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Can Fluctuating Asymmetry in Adult Burrowing Mayflies (*Hexagenia rigida*, Ephemeroptera) be used as a Measure of Contaminant Stress?

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ABSTRACT. *Fluctuating asymmetry (FA) in bilateral organisms may provide an early warning for detrimental effects of environmental stress in organisms well before populations are affected. Adult* Hexagenia rigida *were collected (17 June to 1 July 1996) at 6 sites around Lake Erie, representing areas with high (Monroe, MI; Colchester, ON; Middle Sister Island, ON) and low (Windsor, ON; Kingsville, ON; Catawba, OH) exposure to PCB contaminants. Balsam Lake, ON served as a reference site. Initially 27 structures were examined on the left and right sides of 30 males from both the most contaminated (Monroe) and least contaminated (Balsam Lake) sites using image analysis. Traits were autocorrelated; therefore, 7 independent traits were identified using principal component analysis. It was anticipated that there would be greater variation (i.e., greater FA) in organisms collected from sites known to exhibit high PCB body burdens than in organisms having low levels of contaminants. However, there were no significant differences in FA for male or female adults of* H. rigida *among the sites for the morphometric traits measured. There were significant differences in FA for two traits in* H. rigida *males among years (1991, 1994 to 96) at Windsor, but trends were not consistent. Although measurement error was low (1 to 6 %), results suggest that contaminant effects may be difficult to detect in the field because either the stress (PCBs) in the field was not strong enough to elicit a response by* Hexagenia *or PCB body burdens did not alter development in the insects.*

INDEX WORDS: *Fluctuating asymmetry, adult insects,* Hexagenia rigida*, contaminant stress, biomonitoring, Great Lakes.*

INTRODUCTION

The objective of this study was to determine if asymmetry between the left and right sides of wing and appendage traits in adult *Hexagenia rigida* (a burrowing mayfly) exists and if it relates to contaminant stress. The improved water quality in Lake Erie has resulted in the reappearance and expansion of burrowing mayflies in the western basin since the late 1980s (Krieger *et al.* 1996, Corkum *et al.* 1997). Decades earlier, mayflies were eradicated from Lake Erie by a combination of eutrophically-induced hypoxia and sediment contamination. The presence of *Hexagenia* nymphs in sediment signifies the existence of oxygen in surface sediments (Rasmussen 1988, Winter *et al.* 1996). In addition, both nymphs and adults are known to retain contaminants such as PCBs (Corkum *et al.* 1997). If a significant relationship between asymmetry in morphological traits and contaminant stress exists, the inexpensive measuring technique used to determine asymmetry would enable biologists to identify polluted areas.

Developmental stability in organisms represents a process that buffers development against environmental and genetic disturbances to produce an assumed ideal form (i.e., perfect symmetry) (Zakharov 1992, Palmer 1994, Rabitsch 1997). Departures from symmetry are grouped commonly into directional asymmetry, antisymmetry, or fluctuating asymmetry on the basis of frequency distributions of the absolute value of differences between the right and left sides of a structure (Van Valen

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1962, Palmer 1994). Directional asymmetry exhibits greater development of a character on one side of a plane of symmetry than on another, e.g., the human heart. Antisymmetry is asymmetry without directional bias (e.g., the enlarged claw of the male fiddler crab, *Uca lactea*, may be present on either the right or left side). Fluctuating asymmetry (FA) is the random departure from perfect symmetry of any bilateral anatomical character and shows a normal distribution about a mean of zero (Palmer 1994). These deviations are by chance just as likely to occur on the left or right side of an individual organism. Because departures from symmetry typically result when environmental or genetic stressors destabilize or disrupt developmental processes (developmental instability), measures of FA have been used to identify stress (Parsons 1990, Polak and Trivers 1994).

Developmental effects may occur before contaminants in food, water, or sediment reach levels high enough to cause widespread biological changes in species diversity or abundance (Valentine *et al.* 1973). In some cases, FA has been able to provide an early warning system for detrimental effects of environmental stresses on organisms well before local populations are endangered (Clarke 1993). Evidence for a link between the occurrence of structural abnormalities has been shown in several dose response studies. For example, Valentine and Soulé (1973) observed increasing fluctuating asymmetry of fin rays in grunion fry, a marine fish, exposed to increasing levels of p,p′ –DDT levels in the laboratory. Exposure to fertilizer effluent and diazinon has been associated with asymmetry in Neuroptera (Clarke 1993) and sheep blowflies (McKenzie and Clarke 1988), respectively.

Drouillard *et al.* (1996) showed that bioaccumulation of 17 organochlorine compounds (including 14 PCB congeners) by *Hexagenia* nymphs in Detroit River (Trenton Channel) sediments occurred as a result of contamination sorbed to sediment organic carbon and that sediment was the primary exposure route for the chemicals. In *Hexagenia*, all growth occurs during the nymphal stage (1 to 2 years) and the adults, which do not feed, seldom live more than 3 days (Corkum *et al.* 1995). The short-lived adults reflect the life-long exposure of nymphs to contaminants in the sediments in which they dwell.

In this study, it was determined if FA in insect wings and appendages of *H. rigida* imagos (sexually mature adults) would serve as a useful biomonitor in aquatic habitats. Spatial and temporal variation were considered. Corkum *et al.* (1997) have identified sites in the Detroit River and western Lake Erie where PCB body burdens of *Hexagenia* adults differ. Six sites characterized by high (n = 3 sites) and low (n = 3 sites) contamination were resampled to collect and measure adults for FA. *Hexagenia rigida* adults also were collected from a reference site, Balsam Lake, ON. It was expected that there would be greater FA in organisms with high PCB body burdens than in those with low PCB levels. The second aspect of the study examined temporal variation in *H. rigida* adults at one site, Windsor. No changes in contaminant levels occurred during this period; therefore, it was expected that patterns in asymmetry would remain constant.

METHODS

Field Sampling

Six sites that represented areas with high (Monroe, MI; Colchester, ON; Middle Sister Island, ON) and low (Windsor, ON; Kingsville, ON; Catawba, OH) PCB exposure were selected (Fig. 1). Balsam Lake, ON served as a reference site. Each site was sampled once from 25 June to 1 July 1996. Specimens also were examined from the Windsor site (1991, 1994 to 1996) to determine temporal variation, if any, in body size and asymmetry.

Light traps (Kovats and Ciborowski 1989) were used or lakeside dock lights were positioned within 5 m from shore to collect imagoes (sexually mature adults) for 2 h beginning at sunset on warm, calm evenings. This collection time corresponded to periods of greatest flight activity of adult *Hexagenia*. Specimens were preserved in 70% ethanol.

Laboratory Analysis

Specimens were sexed and identified to species using male genitalia (Burks 1953) or the chorionic sculpture of eggs (Koss 1968). Overall, the ratio of *Hexagenia* species for males was similar among sites in Lake Erie (*H. limbata* : *H. rigida*; 1.1:1.0). However, *H. rigida* was used in the study because it was the only species collected at Balsam Lake. *Hexagenia rigida* also was the dominant species at the Windsor site in each of 4 years (1991, 1994 to 1996).

Body length (excluding cerci) was measured using a digital caliper (to the nearest 0.01 mm) before anatomical parts were removed for morphological trait measurements. Body length frequency distributions were calculated for specimens from all

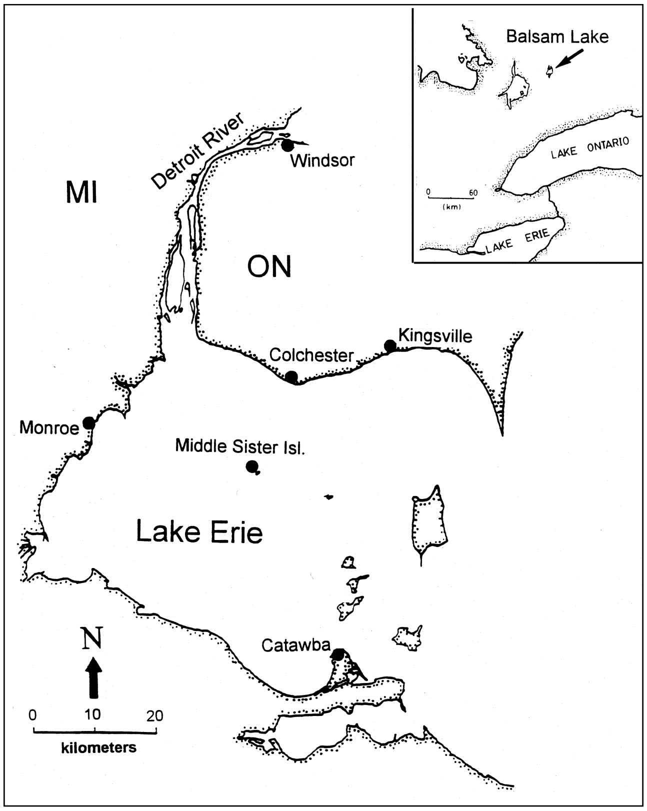

FIG. 1. ***Sample site locations for* Hexagenia rigida *in the western basin of Lake Erie (Monroe, Colchester, Middle Sister Island, Kingsville, Catawba), the Detroit River at Windsor, and a reference location in Ontario (Balsam Lake).***

sites. A common index of FA (Palmer 1994) was used: |Left - Right| ÷ Right. The division by R is to take into account allometric relationships between asymmetry and size. One investigator performed all measurements.

Initially, 27 morphological traits (including forewing and hind wings, veins within wings, delineated areas within the wing, appendages, and forceps) were measured on 30 males (left and right aspects) of *H. rigida* collected from each of two sites representing the extremes in contaminant stress (Monroe, MI, in western Lake Erie and Balsam Lake, ON). The selection of 27 traits was based on ease of identification. All wings and appendages were mounted on microscope slides using CMC medium (Pennak 1953). A grid (crosshairs) was superimposed on the forewing slides for reference points in measuring wing area. All structures used to calculate asymmetry were measured (at 6x or 12x magnification depending on the structure) using a Wild dissecting microscope equipped with a Panasonic WV 1854 high-resolution video camera. Video signals were digitized using a Targa 64 grabber card. Surface areas and lengths were quantified using a Mocha image analysis system (Jantel Scientific®).

To examine variability in measurements using image analysis, three males were randomly selected from Balsam Lake and Monroe sites and 27 traits (left and right aspects) were remeasured five times.

Tests for directional asymmetry and antisymmetry were performed on each of 27 traits measured for male *H. rigida* collected at Balsam Lake and Monroe using the software program, Statistica. To test for directional asymmetry, a t-test was used to determine if the R-L measures of each trait differed significantly from zero. Antisymmetry was tested by examining departures of the frequency distribution of R-L from normality using the Shapiro Wilk's W test. Outliers (the maximum and minimum values) were deleted before analysis. Traits that were indicative of either directional asymmetry or antisymmetry were not used in spatial or temporal comparisons.

After measurements were obtained for all 27 traits of adult male *H. rigida* at Balsam Lake and Monroe, a principal component analysis (PCA) was used to reduce the number of structures that were highly correlated. For example, the dark band along the leading edge of the forewing was significantly correlated with the total surface area of the forewing on specimens collected from Balsam Lake (R^2 = 0.82) and Monroe (R^2 = 0.78). The 27 traits were reduced to 7 independent traits (4 wing features, 2 appendage aspects, 1 forcep length) (Table 1). Subsequently, measurements on the reduced number of 7 structures were made using male *H.*

TABLE 1. ***Description of the 7 traits of adult male* H. rigida *used for comparison among sites. Nomenclature follows Edmunds* et al. *(1976).***

Code	Description
FWDB	Area of dark band of forewing along the costa and subcosta
FWAC	Cell area of forewing closest to the lower margin between $anal_1$ and cubitus posterior veins
HWTA	Total area of hind wing
HWDL	Dark line along subcosta of hind wing
FLTA2	Second tarsal segment of foreleg
MLTI	Tibia of middle leg
FO2	Second segment of forceps

rigida at the remaining five sites. Wing area and length of appendage traits on female *H. rigida* also were compared. Since female specimens cannot be identified to species, slides of eggs from individual females were prepared and Koss's (1968) taxonomic key was used. To determine temporal variation in asymmetry, the reduced number of traits also were measured on 30 male *H. rigida* collected from the Windsor site in each of 4 years (1991, 1994 to 1996).

RESULTS

Size Frequency Distribution of *Hexagenia*

Mean body length (excluding cerci) of adult male *H. rigida* differed among the 7 sample sites (1-way ANOVA, $p < 0.001$) (Fig. 2). The Student-Newman–Keuls multiple comparison test showed that mean body lengths of *H. rigida* adults did not differ among the Lake Erie/Detroit River sites; but, males collected from Balsam Lake were significantly larger than males collected at the six other sites ($p < 0.05$). Probability plots revealed that body lengths of mayflies from each site were normally distributed.

Directional Asymmetry and Antisymmetry

Directional asymmetry and antisymmetry were tested in adults collected from Balsam Lake and Monroe. At the Balsam Lake site, mean R-L values for 2 (proximal appendage length of male forcep and fourth tarsal segment of the hind leg) of the 27 traits were significantly different from zero (t-test, $p < 0.05$). At the Monroe site, mean R-L values for 2 (tibia length and length of first tarsal segment of the hind leg) of 27 traits differed significantly from zero (t-test, $p < 0.05$).

Antisymmetry was tested by examining departures of the frequency distribution of R-L from normality using the Shapiro Wilk's W test. In Balsam Lake specimens, data for 5 [total area of the forewing ($p < 0.001$), femur length of both fore- ($p < 0.0001$) and hind ($p = 0.0004$) legs, the length of the first tarsal segment of the foreleg ($p = 0.0120$), and length of the third tarsal segment of the hind leg ($p = 0.0255$)] of the 27 traits were not normally distributed. At the Monroe site, 3 traits [a cell area of the forewing ($p = 0.0074$), length of the first tarsal segment of the middle leg ($p = 0.0008$), and third tarsal segment of the hind leg ($p = 0.0518$)] were not normally distributed.

Measurement Error

Measurement error is a concern in detecting FA because comparisons of data among sites are measures of the variation between left and right sides and differences in FA among samples are often small (Palmer 1994). Measurement error was responsible for 1 to 6 % of the variability in the data [3-way ANOVA (2 sites, 3 males per site, left and right side of each specimen)]. High variability existed for the dark band along the forewing (6%), the second forcep length (6%), and the tibia of middle leg (5%). Variability was low (1 to 3%) for the other traits.

Spatial Variation

Mean FA in male *H. rigida* did not differ among sites (1-way ANOVA, $p > 0.05$) for any of the reduced set of 7 traits (Table 1, Fig. 3). Thus, no difference in asymmetry existed among specimens collected from sites differing in contaminant stress levels. The mean FA value of 1 trait (FWAC, forewing cell area) was larger than the other 6 traits (Student-Newman Keuls test, $p < 0.05$, Fig. 3) for all sites. Although few females (n = 4 to 21) of *H. rigida* were collected from each of the 7 sites, variation in asymmetry in females among sites was also examined. As expected, no differences in FA occurred among sites for any of the traits (1-way ANOVA, $p > 0.05$).

Temporal Variation

Size frequency distributions of body lengths of male *H. rigida* collected from the Windsor site were consistent from 1991 until 1996. There were no significant differences in FA among years for 5 of 7 traits (ANOVA, $p > 0.05$). Although significant differences in FA occurred among years for both the dark line on the leading edge of the hind wing (HWDL) ($p < 0.01$) and the second forcep segment (FO2) ($p < 0.05$), there was no consistent trend from year to year (Fig. 4).

DISCUSSION

Fluctuating asymmetry has been proposed as an early warning biomarker related to stress during development or to fitness of organisms (Clarke 1993). Stress is any factor causing a potentially injurious change in a biological system (Hoffman and Parsons 1991). In this study, it was assumed that the level of PCB contamination in the body burdens of

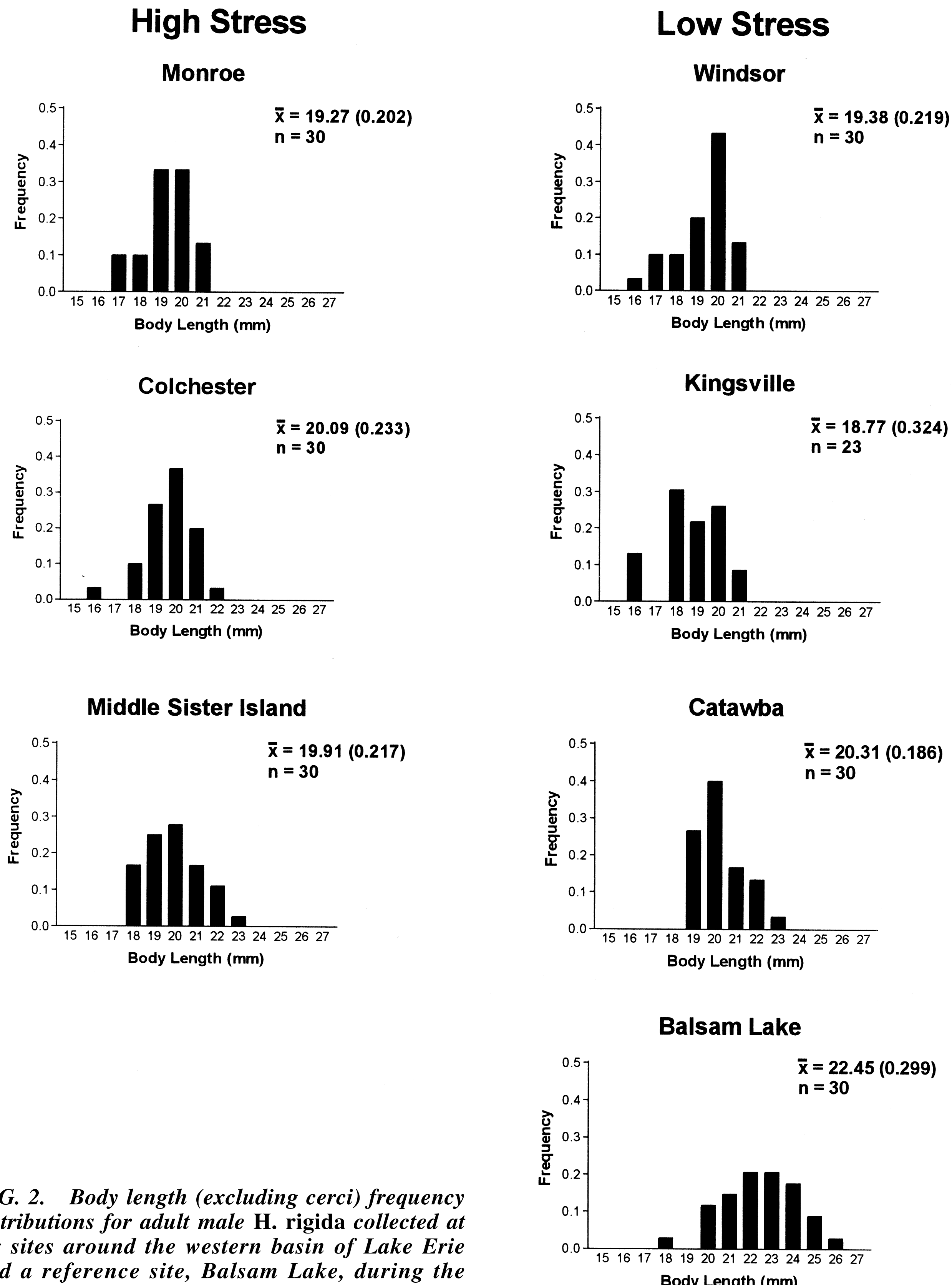

FIG. 2. Body length (excluding cerci) frequency distributions for adult male H. rigida *collected at six sites around the western basin of Lake Erie and a reference site, Balsam Lake, during the summer of 1996.*

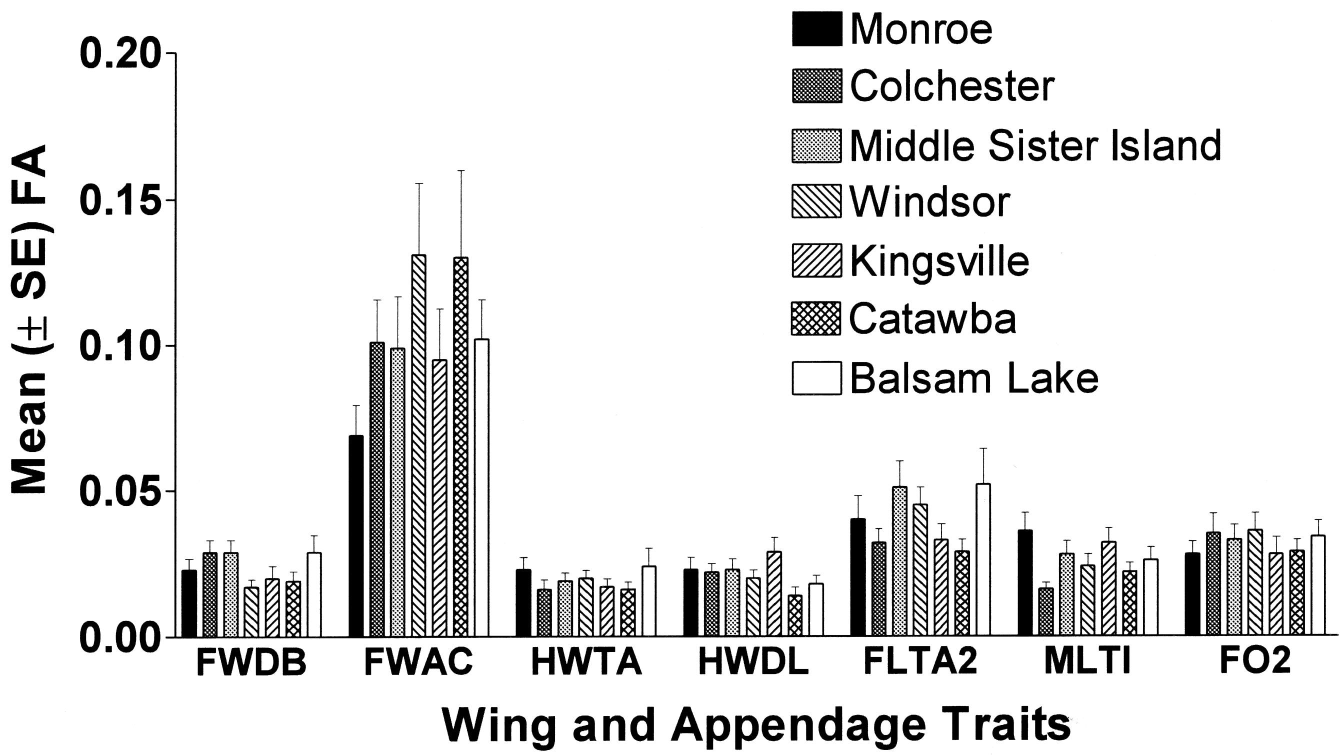

FIG. 3. Mean fluctuating asymmetry (FA) values (±SE) for wing and appendage traits of male* H. rigida *collected at six sites around western Lake Erie representing high (Monroe, Colchester, Middle Sister Island) and low (Windsor, Kingsville, Catawba) levels of PCB contamination and a reference site, Balsam Lake, in 1996. Code descriptions are presented in Table 1.

Hexagenia provided the stress to which *Hexagenia* would respond. Corkum *et al.* (1997) demonstrated differences in concentrations of PCB body burdens in adult *Hexagenia* between "high stress" and "low stress" sites. The presence of stress during ontogeny may alter the normal developmental process and be reflected in an increase in asymmetry (Clarke *et al.* 1995). It was hypothesized that FA would be higher at "high stress" sites than at the other less contaminated sites.

There were no significant differences in FA for male or female imagoes of *H. rigida* among the 7 sample sites for any of the morphometric traits. Given the controversy about the types of traits to be used (Leung and Forbes 1997), our use of multiple measures was a good approach (see Palmer 1994). Of the traits examined, asymmetry was higher for one measure of forewing cell area (FWAC) than for the other traits (Fig. 3). Asymmetry was low for variables related to fitness, forewing area (dispersal) and forcep length (reproduction). Because of the variability in the data, no differences were discerned between local areas of high and low body burdens of PCBs. Evidently, PCB concentrations in western Lake Erie do not affect development in *H. rigida*. Moreover, patterns in symmetry for *H. rigida* populations at Windsor (Detroit River site) changed little from 1991 to 1996. These results are consistent with the contaminant history in the area. Values obtained by Corkum *et al.* (1997) for 1994 were similar to values obtained by Kovats and Ciborowski (1989) for this site in 1987.

Because asymmetry may be affected by several factors within a population, it may not be possible to identify a specific causative agent (Clarke *et al.* 1995). Changes in water temperature, nutrients, food resources, parasites, and weather may have masked any association between FA in *Hexagenia* and sites with PCB contamination in this study. Moreover, stressors may affect development individually or synergistically.

Some morphological traits required for critical functions are often well canalized and correspondingly are symmetrical (Waddington 1942, Clarke *et*

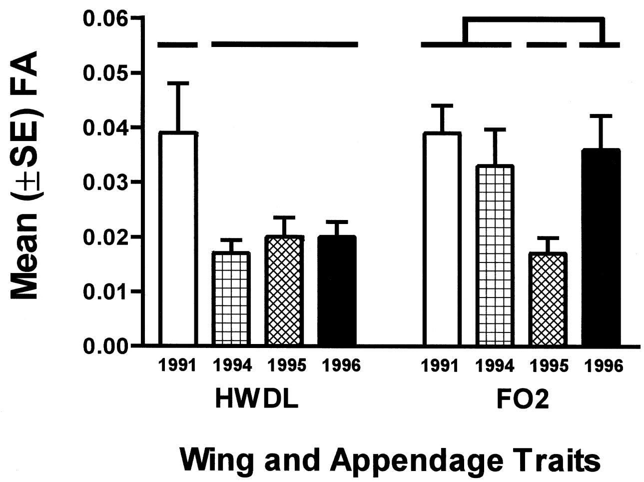

FIG. 4. Mean fluctuating asymmetry (FA) values (±SE) for two traits (HWDL, FO2) of male* H. rigida *collected on the Detroit River at Windsor (1991, 1994 to 96) in which significant differences occurred over time. Solid lines indicate cases in which no significant differences occurred. Codes are listed in Table 1.

al. 1995). Perhaps the traits that were measured (although comprehensive) simply were not reliable measures of stress. Additionally, PCB body burden in *H. rigida* may have been a poor choice of stress in this empirical study. Instead, a laboratory study in which asymmetry is examined in *Hexagenia* nymphs reared in formulated sediment spiked with increasing PCB concentrations would demonstrate if departures from symmetry resulted from contaminant stress.

In contrast to these findings, others have reported significant associations between FA in biota and levels of organochlorines (Valentine and Soulé 1973, Zakharov and Yablokov 1990). Increases in pectoral fin ray asymmetry in grunion (*Leuresthes tenuis)* fry were associated with increasing concentrations of pp′-DDT in a dose response study (Valentine and Soulé 1973). Similarly, FA in grunions was higher in fish collected from pp′-DDT-exposured sites than reference sites. In another study, Zakharov and Vablokov (1990) measured museum specimens and showed that levels of skull asymmetry in Baltic grey seals (*Halichoerus grypus*) were higher in animals born after 1960 (pollution group exposed to organochlorines) than animals born before 1940 (pre-pollution group).

Although significant associations between FA and stress or fitness relationships are common in the literature (Markow 1995), negative results are rarely published. Fluctuating asymmetry in chironomid larvae measured in response to chronic doses of pesticides in artificial channels revealed inconclusive results (Clarke *et al.* 1995). The authors cited problems with experimental design (lack of a true control, too few samples, unknown developmental time, and a range of low doses). In another study, Rabitsch (1997) examined asymmetry in worker ants, *Formica pratensis*, among sites differing in metal contamination (the lead/zinc smelter had been in operation for nearly 500 years) in both mature and pre-mature ant colonies in Austria. Although Rabitsch (1997) showed that there were significant differences in character asymmetries, they were not correlated with lead or zinc. In a recent literature review examining relationships of FA to stress and fitness, Leung and Forbes (1996) concluded that FA might be a poor predictor of either stress or fitness. They determined that measurement error could reduce the strength of the relationships and account for the low effect sizes observed.

Although FA has been used successfully in biomonitoring, results from this study suggest that contaminant effects may be difficult to detect in the field because the magnitude of the stress needed to detect an effect may be unrealistic. Furthermore, numerous other stressors may confound morphometric measures. In the present study, either the body burdens of PCBs in field-collected *Hexagenia* were not strong enough to elicit a response or PCB body burdens did not alter development in the insects.

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