Fluctuating Asymmetry in the Flying Barb (*Esomus metallicus*), the Striped Croaking Gourami (*Trichopsis vittatus*) and the Three-Spot Gourami (*Trichogaster trichopterus*) when Exposed to Insecticide Residues in the Lime Orchard

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Abstract

Fluctuating asymmetry (FA) in three species of fish, the flying barb (*Esomus metallicus*), the striped croaking gourami (*Trichopsis vittatus*) and the three-spot gourami (*Trichogaster trichopterus*), was examined from ditches in a lime orchard and from control sites. FA of *E. metallicus* and *T. vittatus* from the lime orchard was higher than that of the control in all characters. In *E. metallicus,* significantly higher levels of FA were detected in asymmetry of the otolith width and the premaxilla length. No significant differences in FA were detected in any characters of *T. vittatus*, however FA of fish from the lime orchard was higher than that of the control in all characters. No clear relationship between FA and insecticide residues was found in *T. trichopterus* as higher levels of asymmetry were detected in fish from both sites. Results from this study suggest that FA could be used as an indicator for detecting stress from insecticide residues when sensitive species are used.

Keywords Fluctuating asymmetry, FA, indicator, fish, insecticides, lime orchard

1. Introduction

A number of studies have revealed the relationships between fluctuating asymmetry (FA) and pollutants [1,2,7,12,14], however, an ambiguous relationship between FA and environmental stress has also been reported. For example, Jagoe and Haines [8] found only a weak correlation between FA and acidity in the brooke trout *Salvelinus fontinalis*, the white sucker *Catostomus commersoni* and the lake chub *Couesis plumbeus*. A similar result was also obtained by Wiener and Rago [10], who found a weak relationship between FA and low pH in the bluegill *Lepomis macrochirus*. While Ames *et al.* [3] found no differences in the levels of FA in the largemouth bass *Micropterus salmiodes*, the redbreast sunfish *Lepomis auritus* and the bluegill sunfish (*L. macrochirus*) sampled from heated and non heated ponds, fish from areas contaminated with mercury and low pH revealed greater levels of asymmetry, than other populations sampled. Moreover, the relationship between FA and pollutants was not found in the study of FA in the mosquito fish *Gambusia holbrooki* in Perth wetlands [17].While it is now known that various pollutants are capable of causing considerable changes in fish asymmetry of some species, the aim of this study was to investigate whether FA is a good indicator of insecticide residues in aquatic ecosystems in Thailand.

2. Materials and Methods

2.1 Sampling sites

The lime orchard that was the focus of this study was located in Inburi District, Singburi Province, Thailand. The fish were collected from the ditches in the lime orchard.

Each ditch was 0.65 m wide, 120 m long, 1 m deep and located approximately 1 m from the lime trees. The main insecticides used in the lime orchard include a combination of Methamidophos 100 ml, Profenofos 100 ml, Carbosulfan 100 ml and Trigrocide 0.5 kg and are mixed and dissolved in 1 gallon of water before spraying. Insecticide treatments were applied every 2 weeks so the fish in these ditches were exposed to insecticides at regular times, i.e. during spraying and leaching.

The control pond was 32 m wide, 120 m long and 2 m deep, and was located in front of the orchard. This pond was used as a reservoir for water used in the orchard.

Physico-chemical characteristics of the sample sites are given in Table 1. Samples of water were collected from the ditches and the reservoir pond at 1 day and 1 week after insecticide application. The water samples were transported to the Agricultural Toxic Substances Division, Ministry of Agriculture and Co-operatives for analyses of insecticide residues in the water. The results of analyses are given in Table 2.

Since only one species of fish (*T. vittatus*) was collected from the reservoir pond, the remaining species were collected from an additional control site - the canal near Nongkae Temple, Uthaithani Province. This natural canal has only slight disturbance from human activities, and was thus also used as a control site.

2.2 Sampling methods

Three species of fish, the flying barb (*Esomus metallicus*), the striped croaking gourami (*Trichopsis vittatus*) and the three-spot gourami (*Trichogaster trichopterus*) were collected in February 1998, using a long handled sweep net (40 cm x 55 cm with a 0.5 mm mesh). The fish were cleared and stained with Alizarin red according to the method of Potthoff [6]. Left and right sides of all meristic and morphometric characters were counted and measured under the dissecting microscope at X 4 magnification.

Six meristic characters and three morphometric characters were chosen for analysis of asymmetry:

Characters	Code
Meristic characters	
Number of dorsal ribs	DR
Number of ventral ribs	VR
Number of rays of pectoral fins	PC
Number of rays of pelvic fins	PV
Number of brachiostegal rays	BR
Number of spines on preopercle bone	PB
Morphometric characters	
The length of saccular otolith	OL
The width of saccular otolith	OW
The widest width of premaxilla bone	PM

These characters have been used in other studies and/or they were easy to measure.

2.3 Statistical analyses

All meristic and morphometric characters of these samples were counted and measured twice in order to assess the errors from measurements. The replicated data sets were performed using a factorial analysis of variance (sides fixed, individuals randomised) by following the approach modified by Palmer and Strobeck [9] and Palmer [15]. Measurement errors were obtained as the variance contributable to the error.

Directional asymmetry was detected by using T tests performed on the differences between left and right sides in each character. Antisymmetry was detected using moment statistics by following the method of Sokal and Rohlf [5] for testing significances of kurtosis and skewness. Regression analyses between absolute (L-R) differences and mean character values $(L_i+R_i)/2$ were performed on characters for each sample and all samples in each species in order to determine if FA was size dependent. In order to prevent rejection of valid hypotheses (i.e. Type I errors), results from the above tests were adjusted using the sequential Bonferroni correction [11].

Since directional asymmetry, significant deviations from normal distribution and size dependencies of FA were detected in asymmetry of characters of all species, among sample sites, the data were transformed into log and normalized using Box-Cox transformation recommended by Swaddle *et. al.* [16] by using the formula $d^* = (|d'| + 0,00005)^{0.33}$ [18].When d' is equal to log L - log R.

Differences in asymmetry among sample sites were tested by one way analysis of variance performed on absolute (L-R) value of each character, for each species separately. Multiple comparison tests were performed on characters where significant differences were detected using Student-Newman-Keuls test.

3. Results

Since the water in ditches was drained from the reservoir pond for watering lime trees, the water in both ditches and pond had similar physico-chemical characteristics (Table 1). Carbamate insecticides appeared to be hydrolysed quickly in the water. No insecticide residue was detected in the water sampled from the reservoir pond after 1 day and 1 week of application. Profenofos 0.55 ppb and methamidophos 0.1 ppb were detected in the water sampled from the ditches 1 day after application but no insecticide residue was detected in the water sampled from the ditches 1 week after application.

Significant measurement errors were detected in asymmetry of dorsal ribs of *T. trichopterus* collected from the canal near Nongkae Temple and the ditches in the lime orchard and the premaxilla length of *T. vittatus* collected from the ditches and the reservoir pond in the lime orchard. Therefore, these characters were not used in comparison of FA between sample sites.

Although no significant differences in levels of asymmetry were detected in any character of *T. vittatus* at the $p \leq 0.05$ level (Table 3), FA of fish collected from the lime orchard was higher than that of the control site in all characters. Similar results were obtained in *E. metallicus*. Mean asymmetry of *E. metallicus* collected from the lime orchard was higher than that of the control in all characters, particularly in the otolith width and premaxilla length, where significantly higher levels of asymmetry were detected at $p \leq 0.001$.

No conclusion could be drawn in *T. trichopterus* as no significant differences in FA between sample sites were detected in any character at the level of $p \leq 0.05$. Moreover, the higher mean ranks of asymmetry varied from character to character. FA of fish from the control site was higher than that from the lime orchard in some characters whilst FA of the fish from lime orchard was higher in other characters.

4. Discussion

Results from this study have revealed that FA is a more sensitive indicator for detecting stress from insecticide residues than other indicators, particularly chemical analyses. Since the carbamate insecticides were quickly hydrolysed in water [4], only small levels of insecticide residues were detected in the water collected 1 day after application. No insecticide residues were detected in the water collected 1 week after application. However, stress from insecticide residues in the water was clearly shown in *E. metallicus* and *T. vittatus* collected from ditches in the lime orchard and FA was higher than those of the control sites for all characters examined. Results from this study also agree with that reported by Clarke [13] who found that FA of the Australian bush fly (*Musca vetustissina*), derived from the dung of cattle treated with insecticide-Avermectin, was significantly higher than that of flies from untreated dung. FA of flies collected from 4 weeks after treatments was significantly higher than that collected 8 or 11 weeks after treatments. He also suggested that FA was a more sensitive indicator than other biological indicators such as life history parameters. Although residues of Avermectin in dung eight weeks after treatment had no effect on the survival of flies, the stress can still be revealed by FA of flies and can be detected even 11 weeks after treatment.

Among the three species of fish examined during this study, the relationship between FA and insecticide residues was clearly shown in *E. metallicus*. Thus, the selection of this species as an indicator of FA is one of the important factors to determine the sensitivity of FA. The results from this study suggested that *E. metallicus* is a sensitive and reliable indicator for detecting stress from the carbamate insecticide residues in small shallow water bodies in Thailand.

5. Acknowledgments

I would like to thank Siripen Yamkgamlua for help in the laboratory, Dr. John Graham, Dr.

Chinnapong Bumrungsap, Kanungnit Saeriwong Jirawee Weerapradit for statistical advice and Dr. David Morgan and Dr. Suyanee Nachaisit for the helpful comments and proofreading the final draft.

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Table 1 Physico-chemical variables recorded among sampling sites.

Sampling sites	Dissolved oxygen (mg/l)	pH (mg/l)	Conductivity (µS)	Temperature (°C)
Reservoir pond in the lime orchard	8.7	7.37	307	31.8
ditches in the lime orchard	10.1	7.63	305	30.5
canal near Nongkae Temple	7.2	6.88	251	27

Table 2 Insecticide residues in the water sampled from ditches and the reservoir pond in the lime orchard.

Sampling sites	Sampling times	Insecticide residues
Reservoir pond in the lime orchard	1 day after insecticide application	-
	1 week after insecticide application	-
Ditches in the lime orchard	1 day after insecticide application	Profenofos 0.55 ppb Methamidophos 0.01 ppb
	1 week after insecticide application	-

Table 3 Results of ANOVA used to compare FA of *T. vittatus*, *E. metallicus* and *T. trichopterus* between the ditches in the lime orchard and the control sites and mean asymmetry of fish from the two sites ranked from the lowest to the highest.

Species	Characters	Degree of Freedom	F-values	Probability	Mean ranks	
T. vittatus	PC	1,120	.308	.580	Ditches in the lime orchard	Reservoir pond in the lime orchard (Control)
	DR	1,122	.530	.468	Reservoir pond in the lime orchard (Control)	Ditches in the lime orchard
	VR	1,122	.252	.617	Reservoir pond in the lime orchard (Control)	Ditches in the lime orchard
	PV	-	-	-	No asymmetry detected	
	BR	-	-	-	No asymmetry detected	
	PB	1,121	3.906	.050*	Reservoir pond in the lime orchard (Control)	Ditches in the lime orchard
	OW	1,121	1.633	.204	Reservoir pond in the lime orchard (Control)	Ditches in the lime orchard
	OL	1.109	2.829	.095	Reservoir pond in the lime orchard (Control)	Ditches in the lime orchard
E. metallicus	PC	1,99	2.239	.138	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	DR	1,99	2.042	.156	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	VR	1,99	.937	.335	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	PV	1,99	2.042	.158	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	BR	1,99	4.261	.042*	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	PB	1,99	6.278	.014*	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	OW	1,96	17.941	.000***	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	OL	1,99	6.901	.010**	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	PM	1,99	48.595	.000***	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
T. trichopterus	PC	1,76	.049	.825	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	VR	1,76	.475	.493	Canal near Nongkae Temple (Control)	Ditches in the lime orchard
	PV	-	-	-	No asymmetry detected	
	BR	-	-	-	No asymmetry detected	
	PB	1,76	1.472	.229	Ditches in the lime orchard	Canal near Nongkae Temple (Control)
	OW	1,76	1.102	.297	Ditches in the lime orchard	Canal near Nongkae Temple (Control)
	OL	1,76	2.109	.151	Ditches in the lime orchard	Canal near Nongkae Temple (Control)
	PM	1,76	3.348	.071	Canal near Nongkae Temple (Control)	Ditches in the lime orchard

* Significance before adjusted by the Bonferroni correction

* Significance after adjusted by the Bonferroni correction

* = $P \le 0.05$, ** = $P \le 0.01$, *** = $P \le 0.001$