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Changes in Intracellular Free Amino Acids in Tissues of the Caged Mussel, *Elliptio complanata*, Exposed to Contaminated Environments

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Abstract. Intracellular tissue concentrations of free amino acids (FAA) were monitored in caged mussels (*Elliptio complanata*) exposed *in situ* for 27–29 days and 77–79 days in the Yamaska River watershed (Quebec, Canada). Total concentrations of FAA (nmol/mg wet weight) increased in both mantle and adductor muscle tissue at several sites impacted by agricultural runoff and urban effluent from municipal sewage and light industries when compared to levels in mussels located at a site with little anthropogenic impact. Consistent changes in the per cent composition of individual FAA to the total FAA pool included decreases in serine, threonine, glycine and valine as well as increases in glutamic acid and glutamine at 27–29 days but results were not consistent with longer exposure and varied amongst sites. Few changes in total or individual FAA were observed in gill tissue. The results suggest that increases and/or decreases in total FAA in some tissues of freshwater bivalves may be indicative of generalized stress induced by a variety of environmental factors and may be useful as an *in situ* biochemical index of toxicity.

A basic premise of toxicology is that all toxic effects in living organisms begin with a reaction between the toxic chemical and some biochemical receptor (Dixon *et al.* 1985). Therefore, the earliest and most sensitive indication of a toxic response in organisms exposed to chemical contaminants should be measured by the detection of biochemical changes at the molecular and cellular level of organization (Graney and Giesy 1988). In the aquatic environment, numerous biochemical indices of stress have been proposed for their potential use in the assessment of the "health" of populations, communities and ecosystems exposed to contaminants (Bayne *et al.* 1985). Various studies of marine invertebrates, particularly molluscs, have reported that total tissue concentrations of free amino acids (FAA) either increase or decrease in response to natural and anthropogenic stresses such as changes in salinity (Matsushima 1988), anoxia (Powell *et al.* 1982), starvation, temperature and exposure to toxic substances (Jeffries 1972; Roesijadi and Anderson 1979; Briggs 1979; Carr and Linden 1984; Kasschau and Howard 1984). In contrast to marine invertebrates, there have been relatively few studies on the patterns of FAA in the tissues of freshwater organisms exposed to stress (Gardner *et al.* 1981; Graney and Giesy 1988). Alterations in FAA may be a more sensitive indication of toxicant exposure than more traditional measures of effects (respiration) but more research, especially *in situ* studies, are necessary before a decision can be made on the usefulness of FAA concentrations as a biochemical index of stress.

The objective of this research was to monitor the FAA pool of the freshwater mussel, *Elliptio complanata*, exposed *in situ*, in cages, to a variety of stressful environments in tributaries of the Yamaska River watershed, Quebec, Canada and to determine if changes occur in the FAA pool as a consequence of stress. The Yamaska River basin is contaminated with a number of chemical pollutants including metals (Tessier *et al.* 1980; Croteau *et al.* 1984), nutrients from both municipal sewage outfalls and agricultural runoff (Campbell *et al.* 1976), pesticides (Muir *et al.* 1978) and various industrial pollutants (Auger *et al.* 1979).

Material and Methods

Study Area

The Yamaska River watershed (45°05′ and 46°05′N latitude and 72°12′ and 73°07′W longitude) is comprised of a number of tributaries draining a basin of approximately 4843 km² in southeastern Quebec towards Lake St. Peter, a widening of the St. Lawrence River between the cities of Montreal and Quebec (Figure 1). The control site, #35, was located in an rural area with low population (<1000), little agriculture, no industry and light sewage input. The agricultural sites, nos. 30, 31, 32, 33, and 34, also had low populations (<1000) but the % land under agriculture ranged from 14–55% and included extensive corn production as well as pig and cattle farming. Sites nos. 4, 8, and 37 were located in areas with medium population densities (2,500–10,000) and low agriculture but with significant inputs from industry (textile factories, electroplating plants) and untreated sewage from the towns of Valcourt, Waterloo, and Cowansville. Site no. 5 was downstream from the town of Granby (population >40,000) with industrial inputs from at least six textile manufacturers as well as sewage input from the municipal primary treatment plant.

Physical parameters i.e., depth (cm) and velocity (cm/sec) of the water, temperature (°C) and conductivity (μmhos/cm), were mea-

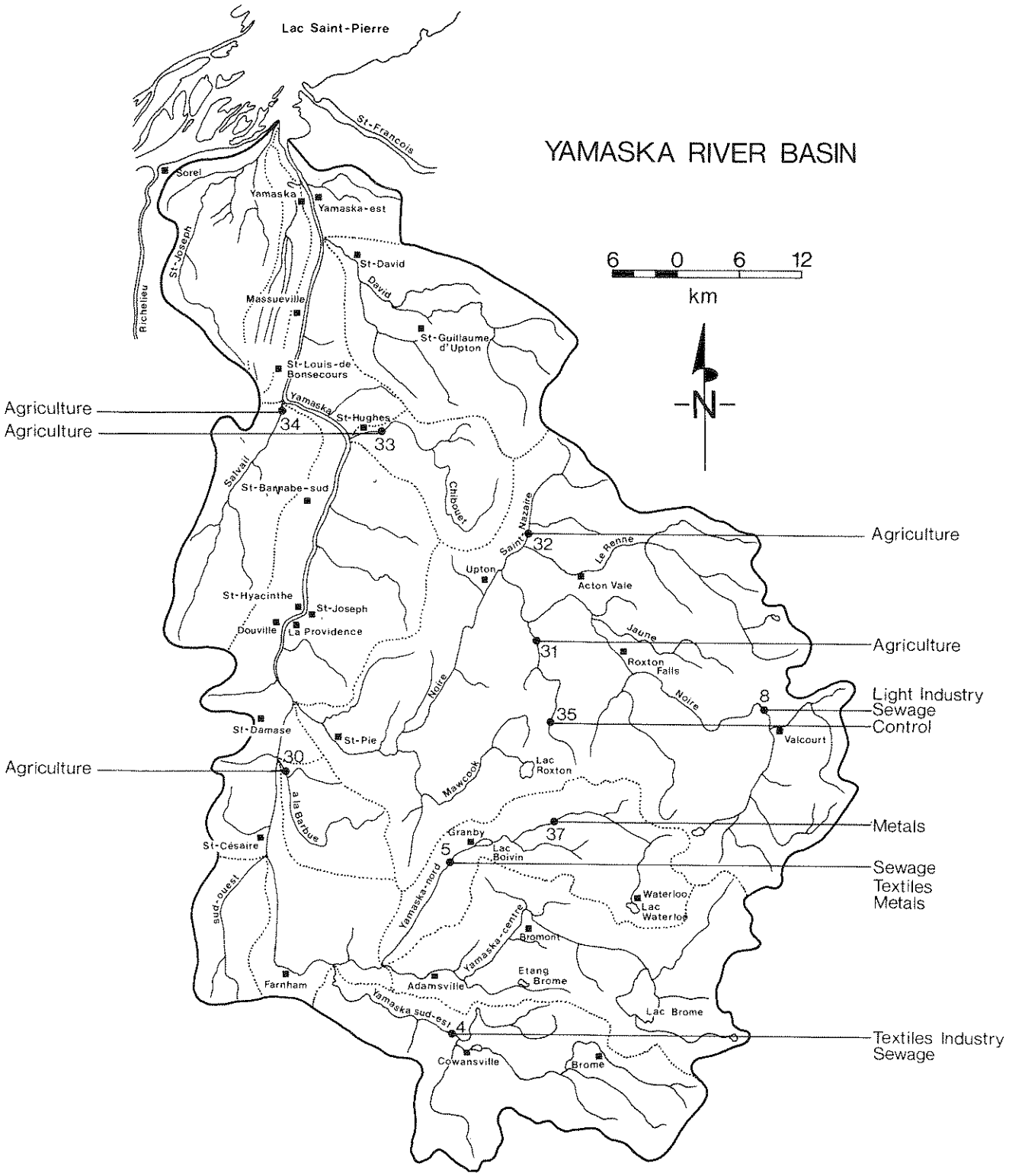

Fig. 1. Study sites for caged mussels and benthic invertebrate samples.

sured at each study site just prior to the placement of the caged mussels and once again during the experiment (August 1987). Suspended sediment was determined at each site by filtering 3–4 L of water through preweighed GF/A filters (15 cm), air-drying the filters and reweighing them. In addition, dissolved oxygen (YSI Model 54), alkalinity, particulate and dissolved organic carbon, major ions (Ca, Mg, Na, Cl), nutrients (NH_3N, NO_3NO_2, total phosphorus) and trace metals (Al, Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) in water were monitored at each study site at least once during the exposure period. Samples were collected and analyzed according to standard

Table 1. Concentration (nmol/mg wet weight) and per cent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 27–29 days

Amino Acid	35 (Control)		4		5		8		30		31	
Alanine	0.56[a]	(0.14)	0.84	(0.21)	0.44	(0.04)	0.50	(0.14)	0.69	(0.04)	0.74	(0.10)
	23.2[b]	(3.3)	21.8	(3.8)	19.2	(1.2)	25.1	(3.4)	22.8	(4.3)	22.0	(1.1)
Glutamic Acid	0.16	(0.06)	0.31	(0.08)	0.75	(0.06)	0.16	(0.02)	0.41	(0.09)	0.46	(0.13)
	6.7	(1.5)	8.1	(0.3)	32.0[e]	(2.1)	8.5	(2.0)	13.0[e]	(1.2)	13.8[e]	(4.6)
Glycine	0.29	(0.03)	0.42	(0.12)	0.19	(0.02)	0.28	(0.10)	0.25	(0.05)	0.28	(0.09)
	12.5	(3.2)	10.8	(1.9)	7.5[d]	(0.7)	13.7	(1.4)	8.0[d]	(0.6)	8.3[d]	(2.0)
Serine	0.27	(0.04)	0.43	(0.12)	0.17	(0.06)	0.26	(0.10)	0.2[b]	(0.08)	0.25	(0.06)
	11.3	(1.4)	11.0	(0.6)	6.7[d]	(1.8)	13.0	(1.0)	8.3[d]	(1.4)	7.3[d]	(1.2)
Arginine	0.26	(0.17)	0.21	(0.12)	0.17	(0.06)	0.09	(0.03)	0.22	(0.02)	0.23	(0.06)
	10.1	(5.1)	5.2	(2.2)	6.5	(2.1)	4.6	(0.2)	7.3	(0.5)	7.0	(2.0)
Threonine	0.19	(0.02)	0.29	(0.05)	0.12	(0.02)	0.18	(0.04)	0.22	(0.03)	0.16	(0.02)
	8.1	(1.4)	7.7	(1.0)	4.9[d]	(0.5)	9.2	(0.8)	7.2	(0.6)	4.8[d]	(0.6)
Valine	0.15	(0.02)	0.25	(0.06)	0.10	(0.02)	0.13	(0.04)	0.17	(0.04)	0.12	(0.02)
	6.1	(1.1)	6.4	(0.8)	4.1	(0.5)	6.7	(0.5)	5.3	(0.4)	3.6[d]	(0.5)
Glutamine	0.07	(0.01)	0.17	(0.08)	0.05	(0.01)	0.04	(0.03)	0.18	(0.05)	0.63	(0.16)
	2.8	(0.2)	4.0	(1.1)	1.9[d]	(0.3)	2.1	(1.6)	5.6[e]	(0.8)	18.9[e]	(5.0)
Proline	0.10	(0.02)	0.18	(0.03)	0.07	(0.01)	0.09	(0.02)	0.14	(0.02)	0.10	(0.05)
	4.1	(0.2)	4.6	(0.9)	4.4[d]	(2.4)	4.7	(0.6)	4.6	(1.3)	3.0	(1.2)
Isoleucine/Leucine	0.20	(0.05)	0.50	(0.16)	0.20	(0.04)	0.19	(0.13)	0.32	(0.09)	0.20	(0.06)
	7.5	(0.8)	12.6[e]	(1.5)	8.0	(1.3)	8.6	(3.5)	10.1	(1.3)	5.2	(1.3)
Others[c]	0.16	(0.02)	0.30	(0.16)	0.14	(0.05)	0.05	(0.03)	0.25	(0.08)	0.18	(0.06)
	7	(0.1)	7.4	(2.5)	4.6	(0.6)	2.1	(1.0)	8.1	(1.9)	5.2	(1.3)
Totals	2.42	(0.39)	3.89[e]	(1.00)	3.30	(1.64)	2.00	(0.64)	3.10	(0.48)	3.36	(0.26)

[a] Concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] Per cent contribution to total FAA pool
[c] Includes aspartic acid, asparagine, methionine and phenylalanine
[d] Significantly decreased from control ($P \leq 0.05$)
[e] Significantly increased from control ($P \leq 0.05$)

Table 2. Concentration (nmol/mg wet weight) and per cent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 77–79 days

Amino Acid	35		4		5		32		33		37	
Alanine	0.52[a]	(0.03)	0.87	(0.08)	0.43	(0.19)	1.56	(0.29)	0.91	(0.22)	0.92	(0.07)
	27.8[b]	(3.2)	24.9	(0.4)	25.1	(3.8)	21.8	(1.8)	24.3	(1.0)	25.1	(1.8)
Glutamic Acid	0.16	(0.03)	0.27	(0.02)	0.17	(0.05)	0.55	(0.09)	0.30	(0.09)	0.29	(0.20)
	8.4	(1.0)	7.7	(1.1)	10.0	(2.0)	7.6	(0.5)	8.0	(1.1)	7.9	(1.2)
Glycine	0.20	(0.05)	0.43	(0.04)	0.20	(0.08)	0.74	(0.09)	0.42	(0.10)	0.42	(0.05)
	10.4	(2.0)	12.3	(2.2)	12.3	(1.9)	10.4	(1.4)	11.2	(0.3)	11.3	(0.4)
Serine	0.17	(0.03)	0.33	(0.02)	0.17	(0.05)	0.72	(0.09)	0.37	(0.08)	0.36	(0.06)
	9.1	(1.5)	9.6	(1.0)	10.4	(2.1)	10.0	(0.4)	10.0	(2.0)	9.8	(2.4)
Arginine	0.11	(0.02)	0.23	(0.18)	0.03	(0.02)	0.34	(0.06)	0.17	(0.04)	0.13	(0.03)
	5.7	(1.3)	6.2	(4.2)	1.4[d]	(0.9)	4.8	(1.1)	4.4	(0.2)	3.7	(0.8)
Threonine	0.16	(0.02)	0.26	(0.03)	0.15	(0.05)	0.50	(0.06)	0.27	(0.07)	0.26	(0.03)
	8.3	(1.2)	7.5	(0.8)	9.6	(0.9)	7.0	(0.2)	7.1	(0.4)	7.1	(0.4)
Valine	0.10	(0.01)	0.20	(0.01)	0.11	(0.03)	0.47	(0.07)	0.22	(0.06)	0.24	(0.04)
	5.4	(0.5)	5.7	(0.4)	6.4	(1.0)	6.6	(0.1)	5.8	(0.5)	6.4	(0.5)
Glutamine	0.05	(0.01)	0.11	(0.03)	0.04	(0.01)	0.27	(0.04)	0.17	(0.09)	0.16	(0.06)
	2.6	(0.2)	3.1	(0.7)	2.5	(0.6)	3.8	(0.3)	4.3	(1.5)	4.2	(1.3)
Proline	0.16	(0.08)	0.19	(0.09)	0.07	(0.03)	0.42	(0.07)	0.22	(0.06)	0.20	(0.04)
	8.3	(4.0)	5.4[d]	(0.6)	4.4[d]	(0.7)	5.9	(0.4)	5.9	(0.2)	5.4	(0.8)
Isoleucine-Leucine	0.16	(0.04)	0.38	(0.07)	0.13	(0.05)	1.00	(0.14)	0.44	(0.18)	0.41	(0.07)
	8.4	(1.7)	10.7	(1.9)	7.7	(1.2)	13.9[e]	(0.3)	11.4	(2.3)	11.0	(1.8)
Others[c]	0.14	(0.04)	0.24	(0.05)	0.04	(0.02)	0.58	(0.15)	0.27	(0.08)	0.31	(0.11)
	7.4	(2.0)	6.8	(1.0)	2.6[d]	(0.6)	8.5	(1.1)	8.5	(2.4)	8.3	(2.4)
Totals	1.89	(0.16)	3.49[e]	(0.36)	1.54	(0.56)	7.15[e]	(0.95)	3.76[e]	(0.97)	3.68[e]	(0.33)

[a] Concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] Percent contribution to total FAA pool
[c] Includes aspartic acid, asparagine, methionine and phenylalanine
[d] Significantly decreased from control ($P \leq 0.05$)
[e] Significantly increased from control ($P \leq 0.05$)

Table 1. (cont'd)

32		33		34		37	
0.49	(0.12)	0.97	(0.24)	0.88	(0.17)	0.84	(0.23)
22.4	(4.3)	25.0	(7.0)	26.8	(3.1)	22.7	(1.1)
0.27	(0.05)	0.47	(0.06)	0.26	(0.03)	0.34	(0.09)
12.4[e]	(0.5)	11.8[e]	(1.1)	7.9	(1.0)	9.1	(2.0)
0.22	(0.06)	0.31	(0.05)	0.34	(0.03)	0.43	(0.12)
9.8	(1.7)	7.7[d]	(1.3)	10.6	(2.1)	11.7	(0.5)
0.20	(0.08)	0.33	(0.07)	0.30	(0.02)	0.40	(0.11)
9.0	(2.0)	8.3[d]	(1.6)	9.1	(0.3)	10.8	(1.4)
0.22	(0.10)	0.33	(0.13)	0.24	(0.06)	0.21	(0.05)
10.7	(5.9)	8.2	(3.1)	7.2	(1.2)	5.8	(2.2)
0.17	(0.06)	0.31	(0.03)	0.25	(0.03)	0.28	(0.08)
7.6	(1.3)	7.8	(0.6)	7.7	(1.0)	7.5	(0.9)
0.12	(0.04)	0.18	(0.02)	0.18	(0.02)	0.24	(0.07)
5.3	(0.9)	4.5	(0.5)	5.5	(0.6)	6.3	(0.8)
0.06	(0.01)	0.28	(0.05)	0.12	(0.03)	0.16	(0.04)
2.7	(0.3)	7.2[e]	(1.3)	3.4	(0.4)	4.4	(0.9)
0.08	(0.03)	0.21	(0.11)	0.23	(0.07)	0.17	(0.06)
3.4	(1.0)	5.4[d]	(3.1)	7.1	(2.0)	4.5	(0.6)
0.23	(0.08)	0.30	(0.10)	0.27	(0.02)	0.41	(0.12)
10.1	(2.0)	7.2	(2.5)	8.1	(1.5)	11.1	(1.1)
0.15	(0.02)	0.28	(0.02)	0.22	(0.08)	0.26	(0.10)
6.9	(0.9)	7.1	(0.5)	11.5	(5.4)	7.0	(2.6)
2.21	(0.39)	3.97[e]	(0.10)	3.29	(0.35)	3.70	(0.93)

methods developed by the National Water Quality Laboratory, Burlington, Ontario (Inland Waters Directorate 1981).

Experimental Design

Specimens of the unionid mussel, *Elliptio complanata,* ranging in size from 7.0–8.5 cm valve length, were collected from a healthy population in Balsam Lake (a pristine lake located in the Trent-Severn River system, southwestern Ontario, Canada) on June 4, 1987. The mussels were transported to the laboratory in Burlington, Ontario in coolers containing lake water (20°C), transferred to a fibreglass "Aquafarms" fish hatchery trough and held under a continuous flow of filtered dechlorinated water for four days. On June 8, 1987, mussels were transferred to a large cylindrical tank and transported to the study basin. No mortality was observed during this time period.

Fifteen mussels were placed in each of two aluminum wire cages (25 × 25 × 11.5 cm) at approximately the same depth at each of the study sites. The cages were weighed down with bricks and attached by cables to iron reinforcing rods driven into the stream bed. Prior to placing the mussels in the cages at each site, each mussel was marked with a number using a utility knife and the valve length was measured to the nearest 1/10 mm using vernier calipers. In addition, 20 mussels were randomly selected, wrapped in pre-fired foil and frozen for analysis of background FAA.

After 27–29 days and 77–79 days exposure, cages containing mussels were retrieved at each site and 10 live mussels were removed from the cages (except where mortality or vandalism had reduced the number available), identified, measured, rinsed clean of mud, wrapped in pre-fired foil, and immediately frozen on dry ice. Mortality in each of the cages was noted and all dead animals were removed from the cages and discarded.

Collection of Benthic Invertebrate Samples

In addition to experiments with caged mussels, benthic invertebrate community assessments were conducted in both riffle and pool habitats at each study site using Surber and Hester-Dendy round multiple-plate samplers, respectively. Three replicate surber samples (area 930 cm^2) were taken at each study site where shallow, fast-flowing riffle areas were present. The contents of each surber net were placed in 500 mL glass jars and preserved with 10% buffered formalin. The multiple-plate samplers were suspended approximately 25 cm from the stream bottom in pools similar to those where the caged mussels were placed and allowed to colonize for seven weeks. Samplers were retrieved, disassembled, and all organisms and debris were scraped into a 200 μm sieve, rinsed and preserved with 10% buffered formalin.

All benthic invertebrate samples were transferred to 70% ethanol, sorted, and identified (Pennak 1978). The Hilsenhoff biotic index (Hilsenhoff 1987) was calculated for each type of sample at each site. This index has been used successfully to assess the water quality in polluted and unpolluted streams in Wisconsin and ranks sites on the basis of the response of the invertebrate community to organic pollution.

Biochemical Analysis

Three mussels from each site/exposure period were partially thawed and the adductor muscles (both posterior and anterior), mantle, and gill tissues were dissected and weighed. Amino acids were extracted by homogenization of each tissue in methanol for 30 sec, followed by centrifugation at 3000 rpm for 10 min and filtration (0.45 Millex/HV) of the supernatant. Prior to extraction, 250 μl of a standard (norleucine) was added to each sample. The filtrates were stored in the freezer (−16°C) until analysis by high pressure liquid chromatography (HPLC). Prior to analysis by HPLC, borate buffer (100 μl) was added to a subsample of each filtrate (400 μl) and the sample was derivatized with 500 μl of 9-fluorenylmethyl chloroformate (FMOC; Sigma Chemical Corp) (Einarsson 1985). After 30 sec reaction time, the excess reagent was removed by extraction with pentane.

The extracted samples were injected into a Waters HPLC system, using a Varian fluorescence detector (λ_{ex} = 260 nm, λ_{em} = 310 nm) and reverse phase elution was employed with a C^{18} column. A constant flow rate of 1.7 mL/min was employed and the mobile phase varied from 25% acetonitrile (ACN) in buffer to 50% ACN in buffer. Water used for the preparation of buffers and amino acid standards (Sigma Chemical Co.) was passed through a Millipore water purification system and Norganic cartridges (Waters Corp). The FAA concentrations were normalized to wet weight (nmol/mg).

Condition Index

Condition indices were calculated for *E. complanata* collected on each sampling date and at each study site. The length of each mussel was measured to the nearest 0.1 mm using vernier calipers and the soft body tissues were removed and weighed. Wet weight was converted to dry weight, using a conversion factor of 0.094 determined from *E. Complanata* collected from the Ottawa River (J. Metcalfe unpublished data). Condition indices (C.I.) were calculated, using Equation 1 as follows:

$$\text{C.I.} = \frac{\text{dry weight (g)}}{\text{shell length (mm)}} \times 100 \qquad (1)$$

Statistical Analysis

Total FAA concentrations, per cent contribution of specific amino acids to the total FAA pool and condition indices of mussels at sites exposed to pollution were compared to those of mussels from a control site using analysis of variance (ANOVA) followed by Dun-

Table 3. Concentration (nmol/mg wet weight) and per cent compositon of FFA in mantle of *E. complanata* after *in situ* exposure for 27–29 days

Amino Acid	35 (Control)		4		5		8		30		31	
Alanine	0.24	(0.01)	0.49	(0.09)	0.33	(0.12)	0.32	(0.22)	0.60	(0.16)	0.50	(0.19)
	23.6	(3.5)	24.4	(1.1)	27.4	(3.2)	19.1	(6.4)	22.0	(0.4)	24.4	(3.5)
Proline	0.21	(0.09)	0.31	(0.08)	0.18	(0.03)	0.49	(0.26)	0.48	(0.15)	0.35	(0.07)
	19.6	(3.6)	15.4	(1.7)	15.3	(2.1)	33.8[e]	(23.5)	17.7	(1.6)	17.6	(2.2)
Valine	0.13	(0.03)	0.22	(0.02)	0.12	(0.03)	0.14	(0.06)	0.28	(0.05)	0.15	(0.05)
	12.5	(0.5)	10.9	(1.1)	10.1[d]	(0.6)	8.6[d]	(1.3)	10.4	(1.1)	7.5[d]	(0.3)
Threonine	0.10	(0.03)	0.18	(0.05)	0.10	(0.03)	0.13	(0.09)	0.24	(0.06)	0.14	(0.04)
	9.6	(0.9)	8.8	(0.9)	8.3	(0.4)	7.7	(3.4)	8.7	(1.2)	6.3[d]	(0.4)
Glutamic Acid	0.04	(0.01)	0.15	(0.06)	0.07	(0.04)	0.12	(0.09)	0.18	(0.06)	0.20	(0.10)
	4.0	(0.8)	7.3	(1.6)	5.9	(2.1)	7.2	(4.3)	6.7	(1.5)	9.8[e]	(2.9)
Glycine	0.07	(0.02)	0.11	(0.02)	0.08	(0.03)	0.11	(0.08)	0.15	(0.06)	0.11	(0.03)
	6.7	(0.4)	5.5	(0.2)	7.0	(0.2)	6.4	(3.2)	5.5	(1.2)	5.4	(0.4)
Serine	0.06	(0.01)	0.13	(0.04)	0.07	(0.03)	0.11	(0.07)	0.17	(0.06)	0.11	(0.03)
	5.4	(1.1)	6.3	(0.8)	5.8	(0.4)	6.4	(2.7)	6.0	(0.8)	5.4	(0.5)
Arginine	0.06	(0.01)	0.08	(0.02)	0.05	(0.01)	0.05	(0.01)	0.09	(0.02)	0.08	(0.02)
	5.6	(1.8)	3.7	(0.1)	4.8	(1.6)	3.7	(1.5)	3.4	(1.5)	4.6	(2.3)
Glutamine	0.02	(0.01)	0.06	(0.01)	0.03	(0.02)	0.03	(0.02)	0.11	(0.03)	0.17	(0.06)
	1.5	(0.3)	3.2	(0.2)	2.7	(0.8)	1.7	(0.1)	4.2	(0.1)	8.3[e]	(0.6)
Isoleucine/Leucine	0.11	(0.03)	0.24	(0.03)	0.13	(0.05)	0.10	(0.07)	0.35	(0.11)	0.17	(0.06)
	10.2	(1.8)	12.3	(2.0)	10.8	(1.9)	5.6[d]	(1.5)	12.7	(2.5)	8.6	(1.1)
Others[c]	0.01	(0.01)	0.04	(0.01)	0.02	(0.01)	0.02	(0.01)	0.07	(0.03)	0.04	(0.01)
	1.2	(0.4)	2.2	(0.5)	1.9	(0.07)	0.3	(0.3)	2.5	(0.7)	1.7	(0.3)
Totals	1.04	(0.23)	2.00[e]	(0.38)	1.19	(0.38)	1.60	(0.72)	2.71[e]	(0.68)	2.02[e]	(0.57)

[a] Concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] Per cent contribution to total FAA pool
[c] Includes aspartic acid, asparagine, methionine and phenylalanine
[d] Significantly decreased from control ($P \leq 0.05$)
[e] Significantly increased from control ($P \leq 0.05$)

Table 4. Concentration (nmol/mg wet weight) and per cent composition of FAA in mantle of *E. complanata* after *in situ* exposure for 77–79 days

Amino Acid	35		4		5		32		33		37	
Alanine	0.44[a]	(0.07)	0.59	(0.12)	0.25	(0.08)	0.60	(0.12)	0.45	(0.10)	0.39	(0.09)
	23.5[b]	(0.5)	17.7[d]	(0.4)	22.9	(0.6)	19.3	(2.5)	19.5	(1.8)	24.0	(0.9)
Proline	0.44	(0.02)	0.74	(0.12)	0.24	(0.08)	0.66	(0.21)	0.45	(0.15)	0.25	(0.07)
	23.8	(3.0)	22.6	(3.9)	21.9	(0.6)	21.5	(7.4)	19.1	(1.0)	15.4	(3.8)
Valine	0.17	(0.05)	0.27	(0.03)	0.12	(0.02)	0.27	(0.02)	0.16	(0.03)	0.16	(0.01)
	9.3	(1.1)	8.1	(1.0)	11.2	(2.1)	8.7	(0.5)	7.0	(0.9)	9.6	(1.3)
Threonine	0.19	(0.02)	0.33	(0.09)	0.10	(0.03)	0.31	(0.03)	0.22	(0.09)	0.14	(0.04)
	10.3	(0.8)	9.8	(0.9)	9.0	(0.3)	10.2	(1.5)	9.3	(1.5)	8.5	(1.0)
Glutamic Acid	0.10	(0.02)	0.22	(0.07)	0.05	(0.04)	0.24	(0.05)	0.20	(0.08)	0.10	(0.06)
	5.1	(0.7)	6.7	(1.4)	4.4	(2.0)	7.8	(1.2)	8.5	(1.2)	5.5	(2.4)
Glycine	0.14	(0.02)	0.27	(0.07)	0.08	(0.04)	0.26	(0.06)	0.19	(0.01)	0.12	(0.05)
	7.7	(0.8)	8.1	(0.4)	7.0	(0.6)	8.2	(1.7)	7.9	(0.4)	7.2	(1.7)
Serine	0.12	(0.03)	0.25	(0.06)	0.07	(0.02)	0.21	(0.05)	0.17	(0.04)	0.11	(0.04)
	6.3	(0.8)	7.5	(0.6)	6.3	(0.2)	6.9	(1.6)	7.2	(0.2)	6.3	(1.2)
Arginine	0.04	(0.03)	0.12	(0.03)	0.05	(0.02)	0.12	(0.02)	0.10	(0.02)	0.05	(0.01)
	2.1	(1.3)	3.7	(0.2)	5.0[e]	(0.9)	3.8	(0.9)	4.2	(0.6)	3.1	(0.2)
Glutamine	0.04	(0.02)	0.13	(0.04)	0.02	(0.01)	0.08	(0.02)	0.12	(0.06)	0.06	(0.01)
	2.0	(0.9)	3.8	(0.6)	1.5	(0.6)	2.7	(0.4)	4.8	(1.0)	3.8	(0.2)
Isoleucine-Leucine	0.16	(0.06)	0.32	(0.07)	0.10	(0.03)	0.29	(0.10)	0.22	(0.03)	0.21	(0.04)
	8.3	(2.3)	9.8	(0.9)	9.1	(2.2)	9.2	(2.8)	9.7	(1.5)	13.3[e]	(3.6)
Others[c]	0.03	(0.02)	0.07	(0.02)	0.02	(0.01)	0.06	(0.03)	0.06	(0.02)	0.05	(0.02)
	1.6	(0.9)	2.2	(0.5)	1.7	(0.5)	1.8	(1.0)	2.8	(1.0)	3.4[e]	(1.7)
Totals	1.88	(0.29)	3.32[e]	(0.66)	1.08[d]	(0.37)	3.09[e]	(0.18)	2.32	(0.64)	1.64	(0.33)

[a] Concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] Percent contribution to total FAA pool
[c] Includes aspartic acid, asparagine, methionine and phenylalanine
[d] Significantly decreased from control ($P \leq 0.05$)
[e] Significantly increased from control ($P \leq 0.05$)

Table 3. (cont'd)

32		33		34		37	
0.44	(0.10)	0.44	(0.12)	0.47	(0.05)	0.30	(0.03)
22.6	(0.8)	22.7	(3.3)	23.5	(1.0)	21.6	(2.1)
0.30	(0.02)	0.25	(0.06)	0.30	(0.05)	0.36	(0.07)
15.8	(3.0)	13.0	(2.3)	15.0	(2.6)	25.8	(4.0)
0.24	(0.08)	0.19	(0.04)	0.21	(0.03)	0.16	(0.02)
11.9	(1.5)	1.0[d]	(2.7)	10.4	(1.3)	11.5	(0.3)
0.18	(0.03)	0.18	(0.07)	0.19	(0.01)	0.11	(0.02)
9.3	(0.7)	9.1	(0.3)	9.6	(0.3)	8.1	(0.5)
0.11	(0.03)	0.17	(0.11)	0.12	(0.01)	0.04	(0.01)
5.7	(1.1)	7.7	(3.6)	5.7	(0.3)	3.1	(0.1)
0.13	(0.03)	0.13	(0.06)	0.12	(0.02)	0.07	(0.01)
6.7	(0.6)	6.4	(1.0)	5.9	(0.4)	5.1	(0.5)
0.12	(0.02)	0.15	(0.08)	0.12	(0.01)	0.06	(0.01)
6.2	(0.8)	7.2	(1.6)	6.1	(0.3)	4.4	(0.2)
0.10	(0.05)	0.09	(0.03)	0.08	(0.02)	0.06	(0.02)
5.2	(1.3)	4.8	(0.5)	3.8	(0.6)	4.0	(0.9)
0.06	(0.01)	0.12	(0.08)	0.06	(0.01)	0.03	(0.01)
3.1	(1.0)	5.7	(2.6)	3.1	(0.3)	2.4	(1.1)
0.24	(0.08)	0.21	(0.08)	0.28	(0.04)	0.17	(0.02)
11.9	(1.7)	9.9	(1.8)	14.0[e]	(2.2)	12.1	(1.3)
0.04	(0.01)	0.05	(0.02)	0.06	(0.02)	0.03	(0.06)
1.9	(0.4)	2.4	(0.2)	3.1	(0.9)	2.1	(0.2)
1.96	(0.40)	1.97[e]	(0.71)	2.00[e]	(0.14)	1.40	(0.11)

nett's multiple range test (Steele and Torrie 1980). All levels of significance are $P \leq 0.05$.

To determine if the measured environmental parameters could be correlated with the observed changes in total FAA, univariate linear regression analysis was performed for total FAA and each variable. All statistical analyses were conducted on a microcomputer using the statistical package, SYSTAT Ver. 4.0 (Wilkinson 1989).

Results

Mortality of E. complanata

The per cent mortality of caged *E. complanata* at most sites was very low (0% at most sites). Mortality was greatest at sites in the Rivière Yamaska nord (downstream from Granby; site 5) and Ruisseau Runnets, an agricultural site near Roxton Falls (site 31) after 27–29 days exposure (23% and 10%, respectively). Few animals died between the first and second sampling dates. Unfortunately, observations on both sampling dates were complicated by the removal and scattering of animals from cages at sites 8 (27–29 days) and the control site (77–79 days) and the withdrawal of cages from the water at several agricultural sites (sites 30, 31, and 34) by vandals (77–79 days).

Changes in FAA pool in Tissues of E. complanata

Concentrations of FAA in background animals were not significantly different from those in the control animals for all tissues analyzed and, therefore, these data are not included in the tables.

Adductor Muscle

Alanine, glutamic acid, glycine, and serine were found in the highest concentrations in adductor muscles (Tables 1 and 2) at most sites on both sampling dates and contributed significantly to the total FAA pool *i.e.*, 19.2–32.8%, 6.2–32.0%, 7.5–13.7%, 6.7–13%, respectively. Other individual FAA found in substantial concentrations (5–10% of total FAA) were arginine, threonine, valine, and isoleucine-leucine. Individual amino acids found in low concentrations (<5.0% of total FAA) were asparagine, glutamine, proline, aspartic acid, methionine, and phenylalanine.

Concentrations of total FAA were increased significantly above control levels at sites 4 and 33 after both 27–29 days and 77–79 days exposure and at sites 32 and 37 after 77–79 days exposure. Concentrations were also elevated at sites 5, 30, 31, 34, and 37 after 27–29 days, although these increases were not statistically significant. No data are available for sites 30, 31 and 34 from the 77–79 days exposure period due to vandalism. The increases in total FAA concentrations at all sites could be attributed to increases in all individual amino acids with the exception of arginine at site 4 after 27–29 days exposure.

The per cent contributions of some individual FAA to the total FAA pool were significantly different at several sites. In general, these changes included decreases in serine, threonine, glycine, and valine and increase in glutamine and glutamic acid at a number of sites (5, 30, 31, and 33), but these changes were not consistent between sampling dates and some occurred at sites which did not show significant increases in total FAA.

Mantle

Individual FAA detected in the mantle of *E. complanata* were similar to those detected in the adductor muscles (Tables 3 and 4); however, the amino acids which were present in the highest concentrations differed. Alanine was again a dominant FAA, accounting for 17.7–27.4% of the total FAA pool but proline and valine were found in high concentrations and contributed 13.0–33.8% and 7.5–12.0%, respectively to the total FAA pool. Glutamic acid, glycine, and serine were less important and ranged from 4.0–9.8%, 5.4–7.8% and 5.4–7.2%, respectively. Per cent contributions of other FAA were similar to those of adductor muscles.

Concentrations of total FAA were significantly increased at sites 4, 30, 31, 33, and 34 after 27–29 days of exposure and at sites 4 and 32 after 77–79 days of exposure compared to animals at the control site. With the exception of site 4 (downstream of Cowansville), these sites again included those considered to be exposed to agricultural runoff. Total FAA were also elevated at sites 8, 32, and 37 after 27–29 days and site 33 after 77–79 days exposure, but these increases were not statistically significant. Concentrations at site 5 were significantly below those of the control animals after 77–79 days of exposure. As with adductor muscles, no data for sites 30, 31, and 34 after 77–79 days exposure were available due to vandalism.

Gill

Individual and total FAA concentrations and per cent contributions to the entire FAA pool in gill tissue of *E. complanata* are presented in Tables 5 and 6. Of the 15 individual

Table 8. Selected chemical parameters in the Yamaska River watershed

Study site	Diss. O_2 (mg/L)	Cond. (μmhos)	Susp. sed. (mg/L)	Part. org. carb. (mg/L)	Nutrients			Trace metals		
					NH_3H (mg/L)	NO_3NO_2 (mg/L)	Tot. phos. (μm/L)	Al (μg/L)	Fe	Zn
35	6.4	136	18.3	0.27	0.033	1.10	51.2	127	284	488
4	—[a]	265	21.4	1.79	0.120	1.43	535.9	102	426	484
5	8.2	320	65.8	2.11	0.220	1.65	194.3	298	439	531
8	10.8	165	6.3	0.74	0.121	0.22	52.6	67	775	508
30	7.7	610	17.3	9.37	0.425	1.24	280.0	1500	2350	536
31	9.9	218	5.6	0.55	0.454	1.17	114.9	125	651	484
32	6.6	380	16.6	1.26	0.044	3.81	157.0	447	1370	521
33	7.8	630	11.7	1.10	0.080	3.24	168.6	343	739	495
34	—[a]	—[a]	—[a]	2.03	0.060	4.39	150.9	1500	2480	493
37	7.7	240	9.3	0.24	0.412	5.68	276.8	117	783	497
Can. Water Quality Guidelines[b]	—	—	—	—	—	<10.0	<30	100	300	30

[a] Data not available
[b] CCREM (1987)

1985). *E. complanata* is ubiquitous in lakes and streams in northeastern Canada and the United States, can live in a variety of environments with substrates ranging from mud and clay to sand and coarse gravel, and is a good candidate for transferral experiments. The present study is the first to measure changes in intracellular concentrations of FAA in caged freshwater mussels exposed to polluted environments. The lack of significant differences in FAA in animals placed at the control site vs. those taken for background samples prior to *in situ* placement indicates that transferral does not stress the animals.

Intracellular FAA Concentrations in E. complanata

The concentrations of total FAA in the various tissues of freshwater bivalves have been shown to range from 0.59–7.14 nmol/mg wet weight (this study; Gardner *et al.* 1981; Graney and Giesy 1988). These levels are substantially lower than those reported for marine and estuarine bivalves which range from 100–600 nmol/mg wet weight (Jeffries 1972; Briggs 1979; Roesijadi 1979). FAA are known to play a role in osmoregulation and the alteration of cellular volume in response to changes in salinity (Bayne *et al.* 1985). Freshwater organisms are not exposed to great extremes of salinity and it is not surprising, therefore, that these organisms have a lesser capacity for compensation and thus a smaller pool of FAA (Dietz 1974; Hanson and Dietz 1976).

In marine organisms, taurine, alanine, and glycine are the individual FAA which contribute most significantly to the total FAA pool (Zandee *et al.* 1980). Taurine was not detected in the present study but alanine was consistently present in the greatest concentrations in all three types of tissue analyzed and contributed significantly to the total FAA pool. Glutamic acid was generally the second most prominent amino acid in adductor muscles and the gill, whereas proline was found in greater abundance in the mantle. Valine and glycine were the third most predominant FAA in the mantle and adductor muscles respectively. Graney and Giesy (1988) found alanine, arginine, and glutamic acid to be the most abundant amino acids in the adductor muscle of the freshwater clam, *Corbicula fluminea*. In the present study, arginine contributed approximately 5–10% to the total FAA pool in the adductor muscles and <5.0% in the mantle and gill. Studies with other freshwater mussels indicate that alanine, glutamic acid, and glycine are predominant in the mantle (Hanson and Dietz 1976; Gardner *et al.* 1981).

A comparison of all data for changes in total FAA in all three tissue types suggests that, in general, total concentrations of FAA became elevated at a number of sites where various sources of contamination were prevalent although not all increases were statistically significant. These sites include site 4, on the Rivière Yamaska Sud-est downstream from Cowansville, and the agricultural sites 30–34. Cowansville (population >10,000) is known to input untreated municipal sewage and industrial effluents from several textile factories and mushroom farms into the river (Croteau *et al.* 1984). Water quality data in the present study showed levels of phosphorus as well as iron and zinc to be above the Canadian Water Quality Guidelines (CCREM 1987) for the protection of aquatic life.

The agricultural sites are in rural areas where pesticides and nutrients from corn production are known to contaminate the watershed (Muir *et al.* 1978; Maguire *et al.* 1989) and water quality is considered to be poor (Auger *et al.* 1978). Levels of nutrients as well as concentrations of aluminum, iron, and zinc, were above those considered adequate for the protection of aquatic biota at most of these sites and may contribute to toxicity.

Several other studies with freshwater bivalves have re-

ported increases in total concentrations of intracellular FAA in organisms exposed to toxicants in both the laboratory and the field. For example, Garner *et al.* (1981), found that total FAA levels in the mantle of natural populations of native *Amblema plicata* were higher in mussels from streams with a history of exposure to acid coal mine drainage and trace metals than those organisms collected from relatively unpolluted streams. Graney and Giesy (1988) showed that acute and chronic exposure of the clam, *Corbicula fluminea,* to sodium dodecyl sulfate caused increases in the total FAA concentrations in both adductor muscle and mantle tissue. These results are in contrast with marine bivalve studies in which animals exposed to a variety of natural and manmade stresses, i.e., oil-contaminated sediments (Roesijadi and Anderson 1979); drilling effluents, anoxia, turbidity (Powell *et al.* 1982); cadmium (Briggs 1979) and various salinities (Matsushima 1988) had lower intracellular tissue concentrations of FAA than those of the controls.

Concentrations of total FAA did decrease at several sites in the present study. For example, total FAA levels decreased in the mantle at sites 5 and 37 after 77–79 days exposure and 5 after 77–79 for adductor muscle. Site 5, on the Rivière Yamaska nord, is located directly downstream from the industrialized town of Granby and is in an area impacted by municipal sewage treatment plant effluents and trace metals such as copper, zinc, lead, nickel, and mercury (Auger *et al.* 1979; Tessier *et al.* 1980). Concentrations of aluminum, iron, and zinc were elevated above the Canadian Water Quality Guidelines set in 1987 for these trace metals for water samples taken in this study.

Other studies with freshwater invertebrates (*Gammarus pseudolimnaeus*) have also observed a decrease in total FAA concentrations following short-term exposure to toxicants (Graney and Giesy 1986, 1987). Whether a specific response to a toxicant is an increase or decrease in total intracellular FAA concentrations may be dependent upon the type of contaminant present, the organism affected and the specificity of the mode(s) of action of the contaminant.

Changes in the per cent contributions of certain individual free amino acids to the total FAA pool have also been reported as indicators of a response to stress. In marine organisms exposed to contamination, an increase in the tissue molar ratio of taurine: glycine is often observed due to a decrease in the concentration of glycine (Jeffries 1972; Bayne *et al.* 1985). A decrease in the sum of the individual amino acids, serine and threonine, is also thought to indicate a response to stress (Bayne *et al.* 1985). Decreases in the per cent contributions of glycine, serine, and threonine as well as valine to the total FAA pool in the present study occurred at site 5 and several of the agricultural sites (30, 31, and 33) and may be indicative of a response by *E. complanata* to stress. Significant increases in the per cent composition of glutamine and glutamic acid were also observed in adductor muscle tissue at these same sites. Increases in glutamic acid levels have also been noted as a consistent response to trace metal pollution in sea anenomes, *Bunodosoma cavernata* (Kasschau *et al.* 1980) and in the adductor muscle of the grass shrimp, *Palaemonetes pugio,* following exposure to PCB's (Roesijadi *et al.* 1976).

Additional data collected during this experiment as well as other studies in this watershed support the concept of toxicants adversely affecting aquatic invertebrates in tributaries of the Yamaska River. The evaluation of sites using the Hilsenhoff Biotic Index indicates that the agricultural sites as well as sites 4 and 5 appear to be the most deteriorated compared to the control. In a study of the incidence of mouthpart deformities in populations of chironomids at all sites in the Yamaska River watershed, Bird (1989) found that the frequency of deformities (>1%) at sites 5, 30, 32, and 33 indicated problems with water contamination.

The mortality of caged *E. complanata* at sites 5 and 31 (23% and 10%, respectively) after 27–29 days of exposure suggests an inability of susceptible animals to cope with deteriorated water quality at these two sites. A decrease in C.I. in organisms at site 5 also suggests that mussels were not as healthy at this site as those exposed at other sites. C.I. is used as a general indicator of an animal's health and reflects the recent physiological history of the animal by measuring energy stored as glycogen, lipid and protein. It is related to the overall metabolic response of an animal to the conditions imposed by its environment and is known to decrease when animals are exposed to environmental stress such as overcrowding or starvation (Peddicord 1977). However, C.I. may not always be a good indicator of stress caused by exposure to toxic chemicals. For example, Roesijadi and Anderson (1979) found that conditions indices in the clam, *Macoma inquinata,* exposed to oil-contaminated sediments were not significantly different from each other after 38 days even though 33% of the clams died in the treated sediments. In the present study, C.I. was significantly reduced at site 8 after 77–79 days, a site which did not show changes in FAA. This decrease could be indicative of poor nutrition rather than toxicity. Site 8 had very low levels of particulate organic carbon and suspended sediment in the water column and thus the filter-feeding mussels may not have been able to obtain adequate food at this site, resulting in the mobilization of energy reserves from the body. An additional explanation may be that poor nutrition as well as moderate toxicity at this site masks the anticipated changes in FAA. Powell *et al.* (1982) suggests that two stresses presented concurrently might superimpose two distinct metabolic phenomena which modify each other to some extent resulting in a FAA pattern different from that produced by either one alone, *i.e.,* some additive or synergistic effects may be found. This could explain why the C.I. in this study indicates a deterioration in the health of organisms at site 8 without a concurrent increase in concentrations of FAA.

The low statistical correlation between the observed changes in total FAA and the water quality parameters indicates that these parameters are not individually responsible for the biotic responses observed. Interactive effects of two or more parameters could be contributing to toxicity but were not specifically studied. In addition, toxicity from unmeasured contaminants could also be responsible for the changes noted. Agricultural practices have a detrimental effect on aquatic invertebrates due to high bacterial and viral concentrations, high turbidity from erosion, and eutrophication from nutrient runoff (Dance and Hynes 1980) and, therefore, a variety of factors could be contributing to mortality and stress at these sites.

The results of this study do not prove that the changes in FAA in molluscs were induced due to toxicity. However, the evidence from this and other studies supports the basic concept that exposure of invertebrates, particularly molluscs, to stressful environments results in either increases (generally in freshwater organisms) or decreases (generally in marine invertebrates) in the entire FAA pool or specific components within that pool depending on species, environmental conditions and the severity and type of stress. Further research in this area is essential before monitoring changes in concentrations of total or individual FAA can become a viable *in situ* ecotoxicological tool.

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