BULLETIN OF MARINE SCIENCE, 70(1): 75–88, 2002

# FLUCTUATING ASYMMETRY AND INTERSEXUALITY IN THE SHORE CRAB *GRAPSUS ALBOLINEATUS* NEAR A COASTAL LANDFILL SITE IN NORTHERN TAIWAN

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## ABSTRACT

Fluctuating asymmetry (FA) is defined as small, random deviation from perfect bilateral symmetry of morphological trait and an increase of FA is proposed to be caused by genetic and environmental stress. In this study, measures of FA in the shore crab, *Grapsus albolineatus*, were used to investigate the impact of a municipal landfill on the surrounding coastal area of northern Taiwan based on a hypothesis that poor environmental conditions can increase degrees of FA. The majority of leg segments examined showed higher mean FA levels in crabs collected from Pa-Dor-Tze than those from either of the control sites. There were significant increases in FA levels in eleven of sixteen leg segments in crabs collected from Pa-Dor-Tze compared to those from Wai-Mon-San, and four of sixteen leg segments in crabs collected from Pa-Dor-Tze compared to those from Red-Ben. Furthermore, there were significant increases in eight of nine composite FA indexes in crabs collected from Pa-Dor–Tze compared to those from Wai-Mon-San and Red-Ben. On the other hand, there was no difference in any composite FA indexes in crabs collected from Wai-Mon-San and Red-Ben. The results of this study support that combining FA information across different characters is a much more reliable tool for detecting differences than analyzing FA in single character. There were larger average carapace widths in crabs collected from Pa-Dor-Tze and Wai-Mon-San compared to those from Red-Ben. The results of this study show significantly increased FA levels of crabs living in the coastal area of Pa-Dor-Tze, immediately adjacent to the landfill, and also provide a support for the use of FA in invertebrate populations as an early warning system to detect the presence of environmental stress.

Fluctuating asymmetry (FA) is an estimate of developmental instability and has been suggested as a potentially valuable and inexpensive tool for detecting stress in natural populations (Leary and Allendorf, 1989; Clark, 1992; Parsons, 1992; Graham et al., 1993; Sommer, 1993; Clark, 1995; Tracy et al., 1995). FA is defined as small, random deviation from perfect bilateral symmetry of morphological trait and an increase of FA is proposed to be caused by genetic and environmental stress. It is believed that poor environmental conditions can cause the disruption of physiological processes and increase energy drain in an individual. These effects are expected to result in an increase of FA in an individual associated with a subsequent decline in fitness of an individual (Graham et al., 1993).

Pollution is one of the most common environmental stresses suspected of increasing FA. Indeed, many field studies of natural populations have reported increases in FA caused by various sources of environmental pollution (Bengtsson et al., 1985; Clarke, 1993; Tracy et al., 1995; Kozlov et al., 1996; Ostbye et al., 1997; Moller, 1998). Therefore, measurements of FA may have potential for application in monitoring environmental quality (Leary and Allendorf, 1989; Clarke, 1993; Parsons, 1992; Graham et al., 1993; Sommer, 1993; Clarke, 1995; Tracy et al., 1995; Ostbye et al., 1997; Moller, 1998; Allenbach et al., 1999). Especially, this approach merits use as a first step of an environmental monitoring program to identify stressed ecosystem and/or populations, regardless of the pollutants involved.

Parasitism is also one of the possible environmental stresses associated with increase in FA. It has been suggested that relationships between parasitism and FA may result from two causes: either that less symmetrical individuals may be more susceptible to parasites or that parasitism may cause physiological imbalances in hosts, causing parasitized individuals to develop imperfectly (Moller, 1996; Ward et al., 1998). Therefore, measures of FA in different morphological traits of host may reflect either the ability of individuals to cope with parasite attacks or the effects of parasitism.

In this study, measures of FA were used as a tool to investigate the impact of a municipal landfill on the surrounding coastal area in northern Taiwan. Shore crabs, *Grapsus albolineatus*, were chosen as test organisms because of their common presence at all sites and for ease of character measuring. During the field collection of shore crabs, a number of collected specimens were found to be infected by rhizocephalan. It provided a good opportunity to examine associations between parasitism and FA in these crabs.

Rhizocephala (Crustacea, Cirripedia) are exclusively parasitic on decapod crustaceans. In the adult state the organism consists of two portions, an internal root-system (interna) and a soft sac-like structure (externa) (Reinhard, 1956). Infection by rhizocephalans begins with the attachment of female larva on the external body surface of a host. The interna of larva then spreads out via connective tissue into a root system which extents throughout various major organs and absorbs its nutrients directly from the host's tissues (Hoggarth, 1990; Takahashi and Matsuura, 1994). As the rhizocephalan continues to grow, it develops a saclike externa acting as a reproductive organ (Reinhard, 1956). The externa contains the ovaries and a mantle cavity in which eggs develop, and occupies the space between the sternum and the abdomen of the host in much the same manner as a crab egg mass. (Takahashi and Matsuura, 1994; Feldmann, 1998). There is some limited laboratory evidence indicated that parasitized crabs may continue to molt with a reduction in the molt frequency and molt increment (Takahashi and Matsuura, 1994). However, it is widely accepted that host crabs usually stop molting after the parasites develop an externa (Reinhard, 1956; Høeg and Lützen, 1995). According to current hypotheses on FA, it is possible that the molting of crabs is affected by parasitism, which in turn indirectly causes increases in the FA levels of infected individuals.

Rhizocephalan parasites in male crab hosts are known to have several effects they cause changes in morphological characteristics which cause their hosts to resemble oviparous females, they alter some physiological processes such as molting and growth, and they also change some behavioral features (Bishop and Cannon, 1979; Weng, 1987; Hoggarth, 1990; Shield and Wood, 1993; Takahashi and Matsuura, 1994). Among these effects induced by rhizocephalans, the commonest and most noticeable change in hosts is the broadening of the abdomen in the male crabs (Reinhard, 1956). The fact that rhizocephalan parasite causes intersexuality, which an individual exhibits morphological characters in opposite sex, in their male crab hosts has been accepted for more than 100 yrs (Giard, 1887). On the other hand, there are little changes on the external morphology of female crabs (Reinhard, 1956).

The main objective of this study is to test a hypothesis that measures of FA levels can be used as a sensitive early warming to identify environmental stress. A refuse landfill site at Pa-Dor-Tze was presumed as a relatively high environmental stress location. I compared FA levels of different leg segments in *G. albolineatus* from three locations with different degrees of environmental stress in northern Taiwan. In addition, associations

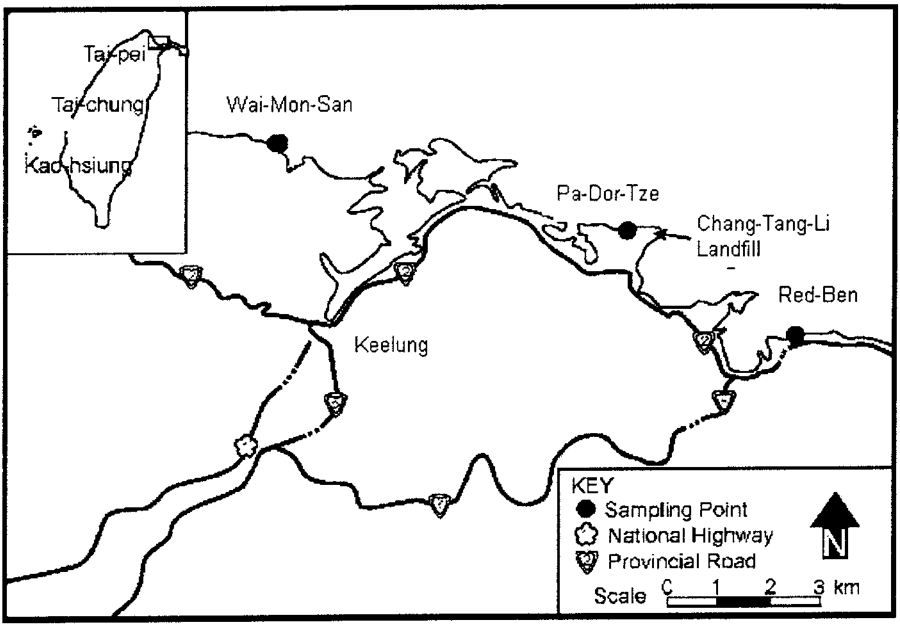


Figure 1. The three sampling locations on the northern coast of Taiwan

between FA and parasitism in male crabs were examined by comparing by comparing FA levels of different leg segments among normal and intersex male crabs for each location.

## Material and Methods

Sampling Sites and Sample Collections.—Crabs were collected from three locations in northern Taiwan, including Wai-Mo-San (25°10′05″N, 121°143'25″E), Pa-Dor-Tze (25°8′56″N, 121°47′24″E), and Red-Ben (25°7′44″N, 121°49′28″E) (Fig. 1). Hung et al. (1984) found some organic wastes and heavy metals (such as lead and copper) as well as inorganic nutrients (such as nitrite, nitrate, phosphate and silicate) were possibly seeping from the Chang-Tang-Li landfill site caused slightly unfavorable stress on biological activities in this marine ecosystem. This landfill was closed in 1992, and the sea dikes protecting this landfill have been damaged by the actions of wave erosion. A recent accident resulting in the massive destruction of the sea dikes at this landfill took place in November of 1997. The trash from this landfill was washed out and deposited on the nearby coast. Therefore, the seashore of Pa-Dor-Tze was constantly littered with a variety of waste products, including plastic and polystyrene materials, car tires, household products, etc. Therefore, this site was considered to suffer from intensive organic pollution. Among three sampling sites, Pa-Dor-Tze was selected as a high environmental stress site, as the Chang-Tang-Li landfill is located on the seashore in Pa-Dor-Tze. The other two sampling sites were considered as low environmental stress sites because there had been no obvious chemical and physical disturbances near these two locations. Except for the presence of the landfill in Pa-Dor-Tze, the other environmental factors in the coastal area of these three sampling sites were very similar (National Museum of Marine Science & Technology Planning Bureau, 1999). Crabs were collected in June and July of 1999 from rocky beaches at all sites and immediately stored in 70% ethanol until transport back to the laboratory from the field. The crabs were stored at 4°C until examined. The numbers of crabs collected at each site were 42, 33 and 64 for Pa-Dor-Tze, Red-Ben and Wai-Mo-San, respectively.

Intersexuality Indentification.—Crabs were sexed by the presence of external secondary sex characteristics on the ventral side of the thoracic area, i.e., a pair of genital pore on the 5th sternum of the female, and a pair of the first pleopod on the 4th sternum of the male. Crabs were not dissected for internae to determine prevalence of infection, and changes in the shape of the abdomens of male crabs were used as an indicator of parasite infection. The widths of the 5th and 7th sternum segments of each crab were measured, and the ratios of the 7th to the 5th sternum widths were calculated as a quantitative index of intersexuality. Each male crab was visually classified as to its status of intersexuality based on the shape of the abdomen according to a classification scheme described by Weng (1987). The differences in the occurrences of intersexuality, the ratios of the 7th to the 5th sternum widths, among male crabs from three study sites were examined by a Kruskal-Wallis nonparametric ANOVA.

FA Measurements.—For every crab with a carapace width greater than 20 mm, a total of 20 leg segment lengths were measured twice with digital calipers to the nearest 0.01 mm, with an interval of 4 wks between the two repeated measurements. All measurements were made by the same person. The length of the merus, carpus, propus and dactylus were measured for each cheliped on both sides of the crab. The 1st chelipeds of many crabs tended to exhibit antisymmetrical. In fact, only FA is considered to reflect developmental instability among three kinds of bilateral asymmetry, and is used as a sensitive indicator of stress. There was no information available on the form of bilateral asymmetry for the 1st chelipeds of *G. albolineatus*, therefore, FA levels of the 1st chelipeds were not considered in analysis of FA. Carapace lengths and widths were also measured twice in each crab.

Statistical Analysis.—The analysis of FA was carried out following the suggestions of Palmer and Strobeck (1986) and Leung et al. (2000). Allometric relations between the absolute length difference between the right and left sides of each leg segment and leg segment size (i.e., the average length of each leg segment from both sides) were determined using linear correlation for each character in all samples. Because there were no significant correlations between asymmetry and leg size for all characters examined, correction for size dependence was not required (Palmer, 1994). In this study, therefore, FA value was calculated as the mean absolute length difference between the right and left sides of each leg segment in crabs. Measurement error was examined by using a two-way mixed-model ANOVA, with sides as the fixed factor, individual as the random factor, and each leg segment as the response variance. Mean measurement error was 18% within the range of 25% accepted as reasonable by Palmer (1994). Before performing FA analysis, it is important to ensure that asymmetry distributions did not show either directional asymmetry or antisymmetry. I examined the distributions of length difference between the left and right sides for each leg segment for departures from normality by testing for skewness (directional asymmetry) and kurotosis (antisymmetry). Because distributions of length difference between the right and left sides of each leg segment were positively skewed for some characters and showed platykurtic for all characters examined, all FA data were transformed to square roots for further FA analysis. Square root transformation was used, because it was the most effective transformation to normality for all characters examined among logarithm, square root and Box-Cox power transformations.

Several authors suggest that combining FA information across different traits is a much more reliable tool for detecting differences than analyzing FA in single trait (Zhivotovsky, 1992; Palmer, 1994; Leung et al., 2000; Windig and Nylin, 2000). Thus, I tested my hypothesis in two ways. First, to retain the maximum information, I compared FA values of each leg segment in crabs among different sites. Second, I evaluated FA levels in crabs in relation to composite FA indexes that should be better reflect asymmetry overall (Palmer, 1994; Leung et al., 2000). There were nine composite FA indexes calculated for each crab. Composite FA indexes were calculated following the recommendations of Leung et al. (2000). In brief, composite scores were computed by dividing each FA value by the average FA level of a given leg segment, then by summing these values across characters to obtain a composite index for each crab. Composite FA indexes were calculated for specific leg segment from the 2nd, 3rd, 4th and 5th legs and for each specific walking leg from merus, carpus, propus and dactylus segments of the same leg. In addition, a composite FA index of sixteen leg segments was calculated for each crab. Then, all FA indexes were transformed to square roots for further FA analysis.

In sum, three analyses were performed on square root transformed FA data. First, I compared the FA levels of normal male, intersex male, and female crabs, for each leg segment in each site. Second, I compared the FA levels of each leg segment in crabs among different sites. Third, I compared different composite FA indexes in crabs among different sites. The FA values were compared using a one-way ANOVA test for each comparison. Differences were considered significant when $P \leq 0.05$. If a significant result was found, the Tukey's multiple comparison test was used to compare all pairs of different groups. All statistical analyses were performed by using the Minitab statistical program (Release 13.1).

## Results

General Characteristics of Shore Crabs.—There was no size different among male, intersex male and female crabs at any site (Table 1). But, there were size differences in the crabs collected from three study sites ($P = 0.001$). The carapace widths of crabs collected from Pa-Dor-Tze ($36.22 \pm 1.09$ mm) and Wai-Mo-San ($34.00 \pm 0.81$ mm) were significantly larger than those of crabs ($30.37 \pm 0.87$ mm) collected from Red-Ben (Fig. 2). In addition, there were no difference in cheliped lengths of five legs among crabs collected from three study sites while analyzing cheliped lengths by ANCOVAs with carapace widths as a covariate (Fig. 3).

Intersexually Occurrence.—The percentage of occurrence in the intersexuality of male crabs collected from Pa-Dor-Tze, Red-Ben and Wai-Mo-San were 40%, 74% and 64%, respectively (Table 2), however, the ratio of the 7th to 5th sternum widths, an indication of parasitism, in male crabs were no significant difference among three study sites by a Kruskal-Wallis nonparametric ANOVA ($P = 0.2$). The ratios of the 7th to 5th sternum widths showed a negative correlation with the carapace widths of the crabs among intersex male crabs (Pearson correlation coefficient $= -0.322$, $P = 0.038$).

FA Value of Single Leg Segment in Shore Crabs.—There were no differences in FA levels for any leg segment among normal male, intersex male, and female crabs for each site (Table 1). All crabs from the same site were therefore pooled into the following analyses. There were significant difference in FA values of 11 leg segments between crabs collected from Pa-Dor-Tze and Wai-Mon-San, including the merus segments of the 4th and 5th legs, the carpus and dactyl segments of the 3rd, 4th and 5th legs, and the propus segments of the 3rd and 4th legs (Table 3). In addition, there were significant difference in FA values of four leg segments between crabs collected from Pa-Dor-Tze and Red-Ben, including the dactyl segments of the 3rd, 4th and 5th legs and the carpus segment of the 3rd legs (Table 3). On the other hand, there was only significant difference in FA values of the merus segment of 4th legs in crabs collected from Red-Ben and Wai-Mo-San (Table 3).

Composite FA Indexes in Shore Crabs.—The composite FA indexes for merus, carpus, porpus and dactyl segments in crabs collected from Pa-Dor-Tze were significantly higher than those in crabs collected from either of control sites (Fig. 4). Crabs from Pa-Dor-Tze also exhibited significantly higher composite FA indexes for the 3rd, 4th and 5th legs than those from Wai-Mon-San and Red-Ben (Fig. 5). On the other hand, comparisons of specific leg segments or specific walking legs of crabs from Red-Ben and Wai-Mo-San revealed no differences in any of their composite FA indexes (Fig. 4,5). Finally, the com-

Table 1. FA values in different leg segments of normal male, intersex male and female crabs at each study site.

| | Pa-Dor-Tze | | | Red-Ben | | | Wai-Mon-San | | |
|---|---|---|---|---|---|---|---|---|---|
| | normal male | intersex male | female | normal male | intersex male | female | normal male | intersex male | female |
| n | 15 | 10 | 17 | 5 | 14 | 14 | 12 | 21 | 31 |
| CW (mm) | 37.51 ± 1.86 | 35.77 ± 1.84 | 35.35 ± 1.91 | 29.62 ± 1.88 | 30.28 ± 1.31 | 30.69 ± 1.46 | 35.69 ± 2.12 | 34.92 ± 1.43 | 32.73 ± 1.09 |
| Leg 2 | | | | | | | | | |
| merus | 0.15 ± 0.05 | 0.32 ± 0.12 | 0.28 ± 0.14 | 0.08 ± 0.02 | 0.12 ± 0.03 | 0.25 ± 0.08 | 0.34 ± 0.21 | 0.12 ± 0.01 | 0.21 ± 0.05 |
| carpus | 0.28 ± 0.09 | 0.45 ± 0.16 | 0.46 ± 0.15 | 0.20 ± 0.05 | 0.24 ± 0.06 | 0.31 ± 0.10 | 0.37 ± 0.17 | 0.22 ± 0.04 | 0.31 ± 0.06 |
| propus | 0.24 ± 0.08 | 0.38 ± 0.16 | 0.39 ± 0.18 | 0.15 ± 0.07 | 0.21 ± 0.08 | 0.40 ± 0.17 | 0.39 ± 0.24 | 0.14 ± 0.02 | 0.31 ± 0.09 |
| dactyl | 0.38 ± 0.12 | 0.62 ± 0.26 | 0.54 ± 0.23 | 0.15 ± 0.05 | 0.32 ± 0.16 | 0.40 ± 0.14 | 0.50 ± 0.28 | 0.12 ± 0.02 | 0.45 ± 0.12 |
| Leg 3 | | | | | | | | | |
| merus | 0.30 ± 0.10 | 0.69 ± 0.22 | 0.47 ± 0.18 | 0.31 ± 0.15 | 0.47 ± 0.33 | 0.23 ± 0.06 | 0.38 ± 0.18 | 0.31 ± 0.13 | 0.32 ± 0.11 |
| carpus | 0.59 ± 0.17 | 1.72 ± 0.48 | 0.81 ± 0.27 | 0.78 ± 0.42 | 0.33 ± 0.15 | 0.27 ± 0.06 | 0.37 ± 0.12 | 0.60 ± 0.19 | 0.33 ± 0.17 |
| propus | 0.76 ± 0.31 | 2.07 ± 0.64 | 0.87 ± 0.35 | 0.90 ± 0.46 | 0.42 ± 0.20 | 0.29 ± 0.06 | 0.47 ± 0.19 | 0.60 ± 0.24 | 0.26 ± 0.25 |
| dactyl | 0.72 ± 0.29 | 2.41 ± 0.72 | 1.32 ± 0.47 | 0.92 ± 0.47 | 0.48 ± 0.19 | 0.29 ± 0.06 | 0.47 ± 0.17 | 0.83 ± 0.29 | 0.39 ± 0.12 |
| Leg 4 | | | | | | | | | |
| merus | 0.70 ± 0.28 | 1.51 ± 0.45 | 0.41 ± 0.14 | 0.20 ± 0.14 | 0.14 ± 0.07 | 0.70 ± 0.35 | 0.19 ± 0.05 | 0.37 ± 0.12 | 0.31 ± 0.11 |
| carpus | 1.26 ± 0.53 | 0.98 ± 0.57 | 0.82 ± 0.25 | 0.97 ± 0.37 | 0.41 ± 0.21 | 0.63 ± 0.15 | 0.57 ± 0.16 | 0.28 ± 0.04 | 0.52 ± 0.17 |
| propus | 1.44 ± 0.60 | 1.03 ± 0.71 | 1.07 ± 0.34 | 1.13 ± 0.52 | 0.31 ± 0.21 | 0.83 ± 0.25 | 0.47 ± 0.17 | 0.25 ± 0.07 | 0.63 ± 0.25 |
| dactyl | 1.67 ± 0.72 | 2.58 ± 1.50 | 1.18 ± 0.37 | 1.26 ± 0.76 | 0.41 ± 0.20 | 0.76 ± 0.27 | 0.65 ± 0.32 | 0.23 ± 0.05 | 0.64 ± 0.25 |
| Leg 5 | | | | | | | | | |
| merus | 0.54 ± 0.17 | 0.35 ± 0.18 | 0.36 ± 0.13 | 0.10 ± 0.01 | 0.27 ± 0.08 | 0.31 ± 0.08 | 0.26 ± 0.12 | 0.17 ± 0.04 | 0.25 ± 0.06 |
| carpus | 0.80 ± 0.30 | 0.56 ± 0.27 | 0.37 ± 0.13 | 0.15 ± 0.04 | 0.32 ± 0.13 | 0.35 ± 0.08 | 0.20 ± 0.06 | 0.20 ± 0.03 | 0.36 ± 0.08 |
| propus | 1.16 ± 0.39 | 0.80 ± 0.47 | 0.55 ± 0.21 | 0.11 ± 0.02 | 0.40 ± 0.21 | 0.57 ± 0.24 | 0.37 ± 0.12 | 0.24 ± 0.07 | 0.61 ± 0.19 |
| dactyl | 1.30 ± 0.54 | 0.90 ± 0.63 | 0.56 ± 0.23 | 0.18 ± 0.06 | 0.36 ± 0.24 | 0.52 ± 0.22 | 0.15 ± 0.04 | 0.19 ± 0.06 | 0.50 ± 0.19 |

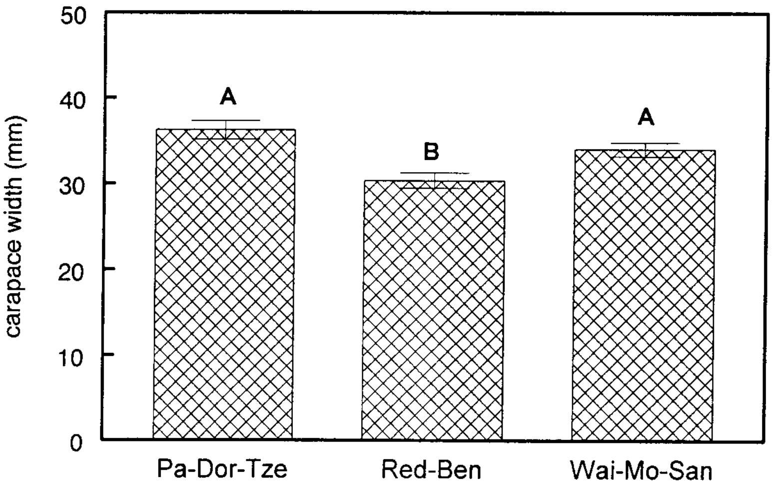


Figure 2. The average carapace width (mean ± SE) of *Grapus albolineatus* collected from three locations in the northern coastal area of Taiwan. Bars with different characters are significantly different by Tukey's multiple comparison test ($P < 0.05$).

posite FA index for all leg segments in crabs from Pa-Dor-Tze was significantly higher than those in crabs from either of the control sites, whereas there was no difference in this composite FA index in crabs collected from both control sites (Fig. 6).

## Discussion

In this study, I examined the hypothesis that environmental stress was negatively related to developmental stability measured as an increase in FA of *G. albolineatus* in an area near a refuse landfill site. As predicted, the result of this study shows an increase on FA levels of leg segments in crabs living immediately adjacent to the landfill in the coastal area of Pa-Dor-Tze. The majority of leg segments examined showed higher mean FA levels in crabs collecting from Pa-Dor-Tze than those from either of the control sites. There were significant increases in FA levels in eleven of sixteen leg segments in crabs collected from Pa-Dor-Tze compared to those from Wai-Mon-San, and four of 16 leg segments in crabs collected from Pa-Dor-Tze compared to those from Red-Ben. Furthermore, the result of this study provides a good support that comparing FA levels of multiple characters is a better method for detecting stress than comparing FA levels of single characters (Leary and Allendorf, 1989; Zhivotovsky, 1992; Palmer, 1994; Leung et al., 2000; Windig and Nylin, 2000). There were significant increases in eight of nine composite FA indexes in crabs collected from Pa-Dor–Tze compared to those from Wai-Mon-San and Red-Ben. On the other hand, there was no difference in any composite FA indexes in crabs collected from Wai-Mon-San and Red-Ben.

FA has been used in many field studies to investigate the effects of acidity and water pollution on fish. Several field studies have shown that FA levels were higher in fishes exposed to water pollution (Valentine and Soule, 1972; Ames et al. 1979; Jagoe and Haines, 1985). However, some field studies measured FA levels in fishes inhabiting acidified and unacidified lakes were no such significant correlation (Wiener and Rago, 1987) or only

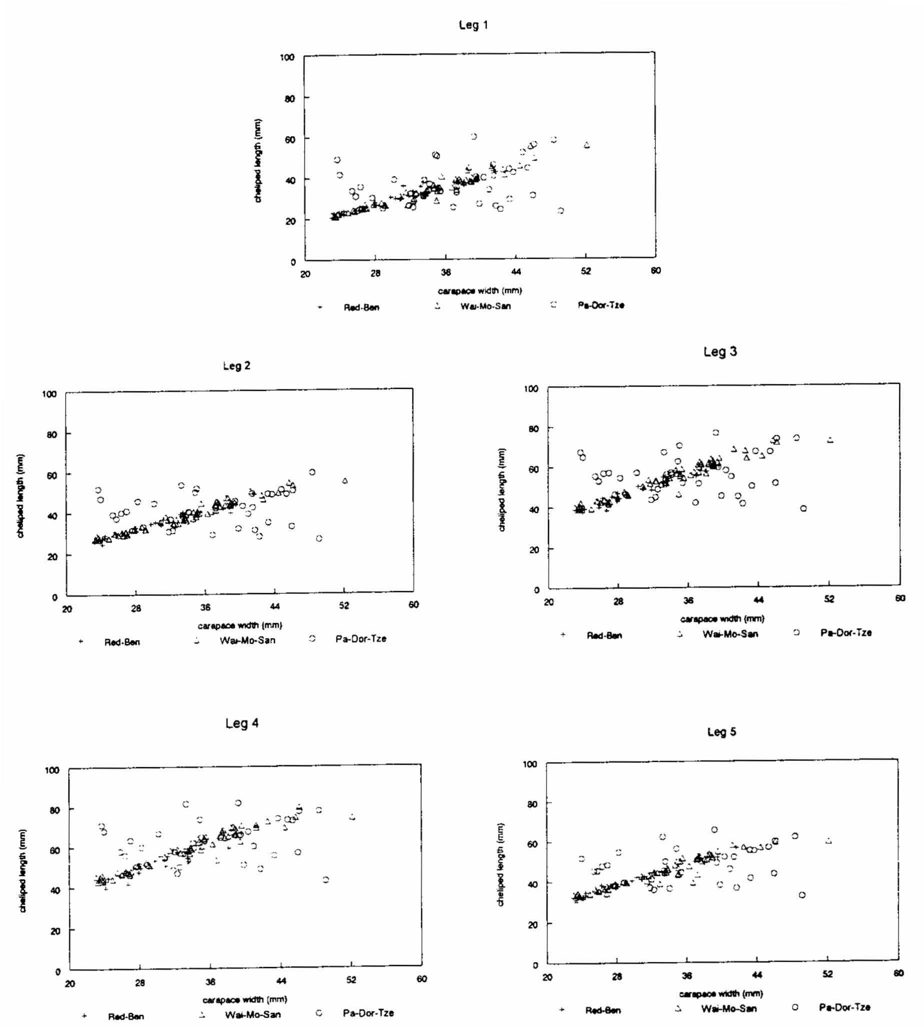


Figure 3. Cheliped lengths vs carapace widths in *Grapus albolineatus* collected from three locations in the northern coastal area of Taiwan.

Table 2. The intersexuality status of male crabs collected from three study sites.

| | Index of the shape of abdomen[1] | Pa-Dor-Tze | | Red-Ben | | Wai-Mon-San | |
|---|---|---|---|---|---|---|---|
| | | n | Ratio[2] | n | Ratio[2] | n | Ratio[2] |
| Male | M1 | 15 | 0.651 | 5 | 0.647 | 12 | 0.656 |
| | M2 | 5 | 0.761 | 9 | 0.769 | 12 | 0.752 |
| | M3 | 3 | 0.858 | 2 | 0.847 | 4 | 0.820 |
| | M4 | 2 | 0.958 | 3 | 0.923 | 5 | 0.958 |
| Female | F4 | 17 | 0.985 | 14 | 0.970 | 31 | 0.968 |

[1]: The shape of the abdomen found in *Grapsus albolineatus*: M1 is of normal males and M2-M4 of the parasitized males with different degrees of intersexuality. M4 is similar to normal females (F4) based on a classification scheme detailed by Weng (1987). M1 can also be defined as the ratio of the 7th to 5th sternum widths below 0.7, M2 is between 0.7 to 0.8, M3 is between 0.8 to 0.9 and M4 is above 0.9 in this study.

[2]: The average ratio of the 7th to 5th sternum widths was calculated as a quantitative index of intersexuality for each crab group.

Table 3. FA values in different leg segments of *Grapus albolineatus* at each study site.

| | Pa-Dor-Tze | Red-Ben | Wai-Mon-San | P value |
|---|---|---|---|---|
| n | 42 | 33 | 64 | |
| Leg 2 | | | | |
| merus | 0.25 ± 0.07 | 0.17 ± 0.04 | 0.20 ± 0.04 | 0.89 |
| carpus | 0.40 ± 0.08 | 0.26 ± 0.05 | 0.29 ± 0.04 | 0.48 |
| propus | 0.34 ± 0.09 | 0.29 ± 0.09 | 0.27 ± 0.06 | 0.89 |
| dactyl | 0.50 ± 0.12 | 0.33 ± 0.09 | 0.35 ± 0.08 | 0.54 |
| Leg 3 | | | | |
| merus | 0.46 ± 0.10 | 0.34 ± 0.13 | 0.32 ± 0.09 | 0.28 |
| carpus | 0.95 ± 0.18[A1] | 0.38 ± 0.10[B] | 0.42 ± 0.08[B] | <0.0001 |
| propus | 1.11 ± 0.24[A] | 0.44 ± 0.11[A,B] | 0.40 ± 0.09[B] | 0.004 |
| dactyl | 1.36 ± 0.28[A] | 0.47 ± 0.11[B] | 0.54 ± 0.11[B] | 0.001 |
| Leg 4 | | | | |
| merus | 0.66 ± 0.15[A] | 0.38 ± 0.16[A] | 0.31 ± 0.07[B] | <0.001 |
| carpus | 1.00 ± 0.24[A] | 0.59 ± 0.12[A,B] | 0.45 ± 0.09[B] | 0.009 |
| propus | 1.19 ± 0.29[A] | 0.66 ± 0.17[A,B] | 0.48 ± 0.13[B] | 0.01 |
| dactyl | 1.63 ± 0.42[A] | 0.70 ± 0.19[B] | 0.51 ± 0.14[B] | <0.001 |
| Leg 5 | | | | |
| merus | 0.43 ± 0.09[A] | 0.26 ± 0.05[A,B] | 0.22 ± 0.04[B] | 0.009 |
| carpus | 0.59 ± 0.15[A] | 0.31 ± 0.07[A,B] | 0.28 ± 0.04[B] | 0.042 |
| propus | 0.83 ± 0.20 | 0.43 ± 0.14 | 0.44 ± 0.10 | 0.08 |
| dactyl | 0.93 ± 0.27[A] | 0.40 ± 0.14[B] | 0.34 ± 0.10[B] | 0.016 |

[1] Different characters in the same row are significantly different by Tukey's multiple comparison test (P < 0.05).

weakly related to water pH (Ostbye et al., 1997). On the other hand, there were few FA studies to investigate the effects of water pollution on invertebrate. Clarke (1993) reported that FA was significantly higher in populations of the shrimp (*Palaemon elegans*) and the blood worm (*Chironomus salinarius*) from the waters near a fertilizer manufacturing facility compared to in those from control sites. Dover et al. (1999) found that aluminum concentration was positively related to FA in water striders (*Rheumatobates rileyi*), but pH was also positively related to FA in water striders. Hardersen (2000) observed an equivocal relationship between the FA in the wings of mature damselflies (*Xanthocnemis zealandica*) and the level of pesticides used in the field. Although the hypothesis of increasing FA in populations at stressful environment still needs more assessments from both field and laboratory studies, the result of this study does provide a good support for a positive correlation between environmental stress and FA. Further analyses, including chemical analyses of organic pollutants and heavy metals in water and crabs, are needed to determine the exact nature of this stress in Pa-Dor-Tze and laboratory-controlled experiments to establish possible cause-effect relationships between FA and this stress.

The finding of the largest average sizes in crabs collected from Pa-Dor-Tze in comparison with those from the other two sites is not too surprising. There are several evidences indicated that some marine molluscs grow larger in polluted area than in control area in relation to intensive organic pollution. For example, Fabrikant (1984) reported the average clam size increased as related to municipal waste effluent off the coast of Palos Verdes, California. Tablado et al. (1994) showed that the densities of *Siphonaria lessoni* in the

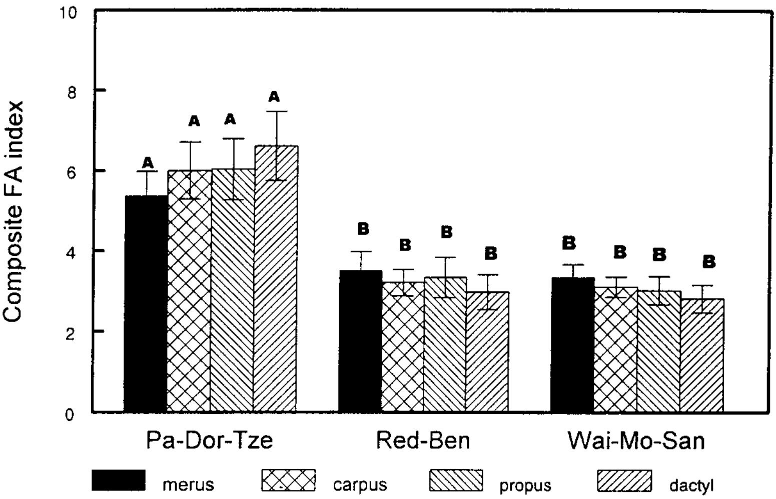


Figure 4. The composite FA indexes (mean ± SE) for specific leg segment by summing FA value of each specific leg segment from the 2nd to 5th legs of *Grapus albolineatus* in three locations in the northern coastal area of Taiwan. Bars with different characters are significantly different by Tukey's multiple comparison test ($P < 0.05$).

vicinity of a sewage outfall near Quequen, Argentina, were lower whereas the individual growth rates were higher than an unpolluted site. Liu and Morton (1998) found that two species of Hong Kong limpets from a heavily polluted site, Stonecutter Island in Victoria Harbor, showed significant larger individual size compared to those from three other relatively cleaner sites. The shore crab *G. albolineatus* collected in this study is known to feed primary on filamentous algae as well as organic matter whenever it is available (Kennish, 1996). Both a higher primary productivity and a lower intraspecific competition were proposed as two possible hypotheses explaining the accelerated growth of organisms in the polluted area (Tablado et al., 1994). Although the relative importance of these two factors could not be assessed in the present study, organic enrichment may be regarded as the possible cause of the larger size of *G. albolineatus* observed at Pa-Dor-Tze.

It is well known that male crabs infested with Sacculina often feminize in their external form, including changes in the form of the abdomen and the length of chelipeds (Giard, 1887; Reinhard, 1956; Weng, 1987; Takahashi and Matsuura, 1994). Parasites do affect abdomen morphology in male crabs collected from three study sites, but there are no differences in FA values of leg segments between normal and intersex males for each study site in the present study. While theory would suggest an association between parasites and FA, the conflicting evidence scattered in literature does not support such a relationship. A number of studies indicate a possible correlation between parasitism and FA in various invertebrates (Polak, 1993; Moller, 1995; Bonn et al., 1996; McLachlan et al., 1999). Some evidence, however, suggests that FA and parasitism are not always associated among different species of insects (Ward et al., 1998; Thomas et al., 1998). The inconsistenceies in currently available literature may be due to different morphological traits having different susceptibility to the effect of parasitism (Moller, 1996). Addition-

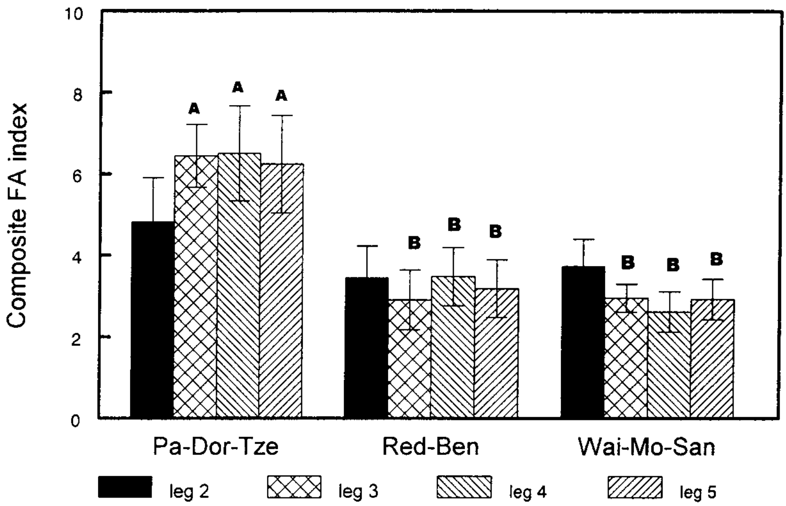


Figure 5. The composite FA indexes (mean ± SE) for a total of the merus, carpus, propus and dactyl segments in each walking leg of *Grapus albolineatus* in three locations in the northern coastal area of Taiwan. Bars with different characters are significantly different by Tukey's multiple comparison test ($P < 0.05$).

ally, the other main reason for the absence of a positive relationship between FA levels and parasitism in *G. albolineatus* may be due to small sample sizes in the present study.

As evidenced by degrees of intersexuality, the infection rate of Sacculina in male crabs was about 40–50 % lower in Pa-Dor-Tze than in either of the control sites, but was no significant difference among three sampling locations. Although it is often believed that environmental changes will affect parasites, the association between parasites and environmental degradation is relatively weak, and the relationship is still not very clear. In fact, environmental stresses can differ dramatically in their effects on parasites, and various parasites may also differ significantly in their susceptibility to environmental changes (Lafferty, 1997). For example, Lafferty (1997), basing his findings on an analysis of 163 comparisons collected from published studies on various environment factors and the effect on parasite numbers, suggests that eutrophication increased parasitism, while heavy metals and unspecified human disturbance reduced parasitism. However, it shall be noticed that the paucity of sites were studied in the present study. Before a defined relationship between pollution and Sacculina parasitism in crabs can be described, it need to conduct a well-define field study to investigate background levels of parasitism in *G. albolineatus* in the coastal area of Taiwan.

In sum, the result of this study shows that value of using FA of invertebrate populations as an early warning system to detect the presence of environmental stress. A composite index containing FA information across a number of characters provides more reliable and sensitive information than a single FA value for one character. The developmental instability approach may have considerable potential for using natural invertebrate population to detect environmental stress. More comprehensive studies should be performed to determine the suitable species and characters as useful biological indicators of environmental stress.

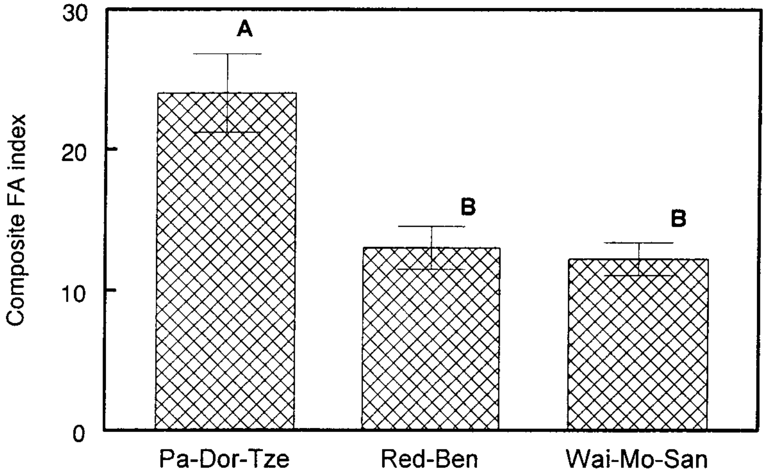


Figure 6. The composite FA indexes (mean ± SE) of all leg segments of *Grapus albolineatus* in three locations in the northern coastal area of Taiwan. Bars with different characters are significantly different by Tukey's multiple comparison test (P < 0.05).

## Acknowledgments

This work was supported by the National Science Council under grants NSC88-2621-B-002-009 and NSC89-2313-B-002-047. I thank S.-L. Yang (Beijing Natural History Museum) for his suggestion on parasite occurrences of *Gapsus albolineatus* and three anonymous reviewers for helpful comments on the manuscript. I am grateful to C.-C. Chang, C.-M. Chu and T.-Y. Tsai for their field assistance in collecting crab samples.

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DATE RECEIVED: June 12, 2000. DATE ACCEPTED: June 12, 2001.

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