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Can we use stable isotopes for ecotoxicological studies? Effect of DDT on isotopic fractionation in *Perca fluviatilis*

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ABSTRACT

Nitrogen and carbon stable isotope analyses are frequently used to assess contaminant biomagnification in animals in the wild. Previous studies, mainly on plant but also on animal tissues, have shown that chemical stress can lead to shifts in $\delta^{15}N$. In order to assess if an exposure to DDT at realistic concentration disrupted stable isotope signature in animals, $\delta^{15}N$ and $\delta^{13}C$ were studied in several tissues (liver, muscle, gill) of *Perca fluviatilis* fed with the same commercial diet uncontaminated or contaminated with DDT. We observed no DDT effect on the $\delta^{15}N$ and $\delta^{13}C$ of fish tissues. Our results show that stable isotopes can remain useful for field ecotoxicological studies despite food-chain contamination. However, correlations between the $\delta^{13}C$ or $\delta^{15}N$ values measured in the different organs were only found in DDT treated fish, suggesting some disruption of major biochemical compound metabolism in tissues.

1. Introduction

Analyses of stable isotopic ratios of carbon ($^{13}C/^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$; $\delta^{15}N$) are a powerful tool for tracing diet habits of organisms. The difference in $\delta^{15}N$ between the consumer and its prey is known as $\Delta^{15}N$. Nitrogen in the protein of consumers is generally enriched in ^{15}N by 3–5‰ relative to prey nitrogen (i.e. $\Delta^{15}N = 3–5‰$). This nitrogen heavy isotope enrichment appears to be caused by isotopic fractionation occurring with transamination during protein catabolism (Doucett et al., 1999). This increase allows determination of an animal's trophic level (TL) in a food web (DeNiro and Epstein, 1981; Post, 2002). Often, the relative isotopic similarity of the $^{13}C/^{12}C$ ratio between diet and consumer ($\Delta^{13}C = 0–1‰$; DeNiro and Epstein, 1978; Post, 2002) allows identification of an animal's diet source (Jardine et al., 2005).

In fish, Stable Isotope Analyses (SIA) are usually performed on white muscle. However, high-turnover tissues (liver, gill), and tissues that can be sampled in a nondestructive manner (fins, scales) are also used (Jardine et al., 2005; Serrano et al., 2007; Vollaire et al., 2007). Mobilization, reorganization, and catabolism of stored lipid and protein reserves, which occur during starvation, are known to increase the isotopic enrichment in tissues (Doucett et al., 1999). During periods of food deprivation, Jardine et al. (2005) observed lower difference between liver and muscle $\delta^{15}N$ in atlantic salmon (*Salmo salar*) and change in correlations between muscle and liver $\delta^{15}N$.

SIA are also increasingly used by ecotoxicologists to elucidate contaminant behavior (bioconcentration and biomagnification) through trophic chains (Borga et al., 2001; Binelli and Provini, 2003; McIntyre and Beauchamp, 2007). Thus, increasing concentrations of contaminants in organisms at increasing TL (i.e. with ^{15}N enrichment) make it possible to estimate biomagnification of a chemical through the food web.

Studies on plant physiology have already provided evidence of change in $\delta^{15}N$ enrichment in plant tissues due to stressors. Enrichment in ^{15}N in needles of Norway spruce (*Picea abies*) from a declining forest was attributed to onset of "catabolism and nitrogen reallocation" (Gebauer and Schulze, 1991). Under ozone exposure, common wheat plants (*Triticum aestivum*) show increase in $\delta^{15}N$ (Hofmann et al., 1997). In animals, Shaw-Allen et al. (2005) found that liver showed greater $\delta^{15}N$ in snowy egrets (*Egretta thula*) fed mercury-contaminated diets while muscle showed lower $\delta^{15}N$ in those birds compared with birds fed control diets. They suggested using the shift in $\delta^{15}N$ as a biomarker of chemical stress because this shift was observed for contaminant concentrations lower than those inducing responses of common biomarkers such as metallothionein and glutathione. We hypothesized that, if low doses of contaminant in diet induce shifts in $\delta^{15}N$ of consumers, the trophic levels assessed with SIA in the field could be biased by the presence of environmental contaminants. Furthermore, this

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shift could cast doubt on conclusions of some studies on biomagnification based on SIA results. Ramos et al. (2009) observed increasing Hg concentrations in tissues of Cory's shearwater (*Calonectris diomedea*) having the highest $\delta^{15}N$. They suggested that this increase in Hg concentrations was due to differences in trophic ecology since Hg can be biomagnified and originate from dietary input. Another hypothesis could be that Hg disrupts nitrogen fractionation in tissues of shearwater as observed in egrets (Shaw-Allen et al., 2005). In lakes, Vander Zanden and Rasmussen (1996), showed a correlation between increasing PCB concentrations and high $\delta^{15}N$ in trout, concluding that PCB concentrations increased with trophic level of trout. Another hypothesis could be that PCB change nitrogen metabolism in trout tissue and alter isotopic fractionation.

Because of their persistence, some organic pollutants are present in almost all ecosystems. DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) was a pesticide widely used over several decades. Despite this compound having been banned in many developed countries, DDT is still detected in most ecosystems because of its persistence (Simonich and Hites, 1995). Bunyan et al. (1970), studying the Japanese quail (*Coturnix japonica*), showed that DDT interfered with protein metabolism in liver. Leffler (1975) reported increased metabolic rate, decreased muscular coordination, inhibited autotomy reflex, and reduced carapace thickness/width ratio in juvenile blue crabs (*Callinectes sapidus*) fed DDT contaminated food. Ramalingam and Ramalingam (1982) reported that the chronic effect of DDT on glycogen utilization in Mozambique tilapia (*Oreochromis mossambicus)* led to the use of liver and muscle proteins as energy sources.

Because of the intensive proteolysis induced by DDT in liver and muscle, and of its presence in almost all ecosystems, we tested whether DDT, at a concentration expected to occur in the natural environment, could increase the $\delta^{15}N$ and the $\delta^{13}C$ of these tissues, and consequently distort conclusions obtained from field studies using SIA. In this case, observations of increased $\delta^{15}N$ at increased DDT concentrations could be due to a disruption of nitrogen fractionation induce by DDT rather than an increase in DDT concentrations with increasing trophic levels. As for nutritional stresses, inducing increases in ^{15}N that can be tissue specific and leading to changes in correlations between $\delta^{15}N$ in muscle and liver (Jardine et al., 2005), we hypothesized that DDT may affect primarily tissues with higher turnover rate (i.e. liver and gill) thereby disturbing the correlation of $\delta^{15}N$ between tissues.

In this study we fed Eurasian perch (*P. fluviatilis*) with controlled commercial diet (CD) and with CD contaminated with DDT (CD*). The two main goals of our study were:

- To assess whether a diet contaminated with DDT at concentrations similar to those found in natural prey items induced disruption of $\delta^{15}N$ and $\delta^{13}C$ in three tissues (gill, liver, and white muscle) having different turnover rates.
- To test whether changes in $\delta^{15}N$ and $\delta^{13}C$ correlations between tissues (e.g. liver vs. muscle) could also be affected by chemical stress altering nitrogen and carbon metabolism in a specific tissue.

2. Materials and methods

Eurasian perch (*P. fluviatilis*) with a mean weight of 2.1 ± 0.1 g were harvested in Lake Neuchâtel (Switzerland). Fish were reared under constant temperature and photoperiod conditions (T = 20–22 °C, 12 h light: 12 h dark) in a recirculating system tank with water purified by a series of filtration treatments including UV and bio-filters. Fish were fed daily on a commercial diet (CD) (BioMar Bioptimal, 46% protein and 11% lipid) made up of fish meal, soy cake, wheat, horse beans, field peas, corn and wheat gluten, fish oil, supplemented with vitamins, minerals and antioxidants (ethoxyquin). Fish received food in the form of dry pellets of different sizes according to fish size (Fontaine et al., 2006).

2.1. Experimental design

Forty-eight fish were randomly assigned to six 180-L fish tanks (eight fish per tank) and acclimatized for 10 d. Two treatment groups of three fish tanks each ("Control" and "DDT") were established. Two random fish were removed from each tank and weighed and measured at the beginning of the experiment (Table 1, t_0). Temperature, pH, dissolved oxygen saturation (DO), NH_4^+, NO_2^-, NO_3^- were monitored and recorded three times a week. Throughout the 45-d experiment, these parameters were held constant across all treatments: temperature = 22.3 ± 0.8 °C, pH = 7.5 ± 0.2, DO = 91.79 ± 2.03% sat, $NH_4^+ < 0.06$ mg L^{-1}, $NO_2^- < 0.03$ mg L^{-1}, $NO_3^- < 50$ mg L^{-1}. In "Control" tanks, fish were fed 10 g kg^{-1} d^{-1} of CD dry pellets without DDT (<0.03 mg kg^{-1} lipid weight). In "DDT" tanks, fish were fed with the same CD dry pellets contaminated with DDT (3.4 mg kg^{-1} lipid weight). This is a realistic concentration, similar to levels of contamination found in top piscine predators in natural habitats (Falandysz et al., 2004; EPA, 2006). The duration of our experiment was fixed at 45 d in order that almost all muscle proteins can be renewed (Sakano et al., 2005). During the first week of experiment, one fish died in each treatment ("Control 1" and "DDT 2" tanks). At the end of the experiment, all fish (n = 34) were starved for 48 h to empty the gastrointestinal tracts, before being sacrificed and stored at −20 °C until analyses.

2.2. Analytical methods

Isotopic analyses were carried out on lipid free fractions of three different tissues with different turnover rates (Haschmeyer and Smith, 1979): muscle, liver, and gill. Lipids have more negative $\delta^{13}C$ values than other major biochemical compounds in animal tissues (Sotiropoulos et al., 2004) and are often removed in ecological studies to avoid discrepancies in ^{13}C depletion due to variation in lipid content between tissues (Persson et al., 2007). Lipid extraction from fish tissue has been found to either have no effect on $\delta^{13}C$ and $\delta^{15}N$ values (Graham et al., 2007) or lead to very small isotope shifts (Sotiropoulos et al., 2004; Ingram et al., 2007). During our study, lipids were removed to be consistent with methods applied to field studies using an accelerated solvent extraction (ASE200) System (Dionex, Voisins le Bretonneux, France). Soft tissues were introduced into a 33 mL ASE cell and the extraction was then performed using dichloromethane:methanol (2:1 v/v) as solvent and under the conditions described by Toschi et al. (2003) – temperature: 120 °C; pressure: 100 bar; heat time: 6 min; static time: 10 min; flush volume: 60%; purge time: 120 s; 2 cycles. The

Table 1
Morphometric data (arithmetic mean ± SD) of fish sampled at the beginning of the experiment from the six tanks (t_0), at the end of the experiment from the three "Control" tanks ("Control" 1, 2, and 3) and from the three treated tanks ("DDT" 1, 2, and 3).

	n	Length	Weight
$t0$	12	17.1 ± 0.80	95.55 ± 14.36
"Control 1"	5	17.93 ± 0.95	113 ± 17.94
"Control 2"	6	18.01 ± 0.59	121.5 ± 10.81
"Control 3"	6	18.18 ± 0.53	126.42 ± 11.38
"DDT 1"	6	17.85 ± 1.17	121.5 ± 31.95
"DDT 2"	5	18.48 ± 1.29	124.17 ± 37.99
"DDT 3"	6	18.26 ± 1.23	123.17 ± 36.32

lipid-free residues were freeze-dried and powdered. One milligram powder sub-samples were packed into 3.3 × 5 mm tin capsules for stable isotope measurements. Mass spectrometer analyses were carried out using a continuous flow isotope ratio mass spectrometer (VG Optima; Model NA-1500, Carlo Erba). Analytical temperatures were: 1020 °C oxidation; 700 °C reduction; and 50 °C oven (column). Stable isotope abundances were expressed in δX notation as the deviation from reference in parts per thousand (‰) according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{reference}}\right) - 1\right] \times 1000$$

where R_{sample} = X/X′ of the sample, and $R_{reference}$ = X/X′ of the reference. X and X′ represent the abundances for heavier and lighter isotopes, respectively. The $R_{reference}$ values were based on the PeeDee Belemnite (PDB) for ^{13}C and atmospheric N_2 (AIR) for ^{15}N.

Organic internal references were used to evaluate analytical error: glutamic acid for carbon (%C = 40.78%, $\delta^{13}C = -28.06‰$) and atropine for nitrogen (%N = 4.91, $\delta^{15}N = 19.92‰$). Precisions for $\delta^{13}C$ and $\delta^{15}N$, based on the standard deviation of internal reference replicates, were better than 0.2‰ and 0.3‰, respectively.

2.3. Calculations and statistics

Statistical analyses were performed using R version 2.4.1 (R Development Core Team, 2006). Computations were carried out using R's languages from the 'lm' and 'nlme' libraries. Differences in $\delta^{13}C$ and $\delta^{15}N$ between treatments were compared using mixed effects model ANOVA with treatment ("Control" vs. "DDT") as fixed effect and tanks as random effect, nested within treatment. Differences among fish from the six fish tanks, in final length and final weight, were compared using ANOVA. Differences in $\delta^{13}C$ and $\delta^{15}N$ δ between tissues or between tissues and diet, were compared using the Tukey HSD test. To evaluate relationships between isotopic signatures measured in the different organs, we carried out correlation analyses using the Pearson correlation test with Bonferroni correction taking into account all fish treated as independent replicates. Differences between Standard Deviations (SD) were tested using the Fischer test.

3. Results

No significant tank effect was observed. Analyses of CD pellets and CD pellets contaminated with DDT (CD*) showed no difference in $\delta^{15}N$ (7.02 ± 0.16 vs. 7.16 ± 0.12), $\delta^{13}C$ (−22.23 ± 0.14 vs. −22.02 ± 0.08), and C/N ratios (4.87 ± 0.18 vs. 4.81 ± 0.08). After 45 d of exposure, the increase in fish weight was not significantly different between treatments and reached 26% and 29% of initial weight in "Control" and "DDT" tanks, respectively (Table 1). Based on SD comparisons, at the end of the experiment, weights of treated fish were significantly more variable than weights of untreated fish (Fischer test; $p < 0.05$). The $\delta^{15}N$ signature was not significantly different between treatments ("DDT" and "Control") for gill ($p = 0.981$), liver ($p = 0.917$), and white muscle ($p = 0.370$) (Fig. 1), nor was the $\delta^{13}C$ for these three organs ($p = 0.264$, 0.093 and 0.983, respectively), and the variabilities of $\delta^{15}N$ and $\delta^{13}C$ were not significantly different between treatments (Fischer test; $p > 0.05$).

All fish tissues (in "Control" and "DDT" tanks) were significantly enriched in ^{15}N and ^{13}C relative to the diet (Fig. 1), with highest ^{15}N enrichments in white muscle ($\Delta_{muscle\text{-}CD} = 3.04‰$ in "Control" and $\Delta_{muscle\text{-}CD}^{*} = 2.84‰$ in "DDT"), followed by gill ($\Delta_{gill\text{-}CD} = 2.39‰$ in "Control" and $\Delta_{gill\text{-}CD}^{*} = 2.27‰$ in "DDT") then liver ($\Delta_{liver\text{-}CD} = 1.56‰$ in "Control" and $\Delta_{liver\text{-}CD}^{*} = 1.37‰$ in "DDT"), the three tissues differing significantly in $\delta^{15}N$ ($p < 0.001$). For

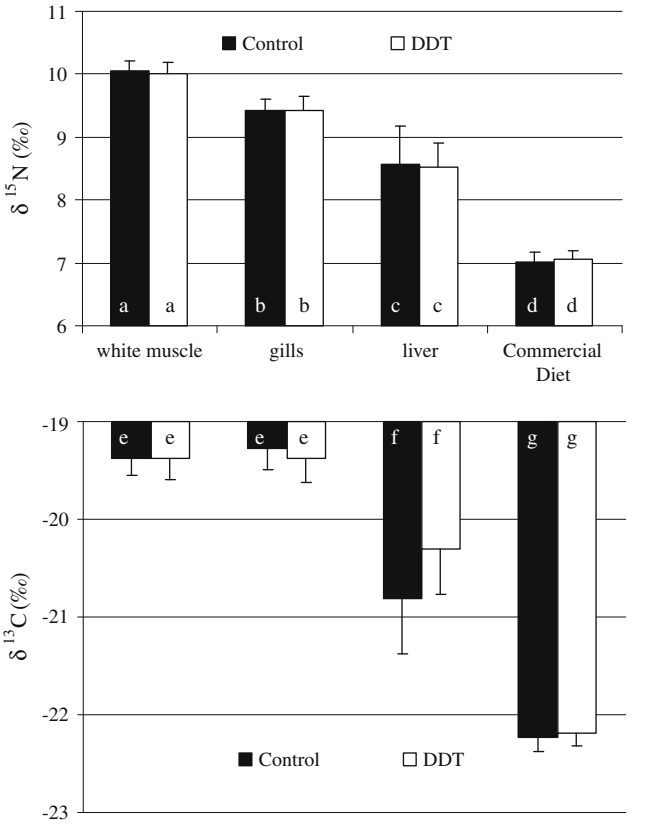

Fig. 1. Arithmetic mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ in lipid-free tissues ($n = 17$) and diet ($n = 6$) of Eurasian perch. Values that share the same letter do not differ significantly (Tuckey HSD test; $p > 0.05$).

^{13}C, the highest enrichments were measured in gill ($\Delta_{gill\text{-}CD} = 2.96‰$ in "Control" and $\Delta_{gill\text{-}CD}^{*} = 2.64‰$ in "DDT") and white muscle (2.85‰ and 2.64‰, respectively). Liver showed significantly lower $\delta^{13}C$ than either white muscle (1.43‰ less in "Control" and 0.93‰ less in "DDT" tanks) or gill (1.54‰ less in "Control" and 0.93‰ less in "DDT" tanks). No significant difference ($p > 0.75$) was found between $\delta^{13}C$ of gill and white muscle.

No significant correlation in stable isotope signatures between tissues was found for the "Control" fish (Figs. 2 and 3). For the treated fish, the gill $\delta^{15}N$ was correlated with the white muscle $\delta^{15}N$ ($r = 0.65$; $n = 17$ $p = 0.004$; Fig. 2d) and with the liver $\delta^{15}N$ ($r = 0.69$; $n = 17$ $p = 0.002$; Fig. 2e). In the treated fish, we also observed significant relationships for $\delta^{13}C$ between gill and muscle ($r = 0.49$; $n = 17$ $p = 0.043$; Fig. 3d). Independently of the tissue and/or the treatment, $\delta^{15}N$ was never correlated with $\delta^{13}C$.

4. Discussion

After 45 d exposure, no significant difference was observed in weight and length between perch from "Control" or "DDT" treatments. Weights of perch from "DDT" treatment were significantly more variable than weights of perch from "Control" treatment. Among the individuals of a population exposed to a contaminant, at the beginning of the exposure, only the most sensitive individuals exhibit symptoms such as reduction of food intake (Ramade, 2007). We can hypothesize that if most sensitive fish consumed less food, these pellets were consumed by the more resistant ones. This difference in rate of food consumption might explain the greatest mass variability among the fish exposed to "DDT" treatment. This hypothesis implies that concentration used was possibly too low to have an effect on the most resistant fish.

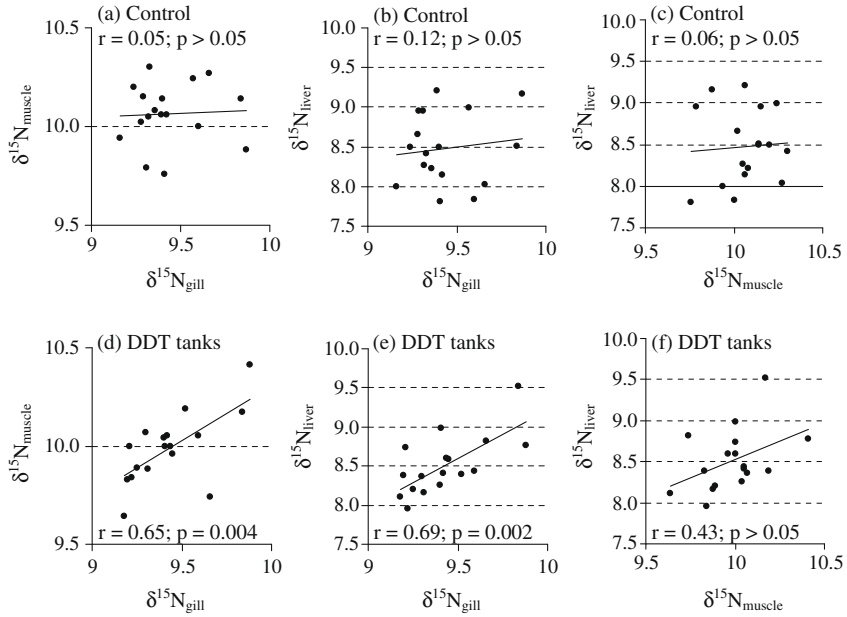

Fig. 2. Relationships between $\delta^{15}N$ of muscle, liver and gill in fish reared in "Control" and "DDT" tanks ($n = 17$; r = Pearson coefficient; $p = p$ value).

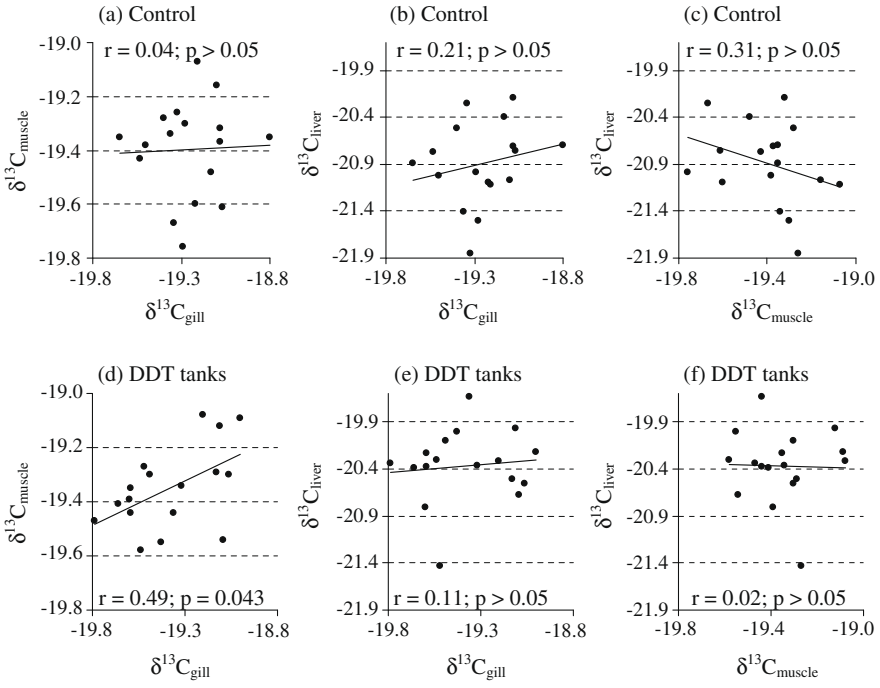

Fig. 3. Relationships between $\delta^{13}C$ of muscle, liver and gill in fish reared in "Control" and "DDT" tanks ($n = 17$; r = Pearson coefficient; $p = p$ value).

For both treatments, the $\delta^{13}C$ and $\delta^{15}N$ values significantly differed between organs. Liver $\delta^{13}C$ and $\delta^{15}N$ were depleted compared with white muscle (difference of 1.48‰ and 1.47‰, respectively, in "Control" vs. 1.47‰ and 0.93‰, respectively, in "DDT"). Pinnegar and Polunin (1999) studying the rainbow trout (*Oncorhynchus mykiss*) found that $\delta^{13}C$ and $\delta^{15}N$ in muscle were respectively 1.92‰ and 1.15‰ higher than in liver. Jardine et al. (2005) showed a reduction of these inter-tissue differences in starved fish.

In studied perch, $\Delta^{15}N$ and $\Delta^{13}C$ ranged from 1.37‰ to 3.04‰ and from 1.43‰ to 2.96‰, respectively. These enrichments were in the range of values observed by Post (2002) in aquatic food web, ranging from 0.5‰ to 5‰ for $\Delta^{15}N$ and from −3‰ to 3.5‰ for $\Delta^{13}C$. However, the ^{15}N enrichments we observed were lower than the mean $\Delta^{15}N$ value (i.e. 3.4‰) estimated from the literature (Post, 2002) whereas the ^{13}C enrichments were in the upper part of the range. Our enrichments in ^{13}C were consistent with $\Delta^{13}C$ observed on the same species by Vollaire et al. (2007). These authors found $\Delta^{13}C$ between 3.44‰ and 5.98‰, depending on the tissues of *P. fluviatilis* feeding on commercial diet with a constant composition.

As the use of muscle and liver proteins as energy source in tilapia (*O. mossambicus)* exposed to DDT was shown by Ramalingam and Ramalingam (1982), we could fear that this metabolic disruption could lead to increase nitrogen fractionation in muscle and/or

liver in fish exposed to DDT and to disruption of relationships between $\delta^{15}N$ of muscle and that of liver or of other tissue. The comparison of $\Delta^{15}N$ and $\Delta^{13}C$ of perch between the two treatments showed that ^{13}C and ^{15}N enrichments in fish tissues were not significantly altered by food contaminated with DDT (Fig. 1). These results indicated that disruption of nitrogen isotopic fractionation observed by Shaw-Allen et al. (2005), as a result of exposure to a heavy metal (Hg), should not necessarily be generalized to exposure to organic contaminants such as DDT. Shaw-Allen et al. (2005) found a significant difference in the $\Delta^{15}N_{consumer\text{-}diet}$ of muscle and acid-soluble fraction of the liver in *E. thula* when fed food contaminated with mercury (0.39 mg kg^{-1} of fresh weight) versus food less contaminated (0.16 mg kg^{-1} of fresh weight). These results were in line with previously reported data on $\delta^{15}N$ increases in plants exposed to stressors (Gebauer and Schulze, 1991; Hofmann et al., 1997).

According to Shaw-Allen et al. (2005), stressors that alter the relative rates of protein degradation and synthesis within organisms can influence the relative abundance of nitrogen stable isotopes in proteins of affected tissue. Disruption of protein metabolism by DDT has been described by several authors (Bunyan et al., 1970; Leffler, 1975). Ramalingam and Ramalingam (1982) observed a decrease of 41% of liver proteins and 49% of muscle proteins in tilapia (*O. mossambicus*) after seven days of exposure to DDT at 10 μg L^{-1}. Although the concentration used in the present study was five times higher than that interfering with protein metabolism (0.8 mg kg^{-1}) in crab (*C. sapidus;* Leffler, 1975), and above the maximum concentrations recorded *in situ* in natural prey of perch (Easton et al., 2002; Minh et al., 2006), we observed no change in $\delta^{15}N$ of perch tissues (gill, liver, muscle). The concentration tested in the present study was higher or close to maximal concentrations recorded in perch and top-predator fish (i.e. <1 mg kg^{-1} in *P. fluviatilis* from the Baltic Sea and <2 mg kg^{-1} in North American Great Lakes top predator fish; Falandysz et al., 2004; EPA, 2006). Therefore, despite the presence of this contaminant in almost all ecosystems, our results provide no clear challenge to the use of SIA for assessing the trophic level of top-predator fish such as perch, even if they are exposed to DDT.

At the end of our experiment, we did not assess DDT concentrations in tissues of studied fish fed with contaminated pellets CD*. Preliminary studies of Macek et al. (1970) and Warlen et al. (1977) showed DDT bioaccumulation in top-predators fish feeding on prey less contaminated than our CD*. After a 90 d exposure period over 90% of food-derived DDT was accumulated by rainbow trout (*O. mykiss*) feeding on contaminated food containing 0.2 or 1.0 mg DDT kg^{-1} (Macek et al., 1970). When Warlen et al. (1977) fed atlantic menhaden (*Brevoortia tyrannus*) with food containing DDT marked with ^{14}C, fish assimilated and retained between 17% and 27% of the cumulative dose from food containing between 0.58 and 93 μg kg^{-1} of DDT.

In this experiment perch were sacrificed and analyzed after 45 d of treatment. Sakano et al. (2005) observed, after 40 d, a 80% turnover of muscle proteins of sockeye salmons (*Oncorhynchus nerka)* weighing 71–170 g. Consequently, even for muscle tissues showing a lower turnover rate than liver and gill (Haschmeyer and Smith, 1979; Tieszen et al., 1983; Gaston and Suthers, 2004), the duration of our experiment should have been sufficient for renewal of the majority of the tissue and to observe any effect induced by chemical stress. However, there is still the possibility that the majority of tissue growth occurred before DDT accumulation to levels resulting in adverse effect. This allows the possibility that accumulated DDT may have an effect on isotope fractionation that we failed to detect.

Jorgensen et al. (2002) showed that the impact of PCB on the stress response in Arctic charr (*Salvelinus alpinus*) is modulated by the nutritional status of the animal. Well-fed fish accumulate lipophilic contaminants into fat, where the toxic effects are low. With nutritional stress, like starvation, these contaminants are remobilized along with the lipids for energy supply. DDT contamination of food was representative of natural perch prey but the contamination level was possibly too low to have an effect on isotopic fractionation. In the field, animals are often exposed to several stresses (e.g. various chemical contaminants, starvation, etc.) and effect could be reached with lower DDT concentrations than in tanks. We can also assume that, since we removed lipids, we may have missed a disturbance of the isotopic signature related to interference between DDT and the metabolism of lipids (Neto et al., 2008). It would be interesting to test the effect of contaminants on the $\delta^{15}N$ and $\delta^{13}C$ of lipid-free tissue and to compare it with that of tissues with lipids, as suggested by Sotiropoulos et al. (2004), to take into account disruptions on the metabolism of various biochemical compounds.

In the present study, correlations between stable isotopic ratios of different tissues were found only for fish fed contaminated food. As for stress due to starvation observed by Doucett et al. (1999) or by Jardine et al. (2005), these results could be due to change in protein metabolism in tissues having high turnover rates resulting in fractionation increase in these tissues. Relationships in $\delta^{15}N$ (Fig. 2d and e) and $\delta^{13}C$ (Fig. 3e) between tissues were higher in fish exposed to DDT and possibly ensue from lower $\delta^{15}N$ and $\delta^{13}C$ differences between tissues in the most sensitive fish. To test this hypothesis, it will be necessary to expose fish to concentrations elevated enough to induce an effect on protein metabolism in all fish, during a duration sufficient for tissue renewal.

Although in the present study, DDT had no clear effect on $\delta^{15}N$ and $\delta^{13}C$ in the three tested tissues, we can nonetheless infer that DDT influenced the relationship in stable isotope ratios between tissues since correlations between the $\delta^{13}C$ or $\delta^{15}N$ values measured in the different tissues were only found in DDT treated fish. It might be useful to complement this study by other experiments taking into account the isotopic signature of biochemical compounds (i.e. lipids, proteins), longer exposure durations and/or exposures at higher concentrations.

5. Conclusion

To our knowledge, the present study was the first study attempting to assess the impact of DDT on the fractionation factor in fish tissues. Since we detected no DDT effect on $\delta^{15}N$ and $\delta^{13}C$ of various tissues, we provide no reason to cast doubt on the use of stable isotope methods for food web and ecotoxicological studies in the field. Nevertheless, the $\delta^{15}N$ and $\delta^{13}C$ relationships observed between tissues in fish fed on commercial diet contaminated with DDT could have resulted from disruptions of protein metabolisms affecting specifically one of the tissues. As disruption of nitrogen isotopic fractionation can result from exposure to Hg at low concentrations, further investigations using metallic contaminants are needed to better estimate the $\delta^{15}N$ difference between animals exposed or not. Although no clear effect was observed using an organic contaminant, it also appears necessary to test the effect of DDT at higher concentrations and to study the nitrogen isotopic fractionation of animals exposed to other contaminants disrupting protein metabolism.

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