Dynamics of metal adaptation in riverine chironomids.

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CHAPTER III

Fluctuating asymmetry and mentum gaps in populations of the midge *Chironomus riparius* (Diptera: Chironomidae) from a metal contaminated river

ABSTRACT

The developmental stability of both metal exposed and non-exposed *Chironomus riparius* populations from the lowland river Dommel was investigated using fluctuating asymmetry (FA) and the incidence of mentum gaps. It was hypothesised that larval development was affected by the influx of metals, directly by chemical stress, as well as through inbreeding of metal adapted and non-adapted specimens. Morphological parameters were, therefore, assessed in field collected larvae and in clean laboratory cultured, first generation (F1) larvae. FA-values and mentum gap incidence at contaminated field sites were significantly higher than at clean, upstream locations. Furthermore, FA-values of clean laboratory cultured F1 larvae fell to reference values mostly, indicating the direct effect of metal pollution on developmental aberrations. Mentum gaps were not observed in clean F1 cultures. Slightly elevated FA-values were, however, still observed in clean F1 generation larvae from polluted locations downstream from the metal input. This residual disturbance was thought to reflect genetic stress emerging from interbreeding between metal-adapted and non-adapted specimens. Fluctuating asymmetry and mentum gaps serve as a useful ecotoxicological marker for metal stress and that combined with in situ studies and F1 cultures allow for analysis of the response of animal populations to spatial and temporal gradients in metal exposure.
CHAPTER III

Introduction

Developmental disturbances in animal populations are of increasing concern, and deformities of both vertebrates (Herkovits et al 1997) and invertebrates (Canfield et al 1994; Janssens de Bisthoven 1995) are more and more used as ecotoxicological endpoints. However, developmental disturbances are not only caused by exposure to toxicants, but also by genetic stress (Leary & Allendorf 1989). The question arises how developmental stability is affected when populations are exposed to these two stressors at the same time. *Chironomus riparius* (Diptera; Meigen, 1804) offers the unique opportunity to study this, because populations of this chironomid in the lowland river Dommel, are subject to both types of stressors. Downstream from a distinct point source, this river is heavily contaminated with metals, especially cadmium and zinc. As a consequence, some of the *C. riparius* populations, originating from the metal-exposed field sites, have been shown to be less sensitive to cadmium compared to unexposed populations (Postma et al 1995a; 1995b). Because site-specific differences were studied using first generation laboratory reared animals, this cadmium-tolerance was assumed to have a genetic base.

Field measurements of drifting non-exposed larvae, a few metres upstream from the point source, showed a steady transport of *C. riparius* larvae into the metal-exposed midge populations (Groenendijk et al 1996). Because the distance between the cadmium-tolerant and non-tolerant midge populations in the vicinity of the point source, is limited to only a few hundred metres, the influence of drifting non-tolerant larvae on cadmium-tolerant midge populations just downstream, can be substantial (Postma & Groenendijk 1999). These drifting larvae normally represent a major part of the total gene flow compared to the minor dispersal of the short living and weak flying imagoes (Davies 1976). We hypothesise therefore, that midge populations downstream from the inlet of metal contaminated water suffer from both environmental stress (metal pollution) as well as intense crossbreeding of tolerant and non-tolerant midge larvae, which may involve genetic stress. To test this, the developmental stability of midge
populations was analysed using fluctuating asymmetry and the presence of mentum gaps as morphological markers of development.

Fluctuating asymmetry (FA) is defined as the occurrence of random differences between the left and right side of a normally bilateral symmetrical organism (Van Valen 1962). When normal developmental processes are affected by genetic stress, such as hybridization or increased homozygosity, or by environmental stress factors, such as pollution or declining habitat quality, FA-levels may increase (Parsons 1992; Clarke 1992). Relationships between the level of FA and environmental or genetic stress have been demonstrated in diverse animal species (Leary & Allendorf 1989; Zakharov & Yablokov 1989; Clarke 1992; Pankakoski et al. 1992). Therefore, it is suggested that FA-measurements might provide a reliable measure of overall population condition or fitness (Palmer & Strobeck 1992).

Mentum gaps were first described by Köhn & Frank (1980) and occur commonly in Chironomus spp larvae. The so called Köhn gaps are severe teratogenic deformities, which cover a relatively large epidermal area on the larval head capsule, and are easily recognised. These gaps are a result of the inhibition of epidermal tissue growth during long-term exposure to mixtures of toxicants (AC Vermeulen, personal communication). In field studies, mentum gaps have been correlated with contaminated sediments containing a mixture of pollutants (Köhn & Frank 1980; van Urk et al. 1992; Janssens de Bisthoven 1995; Vermeulen 1996).

In this study both methods, FA-measurements and counting of mentum gaps, were used to describe the developmental stability of C. riparius populations in the River Dommel. To distinguish between chemical and genetic stress, morphological parameters were not only assessed in field collected larvae, but also in clean laboratory cultured, first generation larvae. In addition, seasonal measurements of FA-values and mentum gap percentages were performed to study temporal variability of these morphological parameters.
Material and Methods

sampling sites

This study was conducted in the vicinity of a zinc factory of Union Minière, in northern Belgium, approximately 80 km north-east of Brussels (figure 3.1). This factory started producing zinc and cadmium from zinc ores in 1888. Yearly production during the 1980s was on average 120,000 t zinc year\(^{-1}\) and 600 t cadmium year\(^{-1}\). The zinc factory is situated on the banks of a small stream, the Eindergatloop, which enters the River Dommel near the village Neerpelt. The Dommel is a second order lowland stream fed by rainwater and is a tributary of the River Meuse. Metal contamination in the Dommel mainly originates from the Eindergatloop, but diffuse input of zinc from the catchment area is responsible for an elevated background of zinc (table 3.1 & 3.2). Average amounts of metals transported through the Dommel, downstream from the Eindergatloop, are 1-3 kg Cd day\(^{-1}\) and 50-200 kg Zn day\(^{-1}\), with a water flow between < 1 and 4.5 m\(^3\) s\(^{-1}\) (Postma 1995).

FIGURE 3.1: Location of the eight sampling sites. The River Dommel is indicated in detailed inset.
FLUCTUATING ASYMMETRY AND MENTUM GAPS

TABLE 3.1: Spatial distribution of metal concentrations in water and detritus (based on dry weight) from the eight sampling sites and the average value for all control (C1-C4) and polluted (P1-P4) sites. All sampling occurred on 16 September and 5 October 1994 simultaneously with collection of the chironomid larvae. The distance from the point source of metal pollution is given in kilometres for each sampling station. Minus signs indicate distances upstream from the point source of metal pollution.

<table>
<thead>
<tr>
<th>distance (km) from point source</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C1-C4</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P1-P4</th>
</tr>
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<tbody>
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<td>dissolved metals</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd (nM)</td>
<td>6.2</td>
<td>2.6</td>
<td>1.2</td>
<td>1.9</td>
<td>3.0</td>
<td>75.9</td>
<td>48.9</td>
<td>29.9</td>
<td>25.8</td>
<td>45.1</td>
</tr>
<tr>
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<td>2.1</td>
<td>1.3</td>
<td>1.4</td>
<td>2.1</td>
<td>7.0</td>
<td>5.2</td>
<td>4.4</td>
<td>3.7</td>
<td>14.0</td>
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<tr>
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<td>17.7</td>
<td>14.9</td>
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<td>13.5</td>
<td>15.0</td>
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<td>10.8</td>
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<td>59.8</td>
<td>88.6</td>
<td>37.8</td>
<td>68.5</td>
<td>112</td>
<td>389</td>
<td>77.2</td>
<td>67.7</td>
<td>161</td>
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<tr>
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<td>2.9</td>
<td>3.9</td>
<td>2.7</td>
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<tr>
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<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
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<td>4.2</td>
<td>5.6</td>
<td>2.3</td>
<td>4.0</td>
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<tr>
<td>Zn (µmol g⁻¹)</td>
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<td>22.4</td>
<td>30.6</td>
<td>56.9</td>
<td>33.5</td>
<td>134</td>
<td>176</td>
<td>184</td>
<td>91.7</td>
<td>146</td>
</tr>
<tr>
<td>Fe (mmol g⁻¹)</td>
<td>1.8</td>
<td>2.1</td>
<td>1.5</td>
<td>1.4</td>
<td>1.7</td>
<td>0.9</td>
<td>0.6</td>
<td>0.6</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Cu (µmol g⁻¹)</td>
<td>3.0</td>
<td>1.5</td>
<td>1.6</td>
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<td>2.3</td>
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<tr>
<td>Pb (µmol g⁻¹)</td>
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<td>0.4</td>
<td>0.5</td>
<td>1.1</td>
<td>0.7</td>
<td>3.7</td>
<td>5.8</td>
<td>3.0</td>
<td>2.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

Eight sampling stations were selected in the Dommel (figure 3.1). Four of them are reference locations situated upstream from the Eindergatloop: Peer, Control 1 (C1), 14.0 kilometres upstream (-14.0 km); Kleine Brogel (C2, -8.5 km); Bemvoortse molen (C3, -3.6 km) and Eindergatloop upstream (C4, -0.1 km). The remaining four sampling stations are situated downstream from the Eindergatloop: Neerpelt, Polluted 1 (P1, 0.5 km); Turfheide (P2, 3.1 km); Achterste brug (P3, 6.0 km) and Borkel (P4, 7.5 km).

Metal sampling and analyses

Water samples were collected from the middle of the stream using acid-washed polyethylene bottles at a depth of approximately 10 cm. These water samples were centrifuged for five minutes at 3000 r min⁻¹ and acidified thereafter. Samples of the top sediment layer were collected in acid-washed polyethylene bottles and were stored frozen prior to preparation. These samples, containing a mixture of sediment and organic material, were sieved using a mesh size of 0.6 mm. Thereafter, sand and other heavy particles were allowed to settle during 60 s. This procedure was repeated
twice. The resulting suspension was carefully suctioned off after an overnight stay. The particles sedimentating out of this suspension were collected and stored freeze-dried until analyses. This material will hereafter be referred to as detritus which is used as food by chironomids. Concentrations of metals in detritus will therefore, characterise the exposure of the different chironomid larvae better than metal concentrations in total sediment. Detritus samples were digested in HNO₃ (Baker Ultrex) using a microwave equipped with a temperature and pressure control programme. Water and detritus samples were analysed (n = 2-4) with Graphite Furnace Atomic Absorption Spectrometry (Perkin Elmer 5100) equipped with Zeeman background correction, or air-acetylene Flame Atomic Absorption Spectrometry (Perkin Elmer 1100B). Quality control of metal analyses was carried out by analysing destruction blanks and reference material (NIST: 2704 Buffalo River Sediment). Measured values agreed with certified values (less than 10% deviation) and destruction blanks were near detection limits. Ranges of metal concentrations in water and detritus for the different study sites are presented in table 3.1 & 3.2. All metal sampling was done on the same dates at all stations and were synchronously taken with the chironomid sampling.

**TABLE 3.2:** Ranges of metal concentrations in water and detritus (based on dry weight) from the C4, P1 and P4 location (n = 8). All sampling occurred simultaneously with collection of the chironomid larvae between January and May 1995.

<table>
<thead>
<tr>
<th>location</th>
<th>C4</th>
<th>P1</th>
<th>P4</th>
</tr>
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<tr>
<td><strong>dissolved metals</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cd (nM)</td>
<td>5.8-12.1</td>
<td>448-1664</td>
<td>305-854</td>
</tr>
<tr>
<td>Zn (µM)</td>
<td>1.6-4.7</td>
<td>13.1-20.9</td>
<td>11.1-17.7</td>
</tr>
<tr>
<td>Fe (µM)</td>
<td>1.6-12.4</td>
<td>0.2-12.8</td>
<td>0.4-26.4</td>
</tr>
<tr>
<td>Cu (nM)</td>
<td>42.5-132</td>
<td>77.1-197</td>
<td>96.0-182.7</td>
</tr>
<tr>
<td><strong>detritus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd (µmol g⁻¹)</td>
<td>0.12-0.24</td>
<td>1.8-4.1</td>
<td>2.2-9.0</td>
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<tr>
<td>Zn (µmol g⁻¹)</td>
<td>24.7-40.5</td>
<td>53.6-106</td>
<td>50.1-120</td>
</tr>
<tr>
<td>Fe (mmol g⁻¹)</td>
<td>0.9-1.9</td>
<td>0.8-1.4</td>
<td>0.8-1.5</td>
</tr>
<tr>
<td>Cu (µmol g⁻¹)</td>
<td>2.3-4.2</td>
<td>3.0-6.5</td>
<td>2.2-5.6</td>
</tr>
</tbody>
</table>
chironomid sampling and preparation

Fourth instar larvae were collected on 16 September and 5 October 1994 at all eight study sites. The reference location C4 and the two polluted sites P1 and P4 were then visited six more times between January and May 1995. However, due to emergence and as a consequence low larval densities, almost no larvae were present during March and April on the C4 and P1 site, resulting in a few missing values. Sampling took place in sedimentation areas of the Dommel, which are a suitable habitat for C. riparius. The upper 5 cm mud layer was scraped over several metres, using nylon nets with a mesh size of 300 μm. Sediment was sieved (400 μm) in the laboratory and larvae belonging to the genus Chironomus were collected. For every sampling location 50-100 chironomid larval heads were removed and placed in a circa 10% potassium hydroxide solution, for at least 12 hours to clear muscle tissues. Cleared heads were then neutralised in glacial acetic acid and placed in 96% alcohol prior to mounting. Heads were mounted in a synthetic resin called Euparal, ventral side up, on glass microscope slides. The preparation method used was slightly modified from Clarke (1993).

culturing chironomids

Field sampled larvae, collected simultaneously with those used for FA-analysis in September and October 1994, were cultured in the laboratory using plastic aquaria, filled with circa 5 liters of clean, synthetic water. All aquaria were equipped with a flight cage on top. Larvae were kept in clean, fine sand (grain size < 300 μm) and were fed a suspension of 10.0 g ground Trouvit and 0.5 g Tetraphyll® in 200 ml water. After emerging, egg masses were collected and allowed to hatch in clean water. Male Chironomus spp imagoes were collected randomly from each location and all identified as C. riparius using the key of Pinder (1978). The first generation of each population was cultured in triplicate, using plastic aquaria supplied with a 1 cm layer of shredded paper as substrate and four liters of clean, synthetic water. The overlying water was aerated constantly and a cage was placed on top of each aquarium. These first generation cultures were started by adding
50 newly hatched first instar larvae to each aquarium. All culturing was done in a controlled climate room at 20 °C ± 1. A 16:7 h light:dark regime was provided, with a dimmed light period of 30 minutes before and after the light period to stimulate reproduction. The water was renewed once a week and larvae were fed twice a week during the experiment with 2.5 ml of suspended food. The above described culturing method rules out environmental stressors like metal pollution and the incidence of mentum gaps and the level of FA, therefore, is thought to reflect genetic stress. After emergence, head capsules of moulted fourth instar larvae were collected and treated identically as the field collected animals.

**fluctuating asymmetry analyses**

The character chosen for FA-analysis was the number of teeth on the pecten epifaryngis, situated at the anterior part of the larval head capsule. Preparations were analysed at 600 times magnification using light microscopy. The number of mentum gaps was counted simultaneously and all counting was done independently by two persons. Repeated measurements of four randomly chosen populations showed that deviations from previous measurements were less than 10%. For each location, asymmetry values were calculated as the sum of the squared differences between the number of teeth left \( L_i \) and right \( R_i \), divided by the number of individuals \( N \) counted:

\[
FA = \frac{\sum (L_i - R_i)^2}{N}
\]

Following Palmer & Strobeck (1986), preliminary steps were taken to investigate whether the conditions for FA-analysis were met. The first step was to examine if directional asymmetry and antisymmetry occurred in the samples. Directional asymmetry occurs when a character on one side of individuals normally has a larger value than its counterpart; eg right gonads in humans are normally larger than those on the left. Antisymmetry is the situation in which asymmetry is the norm, but the side containing the larger character varies; as is the case with the larger signal claw of male fiddler...
crabs (*Uca* spp) (Leary & Allendorf 1989). To check for these characteristics, the frequency of the signed differences between left and right of all populations was plotted in histograms for each sampled population separately. No significant deviations from normal distribution could be traced, indicating that directional asymmetry and antisymmetry did not occur in the pecten epifaryngis of *C. riparius*. The second step was to examine whether the character size did not differ among sampled populations. A small but significant difference was found among the mean character size of all samples counted (Kruskal-Wallis; p < 0.05). However, the observed size variation (S) was relatively small compared to the level of FA (S/√FA < 15). According to Palmer & Strobeck (1986) it was not necessary to scale FA-measurements by character size.

Bartlett's test is the most efficient test for detecting differences among samples, because the index used to calculate FA-values is a variance, and more than two samples are analysed (Palmer & Strobeck 1986). Relevant pairwise comparisons were made using F-tests. Loss of larval head capsules during preparation and missing structures on the glass slides made it impossible to count all the originally mounted 100 individuals. FA-values were only calculated if sample sizes remained larger than 25 counted individuals, because pilot studies showed that this is the minimum number for a reliable FA-calculation.

Analysis of mentum gap frequencies was carried out analysing homogeneity among sample sites tested for goodness of fit. All values of the calculated heterogeneity (Gₜ) equal to or greater than the critical $X^2_{0.05}$ value were considered significant (Sokal & Rohlf 1995). It was tested if a correlation existed between both mentum gap incidence and fluctuating asymmetry values and concentrations of cadmium and zinc in water and detritus (table 3.1 & 3.2) using Spearman's coefficient of rank correlation ($r_s$) (Sokal & Rohlf 1995).
Results

spatial variation

A highly significant difference in FA-values among sampling locations was found (Bartlett; $X^2 = 38.96$, $p < 0.001$) (figure 3.2A). The FA-value at the four control sites varied between 0.68 and 1.28. FA-values at polluted locations were in all cases higher and varied between 1.40 and 1.91. Especially the increase between the C4 and P1 site should be noted, because this increase ($F = 1.48; p < 0.10$) takes place at a distance of only a few hundred metres. Consequently, a significant positive correlation was found between FA-levels and cadmium and zinc concentrations in water and detritus ($0.59 < r_s < 0.64; p < 0.01$).

![Figure 3.2](image)

**FIGURE 3.2:** Values of fluctuating asymmetry (FA), $\Sigma (L_i - R_i)^2/N$, of the pecten epifaryngis and sample sizes of *Chironomus riparius* at eight sites in the River Dommel collected in September and October 1994. C1-C4 represent reference sites (open bars) and P1-P4 metal polluted locations (hatched bars). The distance from the point source of metal contamination is given in parentheses at the bottom in kilometres. Minus signs indicate distances upstream from the point source of metal pollution. Panel A shows FA-values for field-collected larvae, panel B for clean cultured first generation larvae; nd = not determined. Because the index used to calculate FA-values is a variance itself, no error bars can be presented.
Figure 3.2B shows the FA-values and sample sizes for clean, laboratory cultured C. riparius larvae (F1). The number of cultured F1 larvae from the C1 location was not high enough (n < 25) for an appropriate estimation of the FA-index. Furthermore, culturing field larvae from the C3 site failed, resulting in a missing FA-value. For the remaining two reference F1 populations, FA-values were equal to the parental field populations. Three clean cultured F1 generations from polluted sites showed FA-values circa 0.6 units lower than the parental populations, and only in larvae from the P2 location the FA-value remained the same. Consequently, no differences from homogeneity among populations could be detected for the F1 generation of C. riparius larvae (Bartlett; $X^2 = 5.08, p > 0.05$). On the other hand, it should be noted that the first two polluted locations (P1 and P2) still showed the highest FA-values and the increase between the C4 and the P1 location remained visible.

![FIGURE 3.3: Mentum gap frequencies and sample sizes of Chironomus riparius at eight sites in the River Dommel collected in September and October 1994. C1-C4 represent reference sites (open bars) and P1-P4 metal polluted locations (hatched bars). The distance from the point source of metal contamination is given in parentheses at the bottom in kilometres. Minus signs indicate distances upstream from the point source of metal pollution. No mentum gaps were found in clean cultured first generation larvae.](image)

Mentum gap incidence at control locations varied between 0 and 3.1% (figure 3.3). Mentum gap percentages at polluted locations were in all cases higher and varied between 5.6 and 7.9%. Consequently, a significant
difference in mentum gap percentages among sample locations was detected
($G_H = 15.79, p < 0.05$) and a positive correlation with cadmium and zinc
concentrations in water and detritus was found ($0.56 < r_s < 0.58; p < 0.01$). In
contrast, no mentum gaps at all were found in a total number of 641 clean
cultured first generation larvae from upstream control sites nor from
polluted downstream sites.

temporal variation

At the upstream C4 location relatively constant FA-values were found,
varying between 0.90 and 1.34 (total index: 1.15), with no significant
differences among sampling dates (Bartlett; $X^2 = 1.68, p > 0.05$) (figure 3.4).
Although at the polluted P1 and P4 sites, the variation in FA-values was
higher (P1: 1.16-2.31; P4: 1.22-1.92), again no temporal differences in FA-
values could be traced (P1: Bartlett; $X^2 = 4.95, p > 0.05$ and P4: Bartlett; $X^2 =
3.94, p > 0.05$). Among locations however, a highly significant difference was
found in FA-values based on the average seasonal data (Bartlett; $X^2 = 9.33,$
$p < 0.01$), despite the almost identical values in February (figure 3.4). This
difference was found to be significantly correlated with cadmium and zinc
concentrations in water and detritus ($0.59 < r_s < 0.64; p < 0.01$).

![FIGURE 3.4: Temporal variation in values of fluctuating asymmetry (FA), $\Sigma(L-R)_i^2/N$, of
the pecten epifaryngis in three subpopulations of *Chironomus riparius* in the River
Dommel in 1995. Average FA-values based on the pooled seasonal data (sample size
C4: n = 234; P1: n = 176; P4: n = 300) are presented in the right part of the figure. The
shaded rectangle shows the period during which record high water discharge in the
River Dommel was recorded. Because the index used to calculate FA-values is a
variance itself, no error bars can be presented. The dashed lines are for guidance of
the eye only. No midge larvae were present in spring on the C4 (April) and the P1 site
(late March and April).]
Temporal variation in mentum gap frequencies at the three selected sampling locations is shown in Figure 3.5. At the reference site C4, mentum gap percentages varied between 0 and 6.5% (average: 3.4%). At both the polluted locations mentum gaps occurred more frequently, varying for the P1 site between 4.3 and 17.2% (average: 10.3%) and for the P4 site between 3.4 and 8.6% (average: 6.6%). Consequently, a good correlation between mentum gap incidence and metal concentrations in water and detritus was detected ($0.56 < r_s < 0.58; p < 0.01$) and a significant difference in mentum gap frequencies was found among sites, based on the average temporal data ($G_{H} = 13.79, p < 0.01$). However, on the first sample date in March, mentum gap incidence was almost identical at all three sample locations (Figure 3.5).

**FIGURE 3.5:** Temporal variation in mentum gap frequencies in three subpopulations of *Chironomus riparius* in the River Dommel in 1995. Average mentum gap percentages based on the pooled seasonal data (sample size C4: $n = 408$; P1: $n = 300$; P4: $n = 499$) are presented in the right part of the figure. The shaded rectangle shows the period during which record high water discharge in the River Dommel was recorded. The dashed lines are for guidance of the eye only. No midge larvae were present in spring on the C4 (April) and the P1 site (late March and April).

**Discussion**

**Mentum gaps**

There is a distinct, positive correlation between the incidence of mentum gaps in midge larvae and their occurrence upstream and downstream from the point source of metal pollution in the River Dommel. Mentum gaps are
reported after exposure in the field to mixtures of different toxicants (Vermeulen 1995). Several studies indicated that metals are at least one of the toxicant classes which are correlated with the occurrence of mentum gaps in field collected *Chironomus* spp larvae (Köhn & Frank 1980; van Urk et al 1992; Janssens de Bisthoven 1995; Dickman & Rygiel 1996; Vermeulen et al 1996). Therefore, the factor responsible for the increased mentum gap frequencies in midge larvae from the River Dommel, is most likely the high concentration of metals at polluted sites.

In contrast, in first generation midge larvae, reared under clean, laboratory controlled conditions, mentum gaps were totally absent both in reference originated as well as in polluted originated midge cultures (total n = 641). This observation makes clear that the occurrence of mentum gaps in *C. riparius* larvae is not related to a genetic heritability of this deformity, but only to the pollution history of the larvae. Therefore, mentum gaps in field studies could be used as an ecotoxicological marker for exposure to pollution. In addition, the incidence of mentum gaps characterises the fate of dislodged animals. It is hypothesised that migration of larvae from upstream sites will influence the chironomid population downstream from the zinc factory. Settlement of chironomid larvae with low FA-values or non-damaged mentums in downstream areas, will lower the overall FA-level and mentum gap incidence in polluted downstream subpopulations of *C. riparius*. Indications for the role of larval drift can be found in the present study, because equivalent low values for mentum gaps (but also for FA) were recorded in late winter at both the polluted locations P1 and P4 as well as the reference C4 site. These low values concur with high water discharges of the River Dommel during late winter 1995, when North-Western Europe was under the influence of heavy rainfall. During the resulting spate, mass transport of fourth instar larvae has been found (Groenendijk et al 1996). This transport could explain the drop in levels of both morphological parameters at both polluted sites to values normal for reference locations.
fluctuating asymmetry

Our results clearly demonstrate a positive correlation between FA-values and the occurrence upstream and downstream of the point source of metal pollution in the River Dommel. In agreement, Pankakoski et al. (1992) observed a correlation between the level of metal contamination in soils and FA in skulls of Common Shrews (Sorex araneus). Analogously, Zakharov & Yablokov (1990) found a relationship between skull asymmetry in the Baltic Grey Seal (Halichoerus grypus) and the level of pollution, mainly DDT and PCBs. However, chemical stress is not always expressed in asymmetry (Rabitsch 1997). In addition, McKenzie & Clarke (1988) have shown that the FA-levels of a pure breeding insecticide-resistant strain of Sheep Blowfly (Lucilia cuprina) were equivalent to those of a susceptible laboratory strain. This observation suggests that, due to a long exposure time, genetically stable diazinon-resistant populations had developed, with consequently low FA-values. Because Postma et al. (1995a) demonstrated that C. riparius larvae from at least two of the studied polluted sites, the P1 and P4 location, were genetically adapted to cadmium, it was expected that equivalent low FA-values should exist in midge populations sampled at exposed sites. However, developmental stability was clearly still decreased in metal-exposed midges, based on high FA-values in field collected larvae. These results suggest that metal-tolerance in C. riparius in the River Dommel is not stable or sufficient to avoid morphological disorders.

Indications for the instability of these midge populations can be found in the FA-values of first generation, clean cultured chironomids. These culturing methods ruled out environmental stressors like metal pollution and consequently, FA-values represent estimations of the genetic part of the stress factors present in the C. riparius populations. The observations showed that FA-values of clean cultured larvae, are in most cases lowered compared to FA-levels of field collected chironomids, but values for polluted sites were still increased compared to values from control localities. Elevated FA-values, even after culturing the F1 under clean conditions, indicate a genetic stress component. In the present study, such elevated FA-values were found specifically at polluted sites just downstream of the metal...
source. Because the distance between the most upstream polluted (P1) and the most downstream non-polluted (C4) location is only a few hundred metres, it is suggested that non-tolerant larvae from sites located upstream can easily reach polluted sites further downstream (Groenendijk et al. 1996) and interbreed later with local midges. When this interbreeding between non-adapted, upstream specimens and metal-adapted C. riparius takes place regularly, as suggested by the population dynamics (Groenendijk et al. 1996), the process of adaptation will frequently be disrupted. Such disruptions can affect developmental stability of animal populations, resulting in elevated FA-values (McKenzie & Clarke 1988; Leary & Allendorf 1989). Therefore, drift of non-tolerant C. riparius individuals, reaching the adapted populations in the polluted zone can be responsible for the elevated FA-values in the clean cultured, first generation midges of the most upstream located metal-exposed sites. However, in situ, this effect will be overruled by the developmental stability of larvae (increased FA-values), caused by the chemical stress directly.

conclusions
1) Fluctuating asymmetry and mentum gap incidence in C. riparius consistently reflect the chemical stress of metal contamination in the River Dommel.
2) The combined use of fluctuating asymmetry and mentum gaps in parental as well as in first generation midge populations can be used to discriminate between environmental and genetic stress factors in C. riparius. Therefore, we propose this combination of techniques as an ecotoxicological biomarker for coinciding chemical and genetic stress.

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