# Temperature as a key factor determining the regional variability of the xenobiotic-inducible ethoxyresorufin-*O*-deethylase activity in the liver of dab (*Limanda limanda*)

**U. Lange, R. Saborowski, D. Siebers, F. Buchholz, and L. Karbe**

**Abstract**: Water temperature exhibits a strong influence on the regional variability in activity of the 7-ethoxyresorufin-*O*-deethylase (EROD) in the liver of dab (*Limanda limanda*) from the German Bight during the spawning and postspawning seasons. The relationship between EROD activity and water temperature found in situ was, surprisingly, not masked by migrations of dab. Correlations between EROD activity and temperature could not be explained by a direct temperature effect in terms of temperature compensation. Instead, we suppose that temperature influences EROD activity indirectly via its influence on the duration of the gonadal cycle and thus on the time of spawning, which we assume to be coupled with the seasonal variation in EROD activity. Significant negative correlations between EROD activity and the condition factor, gonadosomatic index, and liver–length index could be attributed to the parallel or opposite temporal changes of these parameters. In the liver of spawning females, no linear relationship was detected between the EROD activity and the concentration of the polychlorinated biphenyls (PCB), which have been previously determined.

**Résumé** : La température de l'eau influe fortement sur la variabilité régionale de l'activité de la 7-éthoxyrésorufine-*O*-déséthylase (EROD) dans le foie de limande (*Limanda limanda*) de la baie d'Helgoland (mer du Nord) pendant et après la saison de fraye. Le rapport entre l'activité EROD et la température de l'eau observée in situ n'était pas, chose étonnante, masqué par les migrations de la limande. Les corrélations relevées entre l'activité EROD et la température ne pouvaient pas s'expliquer par un effet direct de la température exprimé sous forme de compensation thermique. Nous supposons plutôt que la température influe indirectement sur l'activité EROD en agissant sur la durée du cycle de développement des gonades, et donc sur le moment de la fraye, qui serait associé à la variation saisonnière de l'activité EROD. Des corrélations négatives importantes entre l'activité EROD et le coefficient de condition, l'indice gonadosomatique et l'indice foie–longueur pourraient être attribuées aux modifications parallèles ou opposées de ces paramètres. Dans le foie des femelles génitrices, on n'a relevé aucune relation linéaire entre l'activité EROD et la concentration de polychlorobiphényles (PCB) conformément aux observations antérieures.
[Traduit par la Rédaction]

## Introduction

The cytochrome P450 enzyme system is involved in the metabolism of lipophilic substrates, such as steroids and unsaturated fatty acids. Because of the multitude and the variety of their substrates, these enzymes play an important role in physiological processes, such as growth, differentiation, homeostasis, and neuroendocrine functions (Nebert 1991). In addition, they metabolize organic xenobiotics (Goksøyr and Förlin 1992; Stegeman and Hahn 1994). In fish, cytochrome P450 1A dependent enzymes are inducible by specific organic xenobiotics such as polychlorinated biphenyls (PCB), polyaromatic hydrocarbons (PAH), dibenzodioxins, and dibenzofurans (Ueng et al. 1992; Lemaire-Gony and Lemaire 1992; van der Weiden et al. 1989).

Based on its inducibility by organic xenobiotics, the P450 1A dependent enzyme 7-ethoxyresorufin-*O*-deethylase (EROD) is often used as a biomarker for monitoring organic pollution in the environment (Goksøyr et al. 1994*a*; Haux and Förlin 1988; McMaster et al. 1991; Payne et al. 1985). In marine environments, increased P450 1A enzyme activities have been found near oil fields (Burns 1976; Davies et al. 1984; Payne et al. 1985; Stagg et al. 1995) and at PAH-contaminated sites (Goksøyr et al. 1991, 1994*a*). Positive correlations between EROD activity and concentrations of several PCB congeners and aromatic hydrocarbons in the liver of flatfish from the North Sea have been established (Eggens et al. 1992; Stagg et al. 1995).

For a reliable interpretation of EROD activity in relation to organic contamination, we need a comprehensive understanding of its natural variability and the biotic and abiotic factors that may influence it. The present knowledge on the natural variability of EROD activity in the liver of dab (*Limanda limanda*) has recently been summarized (Saborowski et al. 1995). In this paper a detailed study of regional and seasonal differences of EROD activity in the liver of dab from the

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**Table 1.** Research vessels, dates of the cruises, number of stations, samples per station, and the stages of maturity of dab (*Limanda limanda*) according to Htun-Han (1978).

| Research vessel | Date | Stations | Samples per station* | Stage of maturity |
|---|---|---|---|---|
| *Valdivia* | April 1992 | 19 | 1–6 | Spawning |
| *Heincke* | August 1992 | 27 | 4–6 | Postspawning |
| *Uthörn* | December 1992 | 6 | 2–8 | Prespawning |
| *Uthörn* | May 1993 | 1 | 42F, 31M | Spawning |

*M, Males; F, females.

German Bight is presented. At different seasons we examined the hepatic EROD activity of dab at 28 stations in the German Bight. To determine the possible factors that might influence EROD activity, we considered water temperature and meristic characteristics, as well as the concentrations of PCB (Landgraff 1995).

## Materials and methods

### Sampling procedure

Male and female dab at different stages of maturity were caught on cruises of the research vessels *Heincke*, *Uthörn*, and *Valdivia* (see Table 1). Nets were trawled for 20–30 min at a speed of 3–4 knots (1 knot = 1.852 km/h) using a beam or a bottom trawl (minimum mesh size 3.5 cm). Fish were killed by a blow to the head and livers were removed and shock frozen in liquid nitrogen for no longer then 8 weeks. All animals were adults with a mean body length of 15–25 cm.

### Meristic characteristics

The stage of maturity and the meristic characteristics condition factor (CF) and gonadosomatic index (GSI) were determined according to Htun-Han (1978). Instead of the hepatosomatic index, we calculated the relative liver weight (liver–length index (LLI)) by dividing the liver weight (g) by the cube of fish length (cm):

$$\mathrm{LLI} = \frac{\text{liver weight}}{\text{length}^3}$$

### Temperature experiments

Fish used in the experiments were kept in 120 × 80 × 20 cm aquaria containing 150 L seawater in a flow-through system at the marine ecological laboratory of the Biologische Anstalt Helgoland. Fish were fed dry food (ALMA-dry food for seabass; Botzenhardt KG, Kempten-Allgäu, Germany) after 1 week of acclimation. After 2 weeks of acclimation at ambient temperature, experiments were begun.

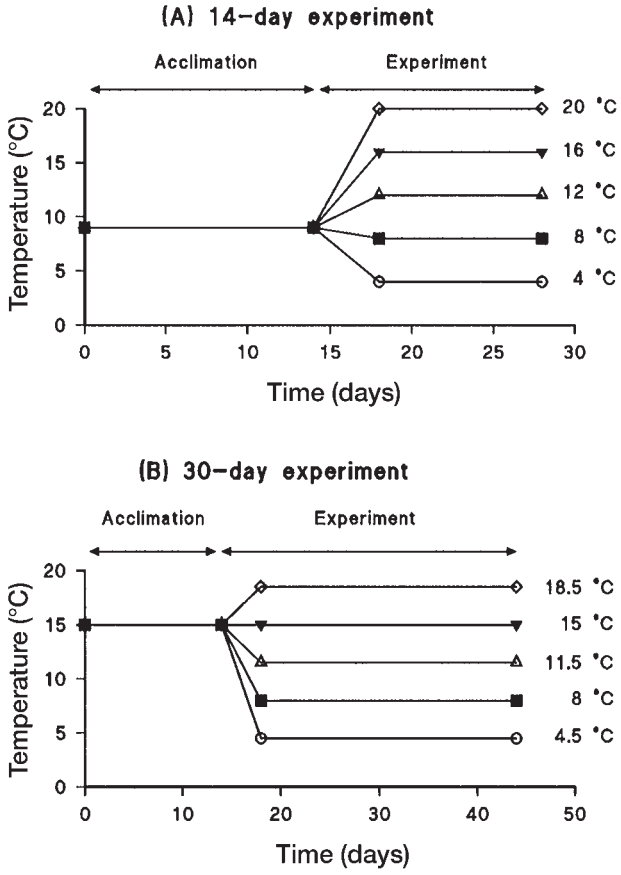
Water temperatures were adjusted in steps of maximum 3°C per day to five different and equidistant temperatures in the range between 4 and 20°C (4, 8, 12, 16, and 20°C) or between 4.5 and 18.5°C (4.5, 8, 11.5, 15, and 18.5°C). The experiments lasted 14 and 30 days, respectively (see Fig. 1).

During the 14-day experiment, all dab were fed daily with dry food at approximately 1% of body weight. For the 30-day experiment, dab received 0.5, 0.625, 0.75, 0.875, and 1% of body weight (at 4.5, 8, 11.5, 15, and 18.5°C) to compensate for the increased energy demand at higher temperatures.

### Biochemistry

For the preparation of microsomes, a maximum of 1500 mg liver tissue was homogenized in two volumes of 0.1 M sodium phosphate buffer containing 2 mM glutathione and 1 mM EDTA tetrasodium salt, pH 6.5, in a Potter homogenizer (Mini Potter, Braun Melsungen,

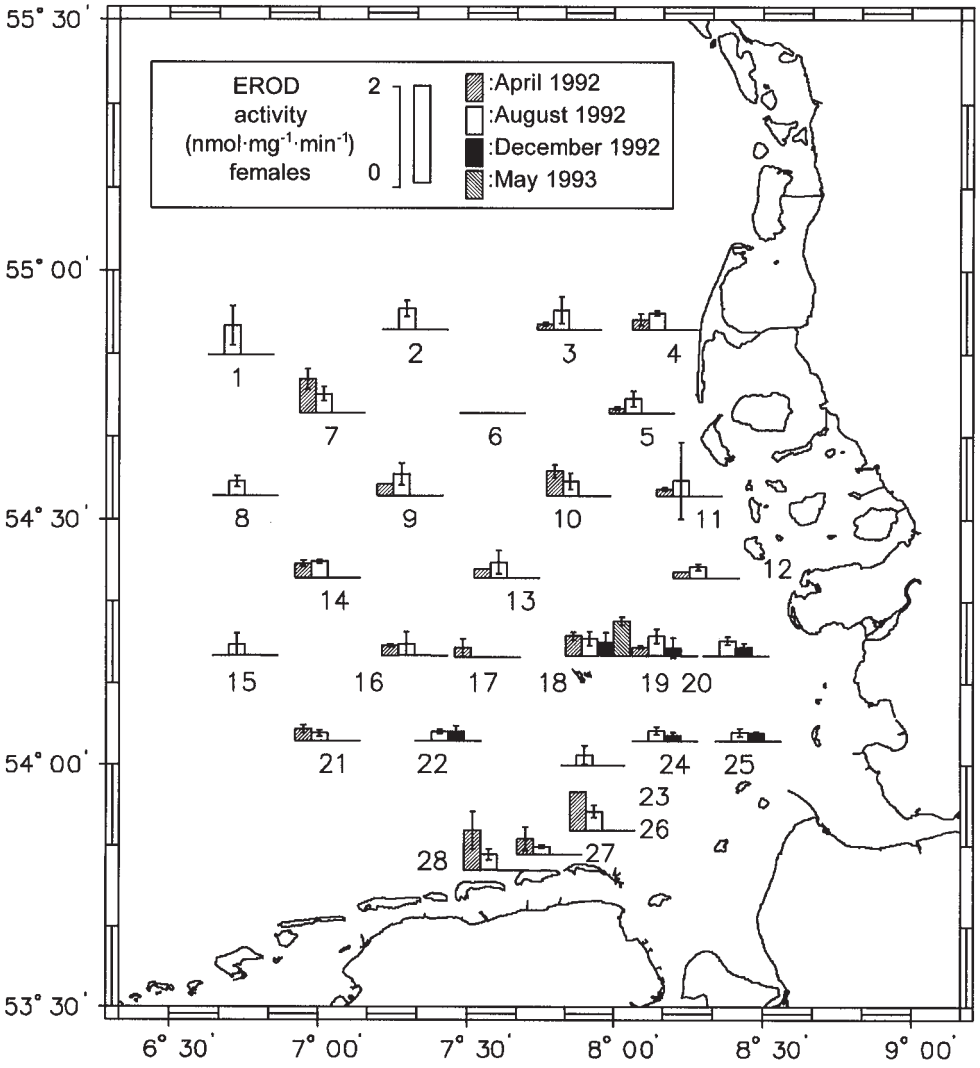
**Fig. 1.** Temperature conditions during the temperature experiments performed over a period of 14 days (December 1992; A) and 30 days (June 1993; B).




950 rpm). Homogenates were centrifuged at 12 500 × *g* for 20 min to remove cell debris and mitochondria. The supernatants were centrifuged for 75 min at 135 000 × *g* to sediment the microsomes. The microsomal pellets were resuspended with two volumes of 0.1 M phosphate buffer (pH 8.0) containing 0.15 M potassium chloride. To microsomal samples, which were stored in liquid nitrogen, 20% glycerol was added. Generally, EROD activity was measured in freshly prepared samples, but for the determination of the Michaelis–Menten constant ($k_m$) and the $Q_{10}$ value, frozen samples were used. Protein concentrations in the microsomal suspensions ranged between 4 and 10 mg·mL$^{-1}$. All operations were performed at 4°C.

Stock solutions of 400 µM ethoxyresorufin were prepared in dimethylsulfoxide. These solutions were kept at 4°C and diluted with nine volumes of 0.05 M Tris–HCl buffer (pH 8.0, 0.15 M KCl, 1 mM dithiothreitol) before use. Eight hundred microlitres of Tris buffer, 50 µL of the diluted substrate solution, and 50 µL of sample were pipetted into a cuvette. After 1 min of incubation the reaction was started with NADPH (100 µL Tris buffer including 0.45 mM NADPH). EROD activity as production of resorufin per unit of time was determined photometrically by monitoring the increase in absorption at 572 nm, using the molar extinction coefficient of resorufin $\varepsilon_0$ = 73.2 mM$^{-1}$·cm$^{-1}$ (Klotz et al. 1984). For the detection limit of EROD activity, we consider twice the detection limit of the photometer (2 × 0.001 = 0.002), because activities were calculated as the difference between the sample and a control. The method used enables us to determine EROD activities ≥13.5 pmol·mL$^{-1}$·min$^{-1}$. Depending on the protein concentration of the microsomal sample, this value corresponds to 30–75 pmol·mg$^{-1}$·min$^{-1}$ (Lange 1995). In the study presented, all EROD activities were higher than 30 pmol·mg$^{-1}$·min$^{-1}$.

**Fig. 2.** EROD activities (nmol·mg$^{-1}$·min$^{-1}$) in the liver of spawning (April 1992 ▨ and May 1993 ▧), postspawning (August 1992 □), and prespawning female dab (*Limanda limanda*) (December 1992 ■). Values are median ± SE of the median from a sample size of 2–8 livers (May 1993: 42 females; $n = 2$ or 3 for bars without error bars).




Protein concentrations, which varied between 4 and 10 mg·mL$^{-1}$, were determined by the method of Lowry et al. (1951), using a stock solution of bovine serum albumin as a standard.

### Statistics

Differences between means obtained from different stations were tested for significance using the single-sided least square difference test (LSD test, STATGRAPHICS). EROD activities at a station were considered to be significantly elevated (depressed), if the mean was significantly higher (lower) than the mean of five other stations ($\alpha = 0.05$).

For the determination of Spearman rank correlations between temperature and EROD activity, data from each fish were considered, and the correlations between the means of the organochlorine concentrations and the medians of EROD activity at each station were tested for significance.

## Results

### Regional variability of EROD activity

In Figs. 2 and 3, the hepatic EROD activities of spawning, postspawning, and prespawning female and male dab from 28 stations of the German Bight are presented. The regional differences are described in Table 2. Trends between coastal and offshore stations were not detected, and stations where significantly high or low activities in the liver of dab were determined varied according to the season.

Generally, EROD activities near the East Frisian islands (stations 26–28) were high (females, 0.3–0.8 nmol·mg$^{-1}$· min$^{-1}$; males, 0.55–3 nmol·mg$^{-1}$·min$^{-1}$). Apart from that, stations exhibiting high or low EROD activities are irregularly distributed throughout the German Bight. In addition, the regional differences in EROD activity differed between sexes. During the spawning season significantly high activities were found at station 5 in the liver of males contrasting with the significantly low activities observed in the liver of females.

### Seasonal variation

A pronounced seasonal variation of EROD activity was established in both sexes; for example, a significant maximum at the

**Fig. 3.** EROD activities (nmol·mg$^{-1}$·min$^{-1}$) in the liver of spawning (April 1992 ▨ and May 1993 ▧), postspawning (August 1992 □), and prespawning male dab (*Limanda limanda*) (December 1992 ■). Values are median ± SE of the median from a sample size of 1–6 livers (May 1993: 31 males; $n$ = 1–3 for bars without error bars).

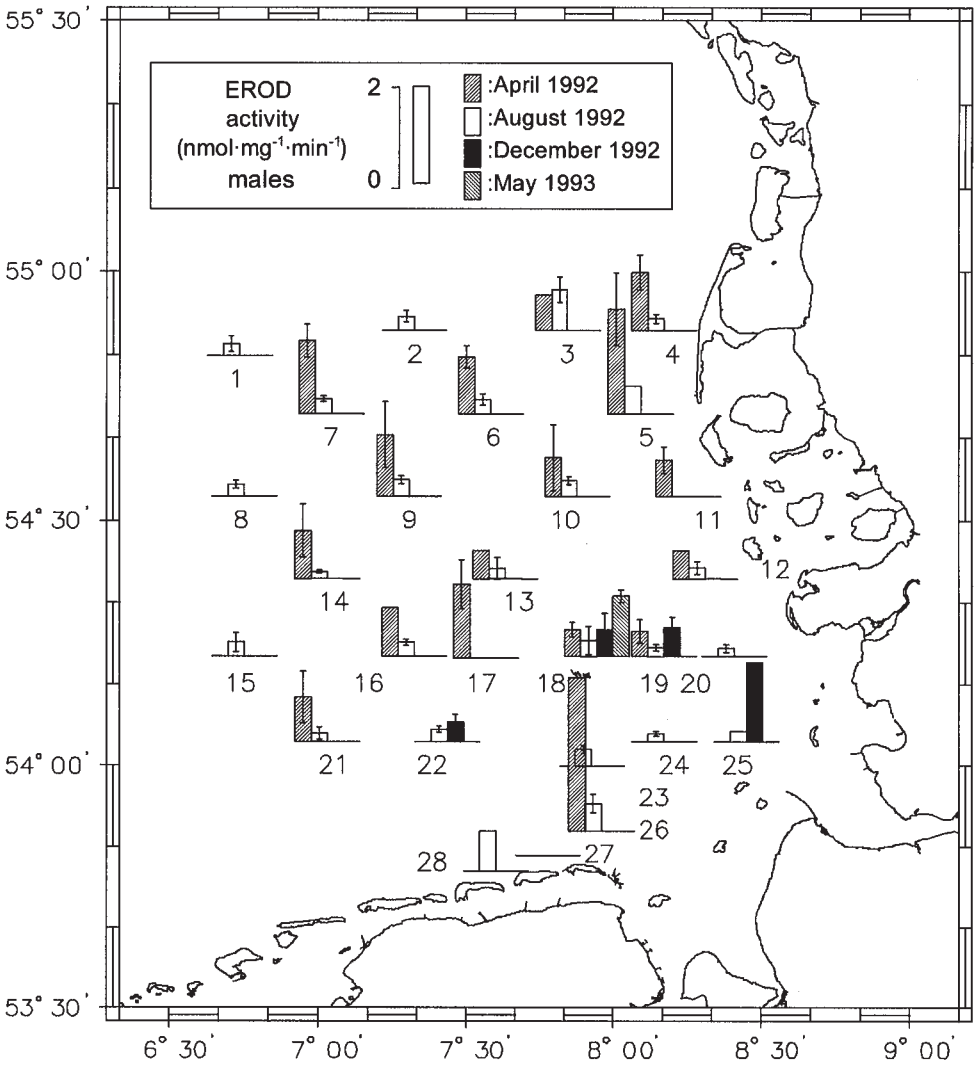



**Table 2.** Stations where male and female dab (*Limanda limanda*) exhibited high and low hepatic EROD activity.

| Date | Stage of maturity | Females | | Males | |
|---|---|---|---|---|---|
| | | Low | High | Low | High |
| April 1992 | Spawning | 5 | **7, 26–28** | 19 | **5, 26** |
| August 1992 | Postspawning | 21 | **1, 11** | 14, 20 | **3, 26** |
| December 1992 | Prespawning | 24 | 18 | 22 | 19 |

**Note**: Stations with significantly higher and lower EROD activities are given in boldface and italic, respectively. Stations with minimal and maximal EROD activities are underlined, even if not significant. For details, see section on statistics in the Materials and methods.

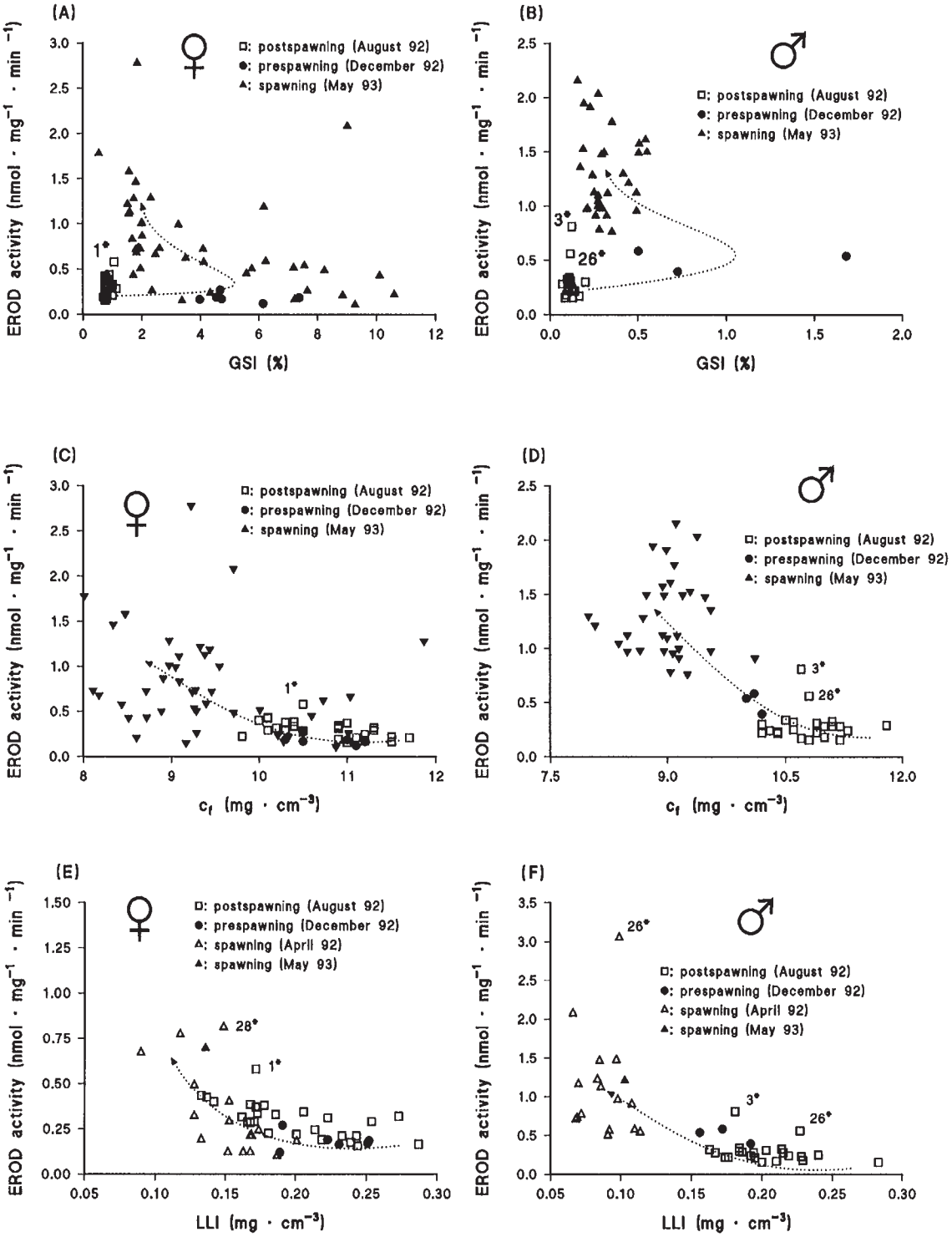
end of the spawning season was measured (April 1992 and May 1993).

During the spawning season (April–May), males exhibited higher EROD activities than during the postspawning season (August), whereby the ratios between spawning and postspawning females differed regionally. At the East Frisian Islands (stations 26–28) and at stations 7, 10, 18, and 21, hepatic EROD activities of females decreased during the period from spawning to postspawning, and at the other stations, activities in the liver of postspawning females were higher than in the liver of spawning females. In May 1993 at station 18, extremely high EROD activities were determined in both sexes, indicating a trend of increasing EROD activity at the end of the spawning season. The ratio of EROD activity between both sexes changed twice. In April and December, activities were higher in the liver of males and in August higher activities were determined in the liver of females.

### Meristic features

In Figs. 4A–4F, EROD activity of both sexes is plotted against the meristic characteristics GSI, CF, and LLI. Seasonal changes in the parameters are indicated by broken arrows. Significantly negative correlations were found between EROD activity and the meristic characteristics (Table 3), which can be explained by the characteristic seasonal variations of the respective parameters.

**Fig. 4.** Seasonal changes of EROD activity (nmol·mg$^{-1}$·min$^{-1}$) plotted against the meristic characteristics (A and B) GSI (%), (C and D) CF (mg·cm$^{-3}$), and (E and F) LLI (mg·cm$^{-3}$) of dab (*Limanda limanda*). Values are medians of the stations examined. For May 1993, single values are presented. Asterisks show significantly high values, which are given with station number.




In autumn (August–December) the GSI of both sexes increased, while changes in EROD activity were minor. During the spawning season (May 1993), the GSI decreased because of the release of eggs and sperm, while EROD activity increased. The opposite development of both parameters during the spawning season resulted in a negative correlation as determined in May 1993 for females (see Table 3). The slight decrease in EROD activity in the liver of females during gonadal maturation resulted in a negative correlation in December. In addition, EROD activity was negatively correlated with the CF and LLI of both sexes (see Table 3). As indicated in Figs. 4C–4F, the CF and LLI decreased during the gonadal

**Fig. 5.** EROD activity (nmol·mg$^{-1}$·min$^{-1}$) in spawning (A) and postspawning (B) female and male dab (*Limanda limanda*) related to the actual water temperature (°C). Values are medians of the stations. Asterisks show exceptionally high values. Spearman rank correlations were calculated using single-sample values (sample sizes: 64 < $n$ < 145).

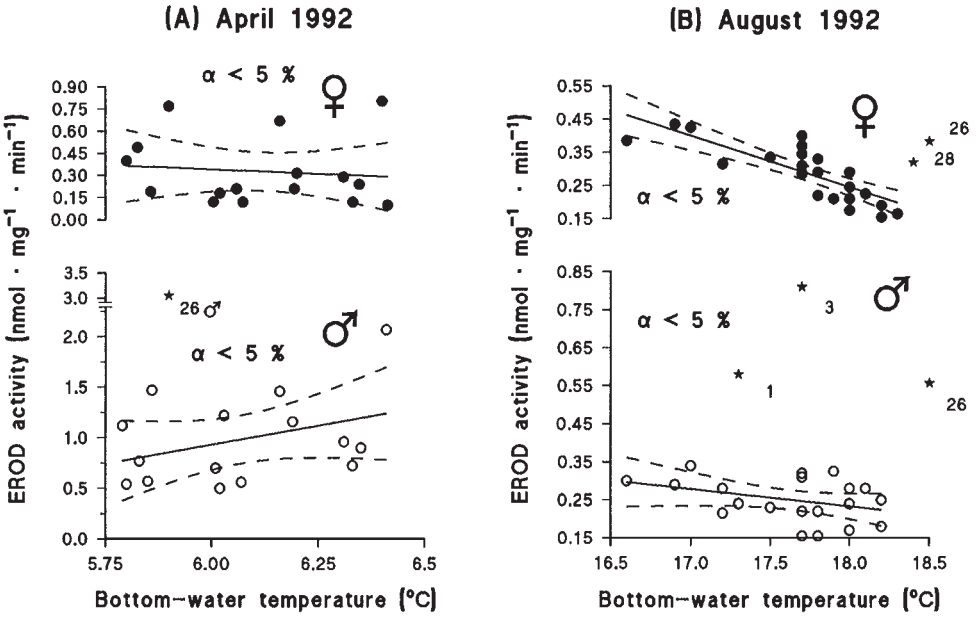



**Table 3.** Spearman rank correlation between EROD activity and the meristic characteristics, gonadosomatic index (GSI), condition factor (CF), and liver–length index (LLI), of spawning (April 1992 and May 1993), postspawning (August 1992), and prespawning (December 1992) female (F) and male (M) dab (*Limanda limanda*).

| Meristic characteristic | April 1992 | | August 1992 | | December 1992 | | May 1993 | |
|---|---|---|---|---|---|---|---|---|
| | F | M | F | M | F | M | F | M |
| CF | nd | nd | – – | – – | – – | ns | – | ns |
| GSI | nd | nd | ns | ns | – – | ns | – – | ns |
| LLI | – – | – | – – | – – | – | – | – – | ns |

**Note**: Correlations were calculated on the basis of single samples. – and – –, significant negative correlation using α = 0.10 and 0.05, respectively; ns, not significant; nd, not determined.

maturation (August–May), while EROD activities increased, resulting in negative correlations between these parameters during the postspawning, prespawning, and spawning season.

## Ambient temperature

In Fig. 5, hepatic EROD activities of spawning and postspawning dab are plotted against ambient water temperature. The relationship between EROD activity and water temperature exhibited sex-dependent as well as seasonal differences. The significant correlations found cannot be explained in terms of a simple negative or positive temperature compensation. A positive correlation was found during the spawning season (April 1992) for male dab and a negative correlation for females. In contrast, during the postspawning season (August 1992), both sexes exhibited a negative correlation between EROD activity and temperature. Interestingly, in the temperature range between 16.5 and 18.5°C, EROD activity in the liver of males decreased by 50%. Results indicate that temperature exhibits a pronounced influence on EROD activity during the spawning and postspawning seasons.

## Temperature experiment

Female and male dab were adapted to different temperatures ranging from 4.5 to 18.5°C over a period of 30 days. In a second experiment, carried out over a period of 14 days, only females were used and temperatures ranged from 4 to 20°C. The results are presented in Fig. 6.

Significantly higher EROD activities were determined in both sexes after acclimation to low temperatures as compared with those acclimated to high temperatures. However, differences between dab acclimated to 16 and 20°C were lower than in our field study (see Fig. 5B). Interestingly, after 14 days of acclimation, females exhibited a maximum in EROD activity at 8°C, but after 30 days, EROD activity decreased with increasing temperature over the total temperature range. EROD activity in control fish at the beginning of the experiment was similar to the activities found in dab acclimated to 11.5 or 12°C (broken lines in Fig. 6).

To find out whether the temperature adaptation of EROD activity was due to qualitative changes, we determined the $k_m$ and the $Q_{10}$ values as well as in vitro temperature optima of EROD activity for dab acclimated to 4.5 and 18.5°C for 30 days (Table 4). The parameters calculated for the two temperature regimes do not differ significantly, except that the $Q_{10}$ value calculated for the temperature range between 5 and 15°C was lower in cold-adapted dab than in warm-adapted dab.

## Organic contaminants

To find out whether organic contamination also influenced EROD activity, the concentrations of the P450 1A inducing PCB in the liver of spawning female dab were considered as determined by Landgraff (1995). Statistical results are

**Fig. 6.** EROD activity (nmol·mg$^{-1}$·min$^{-1}$) in the liver of male and female dab (*Limanda limanda*) after acclimation to different temperatures (°C). Females were acclimated for 14 (A) and 30 days (B), and males were acclimated for 30 days (C) only. The number of asterisks corresponds to the number of samples that exhibit a significantly lower EROD activity than the marked sample. Broken lines indicate the EROD activity of control fish at the beginning of the experiment.

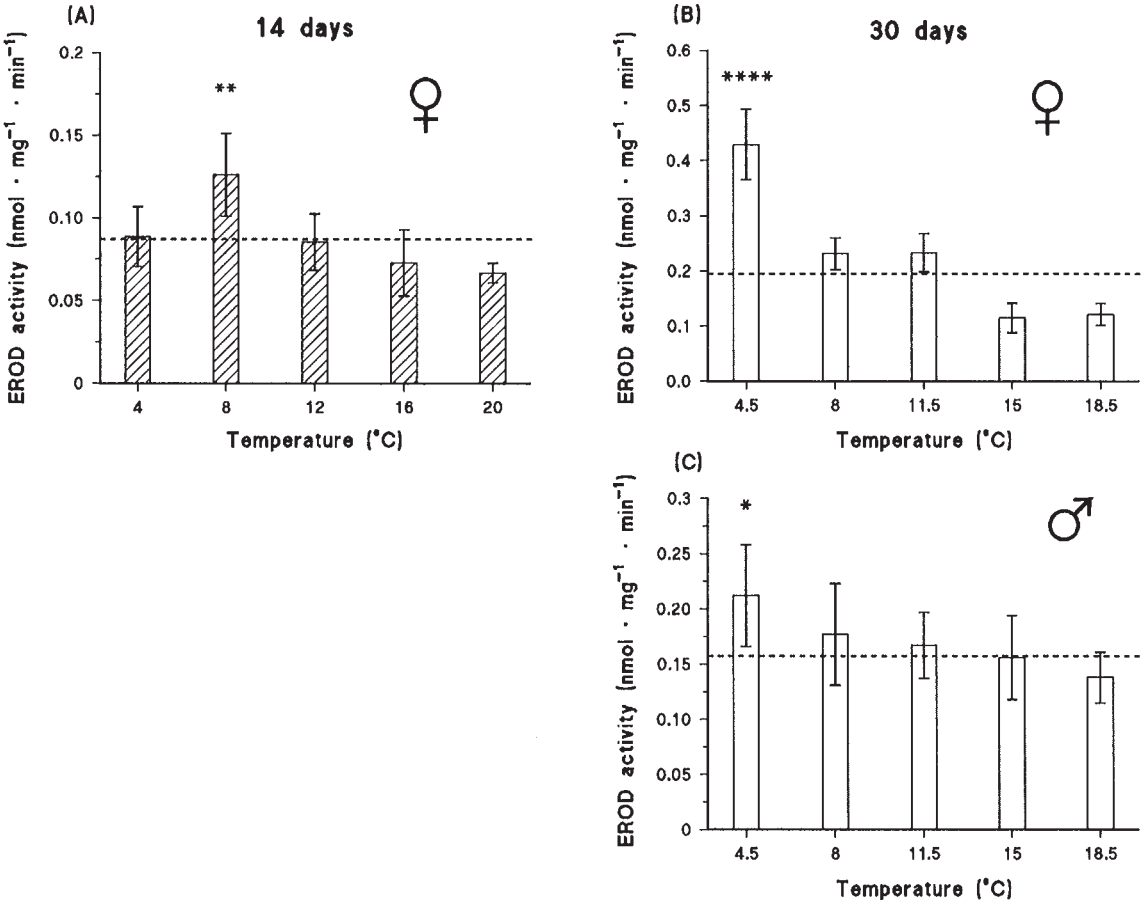



**Table 4.** Values of $k_m$, $Q_{10}$, and in vitro temperature optima of hepatic EROD activity for female and male dab (*Limanda limanda*) after 30 days of acclimation at 4.5 and 18.5°C.

| | | $Q_{10}$ | | Temperature |
|---|---|---|---|---|
| | $k_m$ (μM) | 5–15°C | 10–20°C | optimum (°C) |
| Females | | | | |
| 4.5°C | 2.2 | 2.1 | 2.3 | 29.8 |
| 18.5°C | 2.2 | 2.5 | 2.2 | 29.7 |
| Males | | | | |
| 4.5°C | 2.2 | 2.2 | 2 | 29.5 |
| 18.5°C | 2.1 | 2.8 | 2.3 | 28.8 |

**Table 5.** Concentrations of ICES, non-ortho-, and mono-ortho-PCB in the liver of female dab (*Limanda limanda*) according to Landgraff (1995).

| Organochlorine | Concentration (ng·g lipid$^{-1}$) | $r$ | α |
|---|---|---|---|
| ICES PCB* | 360–4370 | 0.07 | 0.77 |
| IUPAC (excluding congener 128)** | 470–6040 | –0.04 | 0.87 |
| Non-ortho-PCB | 34–137 | 0.13 | 0.60 |
| Mono-ortho-PCB | 85–801 | –0.04 | 0.87 |

**Note**: Correlations between the PCB and EROD activity were tested for significance by Spearman rank correlation. The rank correlation coefficient ($r$) and the error level (α) are given.

*PCB congeners 28, 52, 101, 138 and 180.

**PCB congeners 28, 52, 101, 99, 110, 118, 153, 105, 138, 183, 156, 180, 170, and 194.

presented in Table 5. Data do not indicate a linear relationship between EROD activity and the PCB measured (Spearman rank correlation, using $\alpha = 0.10$).

## Discussion

### Seasonal variation

The seasonal changes in EROD activity in the liver of dab exhibited sex-dependent differences. These differences were characterized by high activities during the spawning season, compared with those measured in the post- and pre-spawning season, and by a change in the ratio of EROD activity in the liver of females to that of males.

Seasonal changes in hepatic EROD activity of fish have often been established. An increase in EROD activity during the spawning season was determined by Eggens et al. (1995, 1996) for plaice (*Platichthys flesus*) and Mathieu et al. (1991)

for striped mullet (*Mullus barbatus*). The seasonal cycle of EROD activity in the liver of dab has recently been studied by Cooreman et al. (1993). They found out that dab from the Belgian coast exhibited far higher EROD activities in March than in April, August, and December. In contrast to this, Larsen et al. (1992) and Lindström-Seppä (1985) established that EROD activities in salmon (*Salmo salar*) and vendace (*Coregonus albula*) decreased during spawning.

Interestingly, during the seasonal cycle the ratio of EROD activity between female and male dab changed. Recent literature indicates that, in general, EROD activities are higher in the liver of male fish than in the liver of females. A reason for this sex-dependent difference may be that, during spawning, the hepatic EROD activity of females is depressed by 17β-estradiol (Hansson and Gustafsson 1981; Stegeman et al. 1982). However, it must be elucidated why postspawning female dab exhibited higher hepatic EROD activities than males as observed in our study.

The pronounced increase in EROD activity during spawning leads to our assumption that EROD activity plays an important physiological role during gonadal maturation. However, a contrasting explanation for the high EROD activities during spawning season is given by Cooreman et al. (1993). They suppose that the increase in EROD activity during spring is related to the mobilization of fat reserves (see section on organochlorines).

In addition, reduced feeding activity during winter can influence seasonal changes in EROD activity. Andersson (1985) and van Veld et al. (1988) found out that EROD activity in the liver of rainbow trout (*Oncorhynchus mykiss*) and spot (*Leiostomus xanthurus*) decreases during starvation.

### Meristic characteristics

Consistent with our results, Sleiderink et al. (1995) determined a negative correlation between EROD activity and condition factor, and Lockhart and Metner (1992) discovered that hepatic EROD activity of burbot (*Lota lota*) decreases with increasing weight of the gonads. As depicted in Figs. 4A–4F, the negative correlations between EROD activity and meristic characteristics correspond to temporal changes in the respective parameters. Consequently, our data are not indicative of a causal relationship, though it cannot be excluded that EROD activity depends on the nutritional status and especially on the fat content of the liver, which should be reflected by the LLI. The dependence of EROD activity in fish liver on the composition of the diet was established by George and Henderson (1992) and Goksøyr et al. (1994*b*).

### Migrations

The significant correlations of EROD activity with the ambient water temperature indicate that dab caught in April and August 1992 in the German Bight had not carried out significant migrations during the preceding months. Otherwise, the correlations should have been masked. This conclusion is supported by Saborowski and Buchholz (1997), who found out that the population structure of dab near the island of Helgoland does not exhibit significant changes during spring and summer. From this result we further conclude that dab caught between April and August in the German Bight is a suitable species for monitoring purposes.

### Organochlorines

During spawning (April 1992), correlations between EROD activity and the concentrations of PCB in the liver of female dab were not significant. Formerly, Eggens et al. (1992) found a positive correlation between EROD activity (ranging from 20 to 156 $nmol \cdot mg^{-1} \cdot min^{-1}$) and the concentrations of several single PCB congeners in the liver of prespawning female dab. They concluded that EROD activity was induced by environmental contaminants. Goksøyr et al. (1991) established 20-fold higher EROD activity in the liver of spawning dab from a contaminated site in the Hvaler archipelago than at a less contaminated site, although fish exhibited similar concentrations of PCB (IUPAC-PCB: 4660–6990 $ng \cdot g\ lipid^{-1}$) compared with those found in spawning female dab from the stations investigated in our study (Table 5; Landgraff 1995). These results are consistent with data from Stagg et al. (1995), who found out that EROD activity in the liver of dab from the vicinity of oil platforms was positively correlated with the concentrations of three- to six-ring polyaromatic hydrocarbons.

In contrast to this, Cooreman et al. (1993) described that EROD activity in the liver of dab from the Belgian coast increased with decreasing PCB concentrations. They discuss this finding as an indication that, during spawning season when fat reserves are mobilized, EROD activity is induced by the inducing capacity of the mobilized xenobiotics.

Considering our results, the question arises whether positive correlations between EROD activity and PCB concentrations can be related to the temporal changes of both parameters. For example, Sleiderink et al. (1995) also found out that, from August 1991 to May 1992, EROD activity and PCB concentrations in the liver of dab increased. The parallel changes of both parameters over time could result in a positive correlation, which does not prove a causal relationship.

### Temperature effects

Results indicate that regional differences in the hepatic EROD activity of dab from the German Bight can be closely related to temperature effects. On the basis of our results, however, the temperature influence cannot be explained in terms of temperature compensation. Instead, we assume that temperature influences EROD activity via its influence on the seasonal cycle of EROD activity. This conclusion is mainly based on the finding that, in April and August 1992, contrasting correlations between EROD activity and temperature were found and that, in August 1992, EROD activity in the liver of male dab decreased by 50% between 16.5 and 18.5°C.

The positive correlation between temperature and EROD activity determined for males in April 1992 is surprising. Field and experimental studies on the influence of temperature on the EROD activity in the liver of fish, as described in literature, revealed that EROD activity generally is negatively correlated with temperature (Stegeman 1979; Koivusaari and Andersson 1984; Andersson 1985; Sleiderink et al. 1995). Sleiderink et al. (1995) found out that, in May–June 1992, EROD activity and cytochrome P450 1A concentrations in the liver of dab from the southern North Sea were negatively correlated with temperature. They supported this finding from a field study by an experimental study, showing that, after 28 days of temperature acclimation, cold-acclimated dab (8°C) exhibited threefold higher EROD activities compared with warm-acclimated dab (16°C).

To explain the different correlations determined for both sexes in April 1992, we considered the long-term hydrographical conditions of the preceding months, because in April 1992, pronounced changes in the temperature of the German Bight occurred. Generally, in spring, winter conditions, characterized by lower temperatures in coastal areas compared with offshore areas, switches to summer conditions, marked by a gradient of decreasing temperature from the coast towards the open sea. These changes are due to the higher temperature amplitude in coastal waters. In addition, in April 1992 a strong inflow of warm Atlantic water from the English Channel was observed leading to a pronounced increase in water temperature in the southwestern German Bight. Thus, the ambient water temperature did not reflect long-term temperature conditions.

Figure 7 shows the hepatic EROD activity of female and male dab caught in April 1992 plotted against the bottom-water temperatures determined in March 1992 (G.A. Becker, Bundesamt für Seeschiffahrt und Hydrographie, Postfach 301220, 20359 Hamburg, Germany, unpublished data). It is interesting to note that the data indicate a positive correlation for both sexes. EROD activity is also positively correlated with the mean bottom temperature of the preceding 4 months (December–March, $\alpha = 0.05$). Results show that long-term temperature conditions have a pronounced influence on the EROD activity in the liver of spawning dab.

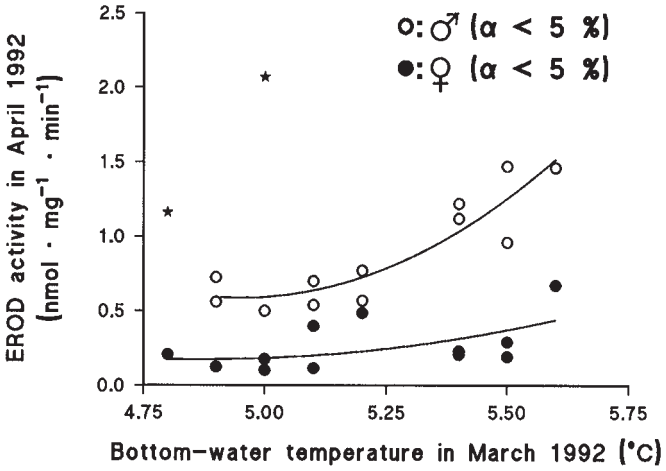
A positive correlation between EROD activity and temperature in the range 5–10°C was detected by Snegaroff and Bach (1990) for rainbow trout, but EROD activities decreased at higher temperatures, i.e., 15 and 20°C. They established a sixfold increase in EROD activity after 8 days of acclimation to 10°C, compared with 5°C. Assuming that the correlations found in our field studies (Fig. 5) represent a temperature adaptation, we have to conclude that EROD activity exhibits a temperature optimum between 6.5 and 16°C (Fig. 5) after temperature adaptation. This assumption could explain the opposite correlations found during the spawning (5.75–6.5°C) and postspawning seasons (16–18.5°C).

However, our experimental studies show that hepatic EROD activity of dab exhibits a significant temperature adaptation in terms of a positive (compensatory) temperature acclimation (Prosser 1991). Thus, the positive correlation found between EROD activity and temperature in April 1992 cannot be attributed to a temperature acclimation. Further, our results indicate that, at 4.5°C, the temperature adaptation lasts longer than 14 days, because EROD activity after 14 days of acclimation showed a significant maximum at 8°C, whereas after 30 days EROD activity was highest at the lowest temperature (4.5°C). This finding suggests that the temperature optimum found by Snegaroff and Bach (1990) for hepatic EROD activity in rainbow trout was possibly due to the short duration of their experiment (8 days).

We cannot estimate the influence of temperature stress during the experiments due to the temperature changes, especially in the 20°C group (14-day experiment) and in the 4.5°C group (30-day experiment). However, the consistency in the results of the two experiments performed indicates that the experimental design was in an acceptable range. In the German Bight, daily temperature changes in the range of 0.1–1°C can occur (Bundesamt für Seeschiffahrt und Hydrographie 1995).

The increase in EROD activity after acclimation to low

**Fig. 7.** EROD activity (nmol·mg$^{-1}$·min$^{-1}$) in spawning male and female dab (*Limanda limanda*) (April 1992) plotted against bottom-water temperature (°C) measured in March 1992 (G.A. Becker, Bundesamt für Seeschiffahrt und Hydrographie, Postfach 301220, 20359 Hamburg, Germany, unpublished data). The asterisks show exceptionally high values, which were not considered.




temperatures cannot be attributed to qualitative changes such as changes in the affinity or the temperature optimum of the enzyme. Between cold- and warm-adapted dab, significant differences in the $k_m$ and $Q_{10}$ values, which could explain the different EROD activities in both groups, did not occur. However, in part, the differences in EROD activity can be attributed to the relatively low $Q_{10}$ value observed for cold-adapted dab in the temperature range 5–15°C. The adaptation could be due to a higher rate of EROD synthesis or to another factor that influences EROD activity indirectly.

To test whether enzyme synthesis is enhanced in cold-adapted fish, concentrations of P450 1A, the main EROD catalyst, or P450 1A mRNA should have been determined. The determination of P450 1A mRNA in the liver of dab was successfully applied by Renton and Addison (1992).

Factors that can modify the activity of the integral membrane enzyme P450 1A are membrane fluidity, phospholipid/cholesterol ratio, and fatty acid composition (Wade et al. 1986; Castuma and Brenner 1989). In addition, EROD activity depends on the activity of the NADPH P450 reductase if the electron transfer is the rate-limiting step (Goksøyr et al. 1994*b*).

## Synthesis

Our results indicate a stronger influence of water temperature on the regional variability of EROD activity compared with the influence of organochlorines. This finding is supported by Sleiderink et al. (1995). They established positive correlations between EROD activity and different PCB congeners in the liver of dab from the southern North Sea, but their multiple regression analysis revealed that the negative influence of temperature dominated over the effect of PCB contamination.

From our experimental results we conclude that the positive correlation found between EROD activity and temperature for spawning dab is not due to temperature adaptation. Therefore, we have to assume that the effect of temperature on EROD

activity changes seasonally or that temperature influences EROD activity indirectly via an unknown parameter. Considering that the spawning time of dab is delayed by decreasing temperatures (Deniel and Tassel 1986; Lange and Greve 1996) and assuming that the seasonal changes in EROD activity are coupled to the gonadal cycle, we propose the following hypothesis: given that the spawning time and thus the increase of EROD activity during spring and the following decrease in summer occur earlier in warmer waters, in spring, EROD activity may increase (earlier) with increasing temperature and in summer it decreases (earlier) with increasing temperature. This hypothesis explains the different correlations found between both parameters in spring and summer as well as the pronounced and significant changes in EROD activity in the liver of postspawning male dab in the temperature range between 16.5 and 18.5°C (see Fig. 5B).

## Conclusions

Our findings suggest that, in the German Bight, the regional variability of EROD activity in the liver of dab is mainly determined by temperature. In addition, the pronounced correlations of EROD activity with the ambient water temperature indicate that, in April and August, dab do not perform significant migrations, supporting the suitability of dab for monitoring purposes. The results of different authors concerning the influence of aromatic hydrocarbons on EROD activity are contradictory. These contradictions show that the influence of natural and of anthropogenic factors on the EROD activity is at present not fully understood.

The temporal changes in EROD activity, meristic characteristics, and the concentrations of organic contaminants have to be studied in more detail. Only a comprehensive knowledge of the seasonal variation of these parameters enables us to determine causal relationships between EROD activity and the respective biotic and abiotic factors.

In conclusion, future studies should emphasize the description of the basal EROD activity level depending on the location and the time of sampling. This knowledge is required to differentiate between possible pollution effects and natural variability. This differentiation is essential in the assessment of anthropogenic impact, since reliable estimation of natural variability allows true interpretations and enables us to make promising recommendations for environmental management and waste treatment.

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