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Significance of the Taurine–Glycine Ratio as an Indicator of Stress

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Concentrations of noxious substances adopted for acute toxicity tests are rarely measured in the environment. Studies on longterm sublethal effects are of major importance (Barrett and Rosenberg, 1981). Environmental changes, due to natural fluctuations or pollution effects, can cause deviations from the normal performance of an organism. Exceeding the signal to noise ratio of a given statistic, these impacts are summarized under the term stress. First defined by Selye (1952) and extended by Bryan (1975) it is now used to describe the environmental impact (the stressor in Selye's concept) which implies an adaptive strategy (the stress response) of the animal (Bayne,1985). For the quantification of stress effects, several methods and systems have been developed. Among others, biochemical techniques seem to be very promising (Jeffries,1972; Lee et al.,1980; Livingstone,1982; Scholz and Theede,1985; Theede,1985).

The following paper deals with the applicability of the taurine - glycine ratio as a biochemical stress index. The effects of heavy metals (Cd, Cu) as well as starvation have been investigated in the blue mussel, Mytilus edulis.

MATERIALS AND METHODS

Mytilus edulis L. (average shell length 65 mm) were collected during spring time prior to spawning, in Eckernförde Bight (Western Baltic) from two neighbouring populations. All specimens were cleaned thoroughly and placed in aereated seawater at 15 °/oo S and 10 °C for defaecation. After 3 days the animals were placed on plastic trays and transferred to clean glass aquaria for 3 to 5 weeks. During this acclimation period, the water was changed every other day. The mussels were fed daily a diet of Chlorella sp. at a final concentration of $1x10^4$ cells ml^{-1} d^{-1}.

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25 mussels were used for each of 3 experiments. The first of the experiments was with an addition of 10 µg Cd l^{-1}, the second with an addition of 20 µg Cu l^{-1}. For the third experiment, mussels were kept without food. For the last two experimental groups a common control was used.

At different time intervals, three individuals were removed from each tank prior to the daily feeding. The posterior adductor muscle was dissected and reserved for amino acid determination, the remainder of the animals was used for heavy metal analysis.

Intracellular amino acids were extracted with 2 ml 80% ethanol in a test tube. After sonification for 20 min, extraction was carried out for 24 h at ambient temperature. The capped vials were then centrifuged at 1800g, and the clear supernatant was stored at -20°C until further analysis. The sediment was dried at 60°C to a constant weight for dry weigt determination.

The determination of total amino acids was carried out according to Habeeb (1966) with glycine and taurine serving as standards. Single amino acids were separated on a Locarte amino acid analyzer MK.5-NF with a 4-buffer system (sodium-tricitrate 0.18-1.50 N; pH 3.24 - 5.20) and ninhydrine (2% in methylcellusolve/ 1M sodium acetate pH 5.50). Sample aliquots of 50 - 100 µl were applied to the column (37 x 0.9cm, Locarte 7 µm resin) by means of an automatic sample injector. Blanks were run throughout all the procedure to check for contamination. Quantification was by means of an automatic peak integrator HP 3385A. All reagents were of ultrapure quality. Heavy metals were determined as previously described (Scholz, 1980).

RESULTS AND DISCUSSION

Heavy metal concentrations used in the present experiments were well below acute lethal concentrations. No obvious signs of an altered behaviour or reaction were detected in the mussels. Cd and Cu concentrations in the whole soft body increased threefold and twofold respectively (Table 1).

Table 1: Mytilus edulis: Heavy metal concentrations in the soft body (µg g^{-1} dry weight) of specimens kept in 10 µg Cd l^{-1} for 15 days or 20 µg Cu l^{-1} for 17 days (mean +/- s_E; n = 3)

	10 µg Cd l^{-1}	20 µg Cu l^{-1}
Start of experiment	4.42 +/- 1.06	10.64 +/- 1.02
End of experiment	15.83 +/- 0.38	22.26 +/- 1.02

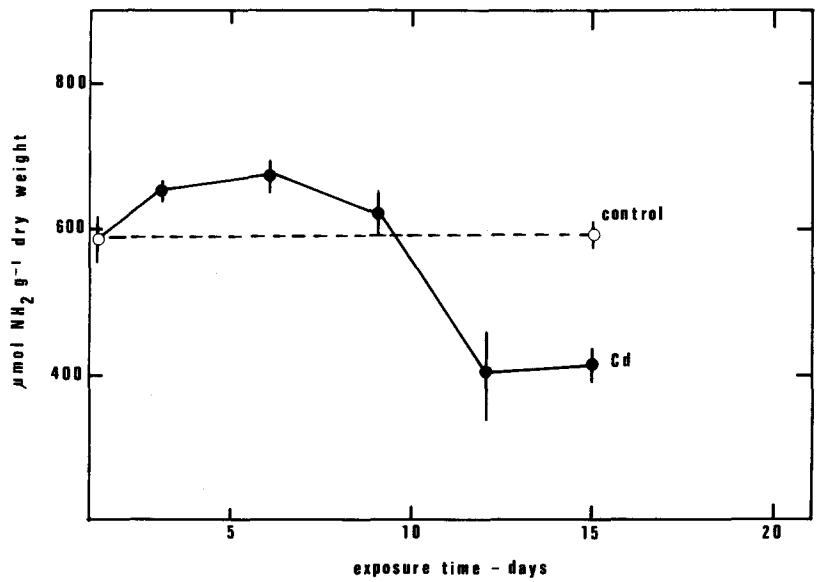

Figure 1. Mytilus edulis: Effects of cadmium (10 µg l^{-1}) on the total NH_2-group content in the posterior adductor muscle

The effects of cadmium on the free amino group content is documented in Fig. 1. After a short increase above control values, the NH_2 - concentration dropped significantly in the metal treated group. This reduction amounted to 30% and is consistent with data from Briggs (1979). Neither copper treatment nor starvation caused a comparable effect.

Results for the taurine - glycine ratio are summarized in Tables 2 and 3. It is evident that in each experimental group the ratio increased with time. In the case of cadmium, a distinct rise could be observed after 6 days of exposure. The mean value of 11.3 means a nearly sixfold increase over normal levels. Though declining the taurine - glycine ratio remained significantly higher than in the control group.

Table 2: Mytilus edulis, posterior adductor muscle. Taurine - glycine ratio in Cd - exposed specimens (mean +/- sd; * = significant difference to controls; n.d = no data)

day	control	Cd
0	1.81 +/- 0.01	1.81 +/- 0.01
3	n.d	2.92 +/- 1.22
6	1.92 +/- 0.20	11.28 +/- 3.14*
10	n.d	5.93 +/- 2.57*
15	2.22 +/- 0.60	4.95 +/- 1.35*

Table 3: Mytilus edulis, posterior adductor muscle. Taurine - glycine ratio in Cu - exposed or starved specimens (mean +/- sd; n.d = no data; * = significant difference to control)

day	control	Cu	starvation
0	2.98 +/- 0.71	2.98 +/- 0.71	2.98 +/- 0.71
3	3.08 +/- 0.52	7.63 +/- 0.40*	3.78 +/- 1.18
7	2.79 +/- 0.55	n.d	9.31 +/- 0.28*
10	3.13 +/- 0.28	2.77 +/- 1.82	n.d
17	2.62 +/- 0.83	3.05 +/- 0.88	n.d
24	2.65 +/- 0.68	n.d	8.31 +/- 0.81*

Copper, on the other hand, produced only a temporary effect. The enhanced level of 7.6 reached after 3 days declined to control values within the next few days.

Starvation, like cadmium, had a distinct influence on the stress index. No "overshoot" reaction however could be observed during the initial phase. The ratio rose steadily and remained constantly high at a level of about 8.3. Control values were, however, slightly higher than in the cadmium group.

This may be due to the fact that the mussels originated from two different sites. Moreover, the Cd - experiment was conducted first and mussels for the Cu - and starvation experiments were kept in the laboratory for a longer period of time (5 weeks instead of 3 weeks). The same may be true regarding the differences to observations of Bayne et al.(1976). They demonstrated an effect of reduced food on the taurine - glycine ratio only at elevated temperatures. Whether the reduced salinity of 15°/oo S in the present experiments was also causitive in this respect requires further verification.

Causes leading to enhanced taurine - glycine ratios are different in each experiment . Starvation causes an intensified mobilization of glycogen for energy demands (Zandee et al.,1980). Consequently, competitive synthesis of glycine via 3-PG and serine, in order to compensate for "leaching" processes, should at least be slowed down if not completely stopped. As can be seen in Fig.2, the glycine level dropped significantly below 10 umol g^{-1}, while the taurine concentration remained constant.

Exposure to cadmium on the other hand caused the inverse effect (Fig. 3). The glycine concentration remains relatively constant, whereas taurine is present in a temporarily fourfold concentration. Though declining towards the end of the experiment, its value remains significantly higher than in the control group.

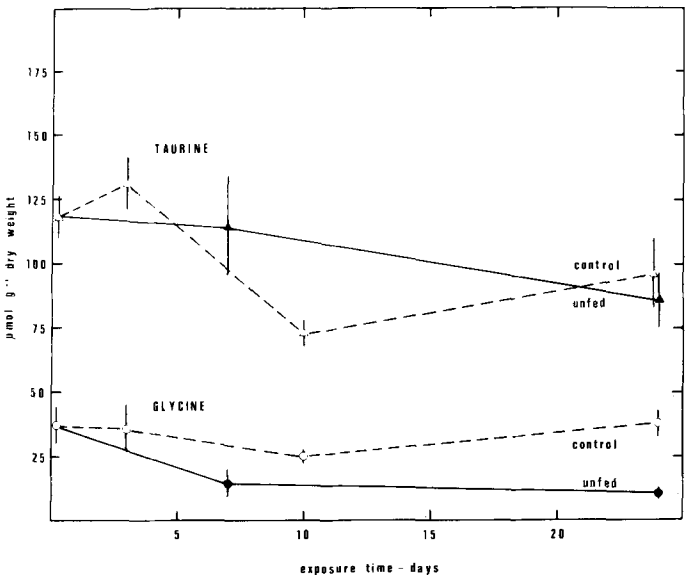

Figure 2. Mytilus edulis: Effecs of starvation on the taurine and glycine concentration in the posterior adductor muscle

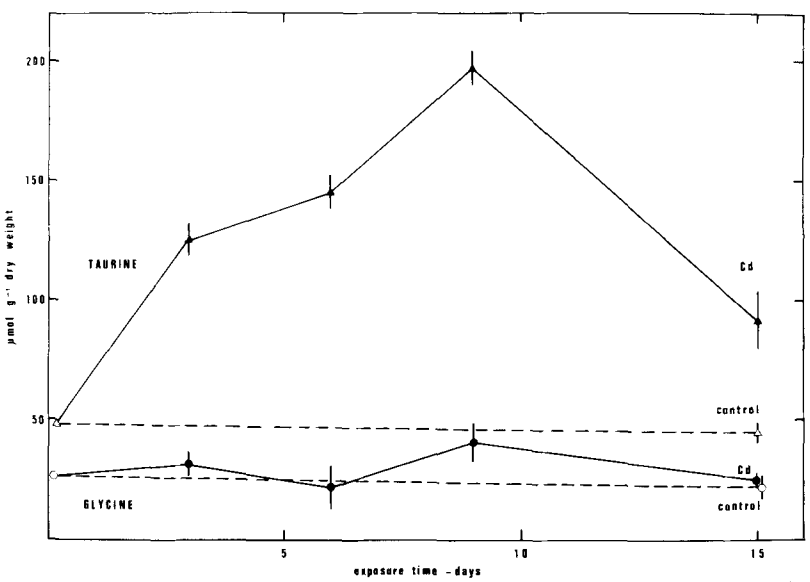

Figure 3. Mytilus edulis: Effects of cadmium (10 µg l^{-1}) on the taurine and glycine concentration in the posterior adductor muscle

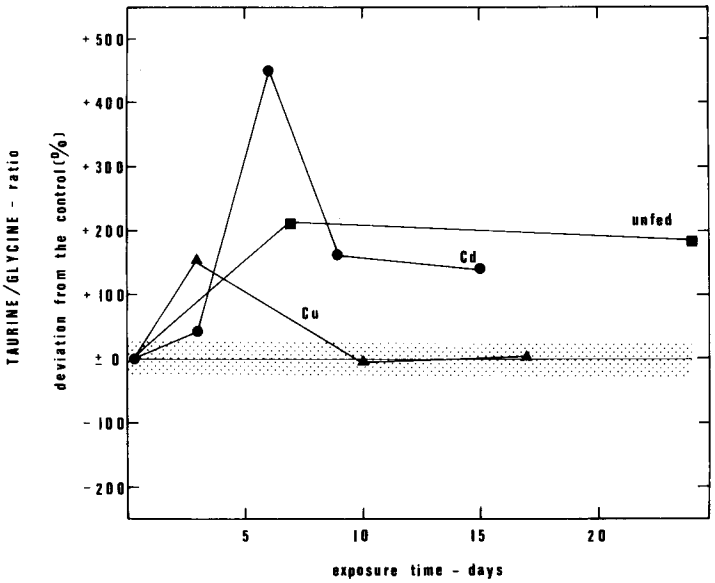

Figure 4. Mytilus edulis, posterior adductor muscle: Effects of exogeneous stress on the taurine - glycine ratio. Values are expressed as deviation from control animals, in %.
Shaded bar: controls with 95% confidence limits

It is not yet clear why taurine accumulates during cadmium exposure. Via cysteine sulphinic acid and hypotaurine, it can only originate from cysteine (Allen and Garrett, 1971). This amino acid, present only in trace amounts in the free amino acid pool, forms the main building block of metallothioneins synthezised in response to cadmium administration (Noel-Lambot, 1976; Scholz, 1980). Therefore one should expect either a constant taurine concentration or a decrease, if taurine is further metabolized (Allen and Garrett, 1971).

To account for the different control values, Fig. 4 shows the taurine - glycine ratio expressed as the deviation from their respective control values. Deviations exceeding the confidence limits of the controls represent significant differences and thus indicate stress.

Enhanced taurine - glycine ratios are not merely a laboratory artifact. Investigations by Jeffries (1972) show this effect to be present in field populations. Hard clam specimens (Mercenaria mercenaria) under pollution stress showed a shortened life span and higher mortality with sublethal stress responses as well. From his investigations, Jeffries concludes that taurine - glycine ratios above 3 are indicative of chronic stress, and values above 5 indicate acute stress. That these limits hold exactly true for Mytilus edulis needs to be verified. More intensive investigations with field populations will highlight the

limits as well as the merits of this biochemical stress index. Constant comparsions of the taurine - glycine ratio between unstressed populations (serving as controls) and possibly endangered ones could be very useful as an early warnig system tool. Extensive diagnostic investigations then could be reduced to a reasonable and justifiable degree.

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