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Cytochrome P450 1A activity in liver and fixed wavelength fluorescence detection of polycyclic aromatic hydrocarbons in the bile of tongue-fish (*Cynoglossus acrolepidotus,* Bleeker) in relation to petroleum hydrocarbons in the eastern Gulf of Thailand

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Abstract: *This investigation was conducted in an area of oil spill along the east coast of Thailand to examine the relations among cytochrome P450 1A activity in liver and PAHs in the bile of the tonguefish and petroleum hydrocarbons in the sediments. PAH sediment concentrations in the reference and oil spill areas were 5.03 ± 0.42 and 0.21 ± 0.043 μg^{-1} dry weight respectively. Cytochrome activity in fish liver from oil spill area was 45.40 ± 3.50 pmoles/min/mg protein, almost threefold higher than that from the reference sites. Flourescense detection in bile metabolites at the oil spill area, 69.8 ± 9.9 flourescense unit was significantly higher than that at the reference sites, 22.9 ± 5.5 and 22.2 ± 3.5 fluorescence unit. A strong correlation was found among cytochrome P450 1A activity in liver, PAH of bile metabolites and petroleum hydrocarbons. Both cytochrome and bile metabolites activity decreased seaward varying to the distance from the oil polluted area. We concluded that both detections in tonguefish can be regarded as a complementary biomarkers for the exposure of PAHs in tropical marine environments.*

Key words: *Cytochrome P 450 1A, Fixed wavelength fluorescence, Polycyclic aromatic hydrocarbon, Tongue-fish*
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Introduction

The aquatic environment is a major sink for many potentially hazardous chemicals from industrial and domestic sources to which resident organisms are exposed. Among these substances are the polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polycyclic aromatic hydrocarbons (PAHs) (Sharma and Cyril, 2007). Exposure to these substances may lead to direct toxicity, hepatic tumors and reproductive impairments to the fish (Nagler and Cyr, 1997) as well as deleterious health effects to those who consume the fish. While some substances accumulate in tissues, others are difficult to monitor as they are rapidly catabolized and excreted such as the low molecular weight PAHs. Recent advances in ecotoxicology have provided a number of molecular biomarkers that allow the exposure of organisms to these substances to be assessed (McCarthy and Shugart, 1990). Of the biomarkers currently available, the induction of cytochrome activity and production of bile metabolites have shown great potential for identifying levels of exposure to PAHs (Burgeot *et al*., 1996; Harvey *et al*., 1997; Aas *et al.,* 1998; George *et al.,* 2004).

The mixed function oxygenase system (MFO) is important to the metabolism of xenobiotics in aquatic vertebrates. One of its components, cytochrome P450s, can be readily assessed in the liver through the *in vitro* measurement of 7-ethoxyresorufin-O-deethylase (EROD) in the liver. Cytochrome P 450 1A (CYP1A) in fish and other vertebrates increases in the presence of xenobiotics, such as PAHs and PCBs and has been advocated as a sensitive indicator of hydrocarbon exposure (Stegemen *et al*., 1988; Burgeot *et al*., 1996). Fixed wavelength fluorescence has proven to be a useful tool for measuring PAHs and their metabolites in bile (Aas *et al*., 1998; Camus *et al.,* 1998; Dissanayake and Galloway, 2004; Fuentes-Rios *et al.,* 2005).

In the Gulf of Thailand the oil and petroleum industry is restricted to the immediate locality around the east coast of Thailand. However, there is growing concern over the impacts of occasional discharges from the oil tanker accidents such as those in 1996 and 2002 that covered long stretches of beach in Sattahip district (Department of Pollution Control, 2003). The present study evaluated the environmental effects of discharges from an oil refinery located on the east coast of Thailand and an oil tanker accident in the Gulf of Thailand based on PAH concentrations in the sediments around the study area. Measurement of PAH metabolites by cytochrome activity (ethoxyresorufin-*O*-deethylase, EROD) in the liver and bile, by fixed wavelength fluorescence, were compared and related to PAH concentrations in the sediment.

Materials and Methods

Tongue-fish were captured by trawling from four areas; Siracha, the location of the oil refinery (Station A), Sattahip, the location of the oil spill (Station B), a Station 100 km south of B (Station C) and a Station 50 km south of C (Station D). Stations C

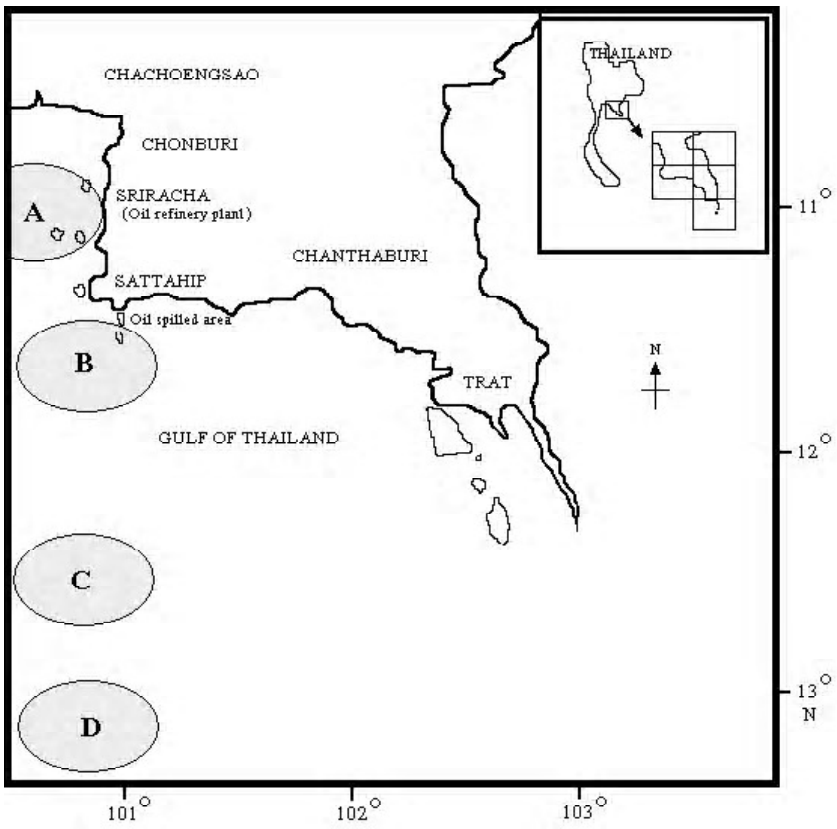

Fig. 1: Map of the Gulf of Thailand and the sampling stations

and D were taken as reference areas. The details of sampling site are given in Fig.1. At each station 15 male fish (25-30 cm, total length) were collected for analyses of PAH metabolites in bile and cytochrome acitivity in liver.

Detection of polyaromatic hydrocarbon (PAH) metabolites by fixed wavelength fluorescence (FF) according to the method described by Aas *et al.* (1998) . Briefly, bile samples were frozen at -20°C and stored until analysis. They were diluted 1:1600 times in 48% ethanol and FF was measured at the excitation/emission wavelengths of 341/383 nm, optimised for the detection of pyrene metabolites. The results are expressed in unit of fluorescence.

Liver samples were frozen in liquid nitrogen, and transferred to -80°C before homogenising and processing of S-9 supernatent. EROD analysis was performed using a modification of the method described by Stagg *et al*. (1995). EROD activity was normalised to protein content and expressed as picomoles/min/mg protein. Protein analyses were carried out on the same liver homogenate as the EROD analysis using a plate reader modification of the method described by Bradford (1976), with a bovine serum albumin standard.

Samples of surface sediment were collected using a Van Veen grab from each station (n = 15/Station), transferred to hexane-rinsed glass jars with aluminum foil inserts and kept at -20°C until analysis. Samples were then freeze dried, ground with a mortar and pestle and filtered (0.14 mm pore size). The ground sediment (20 g) was added to 60 ml of dichloromethane/hexane (1:1, v/v) and held for 50 hr. Samples were then extracted three times by the ultrasonic wave method, each for 20 min and the extracts combined. The dichloromethane solvent in the sample extracts was removed by rotary evaporation and replaced by 20% sodium sulphate. The hexane extract was added to a 10 g Al_2O_3 chromatography column and washed with 60 ml of dichloromethane/hexane (1:1, v/v). The solution was evaporated nearly to dryness to remove the dichoromethane solvent and redissolved in 1.0 ml hexane. Sulphur in the extracts was removed with 1.0 g of Cu and by the ultrasonic wave method. PAH was quantified using GC/MS (HP5989A). Residue concentrations were calculated on the basis of dry weight. Further technical detail of the technique is given by Ma *et al.* (2001).

Results and Discussion

The highest mean (±SE) sediment concentration of PAH, 5.03 ± 0.42 μg g^{-1} dry wt, was found in the oil spilled area of Station B, followed by that from the oil refinery area (Station A: 2.11 ± 0.23 mg g^{-1} dry wt). Concentrations from the reference areas were essentially identical at 0.21 ± 0.043 (C) and 0.21 ± 0.042 (D) μg g^{-1} dry wt, respectively (Fig. 2) and significantly lower (ANOVA; $p<0.01$) than those at Stations A and B.

Cytochrome P450 1A activity (EROD) from the liver of tonguefish collected from the study areas showed a similar pattern

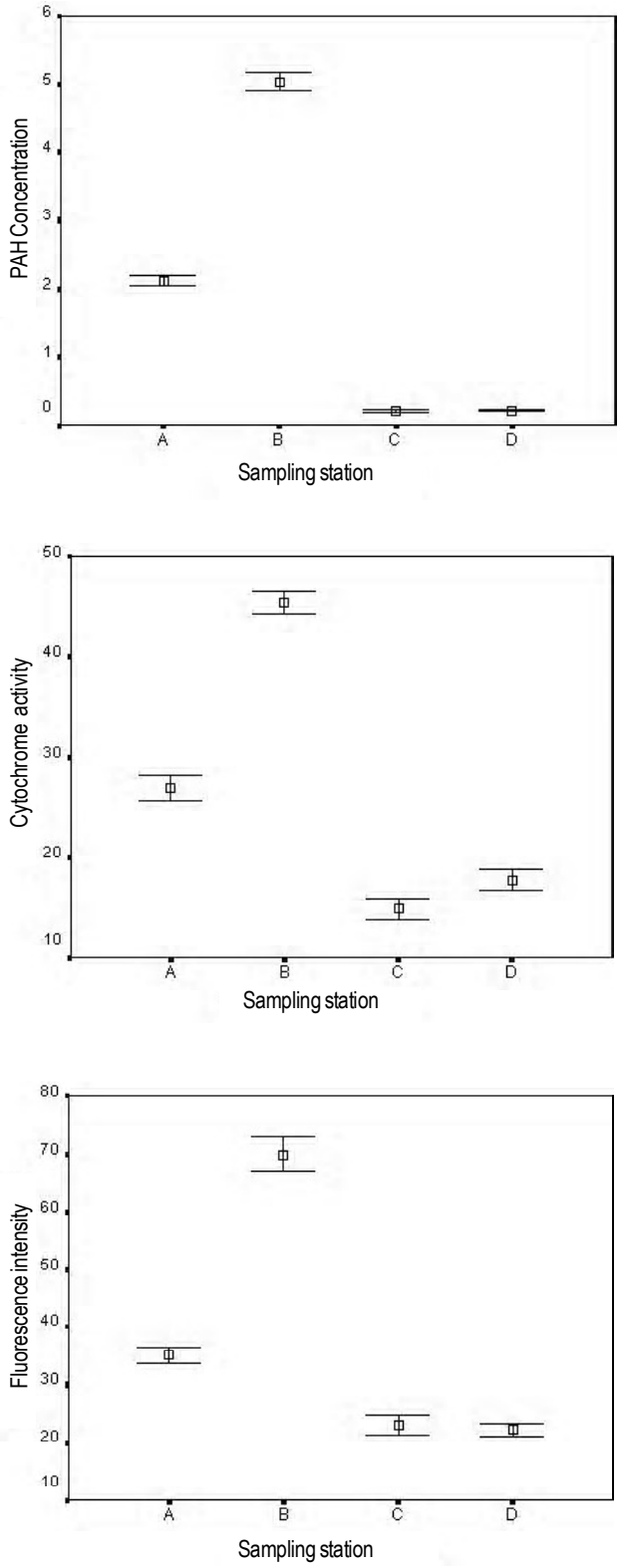

Fig. 2: PAH concentration in the sediments (μg g^{-1} dry wt), cytochrome activity (pmoles/min/mg. protein) in liver and fixed wavelength fluorescence detection (flurorescence unit) in bile metabolites of tongue-fish from various sampling stations

of activity to that found for PAH concentrations in the sediments. Highest cytochrome activity was found in fish liver from Station B at 45.40 ± 3.50 pmoles /min/mg protein, almost threefold higher than that in fish from the reference sites. Cytochrome acitivity from fish captured at Station A, 26.89 ± 4.02 pmoles /min/mg protein, was significantly less ($p<0.01$) than that at Station B but approximately double that at the reference stations. At the reference Stations of C and D, cytochrome activity did not differ significantly ($p>0.01$) at 14.82 ± 3.41 and 17.71 ± 3.26 pmoles/min/mg protein, respectively, but both were significantly lower than concentrations at Stations A and B ($p<0.01$).

PAH metabolites in the bile of tonguefish from the four stations, measured by fixed wavelength fluorescence, followed a similar pattern to that of cytochrome activity in the liver. Highest fluorescence intensity of 69.8 ± 9.9 fluorescence unit was in fish from Station B, followed by that from Station A of 35.0 ± 4.5 fluorescence unit. Flouresence detection in bile metabolites at the reference Stations, C and D of 22.9 ± 5.5 and 22.2 ± 3.5 fluorescence unit did not differ significantly ($p>0.01$) but were both significantly lower than the values at Station A and B.

The relationship between cytochrome activity in the liver and PAH in the sediment was described by a linear regression (Fig.3) :

$$C_l = 14.9 + 5.9\ (P_s) \quad (n = 60)$$

where C_l is cytochrome concentration (pmoles/min/mg protein) and P_s is PAH concentration(μgg^{-1} dry wt) in the sediment. The regression explained 91% of the variation in values and the coefficient, 0.95, was significant at $p<0.01$. Similarly, the relationship between fixed wavelength fluorescence in the bile and PAH in the sediment was described by a linear regression:

$$F_b = 19.2 + 9.6\ (P_s) \quad (n = 60)$$

where F_b is fixed wavelength fluorescence of the bile in fluorescence units. The regression explained 89% of the variation in values and the coefficient, 0.95, was significant at $p<0.01$. Further, values of fixed wavelength fluorescence in the bile and corresponding values of cytochrome in the liver were linearly related:

$$C_l = 5.1 + 0.56\ (F_b) \quad (n = 60)$$

The regression explained 85% of the values and the coefficient, 0.92 was significant at $p<0.01$. In accord with these observations, Beyer *et al*. (1996) found fixed wavelength fluorescence values in the bile metabolites of European flounder, *Platichthys flesus*, and Atlantic cod, *Gadus morhua*, to be well correlated with cytochrome P450 1A concentrations in the liver.

The present study suggests PAH contamination in the fish can be measured with similar sensitivity by cytochrome activity in the liver or fixed wavelength fluorescence in the bile. Importantly the close association between sediment PAH levels, cytochrome activity and bile matabolites suggest that tonguefish are not active migrants and are ideal for studies on the accumulation and impact of contaminants from a localized source.

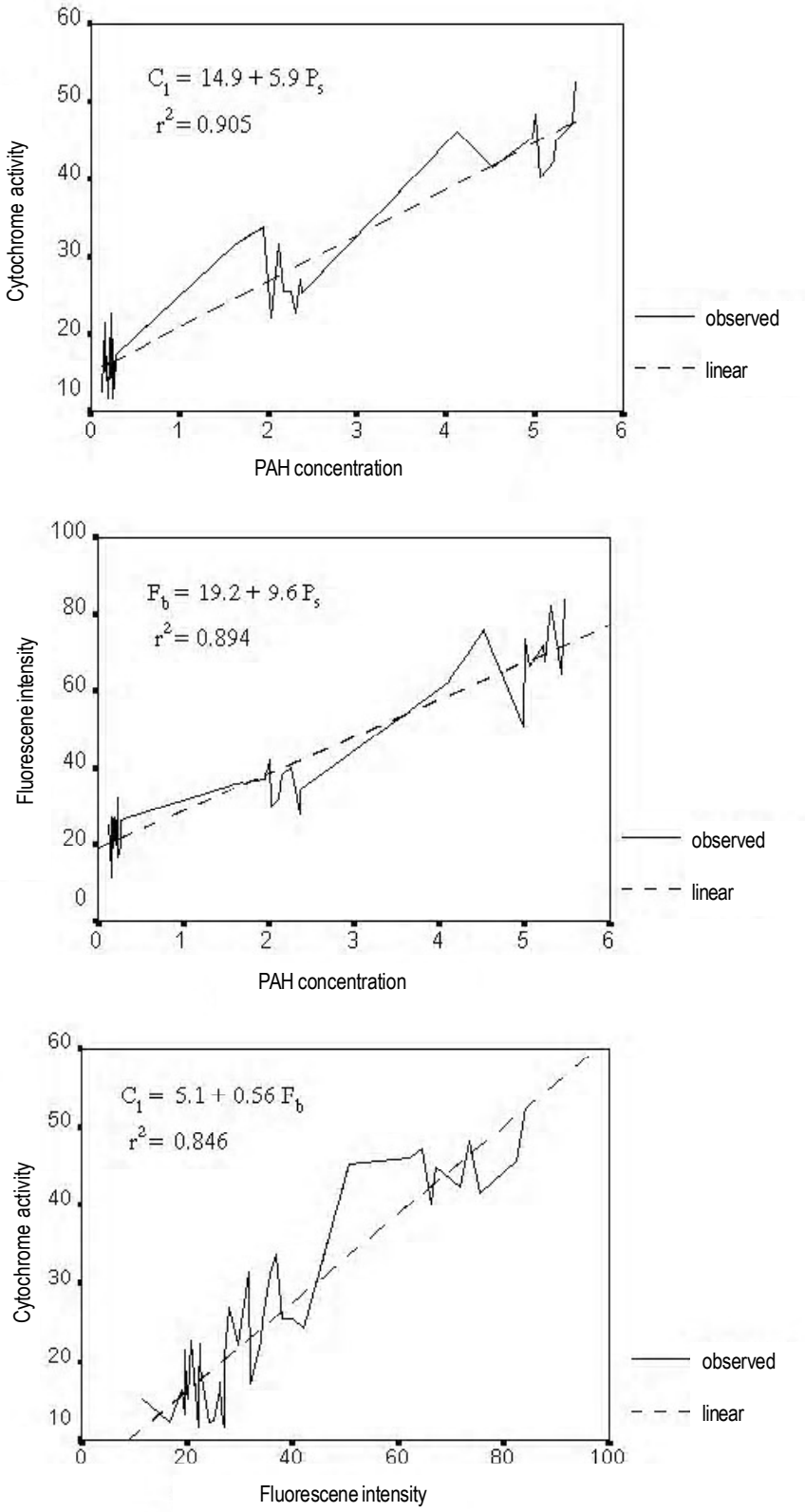

Fig. 3: Correlation between cytochrome activity (pmoles/min/mg. protein) and PAH in sediments (μg g^{-1} dry wt.), fixed wavelength fluorescence detection (flurorescence unit) in bile metabolites and PAH in sediments, cytochrome activity and fixed wavelength fluorescence detection in bile metabolites, of the study area

Although in this study, cytochrome activity was more sensitive than fixed wavelength fluorescence detection in bile metabolites, together with the advantage of simplicity and low cost, we suggest that fixed wavelength fluorescence detection in bile metabolites is also a promising tool for PAHs monitoring. We concluded that the cytochrome activity in liver and fixed wavelength fluorescence detection in bile metabolites of tongue-fish can be regarded as complementary biomarkers in exposure of PAHs in the tropical marine environments of this area.

Acknowledgments

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