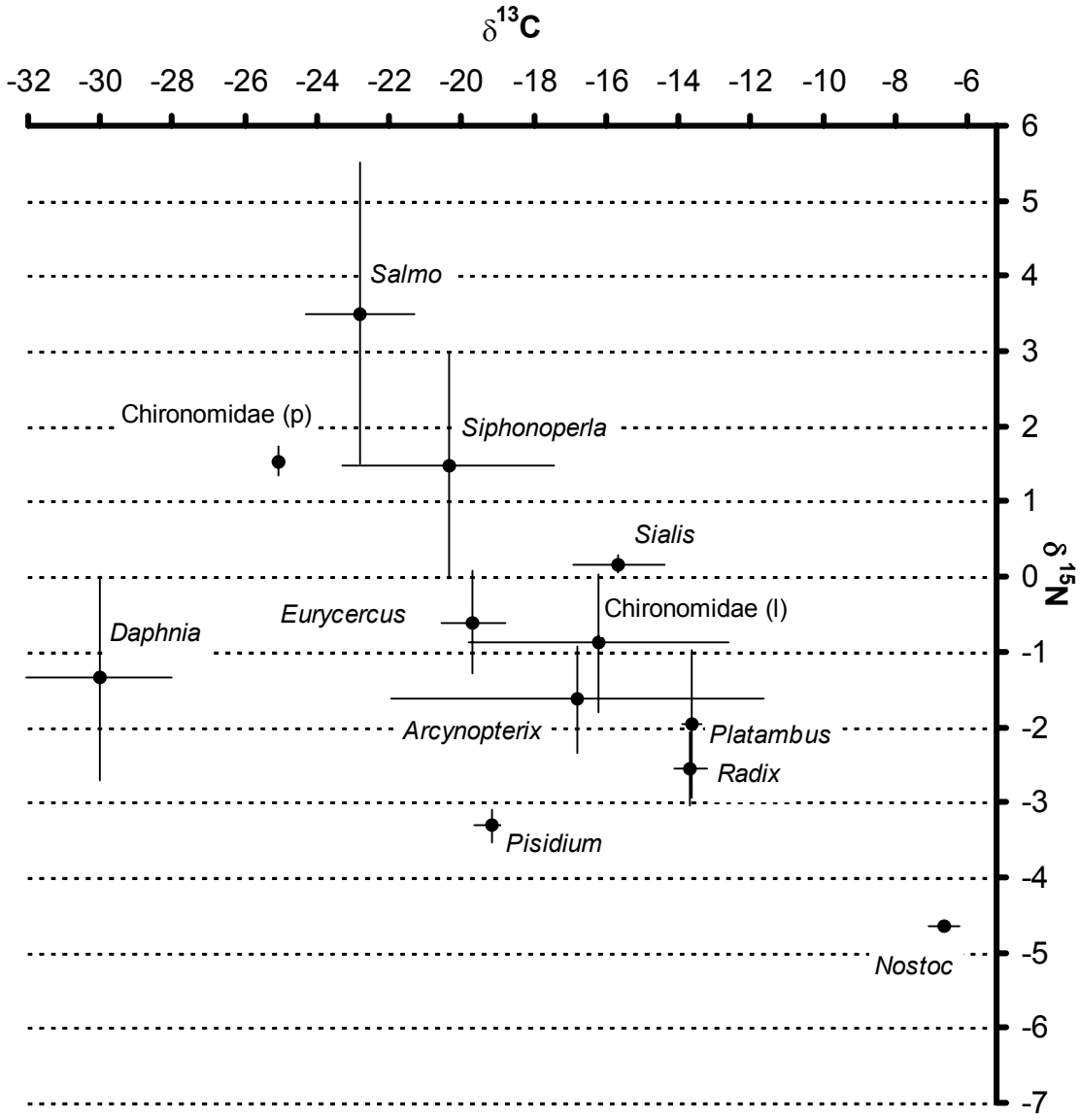

δ13C
-32 -30 -28 -26 -24 -22 -20 -18 -16 -14 -12 -10 -8 -6
6 5 4 3 2 1 0 -1 -2 -3 -4 -5 -6 -7
δ15N
Salmo
Chironomidae (p)
Siphonoperla
Sialis
Daphnia
Eurycercus
Chironomidae (l)
Arcynopterix
Platambus
Radix
Pisidium
Nostoc

Figure 1

Figure 2

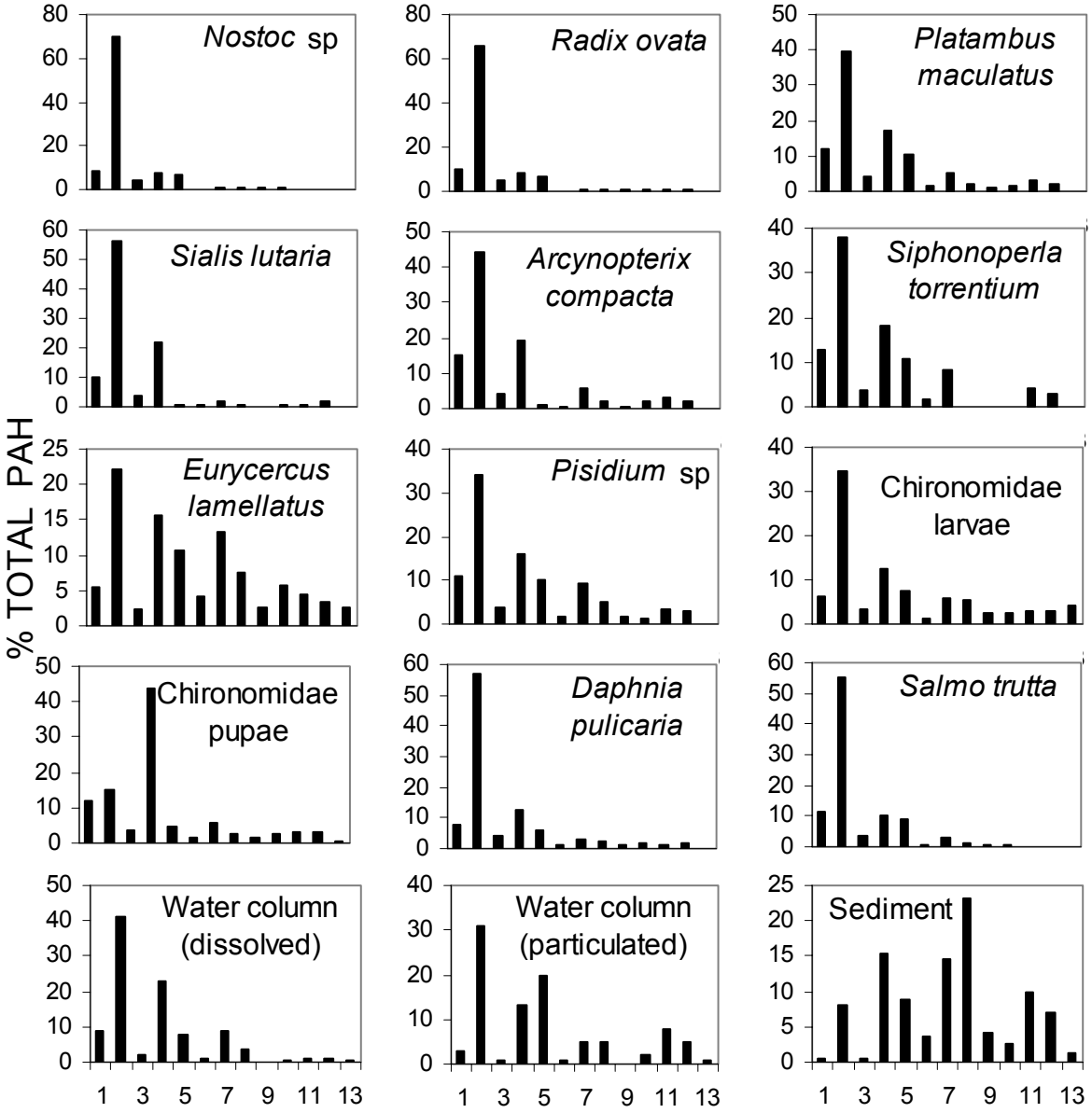

% TOTAL PAH
Nostoc sp
Radix ovata
Platambus maculatus
Sialis lutaria
Arcynopterix compacta
Siphonoperla torrentium
Eurycercus lamellatus
Pisidium sp
Chironomidae larvae
Chironomidae pupae
Daphnia pulicaria
Salmo trutta
Water column (dissolved)
Water column (particulated)
Sediment

Figure 3

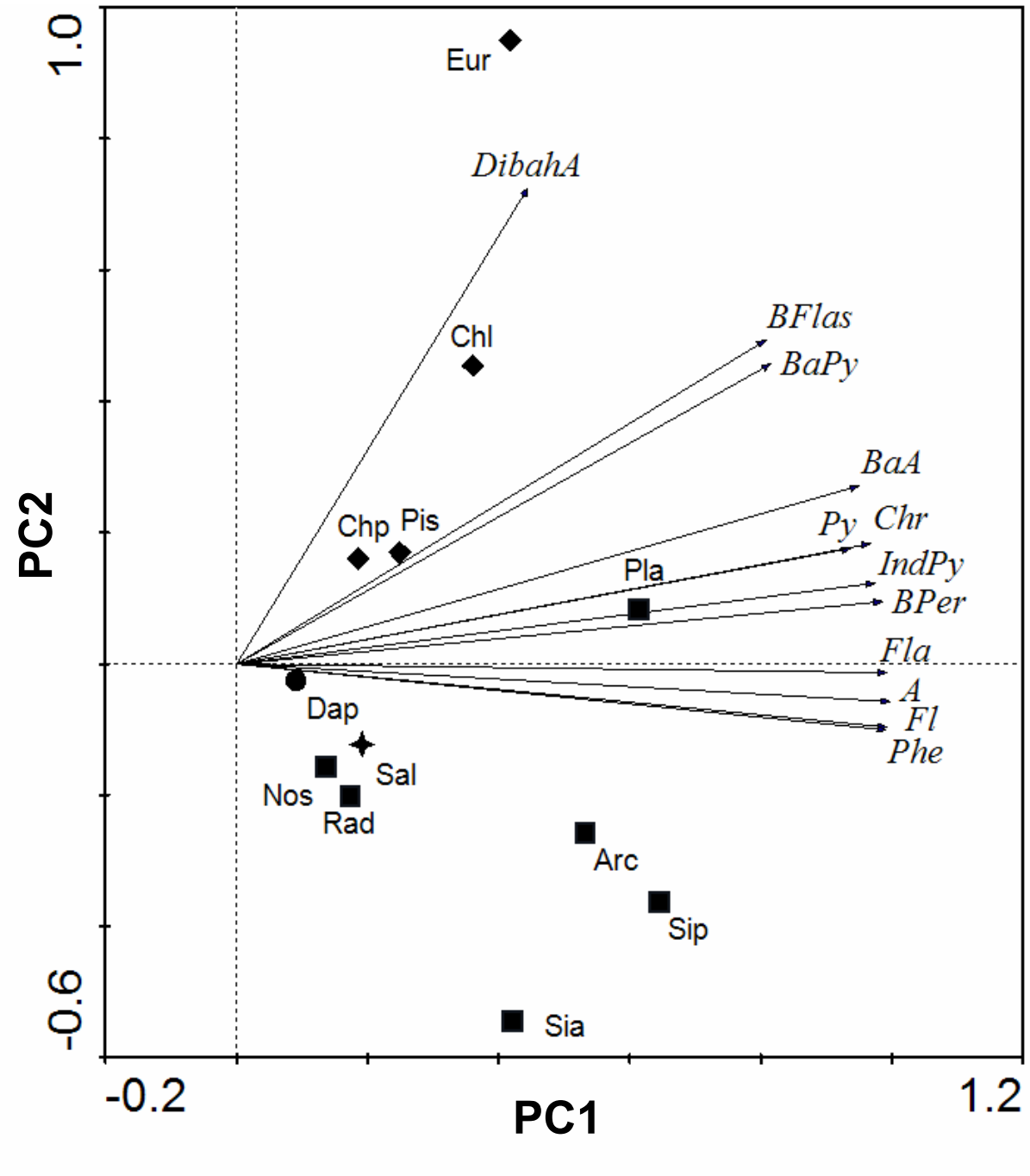

1.0
-0.6
PC2
-0.2
1.2
PC1
Eur
DibahA
Chl
BFlas
BaPy
BaA
Chp
Pis
Py
Chr
Pla
IndPy
BPer
Fla
A
Dap
Fl
Phe
Sal
Nos
Rad
Arc
Sip
Sia

Figure 4

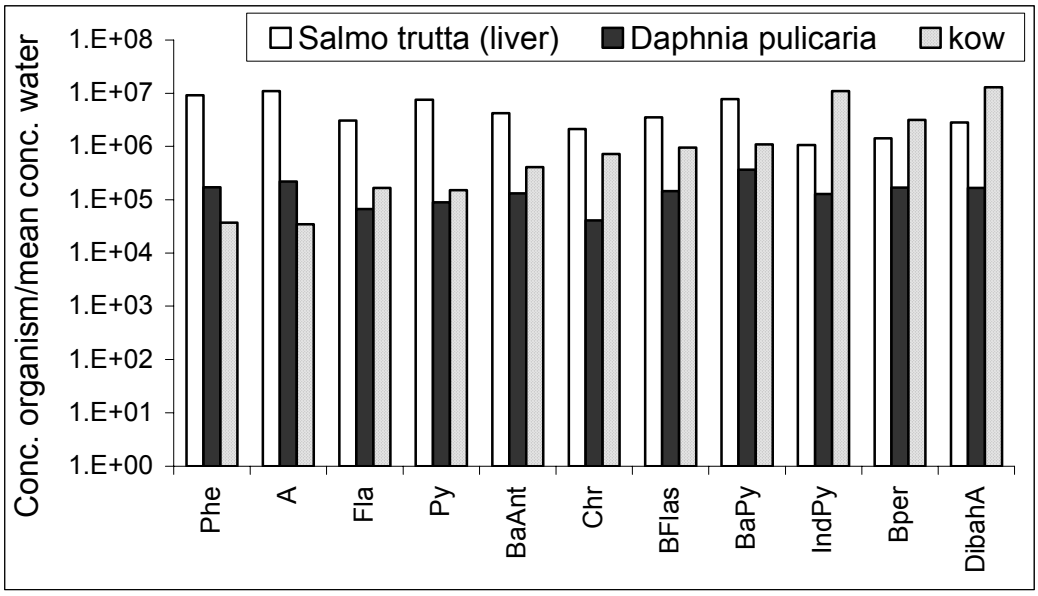
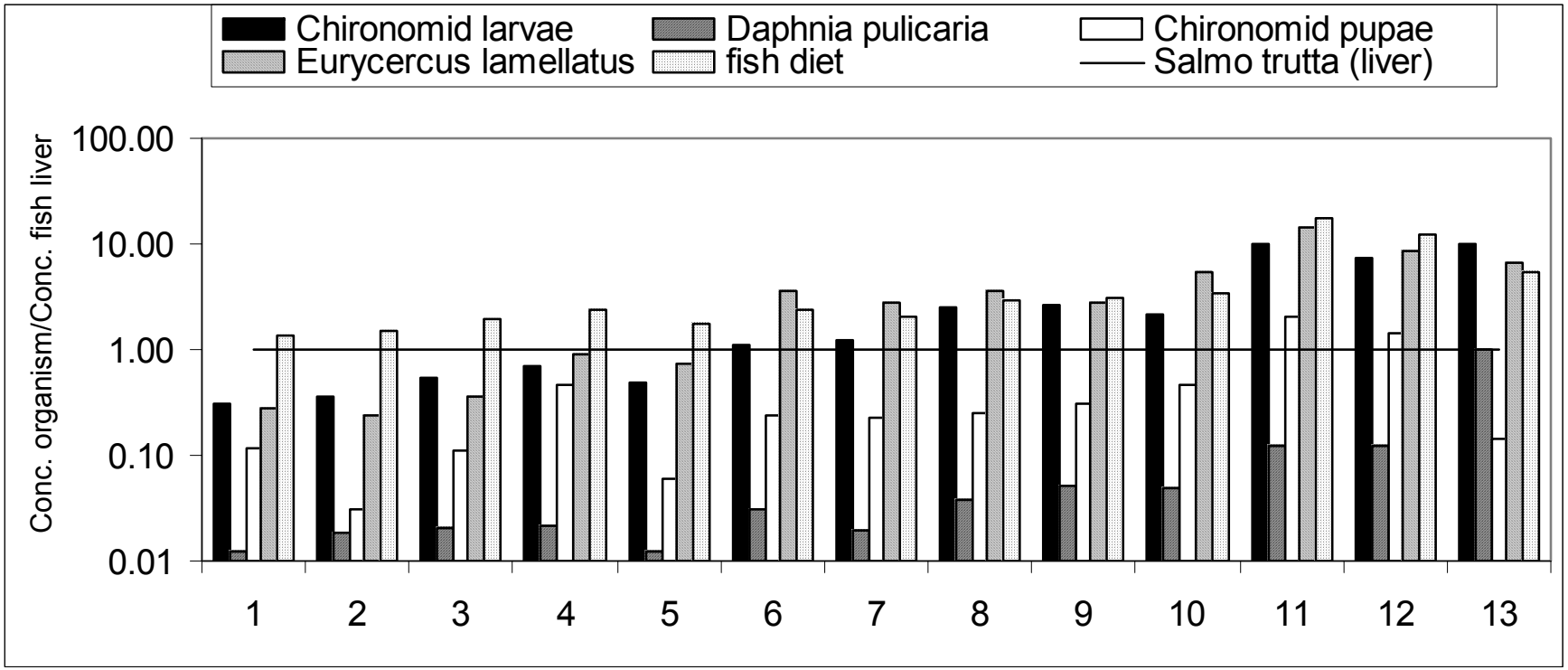

Salmo trutta (liver)
Daphnia pulicaria
kow
Conc. organism/mean conc. water
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
Phe
A
Fla
Py
BaAnt
Chr
BFlas
BaPy
IndPy
Bper
DibahA

Figure 5


Chironomid larvae
Daphnia pulicaria
Chironomid pupae
Eurycercus lamellatus
fish diet
Salmo trutta (liver)
Conc. organism/Conc. fish liver
100.00
10.00
1.00
0.10
0.01
1
2
3
4
5
6
7
8
9
10
11
12
13

Environ. Sci. Technol. **2004**, *38,* 4269−4275

The Roles of Food and Water in the Bioaccumulation of Organochlorine Compounds in High Mountain Lake Fish

JORDI CATALAN* AND MARC VENTURA

Center for Advanced Studies of Blanes (CEAB-CSIC), Accés Cala St. Francesc, 14, 17300-Blanes, Catalonia, Spain

INGRID VIVES AND JOAN O. GRIMALT

Institute of Chemical and Environmental Research (ICER-CSIC), Jordi Girona, 18, 08034-Barcelona, Catalonia, Spain

An integrated study encompassing the distribution of organochlorine compounds (OC) in water, food web (chironomids, terrestrial insects, cladocerans, mollusks, and cyanobacteria), and fish (brown trout) from a high mountain lake (Redon, Pyrenees) is reported. OC distributions in these compartments have been determined to assess their transport routes into fish. Food diets have been estimated by analysis of fish stomach content and food web stable isotopes (δ^{13}C and δ^{15}N). OCs with octanol−water partition coefficient (K_{ow}) higher than 10^6 showed lower concentrations in food than expected from theoretical octanol−water partition, indicating that the distribution of these compounds does not reach equilibrium within the life span of the food web organisms (ca. 1 year). On the other hand, the degree of biomagnification in fish increased with K_{ow}, except in the case of the largest compound analyzed (seven chlorine substituents, PCB #180). OC exchange at fish gill and gut has been evaluated using a fugacity model based on the water, food, and fish concentrations. All compounds exhibited a net gill loss and a net gut uptake. A pseudostationary state was only achieved for compounds with $\log(K_{ow}) < 6$. Calculation of fish average residence times for the compounds in apparent steady state gave values of days to a few weeks for HCHs, 1 year for HCB and 4,4′-DDE, and 2−3 years for 4,4′-DDT and PCB#28 and PCB#52. Residence times longer than one decade were found for the more chlorinated PCB.

Introduction

Chemical pollutants show, in general, higher concentrations at locations closer to the emission sites. However, persistent organic pollutants including some organochlorine compounds (OCs) are found in remote areas without a significant dilution effect. Natural distillation and condensation processes concurrent with atmospheric transport lead to their accumulation in ecosystems and organisms of high latitudes (*1*−*4*) or high elevations (*5*). OCs are mobilized in areas of warm temperatures (ca. mean annual temperature > 5 °C (*6*)). The more volatile compounds, such as hexachlorobenzene (HCB), hexachlorocyclohexanes (HCH), and low chlorinated polychlorobiphenyls (PCBs), show high accumulation in cold areas located beyond 60° N, with mean annual air temperatures below −5 °C (*6*). In contrast, the less volatile compounds, such as more chlorinated PCBs (subcooled liquid vapor pressure $<10^{-2.5}$ Pa) and DDTs, are also selectively trapped in mountain cold areas (*5*), which do not reach such low temperatures as the Arctic zone.

Mountain lakes are relatively small in size and very oligotrophic (*7*), food is scarce, food webs are short, and fish show an opportunistic behavior related to the seasonal availability of food. Unfortunately, the knowledge of the food-web pathways to fish in these environments is scarce (*8*), and no data on OCs distribution in fish food components are available.

In the present paper we report an assessment of the food pathways to brown trout in a mountain lake using stable isotopes, diet evaluation, and OC content of the food web. Concentrations of OC in food and fish are also compared to the theoretical values expected from their bioconcentration from water levels. Finally, a fugacity model based on the measured OC concentrations in water, food, and fish has been used to evaluate the roles of the gill and gut exchanges. The results are discussed in the context of present knowledge of OC bioaccumulation in fish from high mountain lakes.

Materials and Methods

Study Site. Lake Redon (42°38′N, 0°46′E) is situated at 2240 m above sea level in Central Pyrenees (Catalonia, Spain). It has a surface area of 24 ha, a maximum depth of 73 m, a mean water residence time of about 4 yr, and is usually ice-covered from late December to June (*9*). The lake is oligotrophic because most of its small watershed (155 ha) is bare rock, and the rest are alpine meadows with scarcely developed soils. The productivity patterns and seasonal changes in the water column are typical for high mountain lakes (*7*). The lake contains a population of brown trout (*Salmo trutta*) (*10*), from which specimens up to 15 years have been collected (*11*). OC inputs are only related to atmospheric deposition (*12*). The composition of OC in the waters (*13, 14*), sediment, and fish (*5*) of this lake has been described in previous studies.

Sample Collection and Handling. Fish were collected with a series of eight individual bottom gillnets of different mesh sizes (10−46 mm) designed to give the best theoretical catch of brown trout over a range of 10−45 cm. The nets were set perpendicular to the shore at various depths and exposed in the lake for 120 min just at sunrise and sunset. All fish were measured, dissected, and determined for sex on site. Muscle fillets and stomach contents were wrapped in precleaned aluminum foil and kept frozen (−20 °C) until analysis. Brown trout analyzed for OCs ($n = 10$) averaged (mean ±SD) 265 ± 59 mm in length, 204 ± 118 g in weight, 0.99 ± 0.09 in condition factor, and 7 ± 6 years in age.

A survey of the main food chain lake components was carried out in parallel during the same days of fish sampling. Animals were collected from distinct parts of the lake by kick sampling for littoral organisms, Ekman dredges for sediment species, and plankton nets for zooplankton. Samples were kept cold during transport and were later identified and separated in the lab into distinct classes for stable-isotope and OC analysis.

Brown Trout Diet Evaluation. Gut contents were isolated in the field and kept cold until arrival to the lab where they were then analyzed under a dissecting microscope. The food content was determined mostly up to genus or family level, and the relative percentage was estimated on volume basis

* Corresponding author phone: 34 972 33 61 01; fax: 34 972 33 78 06; e-mail: catalan@ceab.csic.es.

10.1021/es040035p CCC: $27.50
Published on Web 07/15/2004

for each fish stomach. The degree of stomach fullness was categorized between 0 (empty) and 5 (full).

Dry Weight and Lipid Content. The percentage of water content in muscle (74.2 ± 1.8%, $n = 8$) was estimated by drying in a vacuum sealed-drier at 20 °C until constant weight. The brown trout lipid content in muscle was determined gravimetrically after extraction with hexane:dichloromethane (4:1, v/v). Lipid content in food web organisms was estimated from C and N elemental analysis assuming that the main body constituents were lipids, proteins, ash, and chitin (*15*). Lipid percentage was estimated from elemental C content after subtraction of protein, ash, and chitin weight. Protein content was calculated from elemental N composition after multiplying by 6.25. Literature values were used for ash (*16*, *17*) and chitin content (*18*). Since cyanobacteria use carbohydrates as energy reserve, the lipid content for *Nostoc* was taken from the literature (*19*).

Stable Isotope Analysis. Stable isotope ratios were analyzed using a Delta C Finnigan MAT mass spectrometer coupled online with a Carlo Erba CHNS elemental analyzer, via a Finnigan conflo 2 interface. Atmospheric nitrogen and Peedee Belemnite carbonate were used as reference. Reproducibility was better than 0.1‰ and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

Organochlorine Compounds Analysis. Fish muscle tissues (5 g wet weight, about 0.75 g dry weight) were extracted and analyzed for OCs as described elsewhere (*20*). OCs in invertebrates and *Nostoc* were determined by grouping individuals in common samples until enough material was accumulated for proper quantification. Combination was carried out at the species level when enough material was available. Organisms that were too small or scarce in the lake were grouped at a higher taxonomical level, and species were composite based on common feeding habits, way of living, and exposure to fish predation (Table 1). Samples were analyzed following a slightly modified method as previously described (*21*). The isolated OC fractions of fish and food web were analyzed in a gas chromatograph equipped with an electron capture detection (GC-ECD; Hewlett-Packard 5890 Series II) and a 50 m × 0.25 mm i.d. DB-5 capillary column coated with 5% phenyl 95% methylpolysiloxane (film thickness 0.25 μm; J&W Scientific, Folsom, CA). The injector operated in splitless mode, and the oven temperature program started at 90 °C (held for 1 min), to 120 °C at 10 °C/min, and then to 310 °C at 4 °C/min (holding time 15 min). Injector and detector temperatures were 270 °C and 310 °C, respectively. Stringent precautions were observed for maintenance of the injector under clean conditions avoiding adsorptions that could deviate the system from linearity and increase the limits of detection and quantification. Helium and nitrogen were used as carrier (0.33 mL/min) and makeup (60 mL/min) gases, respectively. Some samples were examined by negative ion chemical ionization mass spectrometry coupled to gas chromatography (GC-MS-NICI) for structural identification. These analyses were performed in an Agilent Technologies 6890A gas chromatograph equipped with a nonpolar fused silica capillary column HP5-MS (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with 5% phenyl 95% methylpolysiloxane and coupled to a MS detector 5973N. Ion source and transfer line temperatures were 150 and 280 °C, respectively. Ammonia was chosen as ionization gas (1.6 Torr). Helium was used as carrier gas (1.1 mL/min). Procedural blanks were analyzed for every set of six samples. The recovery of the surrogate standards (tetrabromobenzene and PCB #209) was calculated for each sample. Identification and quantification of all studied compounds were performed by injection of external standards at different concentrations. Relative responses to tetrachloronaphthalene and octachloronaphthalene were used in order to correct for instrumental

TABLE 1. Fish Food-Web Components Analyzed for Organochlorine Compounds[a]

operative food-web components	group	habitat	size (mm)	age (years)	food type	feeding mode	comments
Salmo trutta (brown trout)	chordata, fishes	pelagic and littoral	250	7	macroinvertebrates	predator	
Arcynopterix compacta	insecta, plecoptera	benthic	28	2−3	macroinvertebrates	predator	
Siphonoperla torrentium	insecta, plecoptera	benthic	10	1−2	macroinvertebrates	predator	
Sialis lutaria	insecta, megaloptera	benthic	22	2−3	macroinvertebrates	predator	
Polycentropodidae	insecta, trichoptera	benthic	18	1−2	macroinvertebrates	predator	mainly *Plectrocnemia*
Platambus maculatus	insecta, coleoptera	benthic	8	2−3	macroinvertebrates	predator	larvae
Chironomidae (larvae)	insecta, diptera	benthic	6	1−2	algae, debris	collector-gathered	several species but all noncarnivorous
Chironomidae (pupae)	insecta, diptera	pelagic	10		none	nonfeeding stage	mainly Diamesinae
Daphnia pulicaria	crustacea, cladocera	planktonic	2	<0.5	phytoplankton, bacteria	collector-filterer	
Eurycercus lamellatus	crustacea, cladocera	littoral	2.5	<1	algae, debris	collector-gathered	
Radix ovata	mollusca, gastropoda	littoral	6	>1	algae, debris	scraper	
Pisidium sp.	mollusca, bivalvia	littoral	3	>1	phytoplankton	collector-filterer	
Nostoc sp.	cyanobacteria	epilithic	5		autotroph	photosynthesis	included because found in the fish stomachs

[a] The degree of taxonomic resolution was conditioned by the amount of available material for analyses.

TABLE 2. Brown Trout Diet in Lake Redon during Two Distinct Periods of the Ice-Free Season (June and November)[a]

food requirement period	frequency in stomachs (%)		food volume (%)		
	high	low	high	low	annual average
Chironomidae (pupae)	71.4	0	55.9	0	33.7
Daphnia pulicaria	4.8	45.5	3.1	45.8	20.1
Chironomidae (larvae)	38.1	27.3	14.0	10.5	12.6
Sialis lutaria	23.8	18.2	4.7	4.6	4.6
Terrestrial insects	33.0	5.0	5.6	2.3	4.3
Eurycercus lamellatus	0.0	32.0	0.0	14.1	5.6
Radix ovata	0.0	27.0	0.0	11.7	4.7
Polycentropodidae	5.0	0.0	4.8	0.0	2.9
Platambus maculatus	10.0	0.0	4.1	0.0	2.5
Pisidium sp.	29.0	14.0	0.5	1.7	1.0
Siphonoperla torrentium	5.0	0.0	0.6	0.0	0.3
Nostoc sp.	0.0	9.0	0.0	0.6	0.2
other organisms	33.0	18.0	6.5	7.2	6.8
unidentifiable material	5.0	45.0	0.2	1.5	0.7

[a] Commonness in the diet is indicated by the frequency that a certain item was found in the stomachs examined ($n = 21$ and 22, respectively, for high and low food periods). Contribution to the diet is indicated by the percentage of food volume, obtained by weighting percentages of food volume in individual stomachs to the degree of stomach fullness. Annual average contribution to diet was calculated assuming 4 months of high requirements, 4 months of low, and 4 months of very low consumption (stomach fullness <20%) during the ice covered period with a diet similar to the low food requirement period of the ice-free season. Other reasonable assumptions (i.e., different monthly periods for each type of consumption) do not significantly change the average values.

variabilities, and this value was also corrected by the recovery of the surrogate standards.

Results and Discussion

Brown Trout Diet. Sampling was performed in June and November 2000 to cover two extremes of the fish feeding variability during the ice-free period. The availability of some food components (e.g. chironomids) decreases with water temperature as autumn advances. Daily energy (food) requirements also decrease in parallel. These 2 months are therefore representative cases of high and low food demand and availability, respectively. In June, when larvae of aquatic insects were abundant, the average value of the stomach fullness index was 4.4, and no fish with empty stomach was found. In November, during lake overturn and low water temperature, the average value of the index was 1.9, and 9% of the fish had empty stomach. The food items found in the stomachs and their relative contribution were also distinct in the two periods (Table 2). In June, chironomids, either larvae or pupae, were by large the most frequent and abundant. Other organisms, such as terrestrial insects, the bivalve *Pisidium*, or the megaloptera *Sialis*, were often found, but their contribution to the food volume was low. In some stomachs chironomid pupae were nearly the sole content, probably because this transient and passive stage facilitates the capture by trout. In November, the more frequent and abundant food components were cladocerans, the pelagic *Daphnia*, and the littoral *Eurycercus*. However, they were less dominant that chironomids in June. Other items were also relevant either in abundance or frequency (e.g. chironomids, *Radix*, *Pisidium*, *Sialis*). During this period, the number of unidentifiable items increased, although their contribution to food volume remained very low. Unexpected food items such as colonies of the cyanobacterium *Nostoc* were also found.

These two snapshots of the trout diet at contrasting periods of the year suggest that these fish mainly feed on chironomids when they are abundant and on alternative prey, particularly small cladocerans such as *Daphnia*, when they are scarce. The large variety of other food items is complementary.

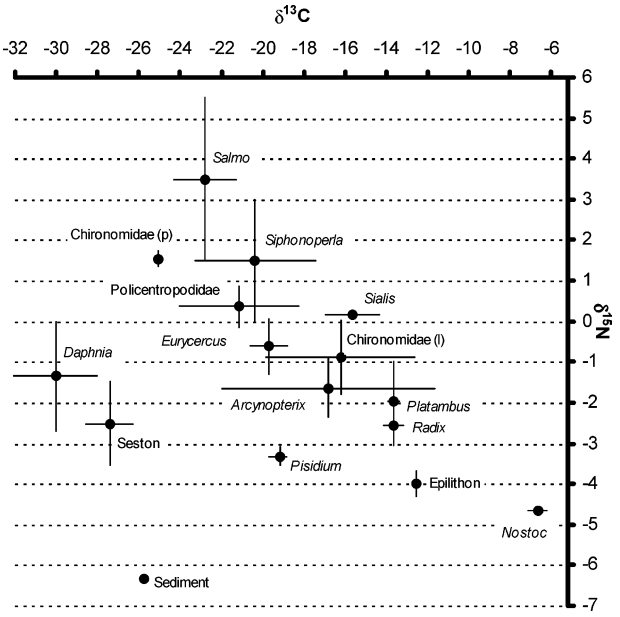


FIGURE 1. Isotopic signature of the main food-web components in Lake Redon. In chironomidae, (p) states for pupae and (l) for larvae. Sample description in Table 1.

Isotope Structure of the Food Web. Differences in carbon and nitrogen stable isotope ratios between the distinct food web components provide information on trophic relationships. Commonly used trophic fractionation values are 1‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ (*22*, *23*), which are similar to mean values found in recent studies of the variation in the trophic fractionation (0.05 ± 0.63‰ $\delta^{13}C$, 3.49 ± 0.23‰ $\delta^{15}N$) (*24*). Due to its lower trophic fractionation, carbon is considered to indicate primary energy sources (e.g. benthic vs pelagic photosynthesis), and nitrogen is used for the discrimination among trophic levels.

The isotopic signatures of the organisms and primary carbon sources involved in the food web pathways to fish in Lake Redon are shown in Figure 1. The main primary carbon sources—namely littoral (epilithon and *Nostoc*), pelagic (seston), and organic detritus (sediment)—showed significantly distinct isotopic signatures. The $\delta^{13}C$ depletion was larger in seston and deep sediment than in littoral algae, which may reflect the predominant occurrence of primary production in the hypolimnion, below the seasonal thermocline, due to the extreme transparency of the water column (*9*). Growing temperature in the hypolimnion is significantly lower than in the littoral (ca. 5−10 °C), and available CO_2 has a larger contribution from within lake respiration (*25*). On the other hand, benthic algae tend to be enriched in ^{13}C, due to a boundary layer effect, involving a limitation of CO_2 diffusion to the cells that favors the use of bicarbonate as carbon substrate (*26*).

Fractionation during nitrogen assimilation by algae (phytoplankton and phytobenthos) can be −4 to −5‰ (*27*). In our data, pooled epilithon (mainly diatoms) and *Nostoc* agreed with these values assuming nitrogen sources close to 0‰ $\delta^{15}N$ as expected from its predominant atmospheric origin. However, seston was slightly richer in the heavy isotope than epilithon, pointing to a mixture of phytoplankton and allocthonous matter in the former. Since *Daphnia*, a planktonic cladoceran mainly feeding on phytoplankton, had a $\delta^{15}N$ similar to other herbivores feeding on littoral algae, a common $\delta^{15}N$ baseline for the herbivore food web around −4‰ was assumed.

TABLE 3. Organochlorine Concentrations (ng g^{-1} Dry Weight) in the Most Significant Organisms Involved in the Brown Trout Diet in Lake Redon (Pyrenees)

Taxa	lipid content (%)	ng g^{-1} dry weight											
		α-HCH	γ-HCH	HCB	4,4′-DDE	4,4′-DDT	PCB-28	PCB-52	PCB-101	PCB-118	PCB-153	PCB-138	PCB-180
Salmo trutta (muscle)	2.8	0.57	3.54	2.40	57.23	4.19	0.72	1.93	2.82	1.52	9.88	8.52	5.86
Chironomidae (pupae)	39.9	1.72	16.29	9.18	275.08	18.76	0.93	1.92	6.45	5.36	22.21	14.43	20.50
Daphnia pulicaria	54.4	0.03	0.16	0.20	0.41	0.11	0.31	0.46	0.11	0.03	0.08	0.08	0.03
Chironomidae (larvae)	30.4	0.17	6.05	3.08	58.56	4.98	0.90	1.94	2.33	1.02	4.35	3.32	3.18
Sialis lutaria	21.1	0.17	9.38	1.58	14.40	4.75	0.97	0.67	2.38	2.12	6.77	4.92	4.22
Eurycercus lamellatus	37.9	0.49	5.19	1.98	60.66	4.34	2.75	1.82	3.07	1.98	2.90	1.64	0.83
Radix ovata	0.6	0.02	0.19	0.23	0.20	0.00	0.47	0.41	0.18	0.08	0.11	0.10	0.10
Polycentropodidae	29.8	0.97	6.79	2.65	3.04	1.43	0.94	1.50	2.15	2.27	5.41	3.52	3.72
Platambus maculatus	9.9	0.00	14.65	2.94	21.00	4.95	1.76	9.34	8.56	3.18	1.72	1.40	2.35
Pisidium sp.	13.2	0.04	1.24	0.87	10.08	1.72	0.73	3.05	2.85	0.97	0.60	0.98	0.13
Siphonoperla torrentium	37.7	0.51	19.26	4.60	25.54	11.29	3.60	14.15	9.41	5.63	2.64	3.05	1.24
Nostoc sp.	1.7	0.03	0.22	0.18	0.10	0.00	0.55	0.25	0.10	0.03	0.01	0.03	0.03
Arcynopterix compacta	26.5	0.99	19.70	3.69	18.17	7.77	4.82	21.72	15.00	7.05	6.77	5.69	4.82

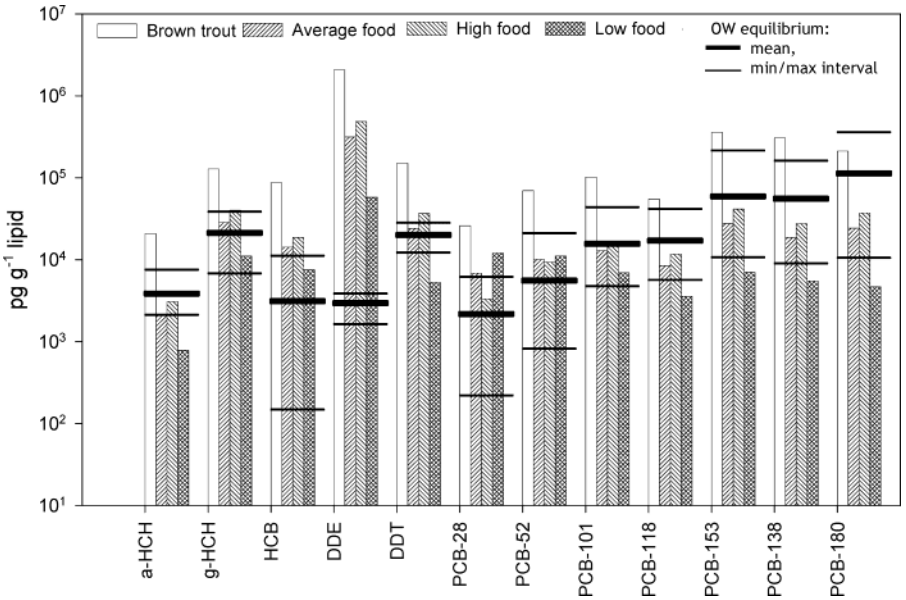


FIGURE 2. Concentrations of the organochlorine compounds in fish and food-web standardized by lipid content. Horizontal bars indicate the expected values according to the concentrations in water ($n = 8$) and K_{ow} (*31*) (values are indicated for high (maximum) and low (minimum) food periods, and mean diet is calculated as described in Table 2).

As expected, brown trout appear to be the unique top predator (Figure 1). However, the average food chain from primary producers to this organism was very short. Assuming an enrichment factor of 3.5‰ δ^{15}N per trophic level change, the average number of energy transfer steps from primary producers to trout is only of 2.2. This is not surprising for a high mountain lake because food availability is scarce due to the oligotrophy of the system, the low inputs from the watershed, and the small lake size (*28*).

The distribution of the distinct organisms throughout the δ^{15}N gradient indicates a high degree of omnivory. The differences between successive organisms in any trophic chain are significantly lower than 3.5 δ^{15}N. Chironomid pupae show higher δ^{15}N values than larvae, although in both cases the species measured were herbivorous. The isotope discrimination could be due to metamorphosis from larvae to pupae. The new form is rebuilt from old tissues, and the transformation may cause an enrichment in ^{15}N in a similar way as it occurs in starving animals (*29*) because there is no food intake during the pupae stage. On the other hand, some consumers show a significant contribution of detritus in their diet, particularly the bivalve *Pisidium*. The δ^{15}N signature of some supposed predators (*Arcynopterix*, *Platambus*) do not agree with an exclusive diet of macroinvertebrates, being closer to scavenger feeding. The narrowing of the δ^{13}C range at increasing δ^{15}N indicates progressive mixing of the pelagic, littoral, and sediment carbon sources at higher food web stages.

The isotopic signatures are consistent with the stomach content observations indicating that trout mainly predate on the herbivore level, constituted by chironomids and cladocerans, with some contribution from other invertebrates. Using the estimated average diet proportions (p_i) from Table 2, and the measured isotopic signatures of the distinct food items ($\delta^{13}C_i$, $\delta^{15}N_i$) the expected isotopic signature for brown trout was calculated as follows:

$$\delta^{13}C_{trout} = \Sigma p_i\, \delta^{13}C_i + 0.05 \text{ and } \delta^{15}N_{trout} = \Sigma p_i\, \delta^{15}N_i + 3.5 \quad (1)$$

The resulting isotopic values are -22.7 ± 1.8‰ δ^{13}C and 3.4 $\pm$ 0.80‰ δ^{15}N, which are quite similar to the direct fish determinations, -22.6 ± 1.5‰ δ^{13}C and 3.5 ± 2‰ δ^{15}N. Thus, the above assumptions on average diet composition are feasible and can be considered to provide a good estimate for the annual average composition.

Food and Fish Organochlorine Compound Levels. Major differences in OC concentrations were observed among the distinct food-web components reflecting in part their large heterogeneity in lipid content (Table 3). However, when

TABLE 4. Concentrations of Organochlorine Compounds Dissolved in Water of Lake Redon[a]

pg L^{-1}	mean[b]	SD	mininum	maximum
α-HCH	483	225	267	952
γ-HCH	2671	1226	856	4846
HCB	9.9	12	0.5	35
4,4′-DDE	7.4	2.3	4.1	9.7
4,4′-DDT	20	5.6	12	28
PCB #28	4.3	5.3	0.4	12
PCB #52	8.8	11	1.3	33
PCB #101	6.2	5.4	1.9	17
PCB #118	3.4	2.5	1.1	8.3
PCB #153	7.5	9.3	1.4	27
PCB #138	8.8	9.5	1.4	25
PCB #180	4.5	5.4	0.4	14

[a] Data from refs *13* and *14* and unpublished. [b] Average values of data collected in July 96 at 1, 5, and 60 m depth; June 97 at 1 m, 5, 25, and 59 m depth ($n = 4$); and November 00 at 1 m depth ($n = 8$).

normalized to lipid concentration, brown trout showed the highest levels in all OC. Among the organisms more common in the trout diet, *Daphnia* showed lower OC concentration values than average, perhaps because of its shorter life span. Combination of the concentrations of the individual organisms to estimate OC content in food shows that the concentrations of these pollutants were slightly lower during the low feeding period (Figure 2). This difference was essentially due to the higher relative contribution of *Daphnia* to the diet. Mean OC intake was therefore calculated by weighting the lipid normalized concentrations in each food item according to the respective mean contributions to diet (Table 2).

Comparison of the mean OC pooled food concentrations with the theoretical values calculated from OC water content and K_{ow} (*30*) (Table 4) provides an estimate of the deviation of OC in food web content (normalized to lipids) from thermodynamic equilibrium. A significant number of OCs show concentrations that are close to those expected at equilibrium, namely HCHs, HCB, 4,4′-DDT and PCB congeners #28 and #52 (Figure 2). 4,4′-DDE exhibit values much higher than expected at equilibrium (Figure 2), but 4,4′-DDT shows the opposite trend which could reflect the conversion of 4,4′-DDT into 4,4′-DDE within the organisms (*31*). In this respect, 4,4′-DDT is always significantly higher than 4,4′-DDE in water (Table 4).

The more chlorinated PCB congeners also deviate from equilibrium since, according to water concentrations, lower values than expected are found. The deviation increases progressively with the degree of chlorination (Figure 2) being larger at higher K_{ow} (Figure 3a). Equilibrium is therefore not reached for OCs with log(K_{ow}) values above ca. 6, indicating that for these OC it could not be reached within the life span (<1 yr) of the organisms more relevant in the trout diet.

In contrast to food, fish show OC concentrations significantly higher than those expected at equilibrium which indicate a biomagnification process (Figure 2) as consequence of food intake. The ratio between the fish and food concentrations, both normalized to lipid content, range between 3.8 for PCB #28 to 16.5 for PCB #138. The ratios between OC in fish and food are also related to K_{ow} (Figure 3b), since the higher is K_{ow} the lower is its loss through the gills. PCB #180 is an exception to this trend (Figure 3b) since it exhibits significant lower biomagnification than expected (Figure 3b). The anomaly could reflect the lower membrane permeation of large molecules (*32*). In consequence, trout uptake efficiency for PCB#180 seems to be significantly lower than for the other congeners.

The Roles of Food and Water in OC Bioaccumulation. OC concentration in fish results from the balance between

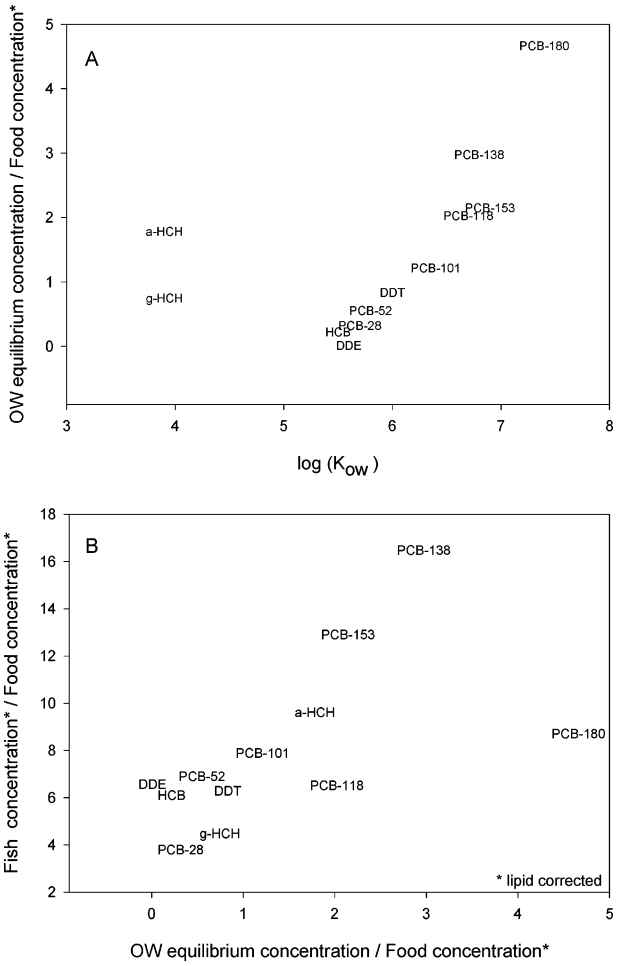


FIGURE 3. (A) Departure from octanol−water equilibrium of the organochlorine compounds in the fish food. (B) Biomagnification ratio of the organochlorine compounds between fish and average diet.

exchanges at the gills during fish respiration, uptake from diet, elimination by fecal egestion, and metabolism and dilution by fish growth. Among these metabolic elimination can be considered of little relevance as compared to the other processes (*33*).

The relative significance of the other flux components can be evaluated using a fugacity approach (*34*) in combination with a brown trout food intake model (*35*) and the measured concentrations in water, food, and fish. For this purpose, a "standard fish" weighting 204 g submitted to the seasonality of feeding over year periods divided in three "weather conditions" each involving 4 months with average water temperatures of 8, 4, and 2 °C in an environment of ca. 9 mg O_2 L^{-1} has been taken as lake representative (*9*). All parts of the fish body were assumed to have the same fugacity. Gill was considered to be a well-mixed compartment in which water flows with oxygen and OCs were transferred inside and outside by diffusion (*30*). Conductivities (Dw) for gill uptake and loss were equally considered to be dependent from gill ventilation rate (Gw). The net flux in the gill exchange (Fg) was determined by the difference between water (f_W) and fish (f_F) fugacities.

$$Fg = Dw(f_W - f_F) \quad (2)$$

The exchanges in the gastrointestinal tract are more complex. Between food uptake and egestion a fraction of matter is removed and there is, in addition, lipid digestion. Therefore, the intestine flux (Fi) required the separate

TABLE 5. Definition of Symbols and Summary of the Parameters Used in the OC Fish Flux Calculations

parameter	units	definition
f_W, f_A, f_F	Pa	water, food and fish fugacities, $f_i = C_i Z_i^{-1}$
C_i	pg L^{-1}	concentration
Z_i	pg L^{-1} Pa^{-1}	fugacity capacity, $Z_i = L_i K_{ow} H^{-1}$
K_{ow}	L·kg^{-1}	octanol−water partition coefficient
H	Pa L pg^{-1}	Henry's law constant
Dw	pg d^{-1} Pa^{-1}	conductivity at gills, Dw = Gw Zw
Gw	L d^{-1}	gill ventilation rate, $Gw = K k_{eo} O_{2w}^{-1} E_{ox}$
K	cal d^{-1}	fish daily intake requirement, K= f(T,W) (*33*)
T	°C	temperature
W	G	fish weight
k_{eo}	mg cal^{-1}	energy to oxygen consumption coeff, 0.047
O_{2w}	mg L^{-1}	water oxygen concentration
E_{ox}		efficiency of oxygen uptake, 0.45
D_A	pg d^{-1} Pa^{-1}	gut uptake conductivity, $D_A = E_A G_A Z_A$
G_A	L h^{-1}	food consumption rate, $G_A = K k_{ef}$
k_{ef}	L cal^{-1}	energy to food volume consumption coeff, 10^{-6}
E_A		gut uptake efficiency, 0.75 (except for PCB # 180, 0.45)
D_E	pg d^{-1} Pa^{-1}	gut loss conductivity, $D_E = G_A (1-\beta) Z_F$
β		fraction of ingested diet absorbed by the fish, 0.8

consideration of uptake (D_A) and loss (D_E) conductivities

$$Fi = D_A f_A - D_E f_F \quad (3)$$

where f_A is food fugacity.

D_A is commonly modeled to depend on food consumption rate (G_A) and gut absorption efficiency (E_A) (*20, 33, 36*). D_E was taken proportional to G_A (1-β), where β was the fraction of ingested diet absorbed by the organism. Development of the two equations according to the definitions in Table 5 provided the following expressions for the two fluxes:

$$Fg = Gw(Cw - C_F/(L_F K_{ow})) \quad (4)$$

$$Fi = G_A(C_A E_A - C_F(1-\beta)) \quad (5)$$

Apart from water and food concentrations, the relative flux differences result from the values of the K_{ow} and E_A coefficients, the latter being particularly relevant for differentiating the behavior of PCB#180. Gw and G_A determine the absolute flux values. The two rates depend on the fish daily energy requirements (*37*), which under optimal conditions result from the body weight and temperature in a nonlinear way. G_A was estimated using Elliot's model for brown trout (*35*) and distinguishing the three feeding periods mentioned above. G_W, in addition to oxygen consumption as determined by the energy requirement, was made dependent on the oxygen in the water and uptake efficiency (*36*) (Table 5).

The resulting calculations show that a number of OCs were close to a steady state, namely HCB, 4,4′-DDE, PCB #28, and PCB #52 (Figure 4). These are the compounds that, in turn, are also in equilibrium between water and food. This parallelism gives ground to the assumptions for the calculations of exchanges in the gills and intestine, at least in relative terms.

Since HCHs and 4,4′-DDT have also $\log(K_{ow}) < 6$, steady state for these OC should be expected, but this is not the case. In 4,4′-DDT gut uptake appears to be higher than gill net loss, which may reflect a metabolic transformation within the fish. In HCHs, gill loss is much higher than gut uptake, which is unlikely unless the assumed water fugacity does not correspond to the one presently experienced by fish. This can certainly be the case as the calculations are based on long time water concentrations averages, and fish renewal time of the more volatile compounds is a matter of days.

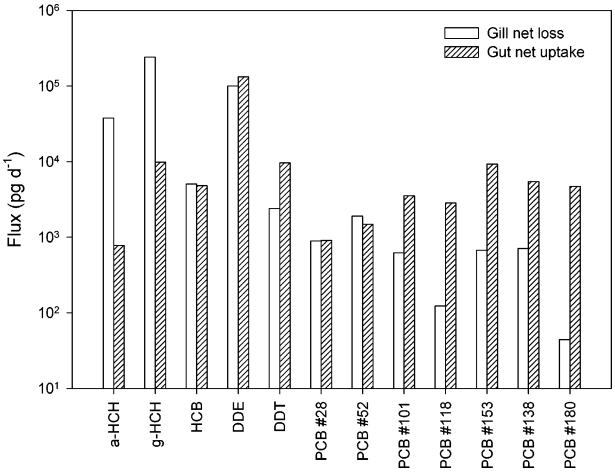


FIGURE 4. Comparison of the calculated net gill loss and net gut uptake of organochlorine compounds in a fish of 204 g in Lake Redon according to the measured concentrations in water, food, and fish.

An average residence time in fish can be calculated for the compounds in apparent steady state. For HCHs it is in the order of some days to a few weeks, for HCB and 4,4′-DDE it is about 1 year, and for 4,4′-DDT, PCB #28, and #52 it is about 2 or 3 years. For the rest of the compounds steady state is not achieved, but the present turnover indicates characteristic times around a decade for PCB #101 and two or three decades for PCBs #110 to #153. In the case of PCB #180, a fish could hardly achieve a steady state at present exposures unless it lived for centuries.

Acknowledgments

This is a contribution of the Limnology Group (UB-CSIC). Financial support from the EU projects EUROLIMPACS (GOCE-CT-2003-505540) and EMERGE (EVK1-CT-1999-00032) and ACA-CIRIT (Generalitat de Catalunya) is acknowledged. One of us, I.V., thanks Generalitat de Catalunya for a Ph.D. fellowship and acknowledges Universitat Autònoma de Barcelona. Guillem Carrera is thanked for the analysis of OC in water. N. García-Reyero (IBMB, CSIC), J.C. Massabuau (University of Bordeaux 1), and R. Lackner (University of Innsbruck) are thanked for their valuable help during sampling.

Literature Cited

(1) Simonich, S. L.; Hites, R. A. *Science* **1995**, *269*, 1851−1854.
(2) Wania, F.; Mackay, D. *Ambio* **1993**, *22*, 10−18.
(3) Blais, J. M.; Schindler, D. W.; Muir, D. C. G.; Kimpe, L. E.; Donald, D. B.; Rosenberg, B. *Nature* **1998**, *395*, 585−588.
(4) Fellin, P., Barrie, L., Doughtery, D.; Toom, D.; Muir, D.; Grift, N.; Lockhart L.; Billeck, B. *Environ. Toxicol. Chem.* **1994**, *15*, 253−261.
(5) Grimalt, J. O.; Fernandez, P.; Berdié, L.; Vilanova, R. M.; Catalan, J.; Psenner, R.; Hofer, R.; Appleby, P. G.; Rosseland, B. O.; Lien, L.; Massabuau, J. C.; Battarbee, R. W. *Environ. Sci. Technol.* **2001**, *35*, 2690−2697.
(6) http://www.ncdc.noaa.gov/oa/documentlibrary/pdf/climate-softheworld.pdf.
(7) Catalan, J.; Ventura, M.; Brancelj, A.; Granados, I.; Thies, H.; Nickus, U.; Korhola, A.; Lotter, A. F.; Barbieri, A.; Stuchlík, E.; Lien, L.; Bitušík, P.; Buchaca, T.; Camarero, L.; Goudsmit, G. H.; Kopáček, J.; Lemcke, G.; Livingstone, D. M.; Müller, B.; Rautio, M.; Šiško, M.; Sorvari, S.; Šporka, F.; Strunecký, O.; Toro, M. *J. Paleolimnol.* **2000**, *28*, 25−46.
(8) Knapp, R. A., Matthews, K. R., Sarnelle, O. *Ecol. Monogr.* **2001**, *71*, 401−421.
(9) Ventura, M.; Camarero, L.; Buchaca, T.; Bartumeus, F.; Livingstone, M. D.; Catalan, J. *J. Limnol.* **2000**, *59*, 97−108.
(10) Encina, L., Rodríguez-Ruiz, A. *Hydrobiologia* **2003**, *493*, 35−45.
(11) Vives, I.; Grimalt, J. O.; Ventura, M.; Catalan, J.; Rosseland, B. *Environ. Pollut.* **2003**, in press.
(12) Carrera, G.; Fernández, P.; Grimalt, J. O.; Ventura, M.; Camarero, Ll.; Catalán, J.; Nickus, U.; Thies, H.; Psenner, R. *Environ. Sci. Technol.* **2002**, *36*, 2581−2588.
(13) Vilanova, R.; Fernández, P.; Martínez, C.; Grimalt, J. O. *J. Environ Qual.* **2001**, *30*, 1286−1295.
(14) Vilanova, R. M.; Fernandez, P.; Grimalt, J. O. *Sci. Total Environ.* **2001**, *279*, 51−62.
(15) Sterner, R. W.; Elser, J. J. *Ecological Stoichiometry. The Biology of elements from molecules to the biosphere*; Princeton University Press: Princeton, 2002; p 439.
(16) Salonen, K.; Sarvala, J. *Verh. Internat. Verein. Limnol.* **1978**, *20*, 1221−1225.
(17) Stelzer, R. S.; Lamberti, G. A. *Ecology* **2002**, *83*, 1039−1051.
(18) Cauchie, H.-M. *Hydrobiologia* **2002**, *470*, 63−96.
(19) Ahlgren, G.; Gustafsson, I.-B.; Boberg, M. *J. Phycol.* **1992**, *28*, 37−50.
(20) Berdié, L.; Grimalt, J. *J. Chromatogr. A* **1998**, *823*, 373−380.
(21) Vives, I.; Grimalt, J. *J. Chromatogr. B* **2002**, *768*, 247−254.
(22) Minagawa, M.; Wada, E. *Geochim. Cosmochim. Acta* **1984**, *50*, 2143−2146.
(23) Michener, R. H.; Schell, D. M. In *Stable isotopes in ecology and environmental science*; Lajtha, K., Michener, R., Eds.; Blackwell Scientific: 1994; pp 138−157.
(24) Vander Zanden, M. J.; Rasmussen, J. B. *Limnol. Oceangr.* **2001**, *46*, 2061−2066.
(25) Rau, G. H. *Can. J. Fish. Aquat. Sci.* **2001**, *37*, 742−746.
(26) Hecky, R. E.; Hesslein, R. H. *Can. J. Fish. Aquat. Sci.* **1995**, *52*, 1195−1201.
(27) Fogel, M. L.; Cifuentes, L. A. Isotope fractionation during primary production. In *Organic Geochemistry*; Engel, M. H., Macko, S. A., Eds.; Plenum: 1993; pp 73−100.
(28) Post, D. M. Pace, M. L.; Hairston, N. G., Jr. *Nature* **2000**, *405*, 1047−1049.
(29) Hobson, K. A.; Alisauskas, R. T.; Clark, R. G. *Condor* **1993**, *95*, 388−394.
(30) Clark, K. E.; Gobas, F. A. P. C.; Mackay, D. *Environ. Sci. Technol.* **1990**, *24*, 1203−1213.
(31) Lund, B. *Organohalogen Compd.* **1998**, *37*, 341−344.
(32) Mackay, D. *Environ. Sci. Technol.* **1982**, *16*, 274−278.
(33) Campens, J.; Mackay, D. *Environ. Sci. Technol.* **1997**, *31*, 557−583.
(34) Mackay, D.; Fraser, A. *Environ. Pollut.* **2000**, *110*, 375−391.
(35) Elliott, J. M.; Hurley, M. A. *Freshwater Biol.* **1998**, *39*, 339−349.
(36) Morrison, H. A.; Gobas, F. A. P.; Lazar, R.; Whittle, D. M.; Haffner, G. D. *Environ. Sci. Technol.* **1997**, *31*, 3267−3273.
(37) Elliott, J. M. *Quantitative ecology and the brown trout*; Oxford University Press: Oxford, U.K., 1994; p 286.

Received for review March 8, 2004. Revised manuscript received June 4, 2004. Accepted June 4, 2004.

ES040035P