Fluctuating Asymmetry in Fishes Inhabiting Polluted and Unpolluted Bodies of Water in Thailand

Poranee Utayopas
Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Campus, Prathum Thani, Thailand 12121.

Abstract

This paper examines the 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*) from polluted and unpolluted bodies of water in Thailand. Highest mean asymmetries were recorded in *E. metallicus* collected from the most polluted water in almost all characters. Similarly, the highest mean asymmetries were also detected in *T. vittatus* from the most polluted site in almost all characters but significant differences in FA were detected in ventral ribs and premaxilla length in which mean asymmetry ranked most highly changed from site to site. No conclusion could be drawn in FA of *T. trichopterus*. Significant differences in FA were detected in dorsal ribs and number of spines on the preopercle bone. However, mean asymmetry ranked most highly varied among locations. Results from comparisons of FA among three species revealed that mean asymmetry of *E. metallicus* was most highly ranked in almost all characters. The relationship between FA and pollutants observed in *E. metallicus* was suggested to result from its feeding behavior and high metabolic rate.

Keywords Fluctuaing asymmetry, FA, fishes, pollutants, water bodies, Thailand

1. Introduction

Due to the rapid growth of agriculture and industry in Thailand, aquatic ecosystems have been, and continue to be, subjected to a range of anthropogenic disturbances including altered hydrological regimes, eutrophication and pollution with pesticides and heavy metals. While high levels of pollutants can have lethal effects, toxic residues stored in sediments may produce sublethal or chronic effects which are often difficult to detect. This raises a need for sensitive and reliable indicators to assess the impacts of pollutants on organisms in ecosystems.

Fluctuating asymmetry (FA) has been recommended by a number of studies as a sensitive indicator for detecting environmental disturbances [1,2,3,4,5]. FA is a morphological deviation from normal symmetry which can be quickly detected at low levels of stress before levels high enough to cause widespread morbidity or changes in community structure are reached [6]. Positive correlations between FA and environmental stresses have also been observed in various aquatic studies [1,2,7,8,9]. The objective of this study, is therefore, to compare the FA of fish from polluted and unpolluted bodies of water and determine the possibility of using FA as an indicator of environmental stress in aquatic ecosystems in Thailand.

2. Materials and Methods

2.1 Sample sites

Fish were collected from unpolluted and polluted bodies of water; the latter containing high levels of organic substances. Fish from the polluted bodies of water were expected to show signs of stress due to eutrophication caused by low oxygen tension. The bodies of water chosen as sampling sites were as follows.

Site One - **Pond 1 and Pond 2 opposite Baossompornchai Temple, Ayuthaya Province.**

These ponds are located near a large cement pad used for drying cassava and residues from a beer factory. Two ponds were sampled. Pond 1 is 20 m long,10 m wide, 3.5 m deep and approximately 5 m *distance* from the pad. Pond 2 is 15 m long, 5 m wide, 4 m deep and approximately 1 m distance from the pad. The water in these ponds was highly contaminated with organic substances leached from the pad. These leaches *resulted* in a large number of fish *dying after rain*, about 1 week before sampling (Utid Pangkum, per. comm.).

***Site Two* - Rice field in Nongkayang District, Uthaithani Province.**

The field has a total area of approximately 32,000 m^2 in which the water is 0.5 m deep. Organic substances contaminating in the water were expected to be the result of eutrophication arising from fertilizers applied in this field.

***Site Three* - Canal in Thammasat University, Rangsit Campus, Prathumthani Province.**

The canal is approximately 2 m wide, 3 km long and 3 m deep. It was contaminated by domestic wastes drained from the camp of workers during the 13^{th} Asian Games.

***Site Four* - Water mimosa (*Neptunia oleracea* Lour.) field, Rangsit District, Prathumthani Province.**

The field is an open water area used for growing the water mimosa (*Neptunia oleracea* Lour.) with a total area of approximately 32,000 m^2 and is 1.5m deep. The fish in this field were subjected to stress from insecticide (Cypermethrin), fertilizers, nutrients and plant hormones (Gibberellin and Auxin) which were applied 3 times per 2 weeks. Insecticide together with other substances may, therefore, directly be toxic to the fish or indirectly produce stress via low oxygen tension arising from eutrophication.

***Site Five* - Pond around and underneath the Predi Pranomyong Library, Thammasat University, Rangsit Campus, Prathumthani Province.**

The pond has a total area of 2765.92 m^2 and *is* 249.6 m long, 11.80 m wide and 2.10 m deep [10]. It is located underneath the library building in which its marginal area, about 1.5 m wide, extended outside and surrounded the building. This area was exposed to the sun light and covered by *Nelumbo nucifera* Gaerth. for about 90 % of the area. This pond was considered as an abundant ecosystem without disturbance from human activities. Therefore it represented the control site for this study.

***Site Six* - Canal near Nongkae Temple, Uthaithani Province.**

This canal runs along the road about 300 m from Nongkae Temple. It is 4 m wide, 20 m long and 1 m deep. This canal is a natural ecosystem and was used as an additional control site because *T. trichopterus* could not be collected from the pond around the Predi Pranomyong Library.

The physico-chemical characteristics of sampling sites are given in Table 1.

2.2 Species examined

Three species of fish, the flying barb (*Esomus metallicus*), the striped croaking gourami (*Trichopsis vittatus*) and the three-spot gourami (*Trichogaster trichopterus*) were chosen for the study because they are abundant and widespread

Since *T. trichopterus* was not found in the water mimosa field, the snakeskin gourami (*Trichogaster pectoralis*) was alternatively sampled from this field. In addition, only a small number of *T. trichopterus,* 7 and 16 fish, were collected from pond 1 and pond 2, respectively. Therefore to prevent an error resulting from small sample sizes, *T. trichopterus* collected from pond 1 and pond 2 were pooled and used as a sample to compare FA among locations.

Fish were collected during February-November 1998 from the littoral zone using a long handled sweep net (40 cm x 55 cm with a 0.5 mm mesh). The fish were cleared by the enzyme Trypsin and stained with Alizarin red according to the method of Potthoff [11]. Left and right sides of all meristic and morphometric characters were counted and measured under the dissecting microscope at X4 magnification.

2.3 Characters examined

Six meristic characters and three morphometric characters were chosen for this study as follows:

Meristic characters	**Code**
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

Characters were chosen on the basis that they had been used in other studies of FA in fish and/or because they were easy to measure.

To estimate the error associated with the measurement process, all meristic and morphometric characters of these samples were counted and measured twice. The replicated data sets were subjected to tested using a two way mixed model analysis of variance (sides fixed, individuals randomised), by following the modified approach of Palmer and Strobeck [12]. The measurement errors were obtained as the variance contributable to the error.

2.4 Preliminary analyses

To ensure that the asymmetry present was truly fluctuating asymmetry, it was necessary to eliminate other alternative possibilities such as directional asymmetry and antisymmetry [13]. T tests were performed to detect directional asymmetry by testing the differences between left and right sides in each character. Significances of kurtosis and skewness were tested using moment statistics by following the method of Sokal and Rohlf [14].

To determine whether asymmetry increased with size, regression analyses between absolute (L-R) differences and mean character values [$|L_i-R_i|$ vs $(L_i+R_i)/2$] were performed on characters for each sampling site and all sampling sites in each species. To prevent a misleading interpretation due to rejection of the hypothesis when it was true (Type I error) [15], significances resulting from all the tests above were adjusted by sequential Bonferroni correction, according to Rice [16], before accepting or rejecting the hypotheses.

Directional asymmetry, significant deviations from normal distribution and size dependencies of FA were detected in asymmetry of characters of all species among sampling sites. Data, therefore, were transformed into log and normalised using Box-Cox transformation recommended by Swaddle *et al.* [17] before being used to compare FA among locations. The transformation performed followed the formula recommended by Cowert *et al.* [18] which was $d^* = (|d'| + 0{,}00005)^{0.33}$, where d' was equal to log L-log R.

2.5 Comparisons of fluctuating asymmetry

Comparisons of asymmetry among sampling sites were determined using one way analysis of variance performed on absolute (L-R) value of each character, for each species separately. Comparisons of FA of three species among samples were performed using Nested ANOVA. Multiple comparison tests were performed on characters where significant differences were detected using the Student-Newman-Keuls test.

3. Results

Significant measurement errors were detected in the width of succular otolith, the length of succular otolith and the length of premaxilla bone of *T. trichopterus* collected from pond 1 and pond 2. These traits of *T. trichopterus* , therefore, were not used in comparisons of FA among sampling sites.

Although mean asymmetry of *T. vittatus* from the most polluted site (water mimosa field) ranked most highly in almost all characters (Table 2), significant differences ($P \leq 0.01$ and 0.001) among sampling sites were detected only in asymmetry of ventral ribs and the premaxilla length. Moreover, the greater asymmetry in these two characters varied among locations. FA of ventral ribs of *T. vittatus* collected from the water mimosa field was significantly higher than those from the other sites whereas asymmetry of premaxilla length of *T. vittatus* from the water mimosa field was significantly lower than those of the other locations.

Similarly, no conclusion could be drawn in the relationship between stress and FA of *T. trichopterus* and *T. pectoralis* as the place producing higher mean ranks changed from character to character (Table 3). Significant differences in FA among locations, at the level of $P \leq 0.01$, after Bonferroni correction, were detected in dorsal ribs and number of spines on the preopercle bone. However, the greater asymmetry in these two characters varied among sampling sites. FA of number of spines on the preopercle bone of *T. pectoralis* collected from the water mimosa field was significantly higher than those from the other locations whereas asymmetry of dorsal ribs of *T. trichopterus* from pond 1 and pond 2 was significantly lower than those of the other sites.

Mean asymmetry of *E. metallicus* from the most polluted bodies of water (pond 1 and pond 2) were ranked most highly in almost all characters (Table 4). In addition, significant differences at the level of $P \leq 0.05$, 0.01 and 0.001, after Bonferroni correction, were detected in FA of *E. metallicus* of all characters except pectoral fin rays and the number of spines on the preopercle bone. However, significantly higher FA of *E. metallicus* from pond 1 and pond 2 than those of other locations were detected in asymmetry of otolith length, otolith width, and premaxilla length. Moreover, asymmetry of brachiostegal rays was not found in all species of all sampling sites, except in *E. metallicus* collected from pond 1.

Comparisons of FA of three species among sampling sites resulting in significant differences at the level of $P \leq 0.01$ and 0.001 were detected in almost all characters except the pectoral fin rays and dorsal ribs (Table 5). No interactions of variations between species and sites were detected in all characters except dorsal ribs and the otolith length. FA of *E. metallicus* were ranked most highly in almost all characters, particularly in brachiostegal rays, number of spines on the preopercle bone, and the width and length of succular otolith where significances were found.

4. Discussion

Relationship between asymmetry and stress seemed to be clearly evident in *E. metallicus*. This result is suggested to correspond to feeding behavior and the high metabolic rate of this fish. *E. metallicus* is an active fish. This kind of fish usually swims together in a school and moves around the water for feeding. The other two species, *T. vittatus* and *T. trichopterus*, on the other hand, like to swim quietly and ambush food near the aquatic plants [19]. The way that *E. metallicus* moves around the water may provide them more opportunity to be exposed to stress than the other two species. Alternatively, *E. metallicus* is an active fish, this fish may be subjected to greater energy demands. The energy is needed both for their development needs and environmental stress. Some energy is needed to be allocated for maintaining homeostasis [20]. If available energy is not sufficient to buffer the stress effects, homeostasis may be impaired, resulting in abnormal development [20,21,22,23,24], Consequently, higher levels of asymmetry were evident in *E. metallicus*.

In almost all characters, mean asymmetry of *E. metallicus* from pond 1 were higher than that of pond 2. It was expected that asymmetry of the fish from pond 2 should have been higher than that of pond 1, as pond 2 is closer to the cement pad than pond 1. Therefore greater amounts of organic substances were expected to be leached into pond 2 than pond 1. As a result, the fish in pond 2 were subjected to higher levels of stress and higher levels of asymmetry should have been observed in the fish from pond 2. Evidence supporting this hypothesis is that the water in pond 2 was still gray in color whilst the color of water in Pond 1 was like the water found in natural ponds. Higher levels of asymmetry detected in fish from pond 1 may be the result of natural selection performed on the fish in these two ponds. Since the death of fish was reported in the two ponds after rain (Utid Pangkum, per. comm.), natural selection probably occurred in both ponds which stronger selection resulting in Pond 2. Fewer fish, but better able to tolerate environmental stress, survived in pond 2. As a result, lower levels of asymmetry were detected in the surviving fish in pond 2. This result is in concordance with those of VIIestad *et al.* [25] and Campbell *et al.* [26] who reported that the use of FA in an ecosystem where stress was so high that death was evident among organisms, may lead to misinterpretation. Lower levels of FA may be detected in an ecosystem with higher level of stress.

In contrast, results from comparisons of FA among locations have revealed that mean asymmetry of *T. pectoralis* collected from the water mimosa field were most highly ranked whilst mean asymmetry of *T. trichopterus* collected from pond 1 and pond 2 were ranked the lowest in almost all characters. This may be explained in that fish from pond 1 and pond 2 were recently exposed to high levels of stress whereas fish from the water mimosa field may have experienced stress for a long time, at least 2 months. The fish in the water mimosa field, therefore, were probably in the process of adaptation to a changing environment. Since adaptation can cause stress to fish [27], high levels of asymmetry were manifested in *T. pectoralis* collected from the water mimosa field. Evidence supporting this hypothesis is the study of Clarke and McKenzie [28] who found that the Australian sheep blowfly (*Lucilia cuprina*) resistant to the insecticide diazinon, when first evolved, possessed a high level of FA of stenopleural bristles. After 20 years of selection and adaptation, the FA of stenopleural bristles of the resistant flies decreased to the same level as those without insecticide resistant genes.

Another explanation for high levels of FA detected in asymmetry of *T. pectoralis* from the water mimosa field may arise from the differences in genetic compositions between the two species, resulting in different tolerance to the stress. Even though *T. trichopterus* and *T. pectoralis* are in the same genus, they are different in species. *T. pectoralis* may be better able than *T. trichopterus* to tolerate to stress and survive while *T. trichopterus* were gradually disappearing from this field. However, stress in this field was still clearly manifested as high levels of asymmetry were revealed in *T. pectoralis* collected from this field. On the other hand, low levels of FA detected in *T. trichopterus* collected from pond 1 and pond 2 may be the result of the death of fish after rain. Few fish, but superior in genetic compositions survived. Very small samples were taken at this site, resulting in low levels of FA detected in *T. trichopterus*.

Fish from the rice field and the canal in Thammasat University seemed to be subjected to lower levels of stress than expected. A possible explanation is that the water in the rice field was shallow. Dissolved oxygen at the water surface may provide a certain amount of oxygen. which could reduce stress tension from low oxygen. In the case of the canal, the canal is rather long, about 3 kg long, organic substances drained from the camp may probably be quickly diluted by the large amount of water so that a low oxygen condition did not result in this canal.

Asymmetry of brachiostegal rays tended to be limited only in *E. metallicus* from the most polluted site (pond 1). This is probably due to high canalization of this trait. This result, therefore, suggested that brachiostegal rays may be one of the characters suitable for the study of FA as its asymmetry was revealed only in high levels of stress, making it easily and clearly interpreted.

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 Daranee and Gary Chenhall for the helpful comments and proofreading the final draft.

6. References

[1] Valentine, D. W., Soul, M. E., and Samollow, P., Asymmetry Analysis in Fishes: A Possible Statistical Indicator of Environmental Stress, *Fishery Bulletin,* Vol. 71, pp. 357-70, 1973.

[2] Valentine, D. W., and. Soul, M., Effects of *p, p'*-DDT on Developmental Stability of Pectoral Fin Rays in the Grunion *Leuresthes Tenuis*, *Fishery Bulletin,* Vol. 71(4), pp. 921-26, 1973.

[3] Leary, R. F., and Allendorf, F. W., Fluctuating Asymmetry as an Indicator of Stress: Implications for Conservation Biology, *TREE,* Vol. 4(7), pp. 214-217, 1989.

[4] Clarke, G. M., Developmental Stability Analysis: an Early Warning System for Biological Monitoring of Water Quality, *Australian Biologist*, Vol. 7(2), pp. 94-102, 1994.

[5] Clarke, G. M., Relationships Between Developmental Stability and Fitness: Application for Conservation Biology, *Conservation Biology*, Vol. 9(1), pp. 18-24, 1995.

[6] Clarke, G. M., Fluctuating Asymmetry of Invertebrate Populations as a Biological Indicator of Environmental Quality, *Environmental Pollution,* Vol. 82, pp. 207-211, 1993.

[7] Zakharov, V. M., and Yablokov, A. V., Skull Asymmetry in the Baltic Grey Seal: Effects of Environmental Pollution, *Ambio*, Vol. 19(5), pp. 266-269, 1990.

[8] Leary, R. F., Allendorf, F. W., and Knudsen, K. L., Genetic, Environmental, and Developmental Causes of Meristic Variation in Rainbow Trout, *Acta Zoologica Fennica*, Vol. 191, pp. 79-95, 1992.

[9] Alados, C. L., Escos, J., and Emlen, J. M., Developmental Instability as an Indicator of Environmental Stress in the Pacific Hake (*Merluccius Productus*), *Fishery Bulletin*, Vol. 91, pp. 587-593, 1993.

[10] Chaungkaew, D., Dumrongsak, S., Ratanawis, W., Siriloirat, P., Paengbangkau, N., Yauprem M., and Modsiri, R., *Building Plans*, Vol. 1, Department of Planning and Corporation, Thammasat University, Rangsit Campus, Prathum Thani, pp. 29-31, 1990. (In Thai)

[11] Potthoff, T., Clearing and Staining Techniques. In Moser, H. G., Richards, W. J., Cohen, D. M., Fahay, M. P., Kendall, A. W. Jr and Richardson, S. L. (Eds.), *Ontogeny and Systematics of Fishe*, American Society of Ichthyologists and Herpetologists Special Publication No 1, pp. 35-37, 1984.

[12] Palmer, R. A., and Strobeck, C., Fluctuating Asymmetry: Measurement, Analysis, Patterns, *Annual Review of Ecology Systematics*, Vol. 17, pp. 391-421, 1986.

[13] Van Valen, L., A Study of Fluctuating Asymmetry, *Evolution*, Vol. 16, pp. 125-42, 1962.

[14] Sokal, R. R., and Rohlf, J. F., *Biometry*. W. H., Freeman & Co, San Francisco, 1981.

[15] Palmer, A. R., Fluctuating Asymmetry Analyses: A Primer, In Markow, T. A. (Ed.), *Developmental Instability and Evolutionary Implications*, Kluwer Academic Publishers, The Netherland, pp. 355-64, 1994.

[16] Rice, W. R., Analysing Tables of Statistical Tests, *Evolution*, Vol. 43, pp. 223-225, 1989.

[17] Swaddle, J. P., Witter, M. S., and Cuthill, I. C., The Analysis of Fluctuating Asymmetry,. *Animal Behaviour*, Vol. 48, pp. 986-989, 1994.

[18] Cowart, M. N., and Graham, J. H., Within-and-Among Individual Variation in Fluctuating Asymmetry of Leaves in the Fig (*Ficus carica* L.), *International Journal of Plant Science*, Vol. 160(10), pp. 116-121, 1999.

[19] Wongkittiwetchakul, S., *Encyclopedia of Thai Fish*, Supply Ltd., Bangkok, 1997. (In Thai).

[20] Mitton, J. B., Enzyme Heterozygosity, Metabolism, and Developmental Stability, In Markow, T. A., (Ed.), *Developmental Instability and Evolutionary Implications*, Kluwer Academic Publishers, The Natherland, pp. 49-68, 1994.

[21] Alekseeva, T. A., Zinichev, V. V., and Zotin, A. T., Energy Criteria of Reliability and Stability of Development, *Acta Zoological Frennica*, Vol. 191, pp. 159-165, 1992.

[22] Ozernyuk, N. D., Dyomin, V. I., Prokofyev, E. A., and Androsova, I. M., Energy Homeostasis and Developmental Stability, In Zakharov V. M. and Graham, J. H. (Eds.), *Acta Zoologica Fennica*, Vol. 191, pp. 167-175, 1992.

[23] Emlen, J. M., Freeman, D. C., and Graham, J. H., Nonlinear Growth Dynamics and the Origin of Fluctuating Asymmetry, *Genetica*, Vol. 89, pp. 77-96, 1993.

[24] Somarakis, S., Kostikas, I., and Tsimenides, N., Fluctuating Asymmetry in the Otoliths of Larva Fish as an Indicator of Condition Conceptual and Methodological Aspects, *Journal of Fish Biology*, Vol. 51, pp. 30-38, 1997.

[25] V llestad, L. A., Ejeld, E., Haugen, T., and xnevad, S. A., Developmental Instability in Grayling (*Thymallus Thymallus*) Exposed to Methylmercury During Embryogenesis, *Environmental Pollution*, Vol. 101, pp. 349-354, 1998.

[26] Campbell, W. B., Emlen, J. M., and Hershberger, W. K., Thermally Induced Chronic Developmental Stress in Coho Salmon Integrating Measures of Mortality, Early Growth and Developmental Instabiliy, *OIKOS*, Vol. 81, pp. 398-410, 1998.

[27] Vermeij, G. J., *Evolution and Escalation: an Ecological History of Life*, Princeton University Press, Princeton, 1987.

[28] McKenzie, J. A., and Clarke, G. M., Diazinon Resistance, Fluctuating Asymmetry and Fitness in the Australian Sheep Blowfly, *Lucilia Cuprina*, *Genetics,* Vol. 120, pp. 213-220, 1988.

Table 1 Physico-chemical variables recorded among sampling sites.

Sampling sites	Dissolved oxygen (mg/l)	pH (mg/l)	Conductivity (µS)	Temperature (◦C)
Pond 1	4.5	7.20	292	30.5
Pond 2	2.5	7.06	263	29.2
Rice field	6.7	7.09	461	26.2
Canal in Thammasat Univ.	6.9	8.36	1631	30.8
Water mimosa field	6.4	8.41	203	29.1
Pond around the library	7	7.13	1612	29.7
Canal Near Nongkae Temple	7.2	6.88	251	27

Table 2 Results of ANOVA and multiple comparisons undertaken to compare FA of *T. vittatus* among sampling sites.

Characters	Degree of freedom	F-values	Probability	Multiple comparisons
PC	2,134	.001	.999	Pond around the library. Canal in Thammasat Univ. Water mimosa field.
DR	2,134	1.406	.249	Pond around the library. Water mimosa field. Canal in Thammasat Univ.
VR	2,134	4.988	.008**	Canal in Thammasat Univ. Pond around the library. Water mimosa field.
PV				No asymmetery detected.
BR				No asymmetery detected.
PB	2,134	1.028	.360	Canal in Thammasat Univ. Pond around the library. Water mimosa field.
OW	2,134	..015	.985	Canal in Thammasat Univ. Pond around the library. Water mimosa field.
OL	2,134	.174	.841	Pond around the library. Water mimosa field. Canal in Thammasat Univ.
PM	2,134	10.136	.000***	Water mimosa field. Pond around the library. Canal in Thammasat Univ.

* Significance before adjustment by the Bonnferroni correction

* Significance after adjustment by the Bonnferroni correction

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

—— No significant differences were detected among groups of samples joined by underlining

Table 3 Results of ANOVA and multiple comparisons undertaken to compare FA of *T. tirchopterus* and *T. pectoralis* among sampling sites.

Characters	Degree of freedom	F-values	Probability	Multiple comparisons
PC	3,172	.205	.893	Canal. Near Nongkae Temple. Pond 1 and 2 Rice field. Water mimosa field.
DR	3,171	5.575	$.001^{***}$	Pond 1 and 2. Water mimosa field. Canal Near Nongkae Temple. Rice field.
VR	3,171	.018	.997	Canal Near Nongkae Temple. Pond 1 and 2. Rice field Water mimosa field.
PV				No asymmetryt detected.
BR				No asymmetry detected.
PB	3,172	5.362	$.001^{***}$	Pond 1 and 2. Rice field. Canal Near Nongkae Temple. Water mimosa field.
OW	2, 149	1.371	.257	Rice field. Canal Near Nongkae Temple. Water mimosa field.
OL	2, 149	.644	.527	Water mimosa field Rice field. Canal Near Nongkae Temple.
PM	2, 149	4.243	$.016^{*}$	Canal Near Nongkae Temple. Water mimosa field Rice field.

* Significance before adjustment by the Bonnferroni correction

* Significance after adjustment by the Bonnferroni correction

$^{*} = P \leq 0.05$, $^{**} = P \leq 0.01$, $^{***} = P \leq 0.001$

—— No significant differences were detected among groups of samples joined by underlining

Table 4 Results of ANOVA and multiple comparisons undertaken to compare FA of *E. metallicus* among sampling sites.

Characters	Degree of freedom	F-values	Probability	Multiple comparisons
PC	4,225	1.273	.281	Pond 2 Pond 1. Canal in Thammasat Univ. Pond around the library. Rice field.
DR	4,227	5.697	$.000^{***}$	Rice field Canal in Thammasat Univ. Pond around the library. Pond 2 Pond 1.
VR	4,227	4.519	$.002^{**}$	Pond 2 Pond 1. Canal in Thammasat Univ. Pond around the library. Rice field.
PV				No asymmetry detected.
BR	4,227	12.498	$.000^{***}$	Rice field. Canal in Thammasat Univ. Pond around the library. ***Pond 2.*** Pond 1.
PB	4,227	2.827	$.026^{*}$	Rice field Pond around the library. Canal in Thammasat Univ. Pond 1 Pond 2.
OW	4,225	11.138	$.000^{***}$	Rice field Canal in Thammasat Univ. Pond around the library. Pond 2 Pond 1.
OL	4,225	13.275	$.000^{***}$	Rice field Canal in Thammasat Univ. Pond around the library. Pond 2 Pond 1.
PM	4,225	32.966	$.000^{***}$	Rice field Canal in Thammasat Univ. Pond around the library. Pond 1 Pond 2.

* Significance before adjusted by the Bonnferroni correction

* Significance after adjusted by the Bonnferroni correction

$^{*} = P \leq 0.05$, $^{**} = P \leq 0.01$, $^{***} = P \leq 0.001$

—— No significant differences were detected among groups of samples joined by underlining

Table 5 Results of nested ANOVA and multiple comparisons undertaken to compare FA of *E. metallicus*, *T.vittatus* and *T. trichoperus* among sampling sites.

Characters	Source of variations	Degree of freedom	F- values	Probability	Multiple comparisons
PC	Fish	2,533	.620	.538	*T. vittatus T. Trihcopterus E. metallicus*
	Sites	7,533	.702	.671	
	Fish x Site	2,533	.209	.812	
DR	Fish	2,534	2.814	.061	**E. metallicus T. Trihcopterus T. vittatus**
	Sites	7,534	4.734	.000	
	Fish x Site	2,534	3.411	.034	
VR	Fish	2,534	5.303	.005	**E. metallicus T. Trihcopterus T. vittatus**
	Sites	7,534	3.281	.002	
	Fish x Site	2,534	.543	.581	
PV					No asymmetry detected
BR	Fish	2,535	9.972	.000	*T. Trihcopterus E. metallicus*
	Sites	7,535	16.781	.000	*T. vittatus*
	Fish x Site	2,535	.000	1.000	
PB	Fish	2,535	15.004	.000	*T. Trihcopterus T. vittatus E. metallicus*
	Sites	7,535	3.743	.001	
	Fish x Site	2,535	.556	.574	
OW	Fish	2,510	31.639	.000	*T. Trihcopterus T. vittatus E. metallicus*
	Sites	6,510	9.489	.000	
	Fish x Site	2,510	.460	.632	
OL	Fish	2,533	23.053	.000	*T. Trihcopterus T. vittatus E. metallicus*
	Sites	7,533	7.731	.000	
	Fish x Site	2,533	3.369	.035	
PM	Fish	2,510	16.664	.000	*T. Trihcopterus T. vittatus E. metallicus*
	Sites	6,510	29.364	.000	
	Fish x Site	2,510	.537	.585	

—— No significant differences were detected among groups of samples joined by underlining