

Stability of Free Amino Acid Levels in Stressed *Abarenicola pacifica*

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JEFFRIES (1972), BAYNE et al. (1976), and ROESIJADI + ANDERSON (1979) have described a change in the composition of the free amino acid (FAA) pool of pelecypod molluscs under stress. This response consists of a significant decrease in the level of glycine, while the taurine level remains constant, leading to a decrease in the total free amino acid level and a decrease in the glycine:taurine ratio. It has been suggested that this alteration in FAA composition may be useful as an indicator of physiological stress in a wide variety of invertebrates. We have tested this suggestion by exposing a burrowing polychaete, *Abarenicola pacifica*, to sediment artificially contaminated with Prudhoe Bay crude oil (PBC).

MATERIALS AND METHODS

Forty-two *A. pacifica* and 84 kg of sediment were collected from the high intertidal area of a mud flat near Sequim Bay, Washington State, U.S.A. Twenty-three mL PBC and 500 mL sea water were added to 44 kg sediment, and the mixture was stirred 10 min with a motor driven impeller. Forty kg control sediment without oil was stirred similarly. The stirred sediment was poured into four mesh-bottomed fiberglass trays, 25 by 47 by 12 cm in size, to a depth of 10 cm. The control and experimental trays, supported on bricks, were placed in separate tubs within a sea table. Raw seawater, at 12°C and 30 ± 1 ‰ salinity, was piped into the tubs under the mesh bottoms and allowed to flow through and around the sediment-filled trays, and out over the edge of the tubs. The rate of flow was two L/min.

The FAA contents of six worms were measured immediately after collection. After the sediment had been flushed with sea water for 24 h, samples of sediment were taken for determinations of hydrocarbon concentration by infrared (IR) spectrophotometry (API 1958) and nine worms were placed in each of the four trays. After one week the worms were removed from one control and one experimental tray, and the sediment hydrocarbon content and tissue FAA contents were measured. The remaining worms were removed and tested after two weeks. A preliminary qualitative

DUCHATEAU-BOSSON et al. (1961), from FLORKIN + SCHEER 1969, reported that glycine made up 68% of the FAA pool of *Arenicola*

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marina, a form closely related to A. pacifica, when it was kept in seawater. When A. marina was exposed to 50% sea water the total FAA level fell to 45% of its original value, but the proportion of glycine remained constant. A preliminary qualitative assay of A. pacifica showed that 82% of its FAA consisted of glycine, and only 3% of taurine. It therefore seems highly probable that any substantial decline in glycine content would be reflected in a drop in total FAA. This parameter was measured in exposed and control groups of the worm.

Immediately after their removal from the sediment, strips of body wall were dissected from the worms, minced, and frozen in 20-mL vials. For the assays, 1 mL 80% (v/v) ethanol per 10 mg tissue was added directly to each vial, and they were shaken at 100 strokes/min for 24 h. The total FAA of the extract was measured by the method of CLARK (1964).

RESULTS AND DISCUSSION

TABLE 1. Hydrocarbon content of sediment (x ppm - wet weight \pm s.e.).

	Control			Contaminated			Net
Day 1	76 \pm 4	n = 3		358 \pm 7	(surface) n = 3		282
Day 7	71 \pm 5	n = 2		437 \pm 2	(below surface) n = 3		366

Table 1 shows the hydrocarbon content of the sediment on the first and seventh day of the experiment. It should be noted that the oiled sediment had been in the intertidal zone for 24 hr previous to the addition of animals. We, therefore, assume that the below surface sample taken of day 7 represents the initial level in these sediments and the decrease exhibited at the surface on day 1 represented leaching of hydrocarbons during the initial 24 hr in the field. The relatively high hydrocarbon content of the control sediment is due to the presence of n-alkane compounds of carbon chain lengths between 22 and 28. The small standard errors (s.e.) associated with the means of hydrocarbon concentrations indicates that the PBC was uniformly mixed into the contaminated sediment, but some leaching into the surrounding water from the surface of the trays appears to have occurred.

After one week of exposure all nine control animals examined were alive and appeared normal. Six out of nine exposed worms were alive, but only one maintained its normal position in its burrow. Three others, one of which responded very sluggishly to touch, were found on the surface of the sediment, and two were found at their burrow entrance with their posterior segments extended into the water column. These five animals were obviously under severe stress, and under natural conditions they would probably have been quickly taken by predators. At this time the mean FAA level of the exposed animals did not differ significantly from that of the controls (Table 2). It

does appear that the variation within the exposed group was greater than the variation within the controls, and that the FAA content is lower in the more severely stressed animals within this group than in the less severely stressed. However, the latter observation is not confirmed by the results after two weeks.

TABLE 2. Free amino acid content of Abarenicola pacifica (x μ mole/g wet weight \pm s.e.)

Time	Control	Exposed
Initial	341 \pm 6 (n = 6)	
1 week	348 \pm 5 (n = 6)	380 (tail in water) 371 (in burrow) 354 (tail in water) 327 (on surface) 318 (on surface) 310 (on surface-sluggish) 343 \pm 12 (n = 6)
2 weeks	357 - injured 344 343 343 336 324 341 \pm 4.4 (n = 6)	326 (on surface) 296 (in burrow) 311 \pm 15 (n = 2)

After two weeks, seven out of nine control animals survived. One of these appeared injured. Of the exposed population, only two out of nine animals survived, and one of these was on the sediment surface. Nevertheless, there was only a slight reduction in the mean FAA content of these survivors, of about nine percent, which was not significant at the .05 level. The levels of the injured and surfaced individuals was higher than the means of their groups.

It therefore seems unlikely that FAA content is negatively correlated with stress in Abarenicola or that reduction in this parameter could be used as an indicator of sublethal stress under milder conditions than those applied in this experiment. While such reductions have been suggested as parameters to be measured in bivalve molluscs (JEFFRIES 1972, BAYNE et al. 1976, ROESIJADI + ANDERSON 1979) their significance in at least one polychaete has not been established.

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