

A biochemical approach to assessment of effects of organic pollution on the metabolism of the non-opportunistic polychaete, *Glycera alba*

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ABSTRACT: Loch Creran and Loch Eil, sea lochs in the west of Scotland, both receive discharges of particulate organic effluent from industrial installations. *Glycera alba* (Müller) is widely distributed in the sediments of both lochs, and assays of activities of enzymes associated with energy-yielding metabolism have been done on crude extracts of specimens collected from variously affected areas. Mean phosphofructokinase activities were low in extracts of *G. alba* collected some 400 m from the source of effluent from a seaweed processing factory, increased to a maximum at 900 m and declined slightly at 1150 m where the sediment is little affected by the effluent. Pyruvate kinase activities exhibited qualitatively similar changes of lesser magnitude and no differences in α -glycerophosphate or malate dehydrogenase activities were observed. In *G. alba* from Loch Eil a relationship was established between phosphofructokinase activity and Eh at 4 cm in the sediment and the maximum change in phosphofructokinase was found at low Eh, below -50 mV. The data are interpreted with reference to results from biological and environmental monitoring in Lochs Eil and Creran. It is suggested that the low phosphofructokinase activities in *G. alba* from the most affected areas of each loch may constitute a consistent biochemical response to effects of the organic inputs.

INTRODUCTION

Inputs of organic material into the marine environment can cause successive changes in the distribution and populations of macrobenthic invertebrates (see Pearson & Rosenberg, 1978 for review). Monitoring of the effects of organic inputs on the fauna has so far been attempted largely by means of biological sampling techniques which relate fluctuations in the benthic populations to the organic inputs, and much of this effort has been directed toward definition of widely applicable indicator species, e. g. *Capitella capitata* (Fabricius) (Reish, 1972), which are relatively resistant to effects of organic enrichment and are able to exploit organically rich sediments, their numbers often increasing to the exclusion of most other species.

Glycera alba (Müller) is a widely distributed predatory polychaete worm which is found in unpolluted areas and areas which may be described as moderately polluted in the Scottish sea lochs, Eil and Creran, which receive inputs of particulate organic effluent from a wood pulp and paper mill and a seaweed processing factory, respectively. It does not compete with opportunistic species, e. g. *C. capitata*, in the most affected areas of these lochs and may therefore be considered to be relatively sensitive with respect to effects of high organic inputs.

Blackstock (1978) found that the activities of phosphofructokinase, pyruvate kinase and α -glycerophosphate dehydrogenase, all enzymes associated with carbohydrate catabolism, decreased in crude extracts of *G. alba* from the area which was considerably affected by the effluent input into Loch Eil. Conversely, malate dehydrogenase activities were highest in specimens from the same area. The investigation was done in association with a programme of biological and environmental monitoring of the effluent effects. Investigations of enzyme activities in crude extracts of *G. alba* from Loch Eil have been continued and a similar investigation has been carried out using *G. alba* from Loch Creran. The results of these investigations are now considered in relation to indication of effects of organic pollution by means of certain enzyme activity assays on suitably sensitive species of macrobenthic fauna.

MATERIALS AND METHODS

Specimen collection locations

The Loch Creran sampling stations are shown in Figure 1. Stations G, H, I, and K have been the subjects of regular biological and environmental monitoring during 1976 and 1977 and some physical conditions and populations of *Glycera alba* in the sediments are summarized in Table 1. The wide range of Eh values and population densities of *G. alba* is indicative of temporal fluctuations which affect the benthic populations. Pearson & Stanley (1977) discuss the monitoring data in terms of spatial and temporal changes in Loch Creran and it is clear that a well defined spatial gradient of organic pollution effect exists. The effect is most pronounced in the vicinity of the point source of discharge of the effluent from the seaweed processing factory and decreases rapidly with increasing distance from the effluent outfall. Station G frequently supports a high biomass of macrobenthic fauna with low species diversity, but is intermittently afaunal as a consequence of temporal fluctuations in sediment conditions. Station H, some 400 m from the outfall, approximates to the reestablishment of "normal" biomass and species diversity, but conditions are occasionally anoxic at the sediment surface. At Stations I, J and K few effluent-related macrobenthic faunal successions have been observed.

Specimens of immature *G. alba* for biochemical analyses were collected from Loch

Table 1. Loch Creran sampling stations: physical, environmental and biological data. Mean values from monthly monitoring from October 1976 to October 1977 with the observed ranges of values in parentheses

| Station | Depth (m) | Distance from effluent outfall (m) | Eh (mV) 4 cm in sediment | T (°C) | S (‰) | <i>G. alba</i> (no./m ²) |
|---------|-----------|------------------------------------|--------------------------|-----------|------------------|--------------------------------------|
| G | 14 | 150 | - 104 (- 302, + 130) | 10 (6-13) | 32.7 (31.6-33.5) | <1 (0- 3) |
| H | 20 | 400 | + 21 (- 173, + 103) | 10 (6-13) | 32.8 (32.0-33.6) | 11 (5-15) |
| I | 22 | 650 | + 74 (+ 14, + 269) | 10 (6-13) | 32.7 (32.0-33.6) | 9 (3-23) |
| K | 18 | 1150 | + 90 (+ 10, + 323) | 10 (6-13) | 32.7 (31.7-33.6) | 6 (3-10) |

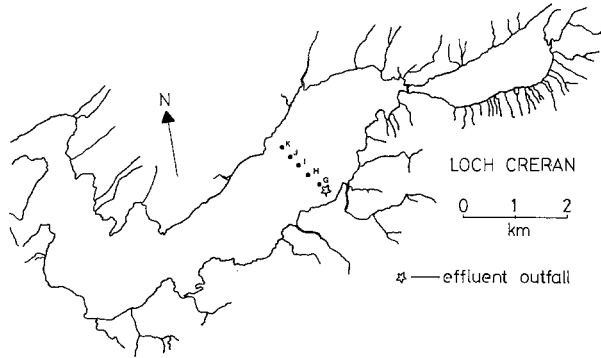


Fig. 1. Outline map of Loch Creran showing sampling stations

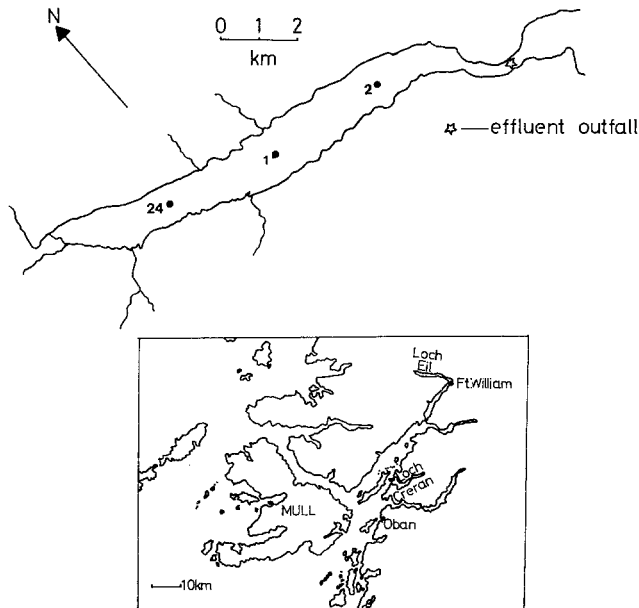


Fig. 2. Outline map of Loch Eil showing the sampling stations and outline map showing the locations of Lochs Eil and Creran in the west of Scotland

Creran Stations H, I, J and K in June 1978. Enzyme activities were estimated in crude extracts of the total tissues. To obtain a measure of variability of enzyme activities within the population, 5–6 specimens of similar size (300 ± 100 mg fresh weight) were collected from each sampling station and individually analyzed.

The specimens of immature *G. alba* from Loch Eil were collected in the vicinity of sampling Stations 2, 1 and 24 (Fig. 2) at irregular intervals between April 1976 and November 1978. Physical conditions at the sampling stations in 1976–77 were described

by Blackstock (1978). In 1977 and 1978 there were considerable effluent-related fluctuations in sedimentary conditions in Loch Eil, and enzyme activities in crude extracts of *G. alba* must now be related to prevalent environmental conditions rather than the comparison of activities in specimens from different sampling stations as was done by Blackstock (1978). Pooled specimens containing a minimum of 10 *G. alba* were used for the analyses of the Loch Eil specimens.

Specimen collection and extraction

The specimens were collected by Naturalist's dredge, extracted in 0.1 M-phosphate buffer, pH 7.5, as described by Blackstock (1978) and the activities of phosphofructokinase (E. C. No 2.7.1.1.), pyruvate kinase (E. C. No 2.7.1.40), α -glycerophosphate dehydrogenase (E. C. No 1.1.1.8) and malate dehydrogenase (NAD dependent, E. C. No 1.1.1.37) were estimated as described by Blackstock (1980) using coenzyme-linked spectrophotometric procedures with substrate concentrations and other reaction conditions modified to obtain maximum rates. Freeze-dried weights were estimated on 0.5 g subsamples of the finely chopped pooled specimens of *G. alba* from Loch Eil and the enzyme activities are expressed in units (U)/g dry weight of tissue, where 1 unit represents the conversion of 1 μ mole of substrate per min under the test conditions. Subsamples of the individual animals from Loch Creran were not taken for estimations of dry weight. Instead soluble protein was estimated by the method of Lowry et al. (1951) and enzyme activities were expressed in milli-units (mU)/mg soluble protein. All enzyme reactions were carried out at 25 °C.

RESULTS

Enzyme activities in crude extracts of *Glycera alba* from Loch Creran

Table 2 shows the mean activities of phosphofructokinase, pyruvate kinase, malate dehydrogenase and α -glycerophosphate dehydrogenase estimated in the crude extracts of *Glycera alba* collected from the four sampling stations in Loch Creran. After appropriate (logarithmic) transformation of the phosphofructokinase and malate dehydrogenase activities, analysis of variance (Snedecor, 1956) indicated that only for phosphofruc-

Table 2. *Glycera alba*. Mean enzyme activities (mU/mg protein) in extracts of individuals collected from Loch Creran

| Sampling station | H | I | J | K |
|--|-------|-------|-------|-------|
| No. of individuals | 6 | 6 | 5 | 5 |
| Phosphofructokinase | 7.7* | 13.9 | 25.2 | 17.9 |
| Pyruvate kinase | 218.7 | 319.2 | 384.0 | 387.6 |
| Malate dehydrogenase | 533.2 | 900.2 | 667.3 | 527.3 |
| α -glycerophosphate dehydrogenase | 51.5 | 47.6 | 54.3 | 44.5 |

* Indicates significant difference (<0.05) from mean activities at Station J

kinase were between-group differences significant at the 95 % level of probability. Subsequent application of Student's *t*-test indicated that the mean phosphofructokinase activity in extracts of *G. alba* from Station H was significantly lower ($P < 0.05$) than the mean activity in specimens from Station J. The results therefore indicate that the lowest mean phosphofructokinase activity was found in extracts of *G. alba* from Station H, which represents the closest proximity (400 m) to the effluent outfall. Increases in mean phosphofructokinase activity are observed with increasing distance from the effluent outfall until Station J, some 900 m from the outfall and considered to be beyond the area of influence of the organic effluent (Peason & Stanley, 1977). At Station K the mean phosphofructokinase activity is some 30 % lower than at Station J, but this difference is not statistically significant at the 95 % level of probability.

Mean pyruvate kinase activity was also lowest in extracts of *G. alba* from Station H, but the differences between the groups were not significant at the 95 % level.

Mean pyruvate kinase activities did, however, exhibit a qualitatively similar pattern of increase in mean activity with increasing distance from the effluent outfall, a maximum being attained some 900 m from the outfall (Station J). Mean pyruvate kinase activity was similar in extracts of *Glycera alba* from Stations J and K (cf. phosphofructokinase).

Mean malate dehydrogenase and mean α -glycerophosphate dehydrogenase activities did not differ significantly ($P > 0.1$) between groups of *G. alba* collected at the different sampling stations.

Phosphofructokinase activities in crude extracts of *Glycera alba* from Loch Eil

Mean activities of phosphofructokinase, and to a lesser extent, pyruvate kinase, have been found to be relatively low in crude extracts of *G. alba* from Station H, which is more affected by the input of organic effluent from the seaweed processing factory into Loch Creran than are Stations I, J or K. Blackstock (1978) has observed significantly low phosphofructokinase and pyruvate kinase activities in crude extracts of *G. alba* from

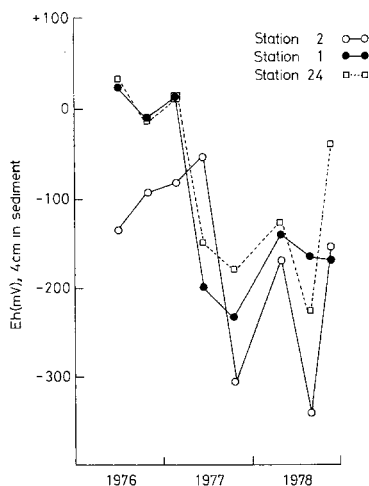


Fig. 3. Eh values at 4 cm depth in the sediments at Loch Eil Stations 2, 1 and 24 from April 1976 until November 1978. Mean values calculated from consecutive pairs of monitoring surveys are shown

Loch Eil Station 2 where conditions in the sediments are considerably influenced by the input of organic effluent from a wood pulp and paper mill. During the latter investigation, in 1976 and 1977 sediment conditions remained relatively constant and conditions at Loch Eil Station 2 were clearly more affected by the organic input to the Loch than were conditions at Stations 1 and 24.

Figure 3 shows Eh values at 4 cm depth in the sediments at Loch Eil Stations 2, 1 and 24 between April 1976 and November 1978. It is clear that major temporal fluctuations in sediment conditions, exemplified by Eh values and thought to be related to the effluent input, occurred from mid-1977 and there was frequent overlap of the ranges of Eh values measured at Station 2 and at Stations 1 and 24. Conditions in Loch Eil improved again late in 1978 and further specimens of *G. alba* were obtained from the vicinity of Stations 1 and 24, but no *G. alba* were obtainable at Station 2. Phosphofructokinase activity, which is thought to provide the most sensitive indicator of effects of the organic input on *G. alba*, was estimated in crude extracts prepared as before (Blackstock, 1978). All phosphofructokinase activities measured in crude extracts of *G. alba* from Loch Eil were then considered in relation to Eh at 4 cm in the sediment at the appropriate time and sampling location, and the data are presented in Figure 4. There is considerable scatter, particularly at high Eh values, but application of a non-parametric statistical test, calculation of the Spearman Rank Correlation Coefficient (r_s) (Siegel, 1956) gave a value of $r_s = 0.404$ ($N = 21$), indicating a significant correlation ($P < 0.05$) between phosphofructokinase in the crude extracts of *G. alba* and Eh at 4 cm in the sediments of Loch Eil.

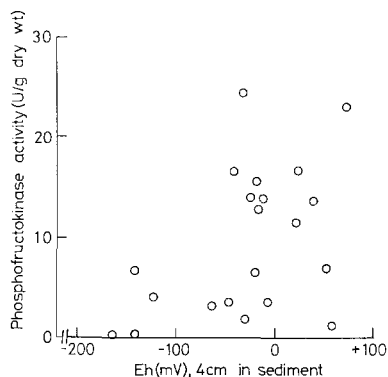


Fig. 4. Relation between phosphofructokinase activity in crude extracts of *Glycera alba* from Loch Eil and the corresponding values (means of 2-3 consecutive survey Eh measurements made at the sampling locations within 1 month of each specimen collection)

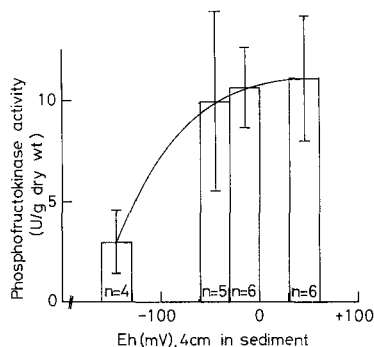


Fig. 5. Mean phosphofructokinase activities (\pm S. E.) in crude extracts of *Glycera alba* from Loch Eil. The four groups were obtained on the basis of Eh values and mean Eh \pm 15 mV ($>$ S. E.) are shown on the horizontal axis

For more detailed examination of the relation between the observed enzyme activities and Eh at 4 cm in the sediments all the data were grouped into 4 groups representing ranges of Eh values (a) below -100 mV, (b) -30 to -70 mV, (c) 0 to 25 mV and (d) $+20$ to $+75$ mV. The mean phosphofructokinase activities \pm S. E. in each group are shown in Figure 5. Mean phosphofructokinase activities in crude extracts of *G. alba* from group (a) (Eh -143 ± 17 S. D.) are clearly significantly lower than in groups (b), (c)

and (d). In addition the smooth curve in Figure 5 can be interpreted as indicating that the relation between mean phosphofructokinase activity in the crude extracts is non-linear and the greatest effect is observed at low negative Eh values.

DISCUSSION

Enzyme activities in *Glycera alba* from Loch Creran

The specimens of *Glycera alba* were collected in a single sampling trip and the enzyme activities can therefore only relate to the well defined spatial gradient of organic pollution effect which is known to exist as a consequence of the input of particulate organic effluent from the seaweed processing factory.

The mean phosphofructokinase activity in crude extracts of *G. alba* was lowest at Station H, some 400 m from the effluent outfall and increased to Station J some 900 m from the outfall. Therefore, in Loch Creran as well as Loch Eil (Blackstock, 1978), mean phosphofructokinase activities in crude extracts of *G. alba* have been found to be lowest in specimens from the sampling stations most affected by the inputs of particulate organic effluent. A relation between environmental conditions in the sediments, primarily a consequence of the organic inputs, and the observed phosphofructokinase activities is therefore suggested.

A quantitative comparison of the changes in enzyme activities in *G. alba* from Lochs Eil and Creran in relation to impact of the effluents is not possible at present since data on sedimentary conditions in Loch Creran at the time of sampling are lacking. However, the mean phosphofructokinase activity from Station J (25.2 mU/mg protein approx. 6 U/g dry weight) is much lower than the corresponding value (14.3 U/g dry weight) of mean phosphofructokinase activity observed in crude extracts of *G. alba* from Loch Eil station 1 (Blackstock, 1978). It is therefore clear that considerable differences in mean phosphofructokinase activity have been observed in the two populations of *G. alba* from Lochs Eil and Creran, and attempts to relate enzyme activity to effects of the particulate effluent must at present be restricted to comparisons within a single population. The technique can therefore only be applied with confidence in relatively small areas. It is probable that differing environmental conditions in Lochs Eil and Creran may affect the observed phosphofructokinase activities but at present the nature of such differences cannot be specified.

The low mean pyruvate kinase activity in *G. alba* from Loch Creran Station H is consistent with the earlier observations in this polychaete from Loch Eil and suggests that pyruvate kinase activities may also decrease in response to the effects of the effluent inputs, but the response is somewhat less sensitive than that observed with phosphofructokinase.

Relation between phosphofructokinase activities and Eh in the sediment

Eh at 4 cm in the sediments of Loch Eil is a reliable indicator of the sediment condition in relation to the effects of the input of particulate organic effluent from the wood pulp and paper mill at Annat Point, and is relatively unaffected by short-term environmental fluctuations at the sediment-water interface (Pearson & Stanley, 1979).

The relation between phosphofructokinase activities in the crude extracts and Eh at 4 cm in the sediment (Fig.4) is therefore an attempt to relate the enzyme activities to medium term fluctuations in sediment conditions which are thought to be largely influenced by variations in the effluent input.

The considerable scatter of points on Figure 4 is thought to be derived from a number of sources of variation including experimental errors in measurement of Eh and enzyme activity, possible effects on *G. alba* of short-term variations in conditions at the sediment-water interface and the inevitable variations in time and location between the Eh measurements and the collection of *G. alba* for biochemical analysis. In spite of these limitations in technique a semi-quantitative relationship between Eh at 4 cm and phosphofructokinase activity has been demonstrated. This relation is interpreted as indicating that sediment condition, as exemplified by Eh, has an effect on *G. alba* which is biochemically demonstrable as a decline in phosphofructokinase activities in the crude extracts. The smooth curve in Figure 5 shows that the effect is most pronounced at low Eh values, below -50 mV at 4 cm in the sediment. Pearson & Stanley (1979) observed that at Eh values between -100 mV and 0 mV at 4 cm in the sediment an increase in species diversity coincided with increase in Eh, and below -100 mV relatively few species of macrobenthic fauna survived. Increased stress of *G. alba* at Eh below -50 mV is therefore consistent with observations of species distributions in relation to effects of organic input on the sediment populations (Pearson, 1975). It is therefore possible that the low phosphofructokinase activities in crude extracts of *G. alba* from locations where the sediment Eh is low reflect a response to medium-term effects of the organic input on sediment conditions.

Metabolic significance of low phosphofructokinase activities

Phosphofructokinase is an allosteric enzyme which is sensitive to modulation of its activity by a number of intermediary effectors including AMP, fructose diphosphate, inorganic phosphate, citrate and ammonia (Mansour, 1972; Newsholme, 1977). It is therefore clear that activities measured in vitro in crude extracts of *Glycera alba* may not accurately reflect activities in vivo. However, Zammit & Newsholme (1976) have indicated that maximum activities of phosphofructokinase in crude extracts of marine invertebrate muscles can provide an indication of the maximum flux of metabolites through glycolysis. Pyruvate kinase also catalyzes a key reaction involved in the regulation of glycolysis and the decline of phosphofructokinase, and to a lesser extent pyruvate kinase activities, in the crude extracts is indicative of a possible decline in maximum glycolytic flux in *G. alba* from areas considerably affected by the organic inputs. It has been observed that phosphofructokinase activities were low in *G. alba* under anoxic conditions when the animals remained immobile and polychaetes from Loch Eil Station 2 (low phosphofructokinase) in 1976 were observed to be less active than *G. alba* from the less affected Stations 1 and 24. Below a certain critical level of phosphofructokinase activity a decreased capability for normal mobility is possible. However, mobility (and enzyme activity) may be rapidly restored (Blackstock, 1978). It is clear that more work is required to provide information on in vivo consequences of the changes in enzyme activities which have been observed in the crude extracts. The ability to maintain normal burrowing and feeding capability when phosphofructokinase activities are low will

depend on various factors including capability for nonglycolytic energy production, e. g. from phosphagens, and the total energy demand, which is considered to be generally low in *G. alba* (Ockelman & Vahl, 1970).

Enzyme activities as indices of pollution effects

Bayne et al. (1976) have suggested that useful indices of the biological effects of environmental change, including pollution, must satisfy two important criteria. The first criterion is that a measurable change in response bears a quantitative relationship with the stimulus. Secondly, the response should have a detrimental effect on growth, reproduction or survival.

Phosphofructokinase activities in crude extracts of *G. alba* have been shown to relate to sediment conditions, which have been quantified as Eh values at 4 cm in the sediment. The established relationship is at present only semi-quantitative and further assessment of the relationship at low Eh values in the range -50 to -150 mV is clearly required. The metabolic and ecological significance of the low phosphofructokinase activities also needs further elucidation; at present detrimental effects of the observed biochemical changes cannot be conclusively proven, although some impairment of energy-yielding metabolism with consequent impairment of feeding or burrowing capability is considered to be a probable consequence in the most affected individuals.

Some apparent inconsistencies of response have been observed, e. g. low α -glycerophosphate dehydrogenase activities and high malate dehydrogenase activities were observed only in *G. alba* from Loch Eil Station 2. This observation may be indicative of differences in the response of the different populations of *G. alba* to the effects of the effluents in Lochs Eil and Creran. The relatively low mean phosphofructokinase activity in *G. alba* from Loch Creran Station K, considered to be out with the area of influence of the effluent, may also indicate natural effects relating to the more impoverished fauna encountered in areas totally unaffected by organic enrichment.

Natural physiological rhythms may be reflected in enzyme activities estimated in crude extracts of polychaete tissues (Blackstock & Pearson, 1979). In *G. alba* the reproductive phase is thought to be of short duration and terminates the life of the worm (Ockelman & Vahl, 1970). In the immature specimens used throughout this investigation there was no evidence of any regular seasonal trends in enzyme activity (Blackstock, 1978) and the enzyme activities are considered to primarily reflect effects of environmental conditions. The wide distribution of *G. alba* in polluted and moderately polluted areas, the absence of regular seasonal rhythms of enzyme activity and the consistently low phosphofructokinase activities in crude extracts of *G. alba* from the areas most affected by the organic inputs into Lochs Eil and Creran are all considered to indicate the potential value of further investigations which may provide rapid, reliable biochemical indices of the effects of organic pollution on sensitive macrobenthic invertebrates associated with the diverse fauna found in unpolluted marine sediments.

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