



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

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ABSTRACT. Hepatic microsomes prepared from 10 fish species from Bermuda were studied to establish features of cytochrome P450 (CYP) systems in tropical marine fish. The majority (7/10) of the species had total P450 content between 0.1 and 0.5 nmol/mg, and cytochrome b_5 content between 0.025 and 0.25 nmol/mg. Ethoxycoumarin *O*-deethylase (ECOD) and aminopyrine *N*-demethylase (APND) rates in these 7 species were 0.23–2.1 nmol/min/mg and 0.5–11 nmol/min/mg, respectively, similar to rates in many temperate fish species. In contrast to those 7 species, sergeant major (*Abudefduf saxatilis*) and Bermuda chub (*Kyphosus sectatrix*) had microsomal P450 contents near 1.7 nmol/mg, among the highest values reported in untreated fish, and had greater rates of ECOD, APND, ethoxyresorufin *O*-deethylase (EROD) and pentoxyresorufin *O*-deethylase than did most of the other species. Freshly caught individuals of all species had detectable levels of EROD and aryl hydrocarbon hydroxylase (AHH) activities. Those individuals with higher rates of EROD activity had greater content of immunodetected CYP1A protein, consistent with Ah-receptor agonists acting to induce CYP1A in many fish in Bermuda waters. Injection of tomtate and blue-striped grunt with β -naphthoflavone (BNF; 50 or 100 mg/kg) induced EROD rates by 25 to 55-fold, suggesting that environmental induction in some fish was slight compared with the capacity to respond. AHH rates were induced only 3-fold in these same fish. The basis for disparity in the degree of EROD and AHH induction is not known. Rates of APND and testosterone 6 β - and 16 β -hydroxylase were little changed by BNF, indicating that these are not CYP1A activities in these fish. Antibodies to phenobarbital-inducible rat CYP2B1 or to scup P450B, a putative CYP2B, detected one or more proteins in several species, suggesting that CYP2B-like proteins are highly expressed in some tropical fishes. Generally, species with greater amounts of total P450 had greater amounts of proteins related to CYP2B. These species also had appreciable amounts of CYP3A-like proteins. Thus, many fishes in Bermuda appear to have induced levels of CYP1A; some also have unusually high levels of total P450 and of CYP2B-like and CYP3A-like proteins. These species may be good models for examining the structural, functional and regulatory properties of teleost CYP and the environmental or ecological factors contributing to high levels of expression of CYP in some fishes. Copyright © 1997 Elsevier Science Inc. comp biochem physiol 116C:1:61–75, 1997.

KEY WORDS. Cytochrome P450, microsomal enzymes, tropical fishes, CYP1A, CYP2B, CYP3A, hydrocarbons, enzyme induction, monooxygenases

INTRODUCTION

Monooxygenases in the cytochrome P450 (CYP) gene superfamily (35) are important in detoxifying lipophilic drugs and pollutants but can also activate chemicals that are pro-toxicants and procarcinogens (12,20,43). CYP also metabolize toxic natural products present in the diets of many ter-

restrial vertebrates and insects (40). The abundance and function of specific CYP proteins can determine the susceptibility of individuals or species to toxic effects of those natural products or anthropogenic xenobiotics. Members of CYP gene families 1–4 that are prominent in xenobiotic metabolism also are inducible by substrates. Expression of novel CYP forms has been linked to the ability of some insects to metabolize toxic allelochemicals and consequently to feed on plants that produce those chemicals (11,39). Thus, CYP enzymes are important in the mechanisms underlying chemically induced toxicity or disease and chemical–ecological interactions.

CYP proteins purified from several fish species

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(9,15,26,62) appear to represent teleost members of the CYP gene families 1–4 (46). CYP enzymes studied most extensively in fish are those in the CYP1A subfamily. Molecular cloning and phylogenetic analysis has confirmed that fish express CYP1A genes (34). In mammals, there are two CYP1As expressed, CYP1A1 and CYP1A2. Fish CYP1As have regulatory, immunochemical and functional similarities to each other and to mammalian CYP1A1 proteins (45). Teleost CYP1A genes are inducible by polycyclic aromatic hydrocarbons (PAH) and by planar halogenated aromatic hydrocarbons (pHAH). These are ligands for the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor found in vertebrates (17), that when bound dimerizes with a second helix-loop-helix protein (ARNT) (18) alters expression of CYP1A and other genes (18). The AhR may be involved in toxicity of pHAH (38). CYP1A induction is used widely as a marker of exposure to these toxic AhR agonists.

CYP genes other than 1A have been identified in fish, but apart from CYP2K1 (8), little is known of their function or regulation. Possible occurrence of CYP2B- and CYP3A-like proteins is particularly intriguing. Phenobarbital (PB) and other chemicals that induce CYP2Bs in mammals have not been found to induce CYP in fish (23), although CYP2B-like activities do occur in fish. Slight changes in rates of aldrin epoxidase or in benzo[a]pyrene metabolite profiles have been interpreted as indicating a PB-type response in fish (6,41), but this has not been substantiated (13). Yet, proteins related to CYP2B have been identified in fish (31,55), and there may be species in which PB-type responses do occur. Similarly, CYP3A-like proteins are expressed in fish liver (32,46). In mammals, CYP3As are the major hepatic CYP, catalyze steroid 6 β -hydroxylase and are induced by and metabolize glucocorticoids and natural products (42). Putative CYP3As in fish appear to be largely constitutive, although response to inducers has been little studied (10).

Characterization of CYP systems in fish from diverse environments might reveal species in which chemical induction of CYP2B-like and/or CYP3A-like proteins is indicated. In 1982, we began studies of hepatic CYP systems in tropical fish species from Bermuda. These studies were undertaken to evaluate the capacities of coral reef fishes to metabolize xenobiotics and to determine whether CYP systems in these species have features distinct from those of other fishes. A further objective was to determine the degree of CYP1A expression in fish from Bermuda, to indicate the degree of biochemically significant exposure to environmental chemicals there. Except for occasional cold-core Gulf Stream rings (61), Bermuda is hydrographically isolated from the East coast of North America where high levels of CYP1A occur commonly in fish (33,49). CYP1A expression also was examined to determine whether chemical exposure might be great enough to mask other sources of variation in expression of CYP genes. Our results indicate

that CYP1A is chronically induced in Bermuda fishes. However, some species were found to have hepatic microsomal enzyme profiles reminiscent of those induced in mammals by PB or PB-like compounds and to have high levels of putative CYP3A. These species may be useful in examining the possible inducibility of CYP2B-like and CYP3A-like proteins in fish and the similarities or differences in CYP regulation between fish and mammals.

MATERIALS AND METHODS

Chemicals

Nicotinamide adenine dinucleotide phosphate (reduced; NADPH), nicotinamide adenine dinucleotide (reduced; NADH), β -naphthoflavone (BNF), dithiothreitol, Tris and buffer salts, horse heart cytochrome c, sodium dithionite and glycerol were obtained from Sigma (St. Louis, MO, U.S.A.). 7-Ethoxyresorufin was synthesized and purified as described previously (27). 7-Pentoxyresorufin was a gift from Dr. Michael Marletta. Immuno-reagents, SDS, and acrylamide were obtained from BioRad (Richmond, CA, U.S.A.). Nitrocellulose membranes (0.2 μ m), nylon filters, alkaline phosphatase secondary antibody and substrate sheets were purchased from Schleicher and Schuell (Keene, NH, U.S.A.). α -Naphthoflavone (ANF), [³H]-benzo[a]pyrene, testosterone, ethoxycoumarin and aminopyrine were from sources named previously (48,28).

Animals and Treatments

The species studied here and numbers of each species examined are listed in Table 1. The species were selected on the basis of availability and dietary preferences to include herbivores and omnivores; Table 1 also gives the common components in the diet of these species. Note that $n = 2$ or more for all species except pinfish. Data are presented for the single pinfish because the results for this individual were somewhat unusual among the fish studied. Fish were collected in August 1982 by angling, primarily at two locations, near pilings at the west end of Ferry Reach (termed Ferry Reach) and over reefs ca. 150 m off Fort St. Catherine, St. Georges (termed Ft. St. Catherine). We are not aware of any point sources of contaminants near these sites. Localized sources of contaminants in Castle Harbor (7) are not likely to have an impact on these collection sites. After capture, animals were transported to the laboratory at the Bermuda Biological Station (BBS) and held in aquaria in flowthrough water for no more than 2 hr before being killed, livers removed and subcellular fractions prepared. Blue striped grunt were collected in Harrington Sound, Bermuda, taken to the Bermuda Aquarium and then transported to the BBS within 10 hr, when some were killed and liver microsomes prepared immediately. Others were held for treatment with BNF. Tomtate used in induction studies were captured in Ferry Reach and were placed in aquaria where they were held for treatment. Other tomtate collected were killed and microsomes prepared from liver immediately.

TABLE 1. Fish sampled from Bermuda waters

Species	Common name	n	Sex (M/F)	Body wt* (g)	Liver wt	Gonad† status	Primary diet‡
					Body wt (%)		
<i>Petrometopan cruentatus</i>	Graysby (rockfish)	2	1/1	138–203	1.28 ± 0.62§	Regressed	Benthic algae and vertebrates
<i>Lutjanus griseus</i>	Grey snapper	5	ind	44–90	0.63 ± 0.04	Regressed	Crustaceans and small fish
<i>Ocyurus chrysurus</i>	Yellowtail snapper	2	2/0	59–60	0.77 ± 0.02	Regressed	Crustaceans and small fish
<i>Sparisoma viride</i>	Parrotfish	6	6/0	705–871	1.28 ± 0.62	Regressed	Scleractinian corals and epifauna
<i>Halichoeres bivittatus</i>	Slippery dick	2	1/1	41–72	0.90 ± 0.10	Regressed	Crustaceans, sea urchins, polychaetes, mollusks
<i>Haemulon sciurus</i>	Blue striped grunt	12	6/6	319–612	0.65 ± 0.08	Regressed	Invertebrates
<i>Haemulon aurolineatum</i>	Tomtate (grunt)	15	7/8	25–33	0.78 ± 0.19	Regressed	Omnivore
<i>Lagodon rhomboides</i>	Pinfish	1	1/0	58	0.69	Regressed	Omnivore
<i>Abudefduf saxatilis</i>	Sergeant major	7	5/2	54–120	1.12 ± 0.11	Regressed	Plankton, benthic invertebrates, algae
<i>Kyphosus sectatrix</i>	Bermuda chub	3	ind	207–366	0.87 ± 0.18	Regressed	Mainly plants, brown algae, some red algae, and invertebrates

*Body weights are ranges, except for the individual value in pinfish. Data for blue-striped grunt are for untreated (freshly caught) fish only.

†Gonads were undeveloped in all individuals. Gonads of species listed as "ind" (indeterminate) could not be identified visually as testes or ovary.

‡Dietary composition is from Sterrer (56).

§Values are means ± SD or range.

Treatment Protocols

The fish used in induction studies, blue striped grunt (*Haemulon sciurus*) and tomtate (*Haemulon aurolineatum*), were held for 24 hr before treatment. Fish were held at a density of four fish per 70-l tank, supplied with flowthrough seawater at ambient temperature (29°C). Blue striped grunt were given a single intraperitoneal injection of 50 mg BNF in corn oil per kg body weight ($n = 4$) or an equivalent volume of corn oil (0.1 ml/kg) ($n = 4$). After 3 days, fish were killed and hepatic microsomes prepared from individual fish as described below. The dose and time used were strongly inducing, based on comparison with dose-response and time course of CYP1A induction by BNF in blue striped grunt, established in later studies (52). Tomtate were treated by intraperitoneal injection with corn oil ($n = 6$) or 100 mg/kg BNF in corn oil ($n = 6$). After 3 days, animals were killed and livers were removed and pooled for preparation of microsomal fractions.

Microsome Preparation and Analysis

Hepatic microsomal fractions were prepared using the buffers and methods used earlier with marine fish (47). Initially, freshly prepared microsomes from each species were analyzed for the content of total protein, for total cytochrome P450 and cytochrome b_5 contents and for rates of ethoxyresorufin *O*-deethylase (EROD) activity. Cytochrome b_5 content was determined from NADH-reduced vs oxidized difference spectra and total P450 content was determined from dithionite difference spectra of CO-treated microsomes, as described previously (47). The spectrophotometrically measured

content of CYP in microsomes is referred to as P450. Individual P450 gene products are designated by their trivial names or as CYP. Protein content was estimated by the procedure of Lowry *et al.* (29). Aliquots of microsomes then were archived in liquid nitrogen and shipped on dry ice to Woods Hole, Massachusetts for further analysis. Control and PB-induced rat liver microsomes were the gift of Dr. David Waxman (Dana-Farber Cancer Institute). Hepatic microsomes from control and induced rats had 1.24 and 1.46 nmol of total P450 per mg of microsomal protein, respectively. Hepatic microsomes from control and BNF-induced scup (*Stenotomus chrysops*), a temperate water teleost species, were obtained as before (49). The properties of these microsomes used for comparison are described elsewhere (49).

Catalytic Activities

EROD activity was analyzed in all samples by the protocol described by Klotz *et al.* (27). Pentoxyresorufin *O*-deethylase (PROD) activity was analyzed using the same spectrophotometric method used for EROD but substituting 5 μ M pentoxyresorufin for ethoxyresorufin. Substrate saturation profiles, pH optima and time dependence for PROD activity were determined empirically for each fish species. This characterization was done in parallel with a characterization of PROD activity in PB-treated rat liver microsomes as a positive reference. Assays were done on 4 or 5 replicates per sample and were repeated by two investigators. Benzo[a]pyrene hydroxylase (AHH) activity was measured radiometrically by the procedure described previously (4). Inhibition

of AHH by ANF was measured in reaction mixtures to which ANF in methanol had been added to achieve a final concentration of 100 μ M. Boiled protein blanks and reactions minus ANF had added solvent carrier only. The concentrations of carrier were empirically determined not to inhibit AHH. Aminopyrine *N*-demethylase (APND) activity was assayed by measuring release of formaldehyde, according to previously described methods (47). Ethoxycoumarin *O*-deethylase (ECOD) activity was analyzed as described before (28). Testosterone 6 β - and 16 β -hydroxylase activities were analyzed by HPLC analysis, as described elsewhere (51). Catalytic assays were carried out at protein concentrations in the range of linear response, determined empirically. Temperatures of incubation with fish liver microsomes were 25°C for EROD and PROD and 30°C for other enzymes. Rat liver microsomes were incubated at 37°C.

Immunoblot Analysis

Microsomal proteins were resolved on 9% or 12% homogeneous or 6–13% gradient SDS-polyacrylamide gels, with 10–30 μ g of microsomal protein per lane. Proteins were transferred electrophoretically to 0.2 μ m nitrocellulose (Schleicher and Schuell). The protocol used was a modification of the protocol of Towbin *et al.* (58) as described previously (25). Non-specific binding sites were blocked with non-fat dry milk or 1% blocking powder (Schleicher and Schuell) in Tris buffer or saline, and blots then were reacted with primary antibodies. These included monoclonal antibody 1-12-3 to P450E from the teleost fish scup (36) and polyclonal antibodies to rat P450 PB-4 (CYP2B1), to scup P450B, scup P450A and trout P450con. The identity of scup P450E has been confirmed as a CYP1A1 (34). MAb 1-12-3 is highly specific for CYP1A proteins and recognizes putative CYP1A in all vertebrate groups (45). In mammals, Mab 1-12-3 is highly specific for CYP1A1 but not CYP1A2. The polyclonal antibody against rat PB-4 (CYP2B1) was the generous gift of D. Waxman (Dana Farber Cancer Institute). Antibodies against scup P450B and against scup P450A have been described (16). Scup P450B is an apparent CYP2B homologue, based on exclusive and reciprocal immunocross-reactivity with rat CYP2B1, 2B2 and 2B3 and on N-terminal sequence analysis. Scup P450A is a CYP3A-like protein, based on exclusive cross-reactivity with CYP3A1, CYP3A4 and with putative CYP3As from other fish species (10). Antibodies against trout P450con, an apparent CYP3A-like protein and immunological counterpart of scup P450A, were obtained as before (9). Secondary antibody was alkaline phosphatase-linked goat anti-mouse IgG or goat anti-rabbit IgG, using 5'-bromo-4-chloro-3-inolyphosphate (BCIP) and nitroblue tetrazolium (NBT) as the color reagents. Alternately, detection was by enhanced chemiluminescence, according to the directions for the Schleicher and Schuell Rad-Free Chemiluminescence kit. Resulting bands were scanned using a Helena Laboratories' Quick-Scan R&D la-

ser densitometer or by video-imaging densitometry. Relative amounts of cross-reactive protein were determined by comparison with scup microsomes containing known quantities of CYP1A1, P450B (putative CYP2B) or with PB-induced rat liver microsomes in which the content of CYP2B1 had been estimated.

Statistical Analysis

Data analysis from BNF-treated and control fish was accomplished using ANOVA followed by a one-tailed *t*-test.

RESULTS

Size, sex, liver weight and gonadal status of the animals examined are shown in Table 1. All individuals examined in each species had gonads that were regressed. Thus, as expected based on the gonadal status and comparison with other species, no sex differences were evident in any of the measures described below. The data for males and females therefore were pooled.

Temperature optima for monooxygenase activity in fish from these waters was established for EROD in two of the species, blue striped grunt and tomtate. In both species, EROD activity was maximal between 27 and 31°C. These temperatures are like the optima determined for some temperate marine fish (47) but lower than optima reported for some other warm water species, including fish from Florida (22) and *Poeciliopsis* species native to warm water (~40°C) streams in Mexico (14). The temperature in Bermuda waters in summer ranges up to 29°C.

Microsomal P450, b₅ and Reductases

The specific content of total P450 ranged between 0.1 and 0.5 nmol/mg microsomal protein in 7 of 10 species examined (Table 2). In pinfish, the P450-specific content was 0.8 nmol/mg, and in both Bermuda chub and sergeant major, the value was as high as 2.3 nmol/mg and averaged about 1.7 nmol/mg. The Fe²⁺-CO absorption maxima were at 449–450 nm in each species, and there was no evidence of cytochrome P420 (denatured P450) in any sample. In 7 of 10 species, the content of cytochrome b₅ was in the range of 0.02–0.2 nmol/mg, whereas in the pinfish, Bermuda chub and sergeant major, the content averaged 0.6 nmol/mg. Average rates of NADPH-cytochrome *c* (P450) reductase and NADH-cytochrome *c* (b₅) reductase differed by 5- to 7-fold between species. The rates in Bermuda chub were one and one-half to two times greater than those in the other species (Table 2). Rates of both enzymes in the fish here were in the range reported for other fishes (43).

Monooxygenase Activities

The rates of activity with five different substrates for P450 were measured in liver microsomes from each species (Table

TABLE 2. Hepatic microsomal cytochrome P450 and electron transport components*

Species	<i>n</i>	Microsomal protein (mg/g liver)	Cytochrome P450 (nmol/mg)†	Cytochrome <i>b</i> ₅ (nmol/mg)†	NADPH-cyt P450 reductase (nmol/min/mg)†	NADH-cyt <i>b</i> ₅ reductase (nmol/min/mg)†
Graysby bass	2	12.5 ± 0.2‡	0.083 ± 0.014	0.024 ± 0.018	18 ± 4	66 ± 42
Grey snapper	5	12.6 ± 1.3	0.164 ± 0.029	0.033 ± 0.010	76 ± 22	38 ± 16
Yellowtail snapper	2	8.8	0.260	0.054	81	105
Parrotfish	6	25.6 ± 6.8	0.308 ± 0.076	0.082 ± 0.009	50 ± 16	56 ± 16
Slippery dick	2	10.5 ± 2.4	0.312 ± 0.169	0.086 ± 0.075	50 ± 36	66 ± 32
Blue-striped grunt	4	6.9 ± 1.9	0.411 ± 0.046	0.177 ± 0.032	97 ± 17	100
Tomtate	3	7.6	0.520	0.140	116	116
	(pooled)					
Pinfish	1	14.0	0.817	0.213	101	138
Sergeant major	6	12.4 ± 3.2	1.660 ± 0.370	0.241 ± 0.055	90 ± 31	41 ± 23
Bermuda chub	3	23.1 ± 0.1	1.730 ± 0.430	0.611 ± 0.189	156 ± 10	262 ± 71

*Data are for freshly caught individuals. *n* = number of freshly caught fish analyzed.

†Values are expressed as nmol or nmol/min/mg of microsomal protein.

‡Values are means ± SD or ± range where *n* = 2 or greater.

3). Average rates of EROD ranged about 100-fold, from 0.01 to more than 1 nmol/min/mg protein. EROD rates generally were greater in slippery dick, Bermuda chub and sergeant major than in the other species, and the highest EROD rate was seen in the lone pinfish. AHH activities ranged about 80-fold, from about 0.01 to 0.8 nmol/min/mg, and were greatest in sergeant major, chub and pinfish. Inhibition of AHH by ANF was seen with all samples, but the degree of inhibition was not correlated with AHH rates. AHH activity was not stimulated by ANF in any sample. ECOD rates ranged 40- to 60-fold between species, from about 0.2 up to 9 nmol/min/mg, and rates of APND ranged up to 20-fold. Both ECOD and APND rates were greater in chub and sergeant major than in the other species. However, the graysby, which had the lowest rates of EROD and AHH activity, had APND rates that were nearly half those

in chub and sergeant major. The amounts of total P450 and specific activities of EROD and APND in the different species are compared graphically in Fig. 1.

PROD activity was not detected in grey snapper, slippery dick, blue striped grunt and in most parrotfish but was readily detectable in all graysby, pinfish, sergeant major and Bermuda chub. The rates in the sergeant major (0.34 nmol/min/mg) were higher than the PROD rates we measured in PB-induced rat liver microsomes (0.32 nmol/min/mg). Interestingly, one of six parrotfish had a PROD rate that was as high as the rates in sergeant major.

Table 3 shows specific activities or rates per mg protein. Normalizing rates to nmol of total P450 reveals the relative efficiency of the total complement of microsomal CYP to catalyze a given reaction. A higher value can reveal a relative "enrichment" of a given catalyst among the total com-

TABLE 3. Hepatic microsomal monooxygenase activities in Bermuda fish*

Species	EROD*	AHH*	Inhibition† by 10 ⁻⁴ M ANF	ECOD*	APND*	PROD*‡
Graysby bass	0.010 ± 0.002	0.012 ± 0.008	97	0.23 ± 0.11	11.85 ± 0.0	0.040
Grey snapper	0.081 ± 0.020	0.160 ± 0.052	45	0.28 ± 0.10	N.D.	N.D.
Yellowtail snapper	0.068	0.067	87	0.32	0.5	—
Parrotfish§	0.086 ± 0.166 (N.D. - 0.422)	0.065 ± 0.127 (0.004 - 0.324)	—	0.22 ± 0.13 (0.02 - 0.53)	7.3 ± 3.0 (N.D. - 11)	N.D.§ (0.36)
Slippery dick	0.580 ± 0.520	0.249 ± 0.338	33	0.26 ± 0.13	7.2 ± 0.0	N.D.
Blue striped grunt	0.083 ± 0.027	0.395 ± 0.161	71	0.62 ± 0.09	3.2 ± 0.5	—
Tomtate	0.127	0.110	100	0.71	1.6	0.070
Pinfish	2.10	0.109	14	2.13	3.4	0.102
Sergeant major	0.516 ± 0.218	0.817 ± 0.010	33	2.57 ± 0.21	22.6 ± 6.3	0.337 ± 0.067
Bermuda chub	1.00 ± 0.028	0.453 ± 0.138	81	9.13 ± 5.06	24.2 ± 2.9	0.102 ± 0.057

*Activities all are given as nmol/min/mg of microsomal protein. Values are means ± range or SD. Numbers of samples are as indicated in Table 2. Dashes indicate not measured. N.D. indicates not detected.

†Percent activity remaining.

‡Limits of detection for PROD were 0.005 to 0.010 nmol/min/mg protein under the conditions of assay. For comparison, hepatic microsomal PROD activities that we measured in control and PB-induced rat were <0.01 and 0.316 nmol/min/mg, respectively.

§Six parrotfish were assayed, one of which had higher values for most activities. Numbers in parentheses show the range, except for PROD. The PROD value in parenthesis was seen in one fish. Five of six fish had no detectable PROD.

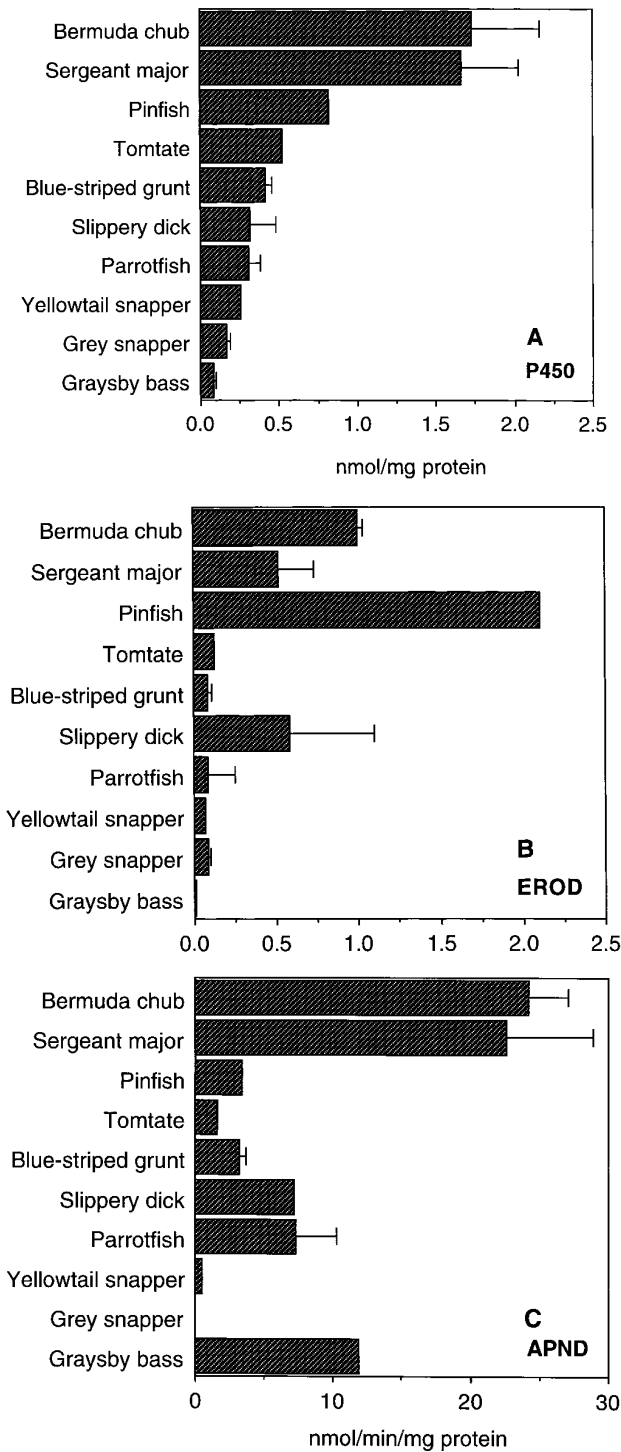


FIG. 1. Hepatic microsomal P450 and monooxygenase activities in Bermuda fishes. (A) Cytochrome P450 content. The results are expressed as nmol of total cytochrome P450, determined spectrophotometrically, per mg of microsomal protein. (B) EROD activity. (C) Aminopyrine *N*-demethylase activity. Enzyme activities are expressed as specific activity (i.e., nmol/min/mg microsomal protein). Values are means of measurements on from 2 to 12 individuals \pm SD or the range, except for the pinfish.

plement of CYP. Chub and sergeant major were among the four species having the highest specific activities of EROD, but the values for EROD per nmol of P450 in these two species were similar to those in most of the other species (Fig. 2A). The EROD catalyst(s) appeared to be enriched in the pinfish and in slippery dick. AHH activities per nmol of P450 ranged nearly 10-fold, with no particular species pattern, but note that this activity was not elevated in the pinfish (Fig. 2B). The ECOD activity per nmol P450 was much higher in Bermuda chub than in any of the other species (Fig. 2C), and APND activity per nmol P450 also was higher in the graysby than in the other species (Fig. 2D). The estimated turnover numbers for PROD were similar among the species for which activity was measured.

Immunoblot Analyses

Western blotting with Mab 1-12-3 to scup CYP1A1 revealed cross-reacting proteins in all species except the graysby. Given the specificity of Mab 1-12-3 for CYP1A proteins, the results indicate the expression of CYP1A in these fishes. In most individuals, this cross-reacting protein was a single band, near the M_r of P450E (Fig. 3). A few individuals had faint bands of lower molecular weight recognized by Mab 1-12-3, presumably minor degradation products.

Polyclonal antibodies to rat CYP2B1 cross-reacted with proteins in several species (Fig. 4A). Those proteins were similar in electrophoretic molecular weight to rat CYP2B1. The samples reacting most strongly were those from tomtate, pinfish, Bermuda chub and sergeant major. Antibody to scup P450B, a possible CYP2B homologue, gave a pattern of staining identical to that obtained with anti-CYP2B1 (Fig. 4B). Samples from several species had multiple proteins cross-reacting with anti-CYP2B1. Chub and sergeant major each had two bands (Fig. 4), and there were three CYP2B-related bands in pinfish. Tomtate and blue striped grunt each showed two or three bands, with the pattern in grunt varying between individuals. The faster migrating band in Fig. 4B, lane 8, may be a degradation product, but the closely spaced bands migrating in the region where CYP migrate suggest the occurrence of multiple CYP2B-like proteins. Generally, samples that had multiple CYP2B-related bands showed no signs of degradation of other CYP forms (see Fig. 3).

The relative intensities of staining of the primary bands recognized by Mab 1-12-3 or by anti-CYP2B in the various species are given in Table 4. Among these freshly caught fish, the highest levels of CYP1A staining were seen in pinfish, slippery dick and sergeant major. Slippery dick, pinfish and grey snapper had the greatest amount of CYP1A staining relative to total P450. Individual parrotfish showed the greatest range in CYP1A staining content within a species, from about 1 to more than 300 area units Mab 1-12-3 stain per nmol total P450. The signal intensity of the major bands showed that several species had similar relative content of

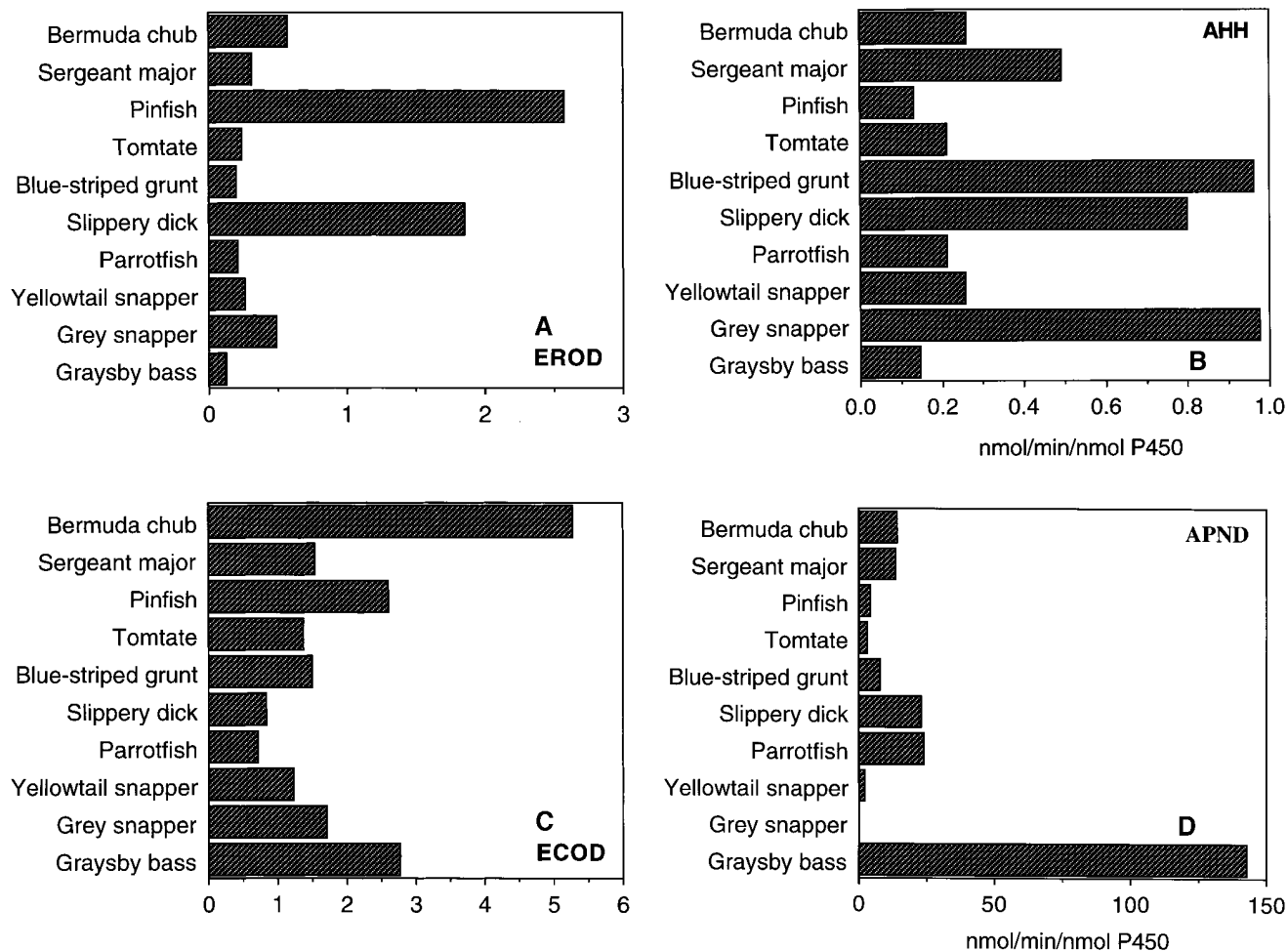


FIG. 2. Hepatic microsomal monooxygenase activities in Bermuda fishes (A) EROD, (B) AHH, (C) ECOD, (D) APND. Rates of each activity are expressed as estimates of turnover number (i.e., nmol/min/nmol total microsomal P450).

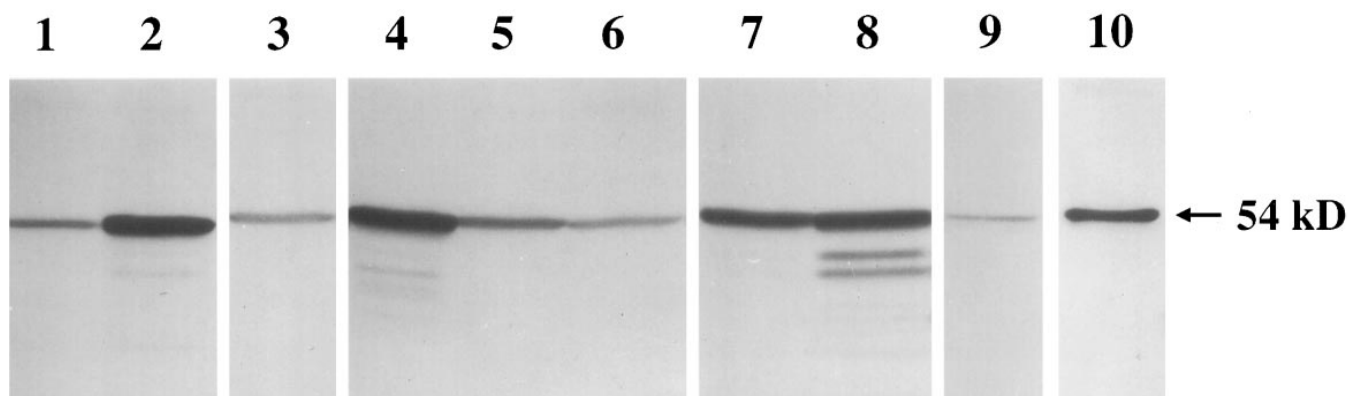


FIG. 3. Cytochrome P4501A in Bermuda fishes. Hepatic microsomes were prepared and proteins resolved as in Materials and Methods, and stained with Mab 1-12-3 to scup CYP1A1. Lanes, left to right: 1, control tomtate; 2, BNF-tomtate; 3, blue striped grunt; 4, pinfish; 5 and 6, Bermuda chub; 7 and 8, sergeant major; 9, gray snapper; 10, 3,3',4,4'-tetrachlorobiphenyl-induced scup liver. Samples in lanes 3-9 are from untreated fish. Similar amounts (30 μ g) of microsomal protein were loaded in each lane. Visualization was with NBT and BCIP. Results with representative species are shown. Immunoblots of CYP1A induced in blue striped grunt were shown previously (52). The migration and intensity of the bands relative to one another and to scup standards are as on the original blots. The apparent molecular weight for scup CYP1A1 (54 kDa) is marked on the blot.

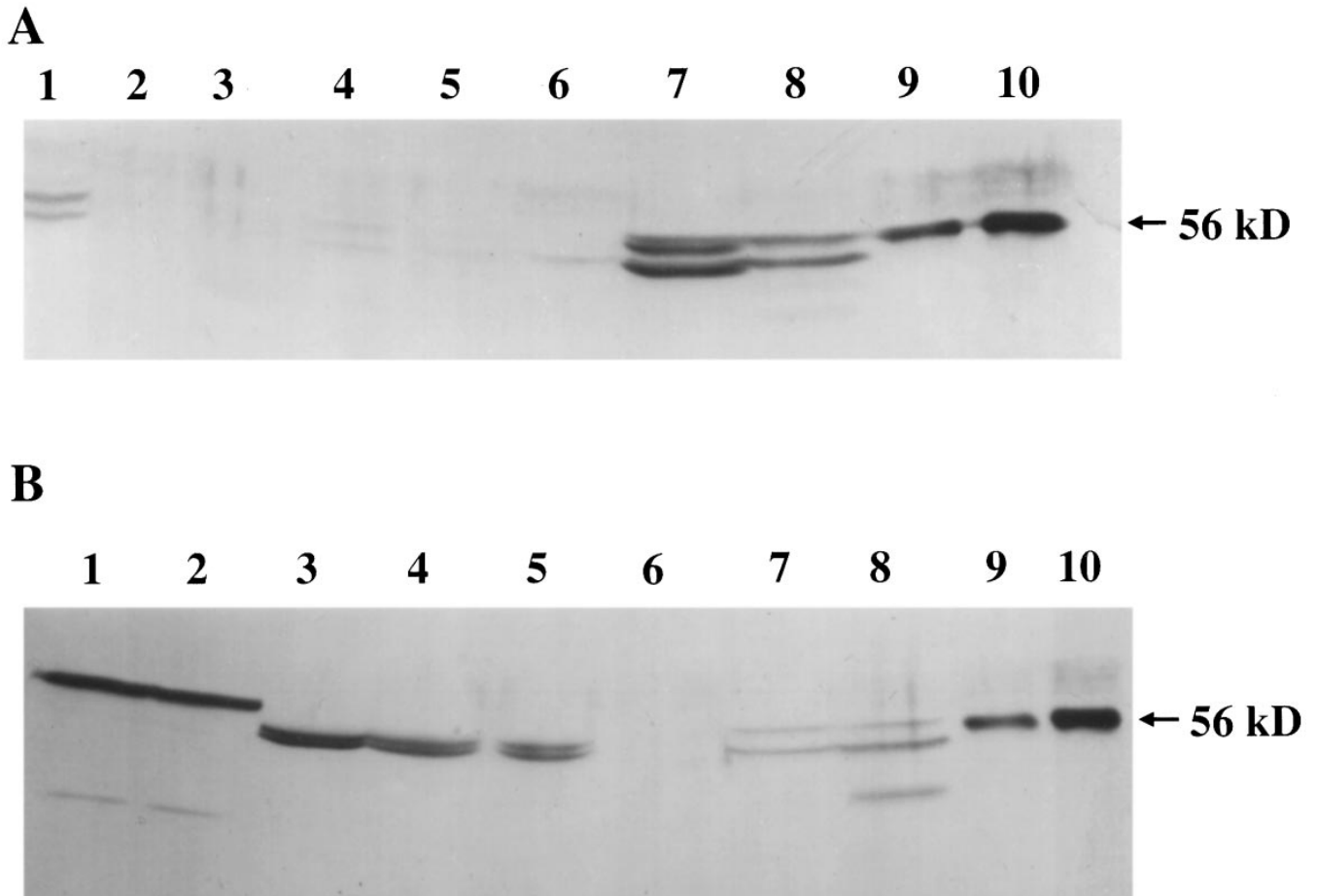


FIG. 4. Cytochrome P4502B-like protein in Bermuda fishes. Hepatic microsomal proteins were prepared and resolved as in Fig. 3. Blots were stained with antibody to rat CYP2B1. (A) Lanes, left to right: 1–5, parrotfish; 6, slippery dick; 7, pinfish; 8, tomtate; 9 and 10, phenobarbital-induced rat liver microsomes. (B) Lanes, left to right: 1 and 2, Bermuda chub; 3–5, sergeant major; 6, graysby; 7 and 8, blue striped grunt; 9 and 10, phenobarbital-induced rat liver microsomes. Microsomal protein was loaded at 30 μ g per lane. Visualization was with NBT and BCIP. The molecular weight for rat CYP2B1 (56 kDa) is marked on the blot.

possible homologues of CYP2B (Table 4). Interestingly, the intensity of the signal normalized to total P450 was less in Bermuda chub than in some of the other species. The staining of both CYP1A and putative CYP2B in the Bermuda species was much less than in the positive controls, BNF-induced scup or PB-induced rat. Some species differences in the amounts of staining could be due to differences in reactivity with these heterologous antibodies.

Microsomal samples from chub, sergeant major, grunt, tomtate and parrotfish were examined for proteins cross-reactive with antibodies to scup P450A and trout P450con, fish CYP3A-like proteins. Antibodies to P450con detected bands in four of the species but did not detect bands in the parrot fish, apart from faint very low molecular weight bands (Fig. 5A). Single prominent bands were detected in each of the other species, and in the chub and sergeant major, weakly staining faster-migrating bands also were detected by anti-P450con. In analysis of chub and sergeant major, the antibodies to scup P450A and to trout P450con both detected the same bands (Fig. 5B). The degree of

staining with anti-P450A was stronger in sergeant major than in chub, a difference not seen with anti-P450con (Fig. 5B).

BNF Treatment

Two species, tomtate and blue-striped grunt, were treated with BNF to compare the level of environmental induction with the capacity for response and to determine the influence of induction on monooxygenase activities other than EROD. BNF elicited a nearly 2-fold increase in microsomal P450 content in blue-striped grunt. In tomtate, the content of total P450 changed little with BNF (Table 5). BNF did not affect the content of cytochrome b_5 in either species. EROD rates expressed per mg protein were induced 25-fold in tomtate and about 55-fold in blue-striped grunt (Table 5). The degree of AHH induction was substantially less than that of EROD, only about 2- to 3-fold in either species; there was only a slight yet significant difference between treated and control fish in sensitivity of AHH activity to

TABLE 4. Densitometric analysis of immunoblots

Species	CYP1A staining		CYP2B-like staining	
	Optical density		Optical density	
	Per μg protein	Per pmol total P450	Per μg protein	Per pmol total P450
Graysby bass	N.D.	—	N.D.	—
Grey snapper	10 \pm 6	64 \pm 52	1 \pm 1	6 \pm 6
Parrot fish*	39 \pm 36	130 \pm 137	3 \pm 6	12 \pm 2
Slippery dick	47	244	2	11
Blue striped grunt	4 \pm 4	9 \pm 8	12 \pm 8	48 \pm 47
Tomtate	89 \pm 11	21 \pm 27	31 \pm 1	67 \pm 14
Pinfish	76	93	53	65
Sergeant major	48 \pm 11	27 \pm 9	29 \pm 5	16 \pm 4
Bermuda chub	15 \pm 8	9 \pm 7	38 \pm 5	23 \pm 9
BNF-scup	832	1320	—	—
PB-rat	—	—	3838	2326

Values are means \pm SD. N.D. means not detected under conditions of assay, with NBT-BCIP color development. Incubation conditions and color development times with a given antibody were the same for all species.

*Values for parrotfish ranged widely. For CYP1A, the area per mg ranged from 0.5 to 80, and for area per pmol, ranged from 1 to >300.

ANF inhibition. The rates of APND and of both testosterone 6 β -hydroxylase and 16 β -hydroxylase per mg protein were slightly (1.3- to 1.6-fold) yet significantly greater in the BNF-treated than in control blue striped grunt (Table 5). There was a similar difference in ECOD between treated and control fish, but that difference was not significant.

Expressing the data as estimated turnover numbers (rates per nmol of total P450) showed that there was a strong enrichment of the catalyst(s) for EROD activity and an enrichment of AHH activity catalyst(s) in the BNF-treated fish (Fig. 6). In contrast, although the specific activities were slightly greater in the BNF-treated fish, the estimated turnover numbers showed no enrichment of ECOD or of APND (Fig. 6). Likewise, there also was no increase in the estimated turnover number of testosterone 6 β - or 16 β -hydroxylase in the BNF-treated fish (not shown). Lack of BNF induction of APND and testosterone 6 β - and 16 β -hydroxylase activities in grunt is consistent with evidence from other fish that these microsomal activities are catalyzed by teleost CYP forms other than CYP1A (53,54).

The levels of CYP1A protein detected with Mab 1-12-3 were induced by BNF treatment in both tomtate and blue-striped grunt (Table 5). There was no evident effect of BNF on the amount of anti-CYP2B1 cross-reacting protein in either species (not shown).

DISCUSSION

CYP systems have been surveyed in numerous fishes from temperate, tropical and deep-ocean environments (3,22, 37,43,44). Here we expand the range of species and proper-

ties of microsomal enzymes examined in tropical fishes. The specific content of total P450 in hepatic microsomes of most of the fish examined here was about 0.5 nmol/mg or less, like the levels in other fish (43). However, Bermuda chub and sergeant major had levels of hepatic microsomal P450 between 1.0 and 2.3 nmol/mg, as great as the content in many mammals treated with CYP inducers and greater than levels reported for most other untreated or induced teleosts. Microsomal P450 content in liver of male winter flounder from sites contaminated by polychlorinated biphenyls (PCBs) ranges up to 2.0 nmol/mg (53), and tomtate sampled in 1988 from Bermuda had ca. 1.6 nmol/mg (52). In flounder, the high P450 content can be attributed largely to CYP1A (16). The identity of CYP contributing to the high P450 content in tomtate from 1988, or in the fish examined here, is not known.

Those species with modest levels of total P450 generally had lower specific activities of several monooxygenase reactions, including AHH, EROD, ECOD, APND and PROD, than did sergeant major and Bermuda chub, the species with the highest microsomal P450 content. When normalized to gram of liver or gram of body weight, the rates of the activities measured were highest in chub. Chub also had the highest rates of microsomal reductases. Chub appears to have a greater capacity for xenobiotic metabolism than do the other species examined.

Despite the higher specific activities, the "estimated" turnover numbers (rate/nmol total P450) for EROD in chub or sergeant major were similar to those in species with lower EROD rates per mg protein. This could occur if the EROD catalyst in chub, for example, is functionally similar to that in other species and accounted for a similar percentage of the total hepatic P450. A similar condition might occur for the PROD catalyst. Microsomal EROD rates per mg protein or per nmol P450 were higher in the lone pinfish than in most of the other species, except for slippery dick. McDonald *et al.* (30) found higher rates of hepatic microsomal EROD in pinfish than in 16 other species they examined from the Gulf of Mexico. Examining pinfish from other areas could indicate whether a high level of CYP1A expression, inferred from EROD rates, is common in this species.

PROD and APND specific activities were highest in chub and sergeant major. The CYP catalyzing these activities have not been identified in fish. In rats, PROD is catalyzed prominently by the PB-inducible CYP2B proteins (5), although CYP1A appears to be a catalyst in some species. APND is also a 2B activity in rat. The estimated turnover numbers for ECOD were higher in chub than in most of the other species, suggesting an enrichment or greater capacity of the ECOD catalyst(s) in chub. ECOD is catalyzed by multiple CYP in mammals and fish, including CYP2Bs in rat and P450A in scup (28). The combination of high total P450 content and high specific activities or enrichment of PROD, APND and/or ECOD catalysts in chub or sergeant major is reminiscent of the profiles of hepatic P450 content and catalytic rates in PB-induced mammals.

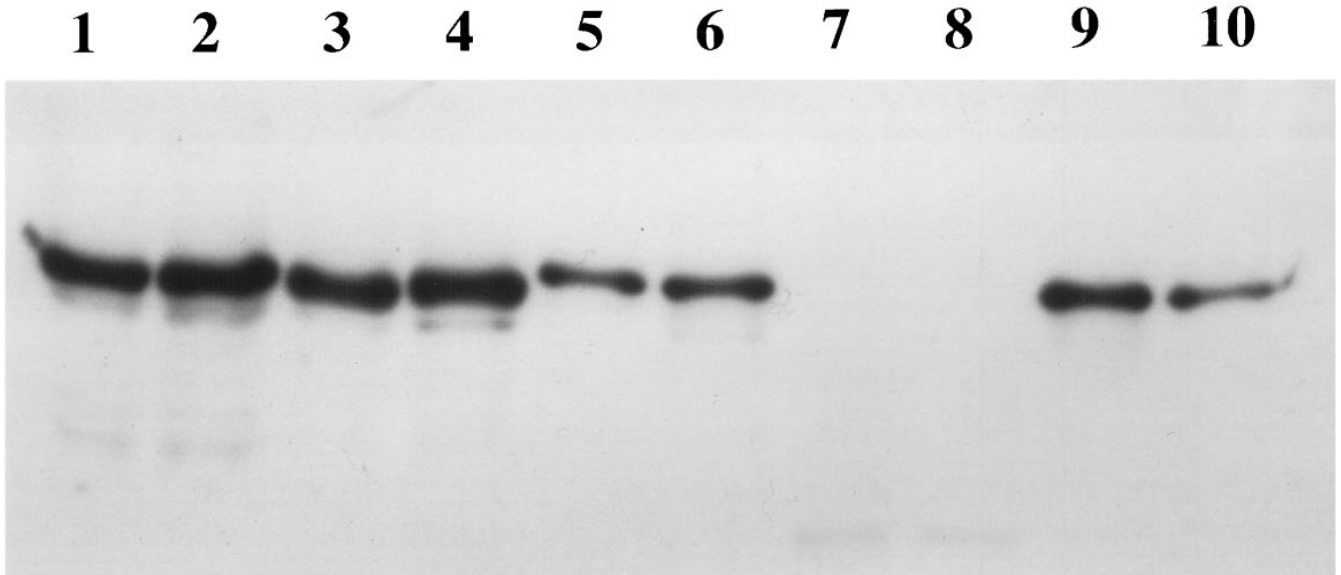
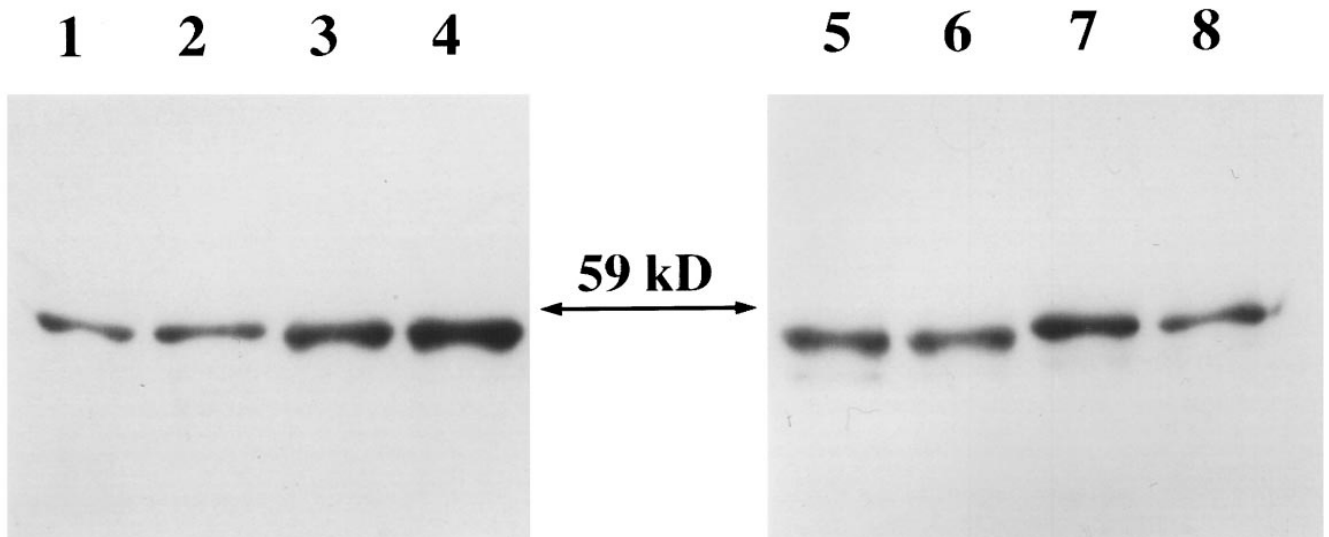
A**B**

FIG. 5. Cytochrome P4503A-like protein in Bermuda fishes. Hepatic microsomal proteins were prepared and resolved as in Fig. 3. (A) Blots were stained with antibody to trout P450con. Lanes, left to right: 1 and 2, Bermuda chub; 3 and 4, sergeant major; 5 and 6, blue striped grunt; 7 and 8, parrotfish; 9 and 10, tomtate. Microsomal protein 10 μ g was loaded in each lane. (B) Blots were stained with antibody to scup P450A (lanes 1–4) or with antibody to trout P450con (lanes 5–8). Lanes, left to right: 1 and 2, Bermuda chub; 3–6, sergeant major; 7 and 8, Bermuda chub. Microsomal protein was loaded in each lane at 10 μ g per lane. Visualization in both A and B was by enhanced chemiluminescence. Trout P450con was run as a standard but is not shown. The apparent molecular weight for trout P450con (59 kDa) is marked on the blot.

TABLE 5. Characteristics of hepatic microsomal systems in control and β -naphthoflavone-treated tomtate and blue striped grunt

Characteristic	Tomtate		Blue striped grunt	
	Control (6)*	BNF (6)*	Control (4)†	BNF (4)†
Liver wt/body wt (%)	0.86	1.02	0.54 ± 0.05	0.71 ± 0.09‡
Microsomal yield (mg protein/g liver)	20.3	12.9	20.0 ± 4.6	18.6 ± 1.6
Cytochrome P450 (nmol/mg protein)	0.408	0.421	0.271 ± 0.084	0.475 ± 0.185‡
Cytochrome b_5 (nmol/mg protein)	0.086	0.080	0.099 ± 0.051	0.086 ± 0.030
Ethoxyresorufin <i>O</i> -deethylase (nmol/min/mg)§	0.025	0.642	0.024 ± 0.020	1.312 ± 0.197
Aryl hydrocarbon hydroxylase (nmol/min/mg)	0.09	0.33	0.17 ± 0.06	0.51 ± 0.19
AHH activity with 10 ⁻⁴ M ANF (% remaining)	51	46	48 ± 4	31 ± 3
Ethoxycoumarin <i>O</i> -deethylase (nmol/min/mg)	—	—	0.54 ± 0.14	0.82 ± 0.27
Aminopyrine <i>N</i> -demethylase (nmol/min/mg)	—	—	5.4 ± 1.4	8.9 ± 1.4
Testosterone 6 β -hydroxylase (nmol/min/mg)	—	—	0.038 ± 0.005	0.074 ± 0.035‡
Testosterone 16 β -hydroxylase (nmol/min/mg)	—	—	0.039 ± 0.013	0.052 ± 0.002‡
CYP1A (% of total P450) [¶]	2.0	10.4	2.5	6.4

*Livers from six fish were pooled for analysis.

†Values for control and treated blue-striped grunt are means ± SD, for $n = 4$. Differences significant at ‡ $P \leq 0.05$ or ^{||} $P \leq 0.005$.

§Units for all enzyme activities are nmol/min/mg microsomal protein.

¶The content of Mab 1-12-3 cross-reactive protein in pooled samples in corn oil or BNF-treated fish. Values are relative to the staining of scup P450E (CYP1A1) standards and are expressed as scup CYP1A equivalents per nmol of microsomal P450.

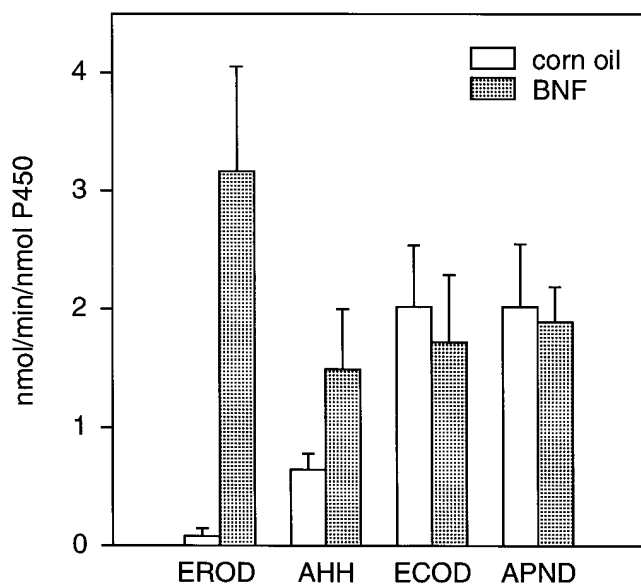


FIG 6. Estimated turnover numbers for monooxygenase activities in control and BNF-treated blue striped grunt. Values shown are nmol of substrate metabolized per nmol of total P450. Values are means of measurements on 4 individuals ± SD. EROD activities in treated fish were significantly different from control fish at $P \leq 0.0005$. AHH activities in treated fish were significantly different from control fish at $P \leq 0.01$. Other activities did not show differences at $P \leq 0.05$.

CYP2B- and CYP3A-Related Proteins

Antibodies to rat CYP2B1 and to the CYP2B-like scup P450B both detected the same protein bands in liver microsomes of fish studied here. In reciprocal immunoblot analysis, the antibodies to CYP2B1 or P450B both specifically

recognize the scup and rat immunogens. Both antibodies also recognize CYP2B1, 2B2 and 2B3 and no other proteins in rat liver microsomes and recognize P450B and no other proteins in scup liver microsomes (55). The present results thus indicate that one or more proteins immunochemically related to CYP2B are strongly expressed in Bermuda chub, sergeant major, pinfish and tomtate. Proteins immunochemically related to CYP2B have been identified also in rainbow trout (31). This trout protein, P450 LMC1, recently has been classified as CYP2M (63), and although the sequence is sufficiently different from CYP2Bs to warrant separate classification, that does not obviate the possibility of a homologous relationship to CYP2B. However, neither CYP2B-like proteins nor CYP2B-related mRNA (24) have been demonstrated to be inducible in fish. If the cross-reactive proteins seen here indeed are homologues of CYP2B, it would indicate either a high level of endogenous expression or an environmental induction of CYP2B-like genes in these fish.

Fish may differ from mammals in the mechanisms of regulation of CYP2B-like proteins. It is possible that fish constitutively express CYP2B-like proteins at levels sufficient to meet requirements for metabolism of compounds that in mammals require induction of CYP2B. However, scup sampled directly from certain Cape Cod waters have as much as a 20-fold greater content of the CYP2B-like P450B than do scup held in captivity (White and Stegeman, unpublished data), suggesting that some dietary or other environmental factor might regulate expression of these genes in fish. The possibility that environmental factors might increase the expression of CYP genes other than CYP1A is suggested also by the 4- to 8-fold differences in P450 content between sergeant major described here and those sampled in 1988 (52).

Earlier it was suggested (43) that the abundance or identity of natural products in the diet might contribute to the evolution or maintenance of PB-responsiveness in vertebrates. The high levels of CYP2B-like proteins in the present study were in species that are primarily herbivorous (Table 1). Natural products in the diet of those fish might be involved in the higher level expression of the putative CYP2B. The possibility that expression of CYP2B-like proteins in fish might be related to dietary natural products was considered also in a study of congeneric species of butterfly fish (59). *Chaetodon capistratus* that preferentially consume gorgonians rich in allelochemicals had a greater content of total P450 and proteins recognized by antibodies to scup P450B (putative CYP2B) than did *C. ocellatus* that avoid eating gorgonians (59). A similar difference between those species occurred in the levels of CYP3A-like protein. In a recent study of tilapia (*Oreochromis niloticus*) from Brazil, the levels of CYP2B-like and CYP3A-like proteins were greater in liver microsomes of fish from a contaminated than from a reference site (Bainy and Stegeman, unpublished data), suggesting that pollutant chemicals might induce these proteins. However, fish from the contaminated but not the reference site had abundant algal residues in the gut, implying that natural products might have contributed to that difference in CYP expression. Thus, CYP2B-like and CYP3A-like protein levels in liver of some fish might be elevated because of effects of dietary natural products.

Although the high content of total P450 could result from elevated expression of homologues of known CYP, there also could be expression of novel CYP in herbivorous species such as the chub. The red and brown algae prominent in the diet of chub contain fucoxanthins and other compounds that might require specialized enzymes for their metabolism. The expression of novel CYP in some insects has been linked to plant secondary metabolites in the diet (11). In some Bermudian fishes, and in *C. capistratus*, several protein bands were detected by antibodies to CYP2B1, suggesting the presence of multiple CYP2B-like proteins. Multiple CYP2B genes are expressed in some mammals (35). If multiple CYP2B-like proteins do reflect products of distinct genes in fish, it will be interesting to determine whether they are regulated differently and whether one or more are induced by natural products or pollutants. These herbivorous fish species with high content of total P450 and apparently high level expression of multiple CYP2B-like and/or CYP3A-like proteins present attractive subjects for examining regulation of these teleost CYP genes and for investigating whether novel CYP genes have evolved in some fish, possibly in response to dietary components.

Induction of CYP1A

A protein detected by Mab 1-12-3 was strongly induced by BNF in blue striped grunt and tomtate. Previously published results also showed that BNF induces a single Mab 1-12-3 cross-reacting protein in grunt (52). Mab 1-12-3 is highly

specific for CYP1A proteins in vertebrates, (25,45), and proteins strongly recognized in the BNF-treated fish or in the untreated fish here are concluded to be CYP1A forms.

Antibody inhibition studies indicate that Mab 1-12-3 cross-reactive protein (CYP1A) in blue striped grunt catalyzes both AHH and EROD activities (54). Accordingly, one could expect both activities to be induced to a similar extent by BNF. Yet in both grunt and tomtate, AHH was induced to a much lesser degree than was EROD. Differences in patterns of EROD vs AHH induction have been seen in other species, for example, in sheepshead (*Archosargus probatocephalus*) (21). There also was a lack of correspondence between estimated turnover numbers for EROD and AHH in the freshly caught fish analyzed here. Such discrepancies raise recurring questions as to whether AHH and EROD are properties exclusively of the same protein or whether there are multiple or distinct catalysts for AHH and EROD in some fish.

If there are distinct inducible catalysts for these activities in fish, it would have important implications for detection and interpretation of environmental induction as a biomarker. Two CYP1A genes have been described in trout (2) (provisionally, CYP1A1 and CYP1A3) (35), but these genes diverged very recently (34). That particular divergence probably occurred only in salmonids. Other teleost lineages would not be expected to have counterparts to the two trout genes, although CYP1A genes certainly may have diverged independently in other fish taxa. A second PAH and pHAH-inducible subfamily, CYP1B, has been described recently in mammals (57). If CYP1B homologues are inducible in fish and have catalytic properties different from CYP1As, they might contribute to the discrepancy in AHH/EROD induction. There also might be functional differences in how the substrates ethoxyresorufin and benzo[a]pyrene interact with a single catalyst. We have evidence supporting the latter possibility (Stegeman, unpublished data), but the possibility remains open that AHH and EROD are catalyzed by different proteins in some fish.

Environmental Induction in Bermuda

The EROD rates and CYP1A content in the freshly caught fish analyzed here indicate a response to inducers of CYP1A. In a later study of fish from Bermuda, we also detected EROD and CYP1A in all fish examined (52), including many of the same species studied here. Data from the two studies are compared in Table 6 and suggest that fish in Bermuda waters are chronically exposed to inducing levels of AhR agonists. The high rates of EROD activity in some of these individuals and species suggest a substantial degree of induction, compared with rates in some experimentally induced fish (e.g., Table 6), although in tomtate and grunt the degree of induction in the environment was slight compared with the capacity to respond to the known CYP1A inducer BNF.

The differences in the levels of CYP1A expression be-

TABLE 6. Hepatic microsomal ethoxyresorufin *O*-deethylase activity in fish from Bermuda*

Species	August 1982		September 1988	
	Location† or treatment	EROD‡ (nmol/min/mg)	Location or treatment	EROD (nmol/min/mg)
Grey snapper	Fort St. Catherine	0.08 ± 0.02	Hamilton Harbor, 1 + 7	0.07 ± 0.03
Blue striped grunt	Harrington Sound	0.08 ± 0.03	Hamilton Harbor, 1 + 7	0.48 ± 0.14
	Corn oil	0.02 ± 0.02	Hamilton Harbor, 2 - 4	1.77 ± 1.70
	BNFS	1.31 ± 0.20	Corn oil	0.49 ± 0.04
Tomtate	BNFS	0.64	BNFS	4.03 ± 0.41
	Ferry Reach	0.13	Castle Harbor, 1	0.69 ± 0.16
	Corn oil	0.02	Castle Harbor, 2	0.81 ± 0.49
Sergeant major	BNFS	0.64		
	Fort St. Catherine	0.52 ± 0.22	Ferry Reach	0.15 ± 0.05
Rainbow trout	Fort St. Catherine	0.52 ± 0.22	Castle Harbor, 2	0.46 ± 0.08
	Corn oil	0.10 ± 0.07		
Scup	BNFS	3.63 ± 0.09		
	Corn oil	0.31 ± 0.09		
	BNFS	4.69 ± 0.06		

*Data from this paper (August 1982 collection) and from Stegeman *et al.* (52) (September 1988 collection). Data are shown for species sampled at both times.

†Details on location are in Materials and Methods or in Stegeman *et al.* (52). Hamilton Harbor 1 + 7 is an average for fish from clean sites 1 and 7. Hamilton Harbor 2-4 is for fish from midway between sites 2 and 4.

‡EROD measurements were made by spectrophotometric (August 1982, and trout and scup) or fluorometric assay (September 1988). Previous comparisons have shown equivalent rates determined by the two methods (27).

§Rates in BNF-treated Bermuda species sampled at 3 days after treatment.

||Data for control and BNF-treated rainbow trout and scup are from Stegeman *et al.* (49) and are included for comparison. Note that these "control" scup have detectable CYP1A1. Depuration for long times (>12 months) reduces EROD activity in these fish to <0.1 nmol/min/mg.

tween species could reflect different degrees of exposure to environmental chemicals, inherent differences in sensitivity to those inducers or possibly an endogenously elevated expression of CYP1A in some fish. Studies continue to show that greater rates of EROD activity and/or content of immunodetected CYP1A reflect exposure to exogenous inducers (50). Consistent with this, in our 1988 study of Bermuda fish, the CYP1A content in several species correlated with the concentrations of PAH and/or PCBs in sediments or biota (bivalves) taken where the fish were caught (52). This does not, however, mean that those compounds are responsible for the induction observed. As suggested above for CYP2B-like and CYP3A-like proteins, natural products could act to induce CYP1A expression in some of these fish. Recent studies suggest that dinoflagellate toxins (brevetoxins) are weak inducers of EROD (60), but the potency with which other marine natural products might induce CYP1A is not known. Nevertheless, the correlations between CYP1A content and environmental chemicals seen in 1988 (52) suggest that the CYP1A content in fish in both collections reflects induction primarily by environmental chemicals. These results provide a background for future studies of these species in other regions and for possible long-term series studies of environmental induction in Bermuda.

Little is known about the origin of PAH or PCBs in waters and sediments of coastal Bermuda. Greater concentra-

tions of such compounds most likely reflect a Bermudian origin (7). However, PCBs at low yet detectable and biologically active levels could come from other regions, by atmospheric or oceanic transport. Atmospheric transport has long been implicated as a major route of anthropogenic chemicals such as PCBs to the open ocean (1), including to the North Atlantic (19). Analysis of open-ocean and midwater fishes for evidence of CYP1A induction could help to distinguish between the local and atmospheric contribution to the burden of inducers in Bermuda waters. Regardless of the origin of the inducers, CYP1A expression in numerous species in Bermuda waters adds to the growing evidence that this biological response to PAH and pHAH is common in fish from coastal waters of the world's oceans. The magnitude and geographic extent of such biological change needs to be examined further in tropical and other regions. The biological significance of low level CYP1A induction, and the Ah-receptor binding it indicates, also remains to be established. Thus, it becomes increasingly important to determine whether the degree of induction like that seen here and occurring widely in other fishes is associated with other persistent effects in these organisms.

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