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EROD MONITORING IN DAB FROM THE BELGIAN CONTINENTAL SHELF

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Abstract

The seasonal variation in the level of hepatic ethoxyresorufin O-deethylase (EROD) in dab (*Limanda limanda*) from the Belgian Continental Shelf was evaluated. The biochemical data combined with the chemical data revealed that (1) a significant induction occurs during the coldest period of the year presumably related to the changing metabolic status of the animal, (2) the hepatic EROD activity is inversely related to the PCB and fat content in the liver, (3) the PCB content in liver fat remains constant during the year, but both PCB and fat content vary in a constant ratio, (4) dab migration makes it difficult to interpret small scale geographical differences in the hepatic EROD level in relation to the chemical content of the sediment, (5) no differences in EROD activities were observed between males and females.

Introduction

Xenobiotic biotransformation in fish can occur via the cytochrome P450-dependent monooxygenase system and various conjugating enzymes. The cytochrome P450 monooxygenase system is a central catalyst in the oxidative "phase I" metabolism of endogenous and exogenous compounds. In animals exposed to xenobiotics the severe biological effects (hepatic damage, thymic atrophy, dermal disorders, reproductive toxicity, immunotoxicity, teratogenicity ...) are preceded by the induction of these defense systems. These biochemical changes are considered to be early warning signals of contamination. Potent inducers include some polychlorinated and polybrominated biphenyls (PCBs and PBBs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans and dibenzo-p-dioxins.

These xenobiotics interact with a common cytosolic aryl hydrocarbon (Ah) receptor inducing the synthesis of Cytochrome P4501A1 protein. Ethoxyresorufin O-deethylase (EROD) activity reflects the presence of induced cytochrome P-450.

More details can be found in articles and reviews by Payne et al. (1987), Buhler and Williams (1988 ; 1989), Foureman (1989), Stegeman (1989), Stegeman and Kloepper-Sams (1987), Stegeman et al. (1990), Hansen and Addison (1990), Jimenez and Stegeman (1990), Duinker and Boon (1986), De Voogt et al. (1990), Addison (1992), Goksøyr and Förlin (1992).

This study reports the seasonal variation in hepatic EROD activity in dab from the Belgian Continental shelf. The chemical data for the organochlorine and fat content in the liver are reported separately by Roose et al. (1993). General conclusions deduced from the combination of biochemical and chemical data are reported.

Materials and methods

Chemicals.

7-ethoxyresorufin was prepared according to Klotz et al. (1984). Its purity was judged similar to that of 7-ethoxyresorufin obtained from Sigma Chemical Co. by a combination of TLC and HPLC. NADPH- Na_4 was from Boehringer Mannheim. All other reagents used were of analytical grade.

Animals

Dab (*Limanda limanda*, body lenght 19 to 30 cm) were sampled along the Belgian coast during scientific cruises of the oceanographic vessel Belgica in March, May, September and December 1992 and in March and May 1993. The sampling area comprised about 3,000 km^2. Dab without dermal diseases nor distortions were selected. EROD determinations in liver extracts were performed immediately after collection. The livers were then stored frozen prior to chemical analysis.

Biochemical measurements

Liver samples were homogenized in 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0 with a motor driven Potter-Elvehjem (1,000 rpm ; 0 °C). The homogenate was processed further by centrifigation to a 10,000 g supernatant (10 min ; 4 °C).

EROD activity was measured fluorimetrically by a slightly modified procedure of Burke and Mayer (1974). The incubation mixture consisted of 100 mM sodium phosphate pH 7.4 , 2.5 µM 7-ethoxyresorufin , 200 µM NADPH and 500 mM methanol. The protein content was between 10 and 50 µg per ml. NADPH was dissolved in water immediately prior to analysis.

7-ethoxyresorufin was dissolved in methanol and diluted prior to analysis. The assay was executed at 20 °C on a Shimadzu RF-5001PC (excitation λ = 510 nm, emission λ = 585 nm) and compared to a standard curve prepared with resorufin (Sigma Chemical Co.) in the incubation mixture.
The EROD activity normalized to protein is expressed as pmoles resorufin . min^{-1} . $(mg\ protein)^{-1}$.
Protein was determined by the procedure of Bradford (1976), with bovine serum albumin as a standard.

Chemical analysis.

All chemical analysis are described in detail by Roose et al. (1993).

Statistical analysis

Differences between means were compared using a one-way ANOVA.

Results and discussion

The sampling sites (stations B07 , 710 , 780 , 340 , 435 , 120 , 230 , 140 , 215 , 435 , 350) are presented in the figures. In the Southern North Sea the dab catches indicate a wider distribution pattern in the summer and fall than in early spring. In March 1992, dab were captured in two sites only, out of eleven sampling areas. The number of sites with dab increased afterwards : 5 in May, 7 in September and 6 in December 1992. The higher abundance in March 1993 (4 sites), compared to March 1992, could be explained by the very mild climatological circumstances. This agrees with the findings of Bohl (1957), Rae (1970) and Creutzberg and Fonds (1971) that dab seems to occur at greater depths in winter time whereas during summer dab favours the shallower regions.

The mean specific EROD activities ± SD (in pmoles resorufin produced per min and per mg protein) in liver samples from females per sampling site are shown in the figures 1 - 6. Fig. 7 shows a clear seasonal variation with highest EROD activities in samples from March 1992 (1892 ± 1882 , min 120 - max 8305, n = 32). The EROD activity was approx. ten times lower in May (142 ± 172, min 0 - max 717, n = 60) and September (63 ± 72, min 0 - max 440, n = 109) and decreased further in December (32 ± 49 , min 0 - max 350, n = 99). The activity level was again elevated in March 1993 (530 ± 884 , min 0 - max 5044, n = 72) and decreased thereafter (163 ± 315 , min. 0 - max 1706 , n = 93). Some authors consider this seasonal variation as a nonenvironmental influence that might be related to the period of reproduction (Vignier, 1985 ; Spies et al., 1989). In many fish species, monooxygenase activity decreases before or during spawning (Koivusaari, 1981 ; Walton et al., 1983 ; Lindström-Seppä, 1985 ; Vignier, 1985 ; Luxon et al., 1987 ; Spies et al., 1989). Nevertheless our study demonstrates the highest hepatic EROD activities in dab during the spawning period which extends from January to August in the North Sea (fig. 7). A similar seasonal pattern in hepatic EROD activities in plaice (*Pleuronectes platessa*) was observed by Galgani et al. (1992).

This phenomenon could be explained by the changing metabolic status of the animal : in the coldest period of the year dab is starving and metabolizes its fat reserves thereby liberating fat soluble contaminants present inducing the biotransformation processes. Indicative of this is the inverse variation between the hepatic EROD level and the fat and PCB content of the liver (Roose et al., 1993) : (1) the EROD activity is highest when the amount of fat in the liver is lowest and decreases to a basic metabolic level in well-fed fish and, (2) a similar pattern was found for the PCB content in the liver

which correlates well with its fat content. Roose et al. (1993) reported that the total PCB concentration in hepatic fat of dab remains constant during the year and that its seasonal variation is related to the variation of the fat amount : both hepatic PCB and fat concentrations decrease in a constant ratio towards the end of the year and early spring and increase again when the temperature raises. These results indicate also that no positive correlation exists between the PCB content and the EROD level in the liver and that PCBs are being eliminated out of it in winter. Liberated PCBs and probably other chemicals may induce the monooxygenase system. The most resistent PCBs will presumably redistribute in and/or outside the organism. The redistribution of PCBs out of the liver in relation to fat metabolism may lead to the assumption that the hepatic fat in dab is saturated with PCBs. These fish could be at greater risk in winter than in summer by an enhanced susceptibility of dab tissues to contaminants.

Our results and those reported by Roose et al. (1993) imply that in the environment starving dab should exhibit greater monooxygenase activities than well-fed dab when inducing factors are present in fat tissue. In other words in polluted environments the overall effect of the combined influences of abiotic and biotic factors affecting the EROD activity is induction in winter. This is somewhat in contrast with earlier reported experiments on the effects of temperature and nutrition on monooxygenase activity. The effect of temperature varies among species. Some species exhibit temperature compensation for their enzyme activities (Stegeman, 1979 ; Egaas and Varanasi, 1982 ; Andersson and Koivusaari, 1985 ; Ankley et al., 1985). Other species exhibit greater hepatic monooxygenase activity at higher acclimation temperatures (Dewaide and Henderson, 1970; Jimenez et al., 1988) and a reduced response to inducers was reported at lower

temperatures (Stegeman, 1979 ; Förlin et al., 1984 ; Jimenez and Burtis, 1988, 1989). Nutrition can also affect the activities of detoxication enzymes in fish. The effect of starvation results usually in a decrease in monooxygenase activity (Walton et al., 1978 ; Andersson, 1986 ; Jimenez et al., 1988). These results imply that fish in unpolluted areas presumably will exhibit lower activities in winter than in summer. Nevertheless, Jimenez and Burtis (1989) have demonstrated that pollutants still can induce monooxygenase activity in moderately starved fish. The overall conclusion might be that a different pattern in seasonal variation in monooxygenase activity in polluted and unpolluted environments may occur. In polluted environments the seasonal variation will depend on the degree and kind of the pollution.

Our results indicate that no differences in hepatic EROD activities between female and male dab were observed. Such differences were first described for trout among which males had greater monooxygenase activities than females (Hansson et al., 1980 ; Stegeman and Chevion, 1980), and are associated with gonad maturation and spawning (Stegeman and Woodin, 1984 ; Williams et al., 1986). Studies on the effects of estradiol on trout confirm that hormonal factors can influence monooxygenase activity in fish (Hansson and Gustaffson, 1981 ; Förlin and Hansson, 1982 ; Stegeman et al., 1982 ; Vodicnik and Lech, 1983). That no sex dependent difference in EROD activity in dab liver was observed was previously reported by Lange et al. (1992). This finding is interesting because no sex distinction will have to be made. Our results indicate that only 10% of the dab catches in the southern North Sea are males. A former study reported a sex ratio of 80/20 for the females (Gillis, 1966). EROD monitoring in male dab would be difficult to perform in some areas of the North Sea.

In a few cases only significant geographical differences in EROD activities were observed : the EROD level in samples from station 340 in May 1992 (fig. 2), stations 230 and 780 in September 1992 (fig. 3), station 350 in March 1993 (fig. 5) and station 215 in May 1993 (fig. 6) were significantly higher than in the other sites. The local induction could not be confirmed by increased PAH and PCB levels in the sediment with the exception that station 780 is a dredge dumping site containing elevated PAH concentrations (Roose, unpublished results). In addition no induction gradient could be demonstrated from the estuary of the Scheldt and the Yser (Nieuwpoort) towards the open sea. This phenomenon is probably caused by the migrational activity of dab. Maximum daily migration speed have been reported from tagging experiments by De Clerck (1984 ; 4 miles) and by Damm et al. (1991; 3 miles). At this speed the animals can easily travel through the whole area in less than 2 weeks. As EROD induction can persist for several weeks (Melancon et al., 1987 ; Kloepper-Sams and Stegeman, 1989) correlation analysis between EROD induction and chemical data in sediment will not be useful. Migration of individuals is probably also the mean reason for the large variation in EROD activities in one sample. In addition the migrational behaviour of dab in the Southern bight is still unclear. De Clerck (1984) reported a mean migration route in a NE direction during the second half of the year whereas Damm et al. (1991) revealed an elevated migratory activity in January through April in a SW direction, probably associated with spawning time.

The overall activity on the Belgian Continental Shelf in September is very low compared to the level in the German Bight. Lange et al. (1992) recorded maximum values of EROD activities of 2.160 pmoles/min/mg protein in dab from the German Bight in August/September 1990. Towards the Dogger Bank these values decreased to 590 pmoles/min/mg protein. Although small scale

geographical differences in EROD activities are difficult to interpret, these results indicate that large scale determinations should be able to distinguish between more or less polluted areas. The EROD activities measured in the German Bight correspond to known high degrees of organic contaminants such as PCBs.

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Fig. 1: EROD activities in dab liver - March 1992
1 cm = 1,000 pmoles resorufin per min and per mg protein

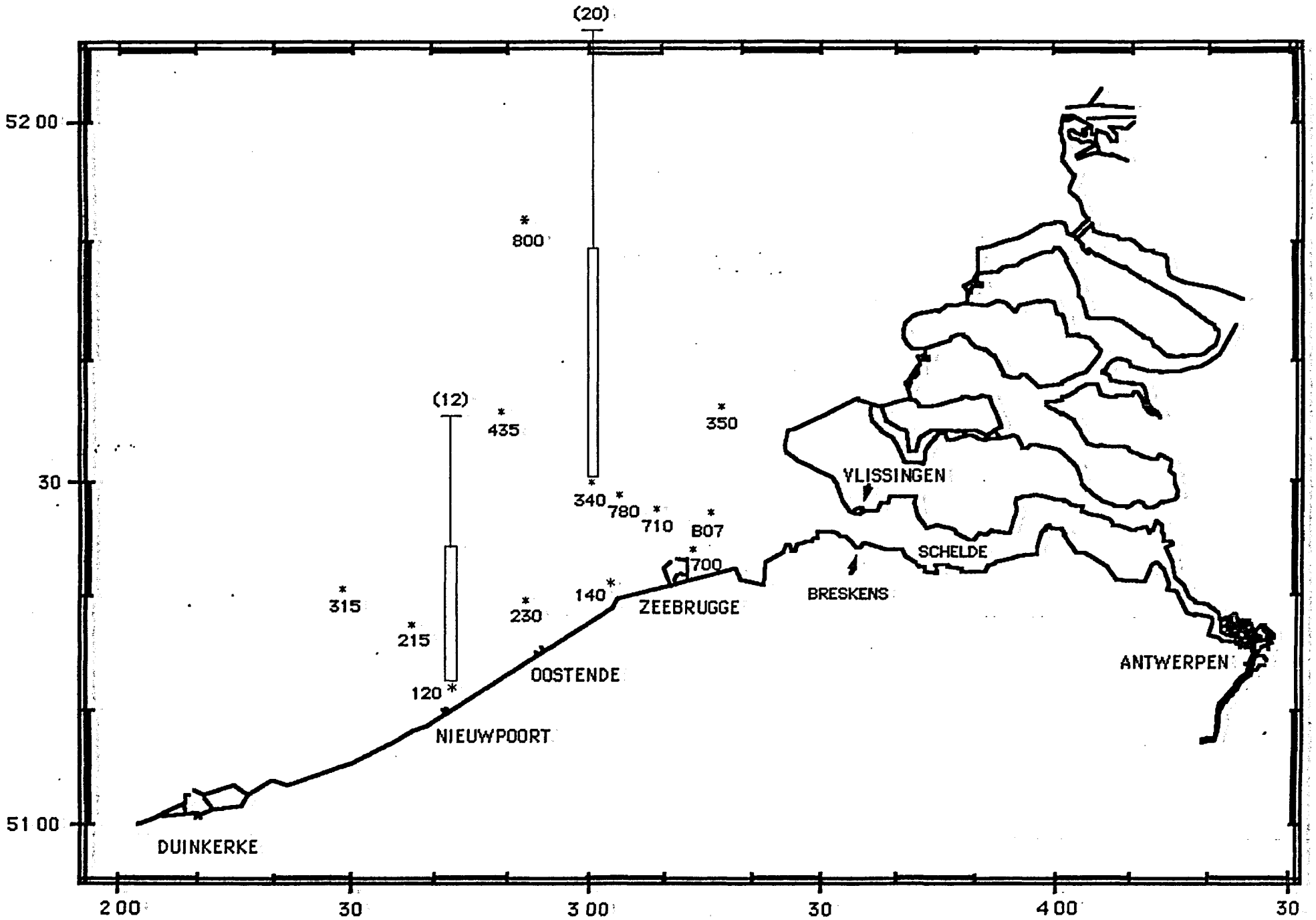

Fig. 2. EROD activities in dab liver - May 1992
1 cm = 1,000 pmoles per min and per mg protein

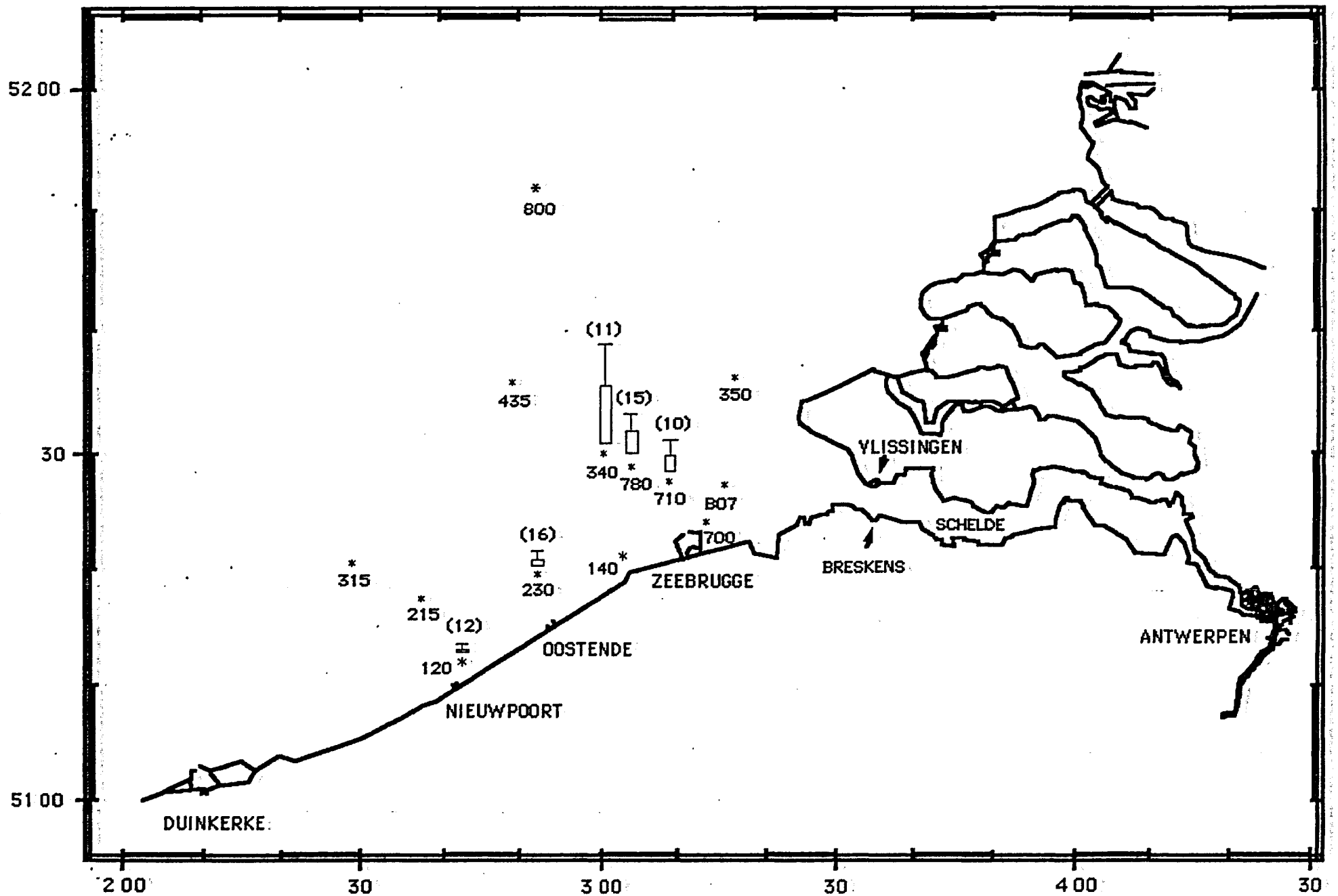

Fig. 3. EROD activities in dab liver - September 199
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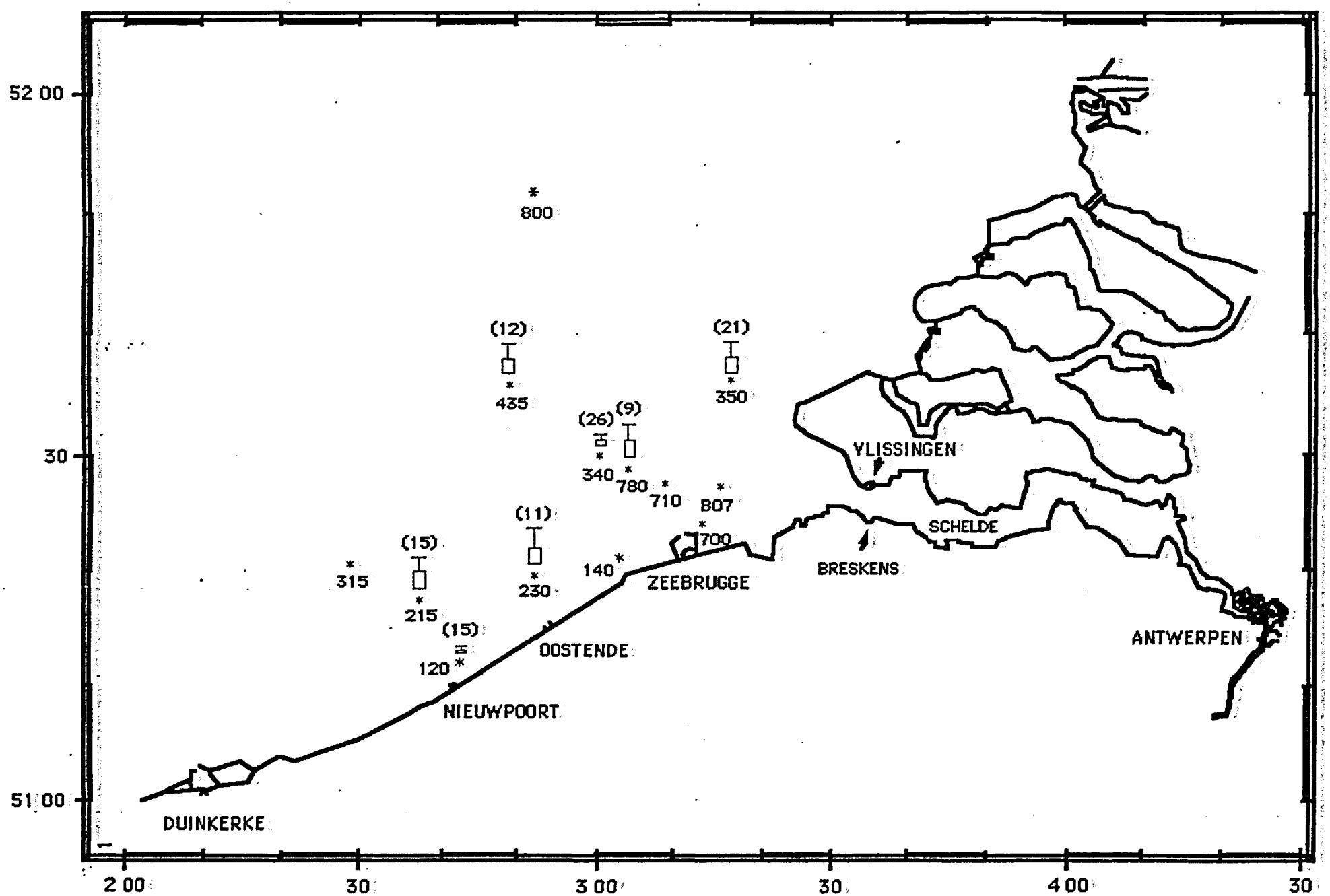

Fig. 4. EROD activities in dab liver - December 1992
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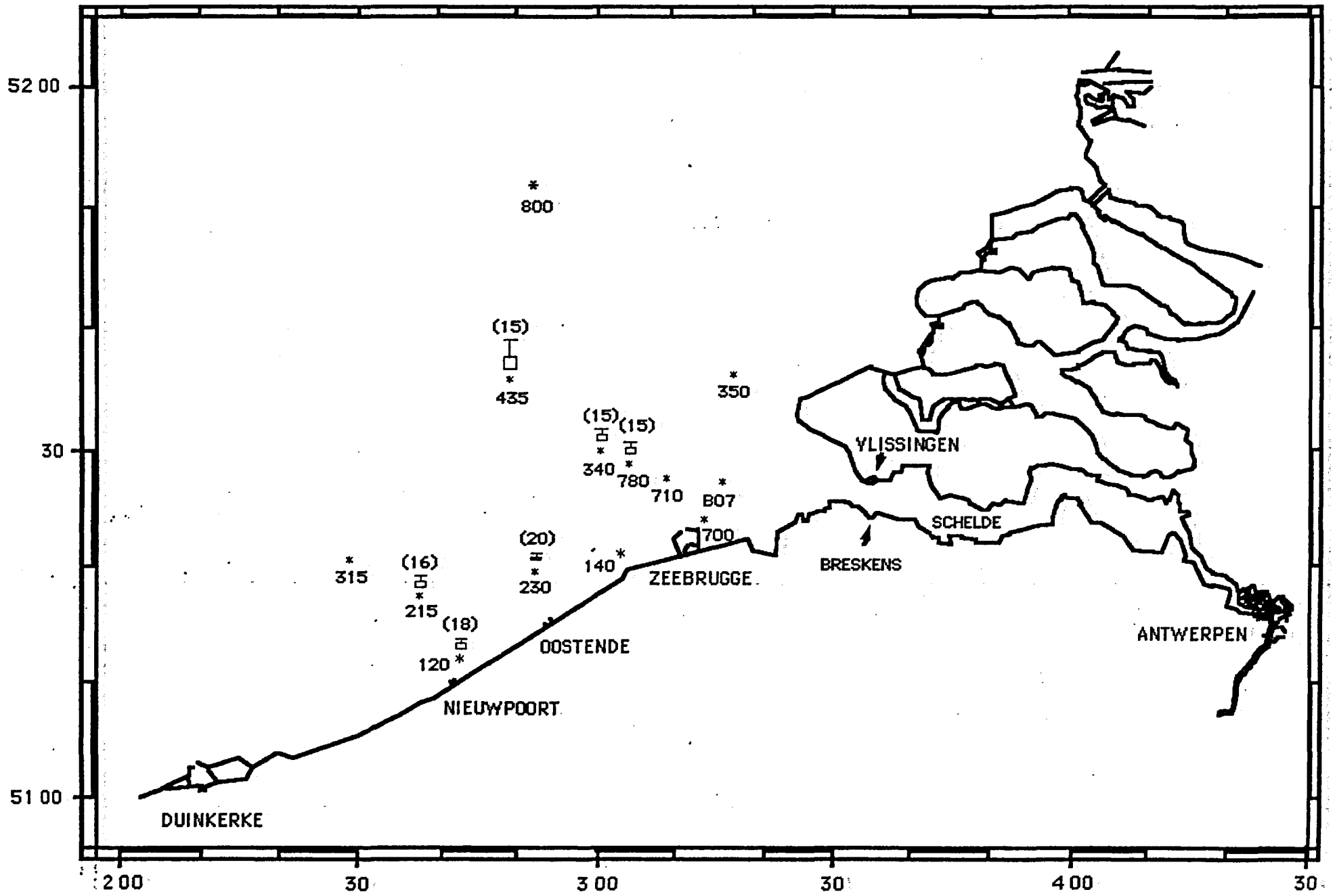

Fig 5. EROD activities in dab liver - March 1993
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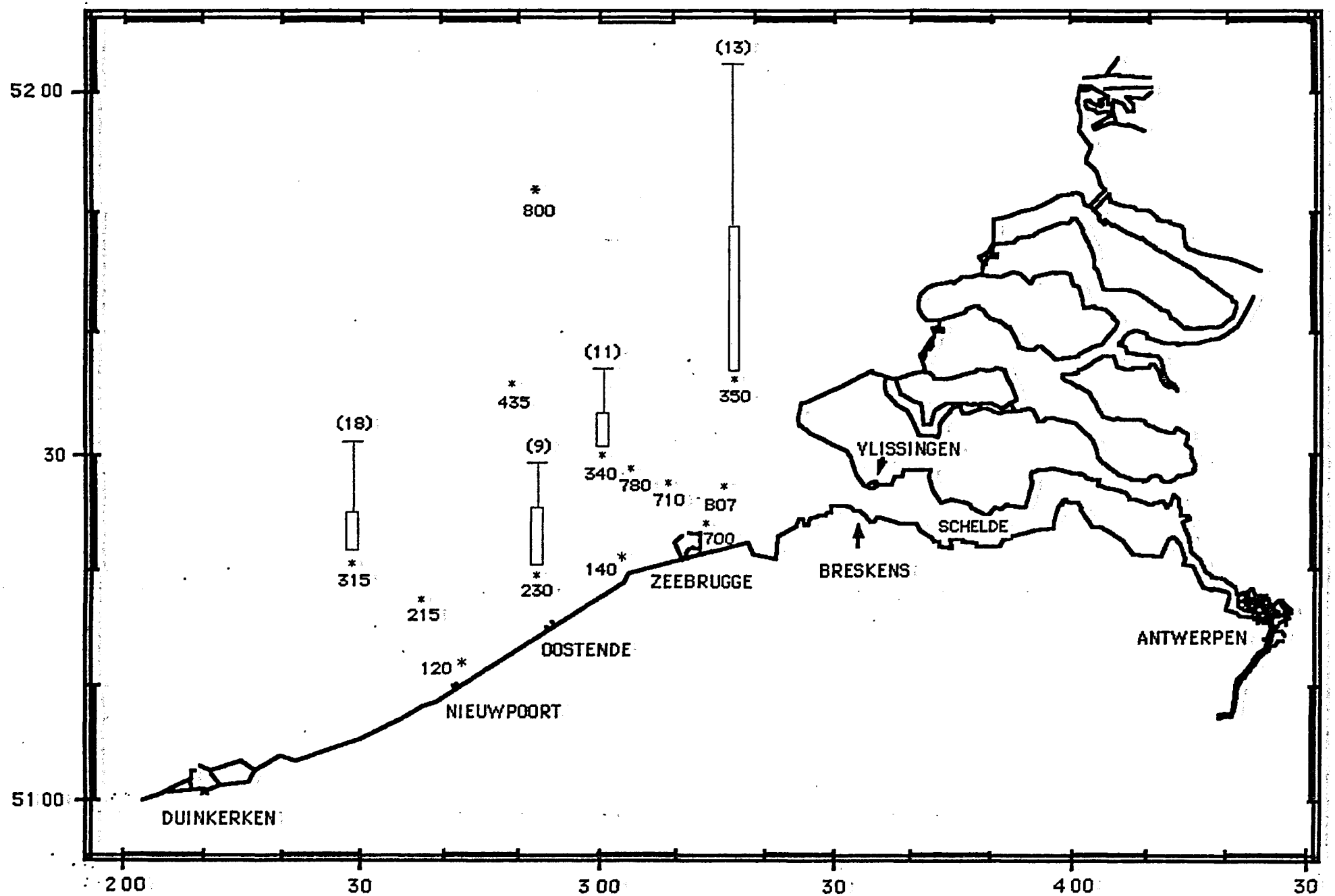
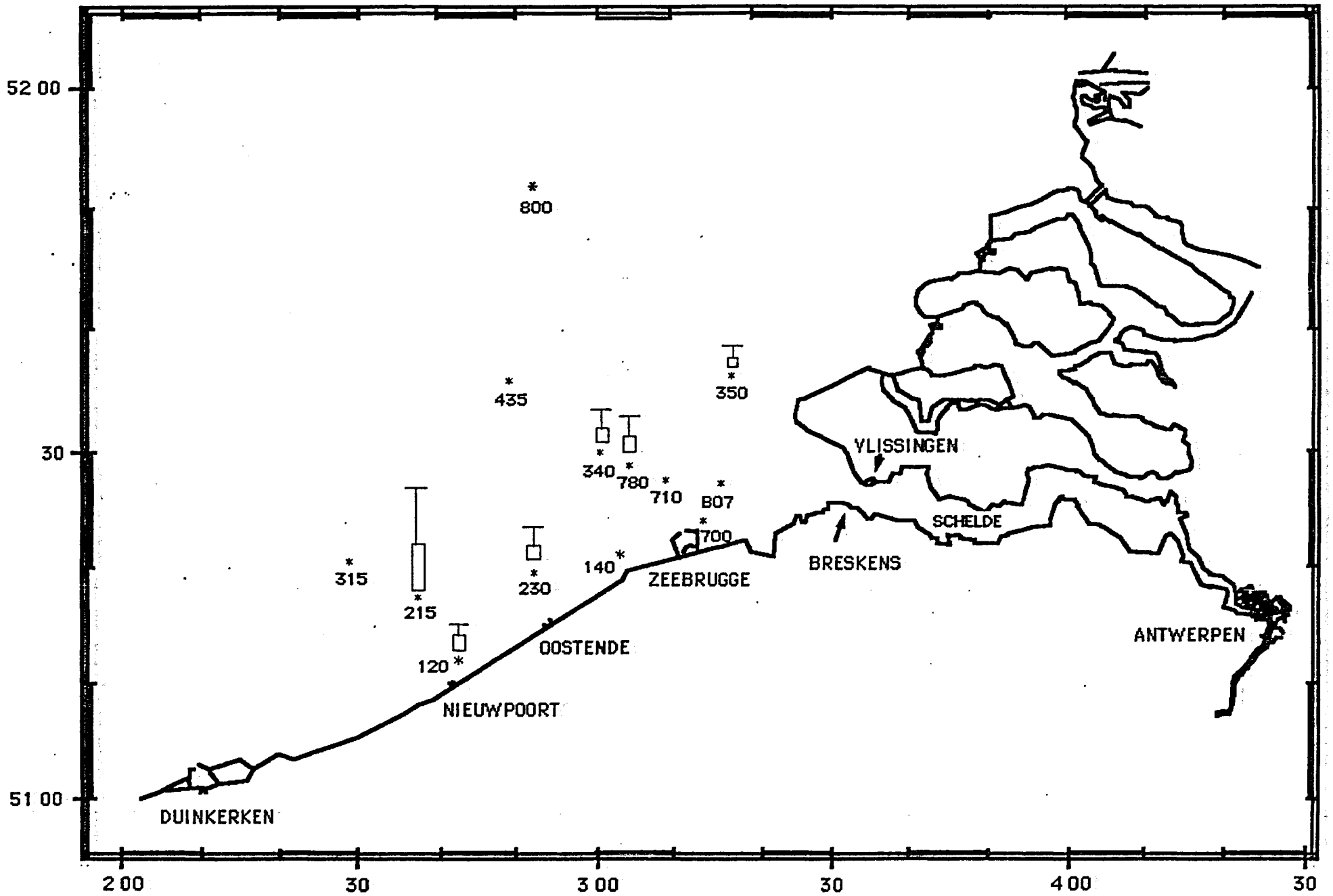


Fig. 6. EROD activities in dab liver - May 1993
1 cm = 1,000 pmoles resorufin per min and per mg protein



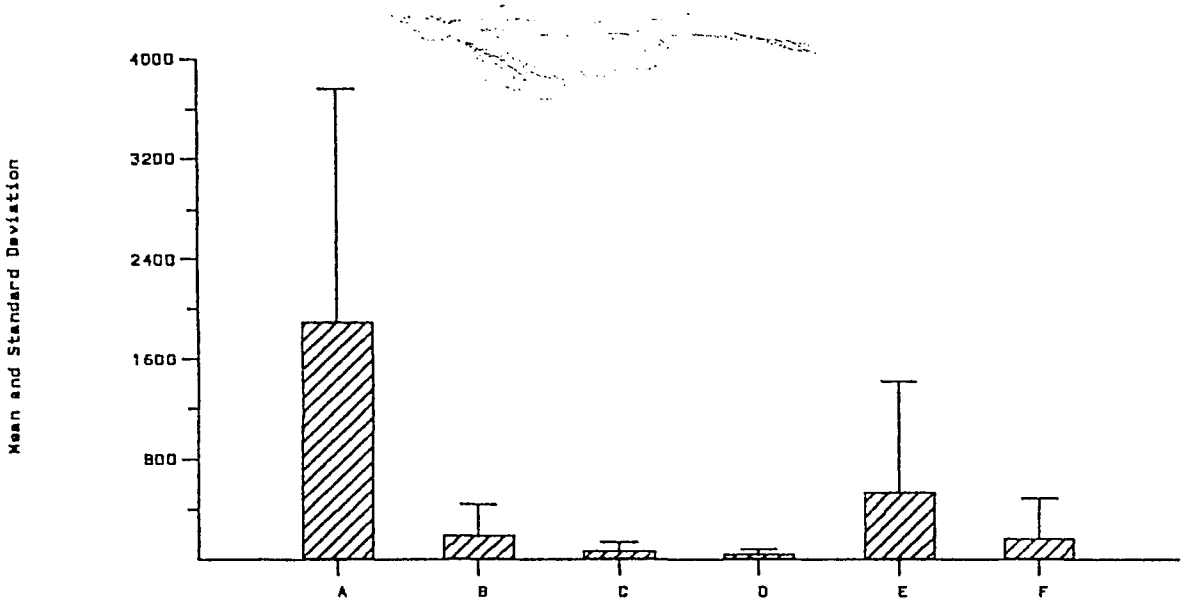
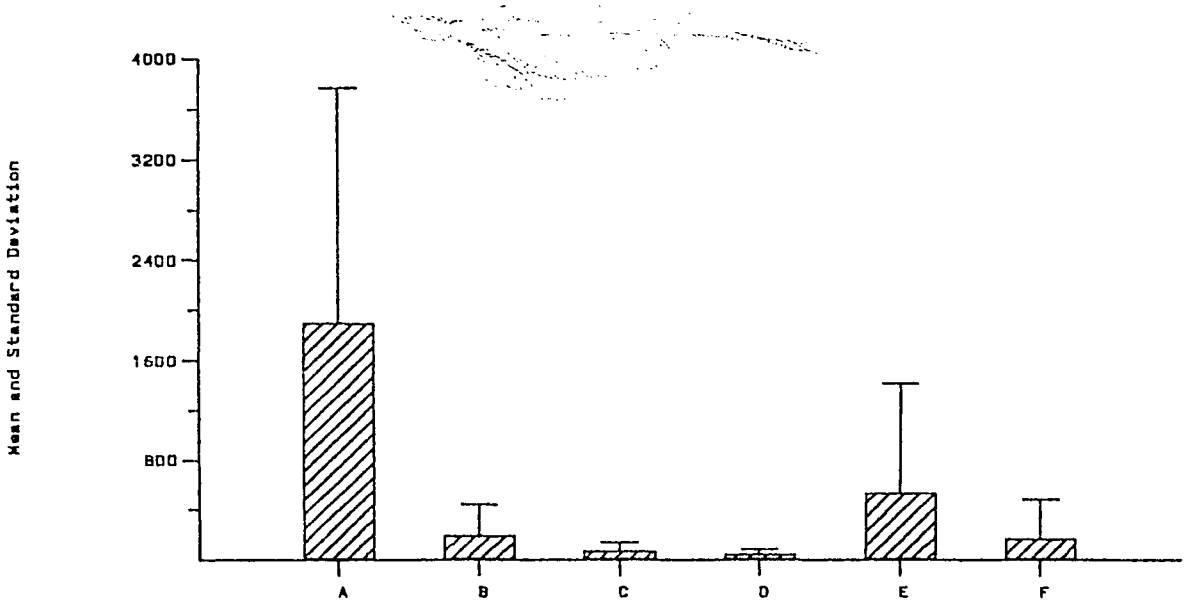


Fig. 7. Seasonal variation of EROD in dab liver from the Belgian Continental Shelf. EROD was determined in March 1992 (A), May 1992 (B), September 1992 (C), December 1992 (D), March 1993 (E) and May 1993 (F).
The activity is expressed as pmoles resorufin/min/mg protein.



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