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Effect of β -naphthoflavone on hepatic cytochrome P4501A activity in the scribbled rabbitfish (*Siganus spinus*) from tropical Indo-Pacific coral reefs

Carmen Kautz^{a,b,*}, Andres Reyes^b, and Jason S. Biggs^{b,**}

^aInstitute of Environmental and Human Health, Texas Tech University, 1207 Gilbert Drive, Lubbock, TX 79416

^bMarine Laboratory, University of Guam, UOG Station, Mangilao, Guam, 96923, USA

Abstract

Several classes of carcinogenic environmental organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins, negatively affect aquatic ecosystems worldwide. Pollutant detection is often difficult and expensive, especially when dealing with complex mixtures and matrices. Biological markers are informative tools to identify living sources that may harbor toxic compounds and areas unsuitable for recreation. Currently, no species have established biomarkers for organopollutant monitoring in Indo-Pacific coral reefs. This study evaluated the time- and dose-dependent induction of the cytochrome P4501A (CYP1A) system in the scribbled rabbitfish, *Siganus spinus* (Siganidae), as a biomarker for organic pollutant exposures in these environments. Results indicate that *S. spinus* hepatic CYP1A enzymatic activity and protein level respond dose-, and time-dependently following a single intraperitoneal (IP) injection of the classic aryl hydrocarbon receptor (AHR) agonist, β -naphthoflavone (BNF). *S. spinus* hepatic CYP1A protein and enzymatic activity rose as function of dose during the first two days and slowly returned to levels close to normal after 16 days, as measured using the 7-ethoxyresorufin-O-deethylase (EROD) and the non-competitive enzyme-linked immunosorbent (ELISA) assays, respectively. These findings support use of the inducible CYP1A system of *S. spinus* as a biomarker for reef fish exposure to coastal marine pollution. Baseline CYP1A expression levels among Guam's wild *S. spinus* populations were also measured and compared.

Keywords

CYP1A induction; *Siganus spinus*; sentinel species; tropical coral reef; β -naphthoflavone; marine pollution

1. Introduction

Over the past 30 years, Guam has undergone substantial urban, commercial, industrial, and military development, and will continue developing for several more years. As a result of anthropogenic activity and natural events, chemical contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and dioxins, may have entered the environment (Denton et al. 2006a/b). Aquatic environments become sinks for many of these contaminants. Marine environments, especially estuaries, harbors, and coastal areas, bear the brunt of contaminant inputs from direct wastewater discharge, agricultural/

^{**}Corresponding author, Tel.: +1 671.735.2190; fax: +1 671.734.6767; biggs.js@gmail.com.

^{*}Present address

industrial/urban runoff, and hydrologic and atmospheric events (Arinc et al. 2000; Walker et al. 2001). As a result, marine organisms inhabiting these areas are exposed to a wide variety of chemical contaminants, which in turn pose considerable risks to entire ecosystems, including human consumers (Whyte et al. 2000; Behnisch et al. 2001). Establishing indicators to determine whether or not coral reef food chains are exposed to organic pollutants is important for evaluating the safety of the cultural food sources of Micronesian communities.

Complex mixtures of contaminants are difficult, time-consuming, and costly to assess analytically. Alone, analytical techniques are unable to determine biological consequences, especially in instances where inert parent compounds transform into active products after entering an ecosystem. To address this, biological indicators (*i.e.* biomarkers) have been developed as tools for detecting exposure to and the persistence of physiologically-relevant chemical pollutants (Depledge, 1993). Biomarkers are evolutionarily conserved physiological responses to cope with natural phenomenon (e.g. forest fires and volcanic eruptions). Biomarkers are normally absent prior to, and become elevated shortly following, exposure. In the case of environmental pollution, this direct relationship between exposure and response makes biomarkers the first detectable and quantifiable measure of exposure to chemicals with potentially detrimental effects on organisms (Depledge, 1993; Walker, 1998; De Matteis, 1994). To date, the most intensively studied biomarkers are a superfamily of enzymes that coordinate the removal of foreign compounds from organisms, the cytochrome P450 system (Siroká & Drastichová, 2004).

The cytochrome P450 (CYP) phase I metabolic system is a superfamily of heme-containing enzymes primarily involved in monooxidative metabolism (Yi et al. 2007; Nelson, 2008). As an enzyme superfamily, CYPs exhibit a broad range of substrate specificity; yet, their general mechanism is remarkably conserved throughout evolution (Prasad et al. 2007). CYP induction can be detected by monitoring changes in gene expression, protein concentration, and/or enzyme activity. By measuring CYP induction, the pharmacological potency of contaminant exposures on an organism can be assessed.

Among the CYPs, the best-characterized and most commonly used biomarker is CYP1A, as it is the isoform responsible for metabolizing the majority of carcinogenic organopollutants (Siroká & Drastichová, 2004; Payne, 1987). Both field and laboratory studies have validated that the CYP1A enzyme systems of fishes respond to many widespread environmental pollutants including PAHs, PCBs, and dioxins (Al-Arabi and Goksøyr, 2002; Bonacci et al. 2007; Addison and Payne, 1986; Collier et al. 1992; Payne et al. 1987). It is also well understood that the mechanism of CYP1A induction is regulated by the aryl hydrocarbon receptor (AhR). Thus, the induction of CYP1A in fish is widely accepted as a sensitive, convenient biomarker of organic contaminant exposure (Goksøyr and Forlin, 1992); and has been adopted in many national and international monitoring programs for aquatic pollution (Al-Arabi and Goksøyr, 2002; Arinc et al. 2000; Machala et al. 1997; Sanchez and Porcher, 2009).

Most biomarker studies investigating the influence of xenobiotics on biotransformation enzymes have concentrated on fish from temperate and cold-water regions, where their presence in freshwater teleost species has been well established (Al-Arabi & Goksøyr, 2002). CYP isoforms have been purified and characterized from several fishes, and their induction by many xenobiotics is also well established (Stegeman, 1990; Williams & Buhler, 1982; Al-Arabi & Goksøyr, 2002; Arinc et al. 2000; Machala et al. 1997; Arellano-Aguilar et al. 2009). However, few biomarker studies have been conducted on fish in tropical environments (e.g. Shailaja & D'Silva, 2003; Shailaja & Rodrigues, 2001, 2003; Gadagbui & Goksøyr, 1996; Pathiratne & George, 1996, 1998; Bainy et al. 1999; Miranda et

al. 2008), and even fewer have studied tropical marine fish (Stegeman et al. 1990, 1997; Al-Arabi & Goksøyr, 2002). To date, no study conducted is applicable to tropical Indo-Pacific coral reefs.

The current study investigated one possible marine fish species (*Siganus spinus*) from the Indo-Pacific region for use as an acute response biomarker sentinel species. *S. spinus* is a common and frequently studied species in the Indo-Pacific region because it is highly abundant and easily captured, has a limited home range, and is a common food resource throughout Micronesia, making it the perfect candidate for use as a sentinel species for coastal zone management in Micronesia. Thus, this study analyzed the dose- and time-dependent induction of *S. spinus* CYP1A following a single exposure to the model organopollutant BNF. Additionally, baseline CYP1A expression levels were measured and compared among wild Guam populations.

2. Methods

2.1. Fish Collection

Adult *S. spinus* were collected for each of three laboratory trials: November 2009, February 2010, and March 2010. For each trial, 90 fish were collected. All animal protocols described herein have been approved by the University of Guam Institutional Animal Care and Use Committee (IACUC # UOG1101). All collections coincided with spring high tides occurring between 20:00–23:00 hours. Fish were collected from Pago Bay (Figure 1) with long minnow nets and maintained in free-floating mesh basins until transported to the University of Guam Marine Laboratory (UOGML). Pago Bay was selected as the collection site for animals used in laboratory studies because of its close proximity to the UOGML. Additionally, previous studies were unable to detect organochlorine or heavy metal contamination in Pago Bay (Denton et al. 2005, 2009). During transportation (approximately two minutes), fish were maintained in plastic basins full of fresh seawater. Upon arrival, fish were transferred to a 3500 L fiberglass tank and continuously supplied with fresh seawater from Pago Bay by the UOGML flow-through seawater system.

2.2. Fish Acclimation

Prior to each laboratory trial, fish were first acclimated to the UOGML flow-through seawater system for seven days and given an unlimited daily supply of fresh *Caulerpa* and *Enteromorpha* algal species. This diet was gradually transitioned to a commercially available algal-pellet diet over the next seven days, with which the fish were maintained for the remainder of the study.

Following this initial 14-day acclimation, fish were haphazardly divided among five 1400-L cement tanks (18 fish per tank), which were supplied with water by the flow-through seawater system. Each of the five tanks represented one of five treatments. To account for potential tank effects, treatments were randomly assigned to different tanks for each trial. To reduce stress, fish were given ample coverage and hiding areas in the form of cement blocks (Portz et al. 2006). Fish were acclimated to their new cement tank for 14 days prior to treatment dosing. Feeding and tank acclimation were identically conducted for all of three laboratory trials.

2.3. Treatments

Following acclimation (day 0 of study), a group of untreated fish (2–4 fish per tank, ~15 fish per trial) was euthanized and their livers dissected for baseline analyses. Euthanasia was carried out by decapitation followed by pithing. Livers were dissected immediately following euthanasia (within six minutes from time of death). Particular care was taken to

avoid rupturing the gallbladder, as bile acids are known monooxygenase inhibitors (Nilsen et al. 1998; Sinal & Bend, 1997). Livers were immediately rinsed with ice-cold homogenization buffer (Nilsen et al. 1998), weighed, flash frozen by submerging them in liquid N₂, and stored at -80° C.

Dose-response trials consisted of tanks where fish were injected (IP) with a single bolus dose (0, 5, 25, and 50 mg/kg body weight, respectively) of beta-naphthoflavone, a commonly used CYP1A inducer (Buhler & Buhler, 1998; Al-Arabi & Goksøyr, 2002; Campbell & Devlin, 1996; Bastos et al. 2004). Injection volumes were based on body weight (i.e., 1.0 mL/kg body weight). All BNF injections were made fresh with soybean oil as the vehicle. To solubilize BNF in soybean oil, sonication was required. The remaining tank served as a no-injection control. To reduce stress, food was withheld for one day prior to injection (Bowker et al. 2011).

To determine the temporal dynamics of CYP1A induction following BNF exposure, fish (2–4 specimens) from each tank were euthanized on days 1, 2, 4, 8, and 16 following injection. Euthanasia always occurred between the 13:00 and 16:00 hours. Fish livers were processed as described previously.

Throughout all phases of each trial, water quality parameters were monitored and remained consistent throughout the study period. Temperature (range = 28.0–30.1°C), salinity (range = 34.0–35.1 ppt), NH₃/NH₄ (range = 0–<0.25 ppm), NO₂ (range = 0–<0.25 ppm), and NO₃ (range = 0–<0.25 ppm) were measured biweekly, and again every day fish were sampled. Following euthanasia, sex and spawning stage of fish were determined by visual assessment. Spawning cycles were recorded for all three trials; however, fish sex was only reliably recorded during the third.

2.4. Microsomal Fraction Preparation

Liver microsomal fractions were obtained using protocols suggested by Nilsen et al. (1998) and the International Organization for Standardization (ISO 2007), except that snap-frozen beads were created by dripping the final supernatants into a Dewar flask filled with liquid N₂. This provides an easy and convenient method for dispensation without thawing. Microsomal fractions were stored at -80° C until needed for kinetic and immunochemical assays.

2.5. Protein Quantification

Total protein concentrations of each microsomal fraction were quantified using the Bradford method (Bradford 1976) and bovine-serum albumin standards (Bio-Rad kit, according to instructions) immediately prior to use. Analyses were measured using a Biotek Synergy HT® multidetection microplate reader with absorbance read at 570 nm.

2.6. Enzyme Assay

Enzyme activity was determined by the ethoxyresorufin-O-deethylase (EROD) assay using a modified ISO (2007) protocol. Each sample was analyzed at random in quadruplicate. The optimum phosphate buffer solution pH, substrate (7-ethoxyresorufin), and cofactor (NADPH) were determined by titration prior to analyses (ISO 2007; Nilsen et al. 1998). Reaction mixtures were prepared in 96-well plates by adding 190 µL phosphate buffer solution (pH 7.8), 2 µL 7-ethoxyresorufin working solution (5µM), and 5 µL of microsomes to each well. Samples were thoroughly mixed for ten seconds using a microplate shaker to allow binding of substrate with enzyme. Then, 5 µL NADPH (30-mM) was added to each well to initiate the reactions. Following another ten seconds of medium shaking, resorufin production was quantified fluorometrically (excitation at 530 nm and emission at 590 nm),

at room temperature (23 ± 1.0 °C), in a Biotek Synergy HT multidetection microplate reader, at one-minute intervals for a total of 16 minutes. For each sample replicate analyzed, the time-dependent fluorescence increase (slope) was calculated at five consecutive points using linear regression.

Resorufin equivalents were calculated by comparing individual slopes to a resorufin standard curve, and the overall CYP1A enzyme activity was calculated for each replicate at steady state. The resorufin standard curve was made from a stock solution of resorufin dissolved in methanol, rather than dimethylsulfoxide as recommended by the ISO protocol (2007). To account for potential microplate reader variability, samples in each microplate were normalized to positive controls included in each microplate (ISO 2007; Nilsen et al. 1998). Mean CYP1A enzyme activities were calculated using measurements from replicate assays.

2.7. Protein Assay

CYP1A protein levels were quantified using an enzyme-linked immunosorbent assay (ELISA) following a modified protocol described by Nilsen et al. (1998). Each sample was analyzed at random in quadruplicate. Sample uniformity was achieved by diluting each sample to a final concentration of 100 µg total protein/mL, which was determined as the optimal concentration under experimental conditions prior to analyses (Nilsen et al. 1998). Optimum concentrations for polyclonal rabbit anti-fish CYP1A primary antibody and for goat anti-rabbit secondary antibody conjugated with the horseradish peroxidase (HRP) were also determined prior to analysis (Nilsen et al. 1998). For detection of the HRP, reactions were initiated through the addition of 100 µL of 3,3',5,5'-tetramethylbenzidine (TMB), allowed to incubate for fifteen minutes at room temperature, stopped by the addition of 100 µL of 1 M H₂SO₄, and then immediately analyzed for absorbance at 450 nm in a Biotek Synergy HT multidetection microplate reader. The mean absorbance was calculated for each sample using the absorbance readings of four replicate wells. To account for potential variability, samples were normalized to a positive control sample included in each microplate (Nilsen et al. 1998).

2.8. Guam Baseline (Field) Study

Siganus spinus were collected from eight commonly fished sites around the island of Guam: Tanguisson Reef Flat, West Hagatña Bay, Agat Boat Basin, Merizo Pier, Cocos Lagoon, Rios Reef Flat, Ipan Reef Flat, and Pago Bay (Figure 1). Eight fish were collected from seven of the sites. Due to dangerous collection conditions, only two fish were collected from Merizo Pier. Collections coincided with spring high tides occurring between 29–31 May 2011 and between 20:00–24:00 hours. At each site, fish were collected with long minnow nets and maintained in free-floating mesh basins until euthanasia. Euthanasia occurred in the field within 30 minutes of capture. Processing of samples, including microsomal fraction preparations, protein quantifications, ethoxyresorufin-O-deethylase (EROD) assays, and enzyme-linked immunosorbent assays (ELISA), occurred within two weeks of collection and followed the previously described protocols.

2.9. Statistical Analyses

All calculations were made using PRIMER 6 and PERMANOVA + for PRIMER (Anderson 2008). Unlike ANOVA, which assumes normal distributions and Euclidean distances, PERMANOVA uses resampling statistics, specifically permutations, to make it distribution free and works with any distance measure that is appropriate to the data (Anderson 2008). Additionally, PERMANOVA allows for unbalanced and heteroscedastic data. PERMANOVA was the chosen statistical method because the data was non-parametric, unbalanced, and heteroscedastic. Additionally, given that permutations were used to obtain

P-values, the statistical inferences made by this method are considered more robust than traditional statistical methods when Euclidean distance matrixes are applied, regardless of normality or homoscedasticity (Anderson 2008).

For laboratory studies examining time- and dose-dependent induction, CYP1A enzyme activity (EROD assay) and protein content (ELISA) were analyzed independently using a nested two-factor, mixed model PERMANOVA with 9999 permutations (where tank effects [trials] were nested within treatment×day). Euclidean distances were used to calculate the similarity matrixes for all data (Quinn and Keough, 2002). Given that tanks had no significant effect on treatments or days (EROD $p = 0.837$, ELISA $p = 0.288$), samples within each treatment×day were considered independent. Given that there was a significant interaction between treatments×days (EROD $p = 0.001$, ELISA $p = 0.001$), pairwise comparisons were computed for each treatment×day. Pairwise comparisons are based on the original student *t* statistic, except that the *P*-value for all pair-wise tests were obtained using permutations, not tables (Anderson 2008). For the pairwise comparisons, Monte Carlo *P*-values were used due to the small number of permutations.

Also during the laboratory study, sex and spawning cycles were determined and analyzed; however, only fish from the third trial were analyzed, as this was the only trial that fish could be reliably sexed. Given that there was not sufficient sample size to test for differences between male and female catalytic activity and protein content within each treatment×day, control fish (day zero and no injection) were pooled. Effects due to fish sex and spawning cycle were analyzed using a two-factor, mixed model PERMANOVA with 9999 permutations, using Euclidean distances to calculate similarity matrixes.

For field analysis, CYP1A enzyme activity (EROD assay) and relative protein content (ELISA) were analyzed independently. Individual sites were compared to each other, as well as compared to the controls used in the previously described laboratory study. To test for baseline level differences, a one-factor PERMANOVA with 9999 permutations was used. Sex was nested within location and fecundity was nested with sex. Euclidean distances were used to calculate the similarity matrixes for all data (Quinn and Keough, 2002).

3. Results

3.1. CYP1A Enzyme Activity (EROD Assay)

Siganus spinus CYP1A EROD activity trends following a single IP injection of BNF are illustrated in Figure 2a. No significant differences were found between the two controls on any of the days (lowest $p = 0.170$). Additionally, there was no significant difference between the medium and high doses on any day (lowest $p = 0.127$). Furthermore, no significant differences were found between treatment and control EROD activity levels on day zero ($p = 0.114$).

The highest significant increase of enzyme activity (EROD) occurred one to two days after injection. All BNF-treated groups returned to baseline (i.e. levels not significantly different from controls) by day 16. The enzyme activity in the low dose group returned to baseline by day four ($p = 0.22$), whereas medium and high dose groups did not return to baseline until day 16 (lowest $p = 0.145$). Finally, when sexual and reproductive cycle differences were analyzed for control fish from the third trial, there was a significant difference in catalytic activity between spawning females and 1) spawning males ($p < 0.001$), 2) non-spawning females ($p = 0.007$), and 3) non-spawning males ($p = 0.002$).

3.2. CYP1A Protein Content (ELISA)

Siganus spinus CYP1A protein concentration following a single IP injection of BNF are illustrated in Figure 2b. No significant differences between treatment and control concentrations were found on day zero (lowest $p = 0.194$). There were no significant differences between the two controls on any of the days (lowest $p = 0.218$), except on day four ($p = 0.019$). Additionally, there was no significant difference between the medium and high doses (lowest $p = 0.263$), except on day 16 ($p = 0.009$). The highest significant increase of CYP1A protein concentrations occurred one to two days after injection for the BNF-treated fish (highest $p < 0.001$). The low dose group returned to baseline CYP1A protein concentrations by day eight ($p = 0.022$); however, the medium and high dose groups did not return to baseline levels before the end of the study (highest $p = 0.022$). Finally, when sexual and reproductive cycle differences were analyzed for control fish from the third trial, there was a significant difference between spawning females and non-spawning females ($p = 0.044$).

3.3. Guam Baseline Levels

No significant differences with regard to CYP1A enzyme activity and relative protein content were found between sites, except at Tanguisson Reef Flat (Figure 3). Tanguisson Reef Flat enzyme activities were significantly lower than those found at four other sites: Agat Boat Basin ($p = 0.004$), Agana Bay ($p = 0.004$), Merizo Pier ($p = 0.018$), and Rios Reef Flat ($p = 0.004$). Additionally, when comparing enzyme activities between controls used in the laboratory study and the eight sites, all sites, except Tanguisson, were significantly higher (highest $p < 0.001$) than controls. Tanguisson Reef Flat was not significantly different from laboratory study controls ($p = 0.255$). When comparing relative CYP1A protein concentrations between laboratory controls and the eight sites, no significant differences were detected (lowest $p = 0.199$).

4. Discussion

In the current study, a marine fish species (*S. spinus*) from the Indo-Pacific region was investigated for use as an acute response biomarker sentinel species. The dose- and time-dependent induction of *S. spinus* CYP1A following a single exposure to the organopollutant -naphthoflavone was investigated. Additionally, baseline CYP1A expression levels in *S. spinus* were compared among eight sites along Guam's coast.

In comparing laboratory controls, day four pair-wise comparisons for protein concentrations were significantly different from all other days. Water quality was an unlikely cause for this variation, as no significant changes in water quality were observed throughout the study period. As fish were fed a commercial algal pellet diet, this may have affected day four protein concentrations; however, given that no other days or treatments were affected, this is unlikely. An induction pathway linked to the stress of injections may also explain the difference between controls on day four; however, for all other days, injections had no statistically detectable effect on CYP1A protein concentration or enzyme activity. These differences could be a result of natural variation. Differences in sex and reproductive cycle have also been shown to exhibit such effects (Linström-Seppä 1985; Förlin & Haux 1990; Nacci et al. 2002).

Although control CYP1A enzyme activities and protein concentrations were low and relatively steady, there was still CYP1A protein and enzyme activity present in the control fish from this study. Induction may have been due to a number of factors, including food quality or other physiological stressors (e.g. captivity) that were similar among all treatments. Another possible explanation is that *S. spinus* constitutively express low levels

of CYP1A to respond to sudden exposure to stressors or as a consequence of diet (Murray 2006).

Based on the laboratory results, the induction of the CYP1A enzyme activity and protein content in *S. spinus* liver is time-dependent. Given that both CYP1A enzyme activity and relative protein levels have the highest significant induction increase one to two days after injection of BNF, it suggests that *S. spinus* responds relatively quickly to BNF. Additionally, following this initial induction spike, CYP1A enzyme activity and protein concentration decreased for all treatment doses, which correspond similarly to other fish species (Al-Arabi and Goksøyr 2002; Sved et al. 1992; Munkittrick et al. 1995). Furthermore, given these results and results found for other fish species, it is suggested that *S. spinus* may respond similarly to other CYP1A inducers and aryl hydrocarbon receptor agonists (Goksøyr & Forlin 1992; Al-Arabi and Goksøyr 2002; Payne et al. 1987). Finally, as not all BNF-treated groups returned to baseline levels by day 16 for ELISA, it is suggested that the life cycle of the protein outlasts the catalytic activity in *S. spinus*.

Based on laboratory results, the induction of CYP1A enzyme activity and protein content in *S. spinus* liver is dose-dependent. In fact, induction factors (*i.e.* protein content and enzyme activity) obtained from this study correspond reasonably well with those obtained for other tropical teleosts (Al-Arabi & Goksøyr 2002; Gadagbui et al. 1996). For both induction factors, there are several possible explanations for the lack of significant difference detected between the medium and high dose groups, such as natural variation or a ceiling effect (*e.g.* due to the AhR pathway being saturated or insufficient cofactors to compensate for the increased demand). Lack of significant differences between medium and high dose expressions could also be attributed to differences in sex and fecundity, thus causing high variability.

The significant differences noted between spawning females and 1) non-spawning females, 2) spawning males, and 3) non-spawning males in the current study suggest that sex and fecundity differences may influence CYP1A catalytic activity and protein content in *S. spinus*. Thus, differences in sex and fecundity could explain the high variation within each treatment×day. To note, this study was cautious in making inferences based on the sex and fecundity statistical findings, given that fish could only be reliably sexed during the third trial. However, this study's findings correlate well with other studies. Other studies have found similar sexual dimorphisms and spawning cycle differences for CYP levels in other fish species (Linström-Seppä 1985; Förlin & Haux 1990; Nacci et al. 2002). Additionally, other studies have found male fish to have higher CYP content than females, especially during spawning cycles (Linström-Seppä 1985; Förlin & Haux 1990; Monostory et al. 1996). Therefore, fecundity and sex are cautiously included as an explanation of variation.

In the field study, enzyme activity levels were significantly higher in natural populations when compared to control fish from the laboratory study, except for at Tanguisson Reef Flat (Figure 3a). Lower CYP1A levels in control fish and fish from Tanguisson Reef Flat could be due to a difference in diet or to different stress factors. In the laboratory study, fish were maintained with a controlled algal pellet diet and were under low predatory stress. In the field, Tanguisson Reef Flat has been documented to have unique algal assemblages not found anywhere else around the island of Guam (S. Abellana, unpublished data). *Siganus spinus* inhabiting Tanguisson Reef Flat may have a different diet than those at other sites. This has been documented with other macroalgivore fish species (*Naso unicornis* and *Naso literatus*) from Tanguisson Reef Flat (S. Abellana, unpublished data). Given that different algae are known to produce different aromatic chemical defense compounds (Paul and Fenicle 1986), different algae may have different CYP1A induction capabilities. Thus, fish inhabiting Tanguisson Reef Flat may be exposed to fewer or less toxic natural product

stressors. Additionally, this unique algal assemblage at Tanguisson could be caused by an increased nutrient or contaminant load. Studies have shown CYP1A inhibition by high nutrient loads, or certain contaminants (e.g. heavy metals). However, no formal studies have been conducted regarding the nutrient or heavy metal load at Tanguisson Reef Flat.

5. Conclusions

Given that BNF induced CYP1A in *S. spinus* in a dose- and time-dependent manner in the laboratory study, inferences can be made. First, because *S. spinus* has the capacity to reflect an acute exposure to various levels of the organic pollutant 1-naphthoflavone through the induction of the CYP1A monooxygenase enzyme system, it is a candidate both as a model species for xenobiotic biotransformation by tropical herbivorous reef fish, as well as a sentinel species for physiologically relevant organopollution in tropical coral reefs. Next, because *S. spinus* reacted to BNF, *S. spinus* may also react to other aromatic pollutants, including PCBs and dioxins (Goksøyr & Forlin 1992; Al-Arabi and Goksøyr 2002; Payne et al. 1987); however, further studies should be conducted to validate this hypothesis in *S. spinus*. Finally, because there is a time- and dose-dependent induction response to a single injection of BNF, it can be inferred that the mechanism associated with aryl hydrocarbon receptor and its associated complex are intact (Fujii-Kuriyama et al. 1992).

Determining a sentinel species that can sensitively and reliably induce CYP1A is the first step in detecting biological effects to pollutant exposure in ecosystems. In the current study, *S. spinus* reacted to the known CYP1A inducer, 1-naphthoflavone, in a dose- and time-dependent manner; thus, *S. spinus* is identified as the first possible sentinel species for tropical Indo-Pacific coral reefs. *Siganus spinus* also has many characteristics that suggest its use as an appropriate ecotoxicological model organism of xenobiotic biotransformation in tropical marine teleosts; however, more studies need to be conducted to further assess natural populations. Future studies are suggested for examining physiological and toxicological effects from trace and chronic exposures, and include seasonal and reproductive variables. Additionally, enzyme induction mechanisms and possible catalytic inhibitors should be investigated. Regardless, *S. spinus* exhibits promise in biomonitoring, and as a model organism for tropical coral reef fish.

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Figure 1.
Field study site locations: 1) Tanguisson Reef Flat, 2) West Hagatña Bay, 3) Agat Boat Basin, 4) Merizo Pier, 5) Cocos Lagoon, 6) Rios Reef Flat, 7) Ipan Reef Flat, and 8) Pago Bay.

Figure 2.

Mean CYP1A a) enzyme activity (EROD) and b) relative protein concentrations (ELISA) ($\pm$ S.D.) for *Siganus spinus* exposed to two controls (no injection and a single vehicle injection) and three single-dose concentrations of β -naphthoflavone (5mg/kg, 25mg/kg, 50mg/kg) over 16 days.

Figure 3. Mean CYP1A a) enzyme activity (EROD) and b) relative protein concentrations (ELISA) ($\pm$ S.D.) for *Siganus spinus* at eight sites around the island of Guam, and compared to laboratory controls from study. * denotes significant difference from other sites.