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The dragonet *Callionymus lyra*, a target species used for evaluation of biological effects of chemical contaminants on French coasts

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ABSTRACT: An oceanographic cruise in 1991 along the English Channel and Atlantic coasts of France indicated the value of dragonet *Callionymus lyra* Linnaeus, 1758 as a target species for monitoring biological effects. Ethoxyresorufin-O-deethylase measurements in this species provided a first assessment of the effects of hydrocarbons (PCB, PAH, dioxins) on the marine environment. The specific behavior of dragonet was defined by biochemical characterization. Biological data were obtained during 3 additional oceanographic cruises to study changes in the abundance and distribution of the species in the English Channel and the Bay of Biscay. This first assessment, carried out in the context of the National Observation Network, indicated that pollutants do not have important chronic effects on marine organisms collected along the Atlantic coasts except at a few heavily contaminated sites.

INTRODUCTION

The use of the ethoxyresorufin-O-deethylase (EROD) parameter to monitor biological effects constitutes a new approach in assessing pollution in marine ecosystems. The utility of measuring hydrocarbon induction of mixed function oxygenase (MFO) enzymes as a monitoring index has been widely demonstrated (Goksøyr et al. 1986, Goksøyr 1987, Stegeman et al. 1987, Payne et al. 1988, Spies et al. 1989). Cytochrome P-450-IA1 is induced in fish by a variety of aquatic pollutants including dioxins (Vodicnick et al. 1981), PAH (Addison & Payne 1986, Van Veld et al. 1990) and some congeners of plane PCB (Gooch et al. 1989). EROD combined with cytochrome P-450-1A1 (Nebert et al. 1989) is an efficient tool for detecting pollutants (Suteau 1987, Stegeman & Lech 1991). The fact that it can be analyzed directly on board oceanographic vessels (Galgani & Bocquené 1991, Eggens et al. 1992) makes this parameter particularly suitable for monitoring purposes. A first oceanographic cruise in August–September 1991 along the English Channel and Atlantic coasts of France validated EROD as a specific biomarker for routine monitoring. We demonstrated that dragonet *Callionymus lyra* Linneaus, 1758, a species of fish uniformly distributed along these coasts, was the most appropriate target species of fish for a monitoring network.

This species is quite familiar to taxonomists, but we found that relatively little was known about its biology along the French coast. Accordingly, we undertook a biochemical characterization of dragonet to understand its specific biochemical behavior better and to determine the optimal conditions for analysis of its enzymatic (EROD) activity. Data collected during 3 other surveys along the English Channel and Atlantic coasts of France from 1987 to 1991 provided information about its seasonal distribution.

This first assessment of EROD activity in dragonet provided an estimate of the chronic toxicity of micropollutants of urban or industrial origin discharged along these coasts. The study constitutes a reference point for the 1992 start-up of a network capable of detecting sublethal effects within the relatively near future.

MATERIALS AND METHODS

Sampling strategy and fish collection. Three surveys along the English Channel and Atlantic coasts of France provided previously unavailable data on changes in the abundance and distribution of *Callionymus lyra* (Figs. 1 to 3). The Channel Ground Fish Survey (CGFS) covered the North English Channel area from the Belgian frontier to Cherbourg from 1988 to 1991. The 'Pêche Cotière' (PECOS) survey covered the area from Cherbourg to the Gironde estuary during April, May and October from 1977 to 1982. Finally, the 'Evaluation des Ressources Halieutiques de l'Ouest de l'Europe' (EVHOE) cruises were carried out in the Bay of Biscay in autumn and spring from 1987 to 1991. Taken together, these 3 series of cruises covered the entire western coast of France.

The sampling strategy used during EVHOE cruises was based on a stratification of the survey area according to latitude and depth. In the English Channel (CGFS survey), each ICES statistical rectangle (30' in latitude by 1° in longitude) was divided into 8 subrectangles, and a haul was done in each subrectangle. Large vertical opening bottom trawl fishing for a standard period of 30 min was performed during the day at each station. The EVHOE survey was carried out from 15 m to 600 m depth, and CGFS from 20 to 50 m, whereas the PECOS cruise was more coastal within the 12 mile limit.

Another oceanographic cruise to determine the biological effects of contaminants by assaying EROD activity in dragonet was conducted along the English Channel and Atlantic coasts from 17 August to 6 September 1991 (Fig. 4). Ten specimens trawl-fished at each station were measured and identified as to sex. From Stns 1 to 24, extractions and analyses were performed on board ship without sex differentiation. Samples from Stns 25 to 34 were frozen in liquid nitrogen and analyzed in the laboratory.

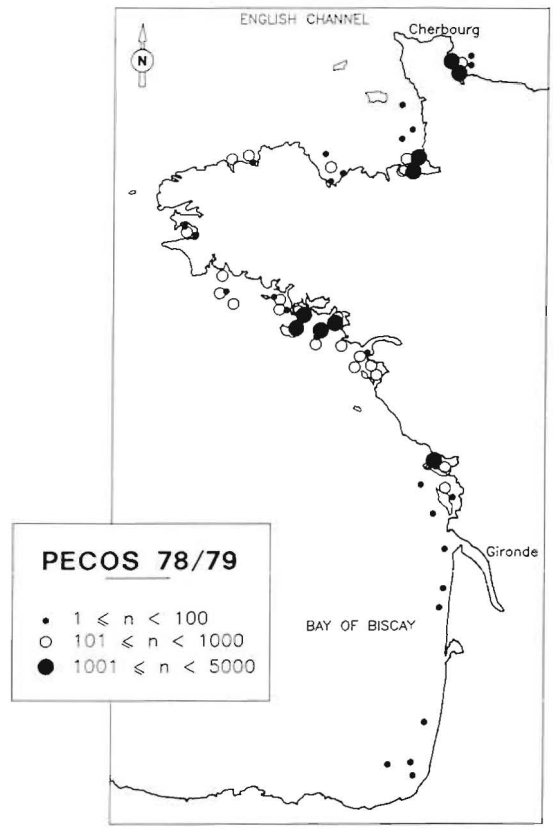

Fig. 2. *Callionymus lyra*. Distribution along the Atlantic coast of France observed during the PECOS cruise, 1978-1979. (n as in Fig. 1)

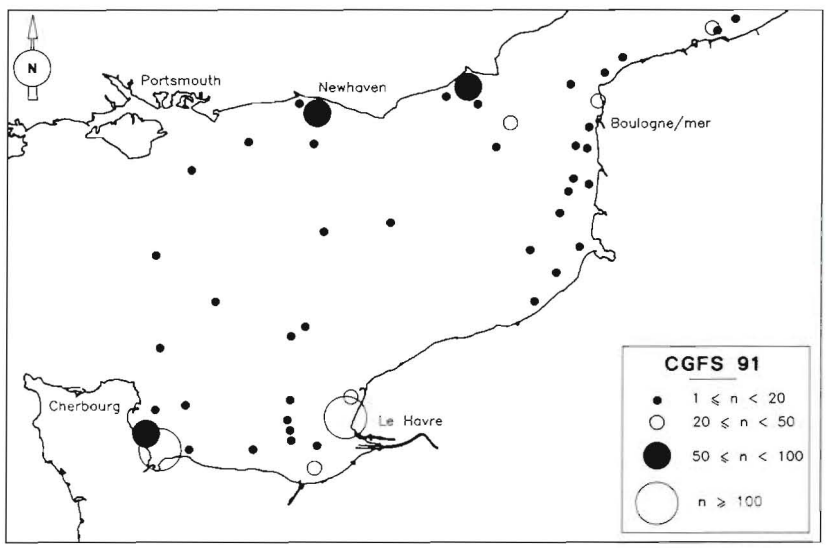

Fig. 1. *Callionymus lyra*. Distribution along the English Channel coast observed during the CGFS cruise, 1991. (n = no. of ind. per 30 min)

Chemical products. Resorufin, 7-ethoxyresorufin, NADPH and metyrapone, a known inhibitor of MFO activities, were purchased from the Sigma Chemical Co.

Preparation of extracts. Livers were washed in buffer (TRIS 50 mM, pH 7.4; KCl 150 mM; EDTA 1 mM; and glycerol 20 % vol) at 4 °C and then minced (5 ml g^{-1} tissue) for 5 to 10 s in a Potter-Elvehjem tube before being centrifuged at 9000 × *g* for 15 min at 4 °C. The supernatant was used as enzymatic solution.

Protein analysis. Proteins were measured in the supernatant according to the method of Bradford (1976),

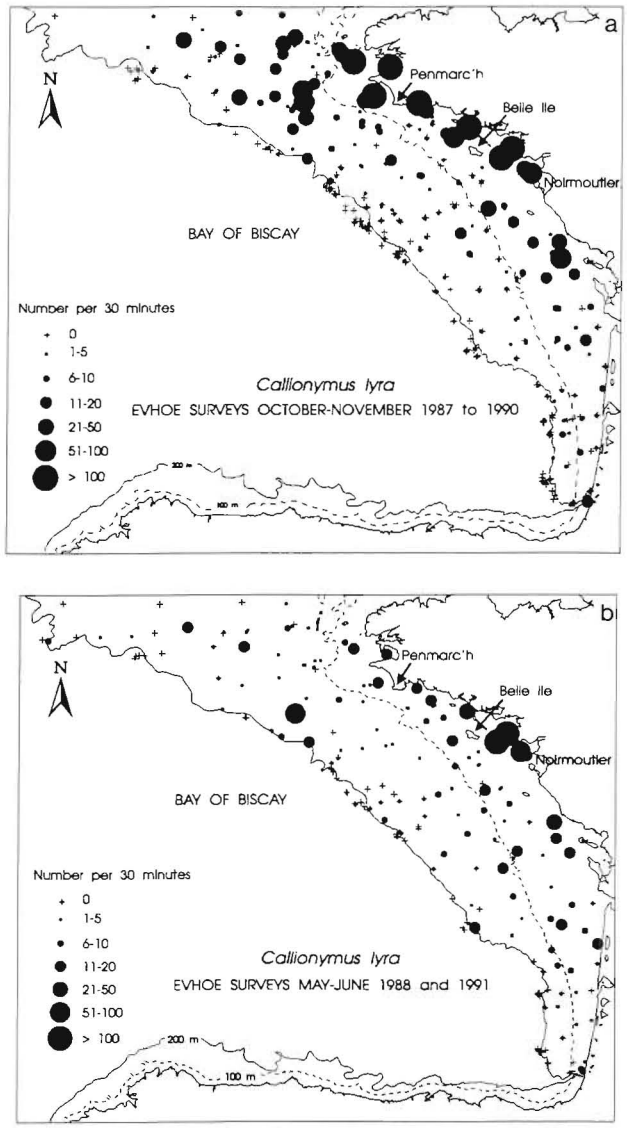

Fig. 3. *Callionymus lyra*. Distribution in the Bay of Biscay observed during the EVHOE surveys in (a) autumn 1987 to 1990 and (b) spring 1988 and 1991

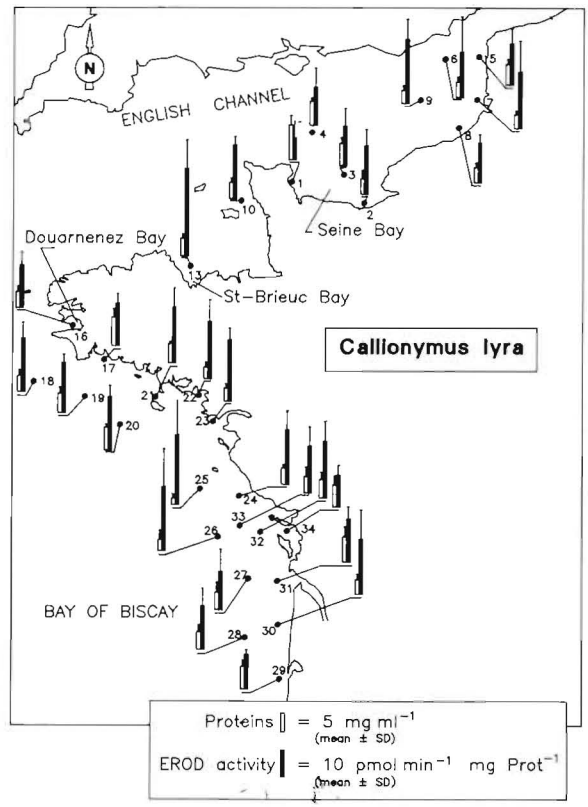

Fig. 4. *Callionymus lyra*. Measurements of EROD activity associated with a protein assay along the English Channel and Atlantic coasts of France

using bovine serum albumin as protein standard. Measurements were done with a platereading spectrophotometer at 595 nm and expressed as mg ml^{-1}.

Analysis of EROD activity. Analyses were performed at 4 °C in a buffer (TRIS 0.1 M, pH 8; NaCl 0.1 M) containing 2 µM of 7-ethoxyresorufin and 0.25 mM of NADPH. Activity was determined by kinetic measurement at room temperature (22 °C) in supernatant according to Addison & Payne (1986), and the quantity of resorufin (the specific product of EROD activity) was determined. Fluorometric measurement (Galgani & Bocquené 1989) was performed according to a modified method of Burke & Mayer (1974), with excitation at 544 nm and emission at 584 nm. Kinetics were determined by using the supernatant extracts diluted to 1 % final. Microplate measurements were done on a Fluoroskan II apparatus (Grzebyk & Galgani 1991). Illumination by excitation light, as well as reception of emission light due to resorufin fluorescence, was done vertically for each well of the plate. Each plate was read in 1 min. Kinetics were linear for 10 min. An external resorufin standard was used to calibrate the apparatus for conversion of fluorescence units into molar quantities (pmol). Results are classically expressed in pmol min^{-1} mg^{-1} protein

Statistics. Means (± SD) at each sampling site were calculated. The differences in EROD activity amongst stations were tested using Student's *t*-test.

RESULTS

Data collected during the surveys showed the distribution of *Callionymus lyra* along the western coasts of France. The main features of species density distribution were observed during the 3 cruises in autumn and spring. It was determined that dragonet are present, regardless of season, along the entire continental shelf.

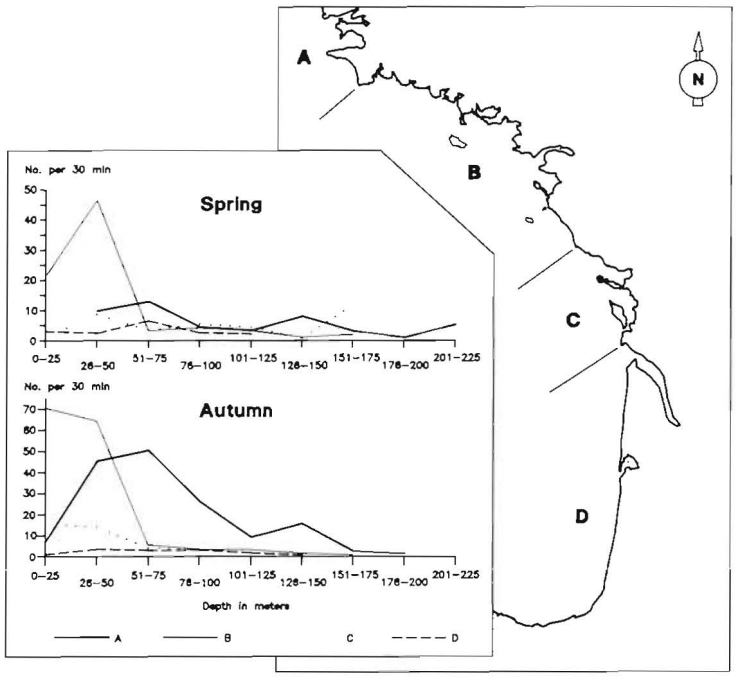

Fig. 5. *Callionymus lyra*. Bathymetric distribution according to geographical areas and seasons in the Bay of Biscay. Data obtained during the EVHOE cruises 1987 to 1991

In the Bay of Biscay, yields are generally lower in the spring, and the species is more dispersed then toward the open sea (Fig. 3b). Seasonal distribution varied less in the middle of the Bay of Biscay than in the northern part.

The distribution was generally more coastal in autumn, and bathymetric dispersion was greater in spring. Bathymetric distribution was less seasonal in Zone B than in Zone A (Fig. 5). The frequency distribution of dragonet length was determined by measuring the total length of all specimens fished in spring 1991. For the entire study area, sizes (Fig. 6) were between 4 and 29 cm, males being markedly larger than females.

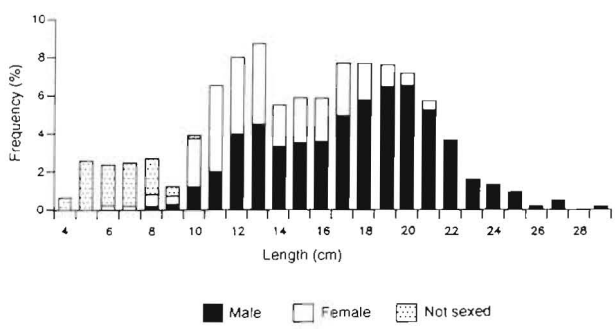

Fig. 6. *Callionymus lyra*. Length frequency distribution in the Bay of Biscay, spring 1991

The histogram for the entire bay shows 2 modes: ca 13 cm and 17 to 18 cm. The sex ratio (no. of males to total no. of fish) was on average greater than 0.5 and slightly higher in spring. Regardless of season, the sex ratio tended to increase with depth.

Changes in the abundance of dragonet according to year and season were studied in the Bay of Biscay. Results were very comparable for the autumn EVHOE cruises from 1988 to 1990 (Fig. 7) with greater abundance in the northern part of the bay and a progressive decrease from north to south, whereas this situation was reversed in 1987. In spring 1988 and 1991, abundance was characteristically low in the northern part of the bay. Results for the last 2 cruises (1990 and 1991) showed an increase of abundances in Areas B and C (Fig. 5) which would seem to be independent of seasonal influence. The very wide confidence interval for the 1987 index (Fig. 5) is attributable to the high concentration (up to 450 ind. tow^{-1}) north and south of Belle-Ile. However, in spring 1988 yields were low and uniformly distributed.

Autumn results for 1988, 1989 and 1990 were very similar, with generally stable abundances.

Biochemical characterization of EROD activity in *Callionymus lyra* was performed to define the specificity of the EROD system for the species. Measurements of EROD enzymatic activity were performed in the liver since most of the activity is centered in that organ (Grzebyk & Galgani 1991). Variations in EROD activity were influenced by the buffer and its pH. Maximal activities were found for TRIS (0.1 M, pH 8.5) and phosphate (0.1 M, pH 7.8) buffers. Use of these types of buffer here as recommended in the standard method (Galgani & Payne 1991) at pH 8 provided optimal conditions for analysis.

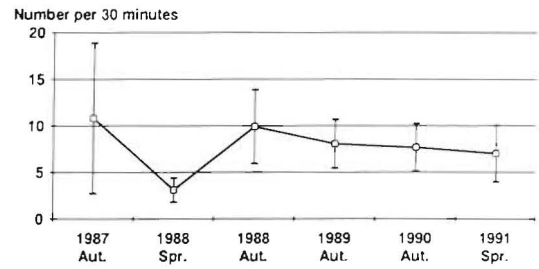

Fig. 7 *Callionymus lyra*. Changes in apparent abundance indices (± 2 SD), spring and autumn surveys, 1987 to 1991. Abundance indices were calculated using the area swept technique, with direct application of the stratified sampling method

Metyrapone was added to the microsomal fraction. This compound is capable of inhibiting EROD activity in the studied standard analytic conditions. EROD activity remained at 90 % (of the EROD activity in standard condition) for concentrations of 0.1 to 0.5 mM of metyrapone but decreased rapidly at concentrations above 0.5 mM.

The influence of temperature on EROD stability was studied to determine the best experimental conditions. Quantities of an enzymatic extract with known activity at 22 °C (standard conditions) were maintained at 22, 37 and 4 °C for 4 h, and measurements were then performed at these temperatures. The survival curve indicating residual enzymatic activity as a function of heating time is given in Fig. 8. The stability of EROD

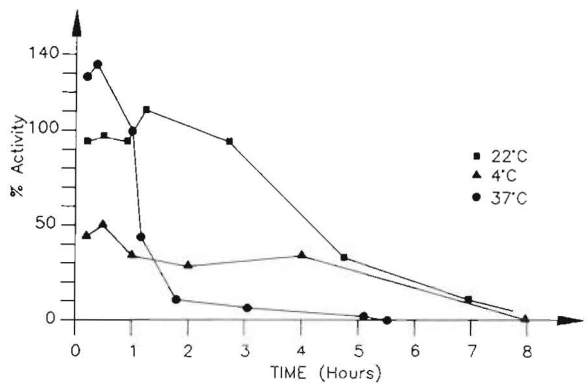

Fig. 8. *Callionymus lyra*. Temperature effect on the stability of EROD activity in dragonet liver

activity at 22 °C allowed 95 % of activity in the standard condition to be obtained after incubation for 24 h. The enzymatic activity of the dragonet is optimal at 37 °C after 30 min incubation of the enzymatic extract but then declines very rapidly.

The kinetic parameters (apparent K_m and V_m) of EROD activity were determined in dragonet liver according to the method of Eadie-Hofster (Galgani et al. 1991b).The absolute value of the slope of the curve gave K_m = 103.76 nM^{-1}, and the ordinate at its origin gave V_m = 24.72 pmol min^{-1} mg of protein (Fig. 9).

The dependence of EROD activity on the NADPH cofactor was also determined. The value indicates that system saturation was reached in our NADPH solution (Fig. 10).

The first results obtained for the western coast of France are relatively homogeneous in the Bay of Biscay and the north of the channel. The absence of significant EROD induction variations observed between the different stations sampled in the open sea and near the coasts does not indicate a significant exposure to the chemical pollutants. The lack of evident

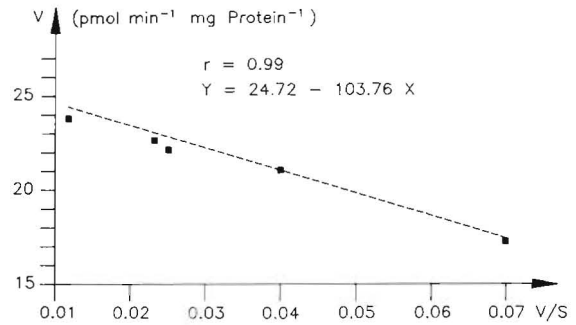

Fig. 9. *Callionymus lyra*. Determination of the kinetic parameters (apparent K_m and V_m) of EROD activity in dragonet liver according to the method of Eadie-Hofster. The absolute value of the slope was K_m = 103.76 nM^{-1}, and the y-intercept was V_m = 24.72 pmol min^{-1} mg $protein^{-1}$

response cannot necessarily be interpreted as meaning there has been no exposure. Interactive effects of other pollutant types (Lee et al. 1980) could severely limit the response and the interpretation. However we have identified significant induction in specific sites such as Seine Bay and Saint Brieuc Bay.

No significant difference was observed in the Seine Bay between Stns 1 and 4 ($p = 0.106$) which seem to be less influenced by the contaminated flux of the Seine River (Salomon 1986). But a significant difference was demonstrated there between Stns 1 and 3 ($p = 0.047$) as well as between Stns 1 and 2 ($p = 0.005$), Stn 2 being located in a zone near the Seine estuary. EROD measurements in dab *Limanda limanda* at Stns 1 and 2 for the same period (Table 1) showed a comparable difference, indicating that the dispersion of contaminants in Seine Bay depends on the dilution flow of the Seine River.

EROD activity was of the same order of magnitude for both dab and dragonet. Induction of EROD activity in the subestuarial zone (Stn 2) showed more exposure of fish to contaminant than in the center and the western coast of Seine Bay. These findings are concordant

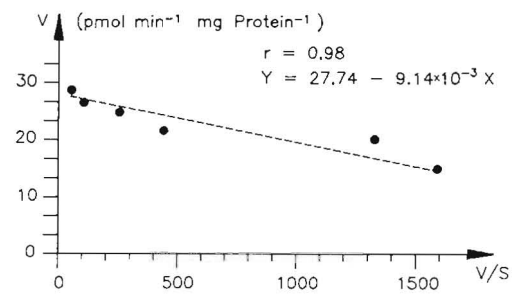

Fig. 10. *Callionymus lyra*. EROD activity dependence for NADPH in dragonet liver. The absolute value of the slope gave the apparent affinity of 9.14 × 10^{-3} mM^{-1} of the EROD system for NADPH

Table 1. *Callionymus lyra* and *Limanda limanda*. Induction of EROD activity in Seine Bay

Species	Stns	EROD activity (pmol min^{-1} mg^{-1} protein) Aug 1991	Mar 1991
Callionymus lyra	1	6.1 ± 1.9	–
	2	18.2 ± 6.3	–
	3	14.9 ± 6.7	–
Limanda limanda	1	4.3 ± 2.7	2.38 ± 2
	2	18.3 ± 10.16	11.15 ± 5.4
	3	–	5.03 ± 3.71

with PCB measurements in Seine Bay sediments and mollusks (Claisse 1989, Galgani et al. 1991a). Assessment of specific PCB congeners demonstrated a higher level of pollutant in the subestuarial zone (5 to 20 µg l^{-1}) than in the center and the western coast (3 to 10 ng l^{-1}) (Abarnou & Simon 1986).

High induction of EROD activity (33 ± 10.9 pmol min^{-1} mg^{-1} protein) was found at Stn 13 Saint Brieuc Bay. This result, based on 5 individuals instead of 10 as at Stn 10, indicates the sensitivity of *Callionymus lyra* to pollutants at a sufficient concentration for induction of EROD activity. These first series of data collected in 1991 require confirmation and comparison with chemical analysis of PCB, in the context of the monitoring network to be set up in 1992.

DISCUSSION

The criteria for biomarker use and the choice of a target species have been defined with respect to assessment of the effect of chemical pollutants on the environment. The conditions proposed during international meetings (Giam 1978, Bayne et al. 1980, Lee et al. 1980, Uthe et al. 1980, Galgani & Payne 1991) were respected in this study. The target species should be widely distributed, indigenous, ecologically important and capable of being maintained in laboratory conditions. Detailed information on its biology, physiology and biochemisty should be known.

The purpose of our study, with respect to these monitoring criteria, was to determine the most suitable species for EROD analysis along the French coasts. An oceanographic cruise along the English Channel and Atlantic coasts indicated the utility of EROD measurements as a biomarker of chemical contaminations (Lech et al. 1982, Lindström Seppä et al. 1985, Buhler & Williams 1989, Burgeot et al. 1992) in *Callionymus lyra*. Since Linneaus first established the genus *Callionymus* in 1758 the classification of this family has been revised several times. The classification of the Callionymidae was examined and its genera are reviewed on the basis of specimens from almost all parts of the world (Fricke 1981, Nakado 1982). Various aspects of the biology of this species have been demonstrated but it has been little studied in France. Works on the behavior of dragonet show that they live in contact with sediment or sand and feed essentially on invertebrates, worms and crustaceans (Davis 1966, Johnson 1973). *C. lyra* is an epibenthic species characterized by a spawning season ranging from April to September, depending on geographical areas (Gibson & Ezzi 1979, Yongshu et al. 1989). A study at a French site (Douarnenez Bay on the Atlantic coast) demonstrated the specific nature of its biology in that region (Hamou-Tahra 1977, Durand 1980). The mating period occurs in January and February, and males can lose up to 70 % of their weight at that time. Dragonet live preferentially in waters with a temperature close to 13 °C. Males may live up to 5 yr, while the females may live 2 yr longer. An analysis of the rates of growth of the spent males showed that the rate of growth is highest in the third-year breeders, and lowest in the fifth-year breeders (Chang 1951).

The stability of dragonet abundance along the western coast in spring and autumn (the periods covered by the different cruises) is a very useful criterion for monitoring the effects of chemical contaminants on the marine environment. A detailed study of the EVHOE cruise in the Bay of Biscay showed a progressive decrease in dragonet abundance from the north toward the south (Fig. 1). The more coastal nature of its distribution in the bay in autumn and its greater bathymetric dispersion in spring (Fig. 5) are helpful criteria for determining a monitoring sampling strategy. As *Callionymus lyra* seemed to be a satisfactory choice in terms of the monitoring criteria defined above, we determined its biochemical characterization. With respect to inhibition of EROD activity, the MFO system appears to be less sensitive to metyrapone in the dragonet than in plaice *Pleuronectes platessa* and flounder *Platichthys flesus* (Grzebyk & Galgani 1991). In the same analytic conditions and for the same concentration of metyrapone (0.5 mM), residual EROD activity was 91 % in the dragonet and only 10 % in the plaice.

Numerous studies involving different species in various parts of the world have shown a correlation between the levels of cytochrome P-450-IA1 induction and the levels of aromatic and chlorinated hydrocarbons in the environment (Payne et al. 1987, Stegeman et al. 1987, 1988, Stegeman & Lech 1991). A study in Seine Bay in the English Channel (Galgani et al. 1991a) demonstrated that PCBs are responsible for cytochrome P-450-IA1 induction in plaice and dragonet. The sampling strategy for EROD activity measure-

ment adopted during that study was then applied along the entire western coast of France.

EROD measurements in dragonet liver using the rapid microplate-reading method (Galgani & Bocquené 1989, 1991) allowed real-time assessment of biological effects in western coastal waters. During a 3 wk period, we integrated the natural variations in this single species relative to biotic and abiotic parameters. The background variation in the level of cytochrome P-450-IA1 was found to be limited and identical for all stations sampled (Goksøyr et al. 1992). Except for the Seine Bay and Saint Brieuc Bay sites where significant variations in induction were observed, EROD activity in the dragonet was relatively homogeneous for the reference stations sampled at sea (around 48 km from the coast) and those near the shore more contaminated by pollutants of anthropogenic origin. The high induction of EROD activity measured in Saint Brieuc Bay (Fig. 4) will be studied in the future to check whether the data are valid. This first study was characterized by a wide sweep along the western coast. Subsequent studies targeted more on sensitive sites such as large estuaries, and involving more frequent sampling, should indicate whether this first assessment of biological effects was correct. A network will be set up in 1992 for biannual monitoring (spring and autumn) along the English Channel and Atlantic coasts of France. Seine Bay, which is heavily contaminated by PCB and PAH (Abarnou & Simon 1986, Claisse 1989), will be the pilot site for monitoring start-up. The objective is to estimate the chronic effects of chemical contaminants (PCB, PAH and dioxins) on marine organisms. Measurements of EROD activity induction in dragonet liver will be performed using a sampling strategy integrating the biological characteristics of this species.

The biology of *Callionymus lyra*, notably its wide distribution, make this fish a particularly useful species for evaluating the biological effect of hydrocarbon contaminants. The use of this target species will probably be extended to the monitoring of other contaminants such as radionuclides.

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