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Molecular Characterization of Cytochrome P450 1A (CYP1A) in Asian Sea bass (*Lates calcalifer* Bloch) and Its Application as a Biomarker in the Gulf of Thailand

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Abstract: This study focusses on aquatic pollution in Thailand, using induction of hepatic cytochrome P450 1A (CYP1A) in Asian sea bass as a biomarker to assess exposure to polyaromatic hydrocarbons (PAHs). Polyclonal antibodies (PAb) were raised against Asian sea bass CYP1A in mice. Western blot analyses revealed that these antibodies recognized a CYP1A protein in Asian sea bass treated with benzo[*a*]pyrene (BaP). The presence of a CYP1A orthologue in Asian sea bass liver was confirmed by isolating a partial cDNA, using a reverse transcriptase polymerase chain reaction (RT-PCR) approach. Quantitative RT-PCR analysis revealed that CYP1A mRNA was 2.5-fold higher in fish injected with BaP. The PAb were next applied on 15 different tropical fish species, caught off the Chonburi from three different stations: (1) Koh Loi, Si Racha, (2) Ao Udom and (3) Laem chabang. Although, species variation in CYP1A protein levels was observed, the data suggest that Si Racha and Ao Udom were more contaminated with PAH-type contaminants compared with Laem chabang. This study confirm studies in temperate waters that CYP1A could be used as early indicators of PAH-type exposure in Thailand, and that PAb against Asian sea bass is suitable for analyses of CYP1A in different tropical fish species.

Key words: Thailand, PAH, benzo[*a*]pyrene, CYP1A, Asian sea bass, tropical fish.

Introduction

The impact of environmental contaminants on the health of aquatic organisms can be analyzed by using biomarkers. Induction of CYP1A in fish is an established biomarker used to assess exposure to polycyclic aromatic hydrocarbons (PAHs), planar halogenated aromatic hydrocarbons (PHAHs), including planar polychlorinated biphenyls (PCBs) and dioxins in the aquatic environment (Stegeman and Hahn, 1994; Siroka and Drastichova, 2004; Miller et al., 1999; Schlenk et al., 2008). Induction of CYP1A expression is mediated by binding of a PAH or PHAH to the cytosolic aryl hydrocarbon receptor and in particular liver and vascular endothelium are important sites for CYP1A induction (Stegeman and Hahn, 1994).

Petroleum oil is a major source of PAH contamination in the aquatic environment and accidental oil spills in particular causes PAH pollution in the marine environment. An earlier lab-study in Sweden, using the marine fish viviparous eelpout (*Zoarces viviparous*), showed that hepatic CYP1A protein levels were highly induced in eelpout exposed to the heavy gas oil fraction of North Sea oil, which contains several different PAHs (Celander et al., 1994). A number of biomonitoring

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programmes have been conducted over the past decades in temperate waters, predominantly in North Europe and North America. However, in tropical waters this is poorly investigated.

The Chonburi coast is a part of the Gulf of Thailand (Figure 1A) that is continuously subjected to petroleum oil pollution through accidental oil spillages as a result of ship collision and grounding. In year 1995, petroleum hydrocarbons were detected in the upper Gulf of Thailand. Thus, the concentrations of PAHs in sea water ranged between 0.12 and 18.25 µg/L chrysene equivalents. In addition, PAHs and other hydrocarbon classes i.e. *n*-alkanes were detected in marine sediments where levels of total PAHs in the sediments ranged between 0.08 and 2.0 µg/g dry weight (Chumchuchan et al., 1998). Later studies showed that PAH contamination is widespread in the Gulf of Thailand, and oil leakage from shipping collision and grounding are suggested to be the dominant sources of petroleum oil pollution in this area (Sarin and Srisuksawad, 2000). Recently, an up to 3-fold induction of CYP1A enzyme activity was reported in tongue-fish (*Cynoglossus acrolepidotus,* Bleeker) captured along the east coast of Thailand. Furthermore, there was a positive correlation between hepatic CYP1A activity and bile PAH metabolites in these fish (Cheevaporn and Beamish, 2007).

Although, this east coast of Thailand is a high-risk area for oil spillage, it is also important for commercial fishing as well as for tourisms. In spite of this, there have been no attempts to perform monitoring programmes to assess the effects in wildlife of oil pollution along this coast. Indigenous Thailand fish species, such as the Asian sea bass, is reared for human consumption in coastal and estuaries areas, where they are directly exposed to polluted water from the Gulf of Thailand. Hence, there is a need from an ecological point of view as well as for safe human consumption to investigate the impact of PAH exposure in wildlife in tropical waters in South East Asia. This requires development of specific biomarkers for tropical fish species. The purpose of this study was to isolate CYP1A from Asian sea bass liver to develop an antibody probe against Asian sea bass CYP1A protein and apply this biomarker on 15 different tropical fish species, caught from three different locations on the Chonburi coast in the Gulf of Thailand (Figures 1A and B).

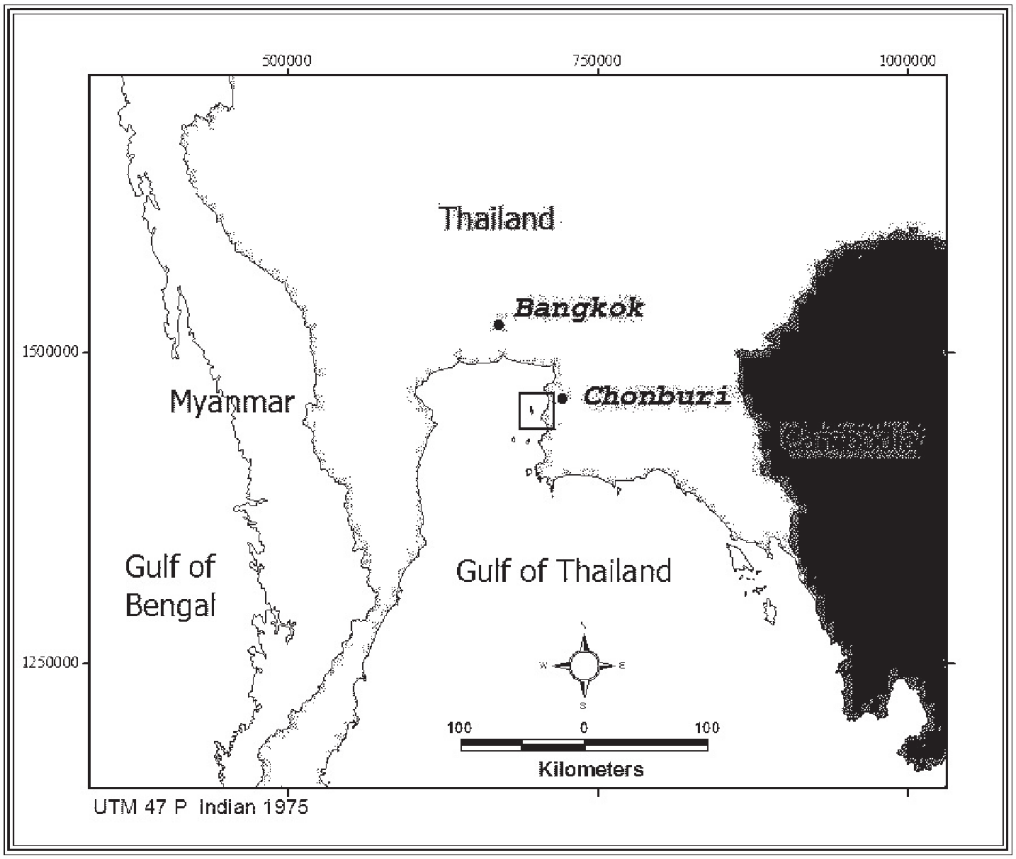

Figure 1A: Map of Thailand.

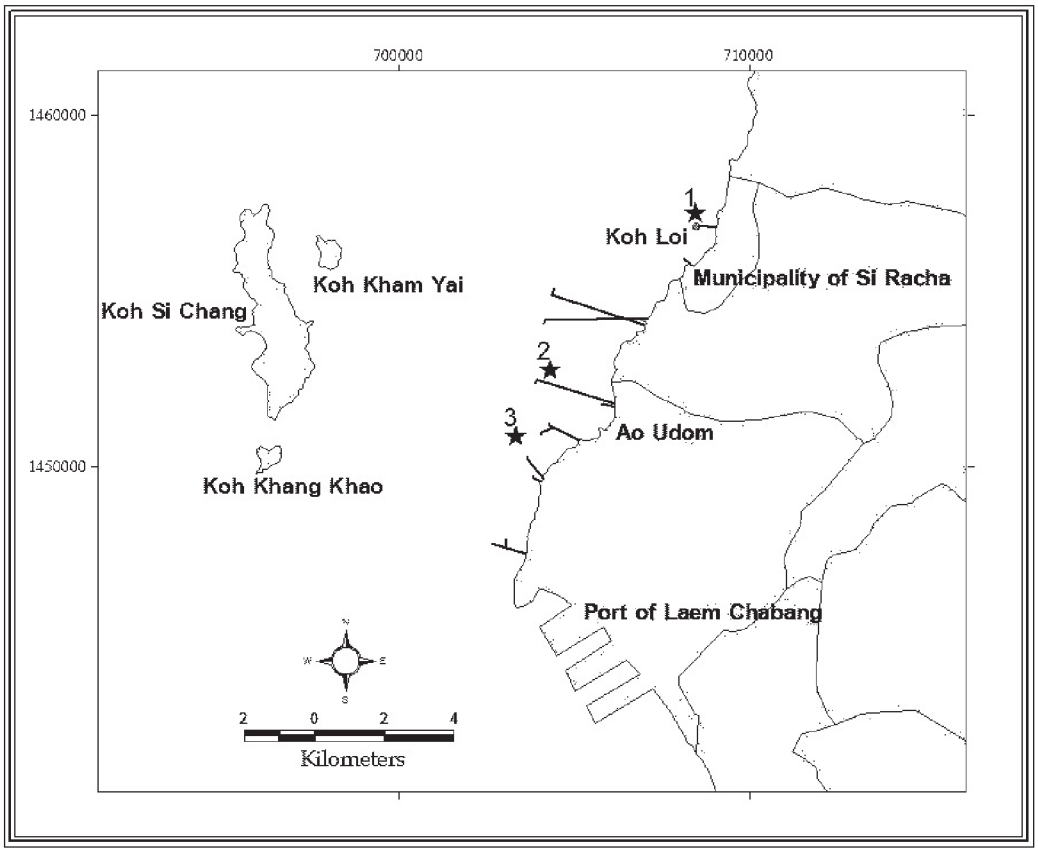

Figure 1B: Map of the Chonburi coast indicating the three sampling stations.
Station 1: Si Racha 13°08′ N, 100°53′ E; Station 2: Ao Udom 13°06′ N, 100°53′ E; and Station 3: Laemchabang between 13° 08′ N, 100°52′ E and 13°07′ N, 100°52′ E.

Materials and Methods

Chemicals

Benzo[*a*]pyrence was purchased from Fluka (product of USA). Rabbit PAb against rainbow trout CYP1A has earlier been described (Celander and Förlin, 1991). Immunoreagents, sodium dodecyl sulphate (SDS), acrylamide, goat anti-mouse IgG conjugated with horseradish peroxidase were purchased from Jackson Immuno Research Laboratories, Inc. (Bangkok, Thailand). The RNA*later* solution and 3,3´Diaminobenzidine-4-HCl were from Sigma-Aldrich (Bangkok, Thailand). The RNeasy® plus mini kit was purchased from Qiagen, UK and Experion™ RNA StdSens Analysis kit and IQ SYBR Green supermix were from BioRad, Sweden. The DNA gel extraction kit (Omega, bio-Tek, China) was purchased from VWR, Sweden; RT-PCR kit was from Applied Biosystems, Sweden and the DNA size marker was from Fermentas, Sweden. All other electrophoresis and Western blot chemicals were of the highest purity available purchased in Thailand from Bioactive Inc., Gibthai Inc., and BioRad and in Sweden from Sigma-Aldrich.

Animals and Sampling

Cultured Asian Sea Bass

Hatchery reared juvenile Asian sea bass (*Lates calcarifer* Bloch) of both sexes and with an average weight of about 400 g were obtained from the earthen pond in the Chachoengsao province in Thailand and kept in indoor concrete basins (2000 litre) in the Department of Aquatic Science animal facility at the Burapha University. The basins were provided with continuously flowing, aerated, filtered, brackish water with 10% salinity at a temperature of 28 ± 1 °C and the basins were covered with black plastic to reduce sunlight exposure. The Asian sea bass were fed with fish meat, approved for human consumption, purchased from a local fish market in Chonburi. There were five fish in each basin and they were acclimatized to these conditions for two months, prior to exposure.

After acclimatization the fish were next i.p. injected with 0.1, 1.0 and 10.0 mg BaP/kg body weight. The BaP was dissolved in corn oil. Control fish received an i.p. of corn oil alone (vehicle = 1.00 ml corn oil/kg fish). There were five fish in each treatment group and after six days the fish were sacrificed by cervical transection and the livers were quickly dissected out and placed in liquid nitrogen.

Feral Fish from the Gulf of Thailand

Fish were caught with hook fishing at Koh Loi, Si Racha (station 1) and boat trawling from off shore at Ao Udom (station 2) and Laem chabang (station 3) along the Chonburi coast (Figures 1A and 1B). All fish species collected are listed in Table 1. The fish were immediately sacrificed by cervical transections and the livers were quickly dissected out and placed in liquid nitrogen, and transported to the laboratory at the Department of Aquatic Science, Burapha University.

Liver Preparations

Microsomal fractions were isolated as described by Rice and Schlenk, 1995. The microsomes were stored in aliquots at –80°C before analyses. Total microsomal protein levels were determined using the BioRad protein determination kit, according to the instructions provided in the kit.

Pieces of liver (30 mg) from Asian sea bass were placed in RNA*later* solution and transported in room temperature to Göteborg, Sweden where it was stored at –20°C upon arrival. The total RNA fractions were isolated and purified using the RNeasy® plus mini kit from Qiagen, according to product specifications. The quality and the concentration of RNA was analyzed using Experion™ RNA StdSens Analysis kit (BioRad, Sweden), according to instruction manual. The samples that yielded RNA concentrations at acceptable concentration and quality for RT-PCR were selected and stored at –80°C prior to RT-PCR analysis.

Table 1: Western blot analyses using mouse PAb (diluted 1:250) raised against Asian sea bass CYP1A in liver microsomes of feral fish (juvenile) caught from three different sites on the Chonburi coast—Koh Loi (Si Racha), Ao Udom and Laem Chabang—that are shown in Figure 1B.

Sampling site	*Number of fish/ sample*	*Weight/length*	*Species*	*Dietary preferences*	*CYP1A staining*
Koh Loi (Si Racha)	6	9-20 g/8-11.5 cm	*Terapon jarbua*	Carnivore	+
(station 1)	3	11-21 g/10.1-11.5 cm	*Terapon jarbua*	Carnivore	+ +
	3	19-21 g/10.1-11 cm	*Terapon jarbua*	Carnivore	+ +
Ao Udom	1	17.5 g/10 cm	*Terapon jarbua*	Carnivore	+ +
(station 2)	4	12-17 g/11.5-12.3 cm	*Sillago aeolus*	Carnivore	+
	1	66.4 g/15.7 cm	*Lethrinus lentjan*	Carnivore	-
	3	30.2-35.2 g/3.5-4 cm	*Ambassis kopsii*	?	-
	4	22.2-37 g/12-13.3 cm	*Dendrophysa russelli*	Carnivore	+
	3	15.3-53.7 g/12.5-18.5 cm	*Grammoplistes scaber*	Carnivore	+ +
	4	16.1-25.5 g/9.5-12 cm	*Acentrogobius caninus*	?	+ +
	1	242 g/35 cm	*Sphyraena jello*	Carnivore	+ +
	1	21 g/18.2 cm	*Himantura signifier*	Carnivore	-
	27	4-5 g/3.2-3.9 cm	*Leiognathus blochii*	Omnivore	-
	17	7-15 g/3.9-7.3 cm	*Monacanthus chinensis*	Omnivore	-
Laem chabang	4	13-25 g/14-20 cm	*Sillago aeolus*	Carnivore	-
(station 3)	16	40 g/12.9-16 cm	*Gerres kapas*	Carnivore	-
	9	19-42 g/12-16 cm	*Ilisha sirishai*	Carnivore	-
	1	12.7 g/28 cm	*Grammoplistes scaber*	?	+ +
	10	13-22 g/17-20 cm	*Depane punctata*	?	-
	2	15 and 16 g /14 and 15 cm	*Nemipterus hexodon*	?	-
	1	260 g/40.5 cm	*Sphyraena jello*	Carnivore	-
	1	38 g/13.2 cm	*Terapon jarbua*	?	-
	2	35, 42 g/12, 14 cm	*Dendrophysa russelli*	Carnivore	-

(– No staining; + Weak staining; + + Strong staining)

Isolation of Asian Sea Bass CYP1A Protein and Immunization

Hepatic microsomal proteins from five individual Asian sea bass i.p. injected with 10 mg BaP/kg fish were separated on a 12% SDS-polyacrylamide gel. The gel was stained with Coosmassie blue and a 56 KDa protein band (CYP1A) was recognized using rabbit-anti-rainbow trout CYP1A in Western blots (not shown). The 56 KDa protein band was excised from the gel and subsequently used for mice immunization.

Six ICR female mice of ten weeks old were sensitized using standard protocols in the animal facilities of Burapha University. Briefly, mice were i.p. injected with an initial dose of 500 µg of purified CYP1A protein from Asian sea bass liver microsomes together with Complete Freund's Adjuvant. Subsequently, booster doses with 300 µg protein CYP1A and incomplete Freund's adjuvant were injected i.p. after two and four weeks, respectively. A final i.v. injection (200 µg protein) in the tail vein concluded the immuno-sensitization procedure. The mice were bled from the tail veins after five days. Serum fractions were collected and stored at –80°C.

CYP1A Protein Analyses

Hepatic microsomal CYP1A protein levels were determined by Western blot analyses using standard protocols and detected as described in Celander and Förlin (1991), with minor modifications. Here, 20 µg microsomal proteins were loaded in each lane and transferred to nitrocellulose membranes. The PAb against Asian sea bass CYP1A raised in mice was diluted 1:250 in blocking solution (5% fatfree dry milk (w/v) in TRIS-buffer saline, pH 7.4). The PAb against rainbow trout CYP1A raised in rabbits were diluted 1:1000 in blocking solution. As secondary antibodies, goat anti-mouse- or goat anti-rabbit IgG conjugated with horseradish peroxidase were used, diluted 1:1000 in blocking solution. The blots were stained with 0.03 % 3,3´diaminobenzidine-4-HCl, 0.006% H_2O_2, 0.05 % $CoCl_2$ in phosphate buffer saline, pH 7.4.

Isolation of a CYP1A cDNA from Asian Sea Bass Liver

Total RNA (1 µg) was reversed transcribed with PCR kit from Applied Biosystems, using random primers. For PCR amplification of cDNA we used primers designed for conserved regions of CYP1A from several different fishes. These were forward primer: 5'-GGC TAC TTC ATT CCC AAA G-3' and reverse primer: 5'-CAG CGT TTG TGC TTC AT-3'. We tested three different annealing temperatures (49°C, 52°C, and 54°C). The PCR cycling parameters were as follow: Initial denaturation at 94°C for 5 min followed by 30 cycles [denaturation at 95°C for 30 s, annealing at 49°C, 52°C, or 54°C for 30 s for each set, polymerization at 72°C for 45 s]. Aliquots of each reaction were separated in an 1.5% agarose gel containing ethidium bromide and the PCR products were visualized under UV light as shown in Figure 2.

The PCR product was excised from the gel and purified using a Gel extraction kit from Omega Biotek. Sequencing of the purified PCR product was performed by Eurofins MWG Gmbtl (Ebersberg, Germany) with the PCR primers used for sequencing in both directions.

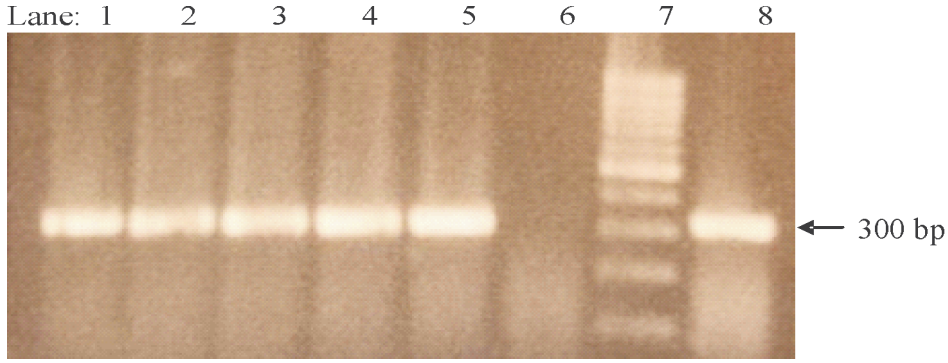

Figure 2: Optimization of RT-PCR protocol for isolation of a 300 base pair CYP1A cDNA fragment from livers from two Asian sea bass treated with 10 mg BaP/kg fish analyzed on an 1.5% agarose gel stained with ethidium bromide and visualized by UV-light.

Lanes 1-3: Fish A with annealing temperature 49, 52 and 54 °C, respectively; Lanes 4-5: Fish B with annealing temperature 49 and 52 °C, respectively; Lane 6: Negative control (no addition of RT enzyme); Lane 7: DNA Size marker (100 base pair) and Lane 8: Liver RNA from Viviparous eelpout (*Zoarces viviparus*).

CYP1A mRNA Analysis

From the Asian sea bass CYP1A partial sequence above, a new pair of gene specific primers was designed for quantitative (Q) realtime PCR analyses using the 'Beacon designer' software (Premier Biosoft International) and synthesized by Eurofins MWG. The Q-PCR primers were: forward 5'-GGA AGG GGA GAA GGT GAT G-3' and reversed: 5'-GAT TGC CAA GAA GAG GAA GAC-3'. These primers give an amplicon size of 94 base pairs which is not restricted to the conserved region and therefore more likely to get 100% efficiency in the Q-PCR reaction.

The Q-PCR was done with IQ SYBR Green supermix and the instrument I Cycler/MyIQ (BioRad) and 25 ng of transcribed total RNA template was used in each reaction volume of 25 µl with 1 µM of each primers in the following protocol; 94°C for 20 s, 55°C for 20 s, 72°C for 30 s in a total of 40 cycles. A meltcurve analysis from 65 to 95°C confirmed a specific reaction (data not shown).

Results

Development of PAb Against Asian Sea Bass CYP1A

Of six mice immunized, two mice had responded strongly to the immunization treatment as judged by Western blot analysis (Figure 3). To confirm the specificity of mice PAb to CYP1A protein from Asian sea bass, rabbit PAb raised against rainbow trout CYP1A was used for comparison (Figure 4). Next, the sensitivity of mouse PAb was tested in serial dilution of liver microsomes from

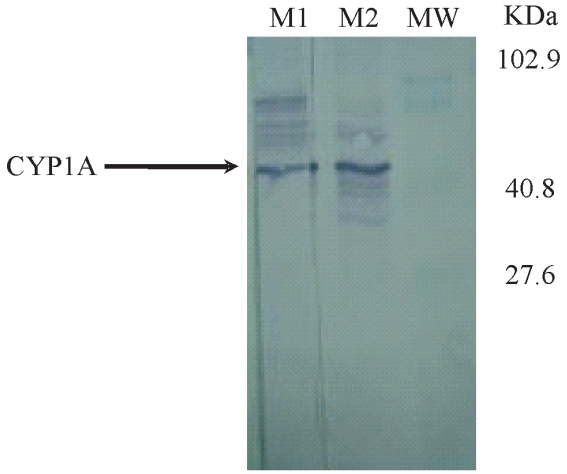

Figure 3: Serum from two individual mice (M1 and M2) immunized with Asian sea bass CYP1A protein diluted 1:250 in blocking buffer was tested on western blots of liver microsomes from BaP-treated Asian sea bass.

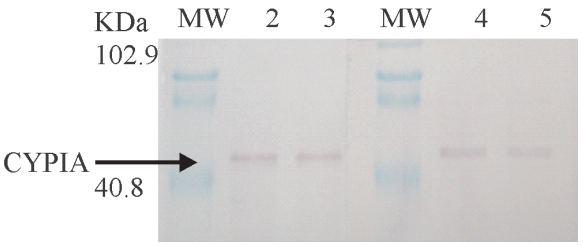

Figure 4: Comparison between mouse PAb raised against Asian sea bass CYP1A and rabbit PAb raised against rainbow trout CYP1A sera in western blots of liver microsomes from two different individuals of BaP-treated Asian sea bass.

Lane 1: Pre-stained molecular weight standard (low range); Lanes 2-3: Asian sea bass liver microsomes from two different individuals stained with rabbit PAb raised against rainbow trout CYP1A (diluted 1:1000); Lane 4: Pre-stained molecular weight standard (low Range); Lanes 5-6: Asian sea bass liver microsomes (same as in lanes 2-3) stained with mouse PAb raised against Asian sea bass CYP1A (diluted 1:250).

Asian sea bass treated with 10 mg BaP/kg fish. Western blot analysis revealed that these antibodies detected CYP1A protein in a dose-response manner in liver microsomes ranging from 2 to 20 µg microsomal protein/lane (Figure 5).

Isolation of CYP1A cDNA from Asian Sea Bass and mRNA Analysis

The 329 bp CYP1A fragment from Asian sea bass shared 88% sequence identity with the corresponding sequence fragment from European sea bass (*Dicentrarchus labrax*) and 79% identity with rainbow trout according to NCBI nBLAST (Table 2).

Table 2: Sequence identity between Asian sea bass CYP1A and CYP1A cDNAs from other fish species according to NCBI nBLAST

Fish species	*Sequence identity*
Japanese amberjack	90%
European sea bass	88%
Japanese medaka	80%
Rainbow trout	79%
Zebra fish	76%

Quantitative (Q) realtime PCR analysis showed a 2.5-fold induction of CYP1A mRNA in Asian sea bass treated with 10 mg BaP in a dose-response study (Figure 6). Although, the Q-PCR data implies a dose-response relationship additional samples are required before this can be statistically verified.

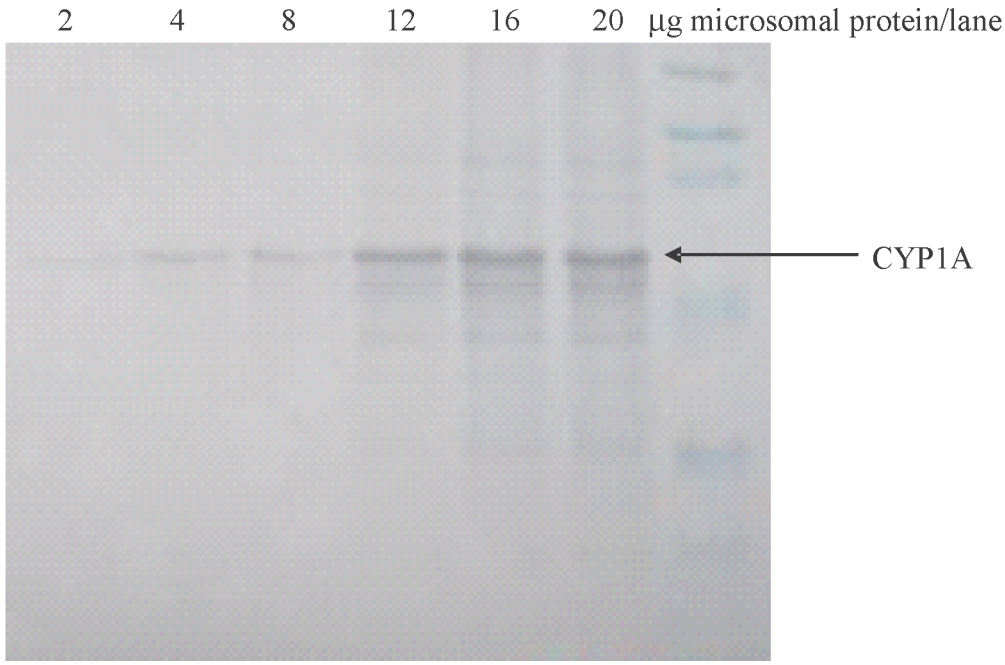

Figure 5: Detection of CYP1A protein in Western blot using mouse PAb raised against Asian sea bass CYP1A (diluted 1:250) of serial dilutions of liver microsomes from BaP-treated Asian sea bass, ranging from 0.2 to 20 µg microsomal protein in each lane.

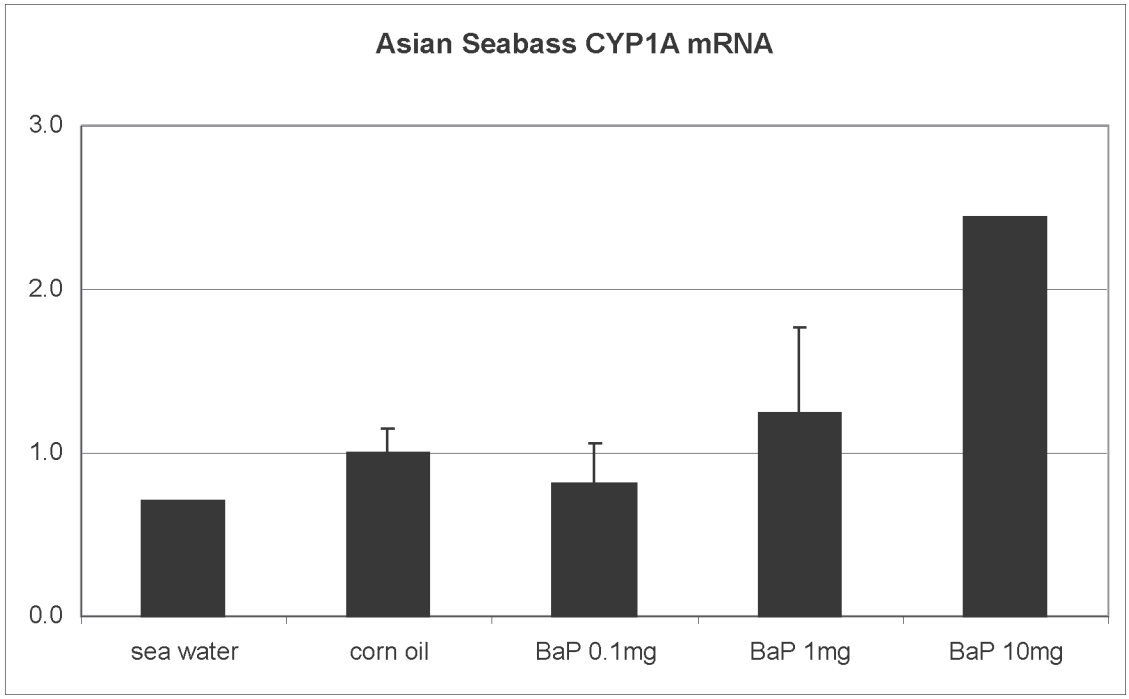

Figure 6: Dose-response study of hepatic CYP1A mRNA levels in Asian sea bass treated with 0, 0.1, 1 and 10 mg BaP/kg fish. The bars represent relative CYP1A mRNA expression, and error bars standard deviation of two fish (except 10 mg/kg, data from one fish).

Detection of CYP1A protein in marine fish from Gulf of Thailand

The mouse PAb raised against Asian sea bass CYP1A was next applied on 15 different tropical fish species, caught off the Chonburi coast. Three different sample sites were selected: Koh Loi, Si Racha (station 1), Ao Udom (station 2) and Laem chabang (station 3). The three stations are shown in Figure 1B.

The intensity of CYP1A protein staining varied between sampling site and species. In station 1, all three fish analyzed had detectable CYP1A levels. In station 2, six of totally eleven fish had detectable CYP1A levels and in station 3, one of totally nine fish had detectable CYP1A protein levels. The results are listed in Table 1. The CYP1A cross-reactivity in these species was confirmed using rabbit PAb raised against rainbow trout CYP1A (data not shown).

Discussion

Biomonitoring programmes are routinely carried out in temperate waters, in particular in Europe and North America, and induction of CYP1A is used as a sensitive biomarker and "early warning signal" to assess exposure to aromatic hydrocarbons (reviewed in: Stegeman and Hahn, 1994; van der Oost et al., 2003). Compared to that in fish from temperate waters, little is known about biomarker responses in fish from tropical waters.

The presence of a CYP1A gene in Asian sea bass was shown by cDNA isolation using a RT-PCR approach and a Q-PCR protocol was developed for determination of CYP1A mRNA levels in Asian sea bass exposed in the lab to BaP. Here, we also describe the development of specific antibodies against CYP1A from Asian sea bass and the specificity for CYP1A was confirmed by comparison with PAb against rainbow trout CYP1A, which recognizes CYP1A orthologues in fish from Swedish waters (Förlin and Celander, 1993). This is, to our knowledge, the first antibody raised against CYP1A from a tropical teleost species, which may be particularly suitable to determine induction of CYP1A protein expression in fish from South East Asia in future biomonitoring programmes.

The antibody was next tested on a suite of 15 different wildlife fish species caught on Chonburi coast in the Gulf of Thailand, which is an area previously shown to be polluted with, among others, PAHs from petroleum sources as a result of heavy shipping traffic. Three different sample sites were selected: Koh Loi, Si Racha (station 1), Ao Udom (station 2) and the more off-shore Laem chabang (station 3). The results imply that feral fish from the Chonburi coast have been exposed aromatic hydrocarbons as indicated by weak to strong CYP1A immunoreactive bands in liver microsomes from several fish species as determined in Western blots. In Si Racha, all fish analyzed had detectable CYP1A levels. In Ao Udom, more than half of the fish collected had detectable CYP1A levels whereas in Laem chabang, only one of totally nine fish had detectable CYP1A levels. Although, the species varied between the stations, the data implies that Koh Loi, Si Racha and Ao Udom were more contaminated with PAH-type contaminants compared with the more off-shore Laem chabang that seems to be less exposed to CYP1A inducers. However, further studies are required including more individuals of each species from all three stations, before this can be verified. Nevertheless, the preliminary data presented demonstrate that there are CYP1A-inducers along this coastline that affect fish which calls for future biomonitoring in this area that is of great economic importance for fishing as well as for tourism in Thailand.

The dietary preferences should also be considered as previous studies have shown that dietary chemicals may affect CYP gene expression in fish. For example, unexpectedly high hepatic CYP level were observed in the tropical butterfly fish *Chaetodon capistratus* feeding on allelochemically-rich gorgonian corals though it appeared to be mostly members of the CYP2 and CYP3 gene families, and not CYP1A, that were high in these fish (Vrolijk et al., 1994). The possible interference of natural dietary chemicals was further suggested in tropical fish from the Bermuda Archipelago where herbivorous fish generally had higher CYP protein levels (i.e. CYP1A, CYP2B and CYP3A) compared to carnivorous fish and omnivorous fish had CYP levels in between (Stegeman et al., 1997). In the present study, the majority of fish are carnivorous and thus less likely to be exposed to allelochemicals. However, for some species the dietary preferences are less clear and the possible impact of natural dietary chemicals should not be overlooked in future biomonitoring programmes in this area.

Conclusion

This paper confirms studies in Europe and North America that the hepatic CYP1A could be used as early indicators of chemical exposure in Thailand waters, and that PAb against Asian sea bass is suitable for analyses of CYP1A in a number of different tropical fish species.

Acknowledgements

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References

Celander, M. and L. Förlin (1991). Catalytic activity and immunochemical quantification of hepatic cytochrome P-450 in b-naphthoflavone and isosafrol treated rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry*, **9:** 189-197.

Celander, M., Näf, C., Bromann, D. and L. Förlin (1994). Temporal aspects of induction of hepatic cytochrome P450 1A and conjugating enzymes in the viviparous Blenny (*Zoarces viviparous*) treated with petroleum hydrocarbons. *Aquatic Toxicology*, **29:** 183-196.

Cheevaporn, V. and Beamish, F.W.H. (2007). 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. *Journal of Environmental Biology,* **28:** 701-705.

Chumchuchan, S., Wattayakorn, G., Tuanwechayan, W. and P. Suthannaruk (1998). Petroleum hydrocarbon contamination in coastal environment of the upper Gulf of Thailand: Extent and sources. *In:* ASEAN-Canada Cooperative Programme on Marine Science. October 26-30, 1998. Langkavvi, Malaysia, 217-224.

Förlin, L. and M. Celander (1993). Induction of cytochrome P4501A in teleosts: Environmental monitoring in Swedish fresh, brackish, and marine waters. *Aquatic Toxicology*, **26:** 41-56.

Miller, H.C., Mills, G.N., Bembo, D.G., Macdonald, J.A. and C.W. Evans (1999). Induction of CYP1A in *Trematomus bernachii* as an indicator of environmental pollution in Antarctica: Assessment by quantitative RT-PCR. *Aquatic Toxicology*, **44:** 183-193.

Rice, C.D. and D. Schlenk (1995). Immune function and cytochrome P450 1A activity after acute exposure to 3,3',4, 4',5-Pentachlorobiphenyl (PCB126) in channel catfish. *Journal of Aquatic Animal Health,* **7:** 195-204.

Sarin, C. and K. Srisuksawad (2000). Distribution of aliphatic and aromatic hydrocarbon in dated sediment cores of The Gulf of Thailand. Regional Training Course (RCA) on Application of Traces to Study Transport Processes and Sedimentation Rates in the Marine Environment. 21 Feb-4 Mar 2000, Thailand. IAEA-SM-354/210P, 563-564.

Široká, Z. and J. Drastichová (2004). Biochemical markers of aquatic environment contamination— cytochrome P450— in fish. A review. *Acta Veterinaria Brunensis*, **73:** 123-132.

Schlenk, D., George, S., James, M., Van den Hurk, P., Willett, K., Kullman, S., Celander, M. and E. Gallagher (2008). Biotransformations. *In:* The Toxicology of Fishes. (Eds.) R. Di Giulio and D. Hinton. Taylor and Francis Publishers, Washington DC. 153-234.

Stegeman, J.J. and M.E. Hahn (1994). Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. *In*: Ostrander, G.K., Malins, D. (eds), Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, Boca Raton, FL. 87-206.

Stegeman, J.J., Woodin, B.R., Singh, H., Oleksiak, M.F. and M. Celander (1997). Cytochromes P450 (CYP) in tropical fishes: Catalytic activities, expression of multiple CYP proteins and high levels of microsomal P450 in liver of fishes from Bermuda. *Comparative Biochemistry and Physiology*, **116C:** 61-75.

Van der Oost, R., Beyer, J. and N.P.E. Vermeulen (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environmental Toxicology and Pharmacology,* **13:** 57-149.

Vrolijk, N.H., Targett, N.M., Woodin, B.R. and J.J. Stegeman (1994). Toxicological and ecological implications of biotransformation enzymes in the tropical teleost *Chaetodon capistratus*. *Marine Biology,* **119:** 151-158.

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