The report can be obtained free from CONCAWE, 60 Van Hogenhoucklaan, The Hague, 2018, The Netherlands.

Rivers Polluters Fined

The Allied Chemical Corporation has been fined more than 13 million dollars—the largest penalty ever imposed for polluting the country's waterways—for contaminating the James River, Virginia.

The chemical firm was said to have shown 'appalling' neglect in pollution control practices while producing the pesticide Kepone—a poison which produced widespread damage to wildlife as well as heavily polluting the river James. Most of the pesticide is exported for use in Europe for the eradication of such pests as the Colorado Beetle.

Counting the Birds

Next month while lesser mortals are trying to keep warm at home, 600 volunteers will brave the icy blasts on Britain's beaches to hunt for dead seabirds.

Most of them, though, will be used to the task. It's now six years since the Beached Bird survey, run by the Royal Society for the Protection of Birds (RSPB) along with the Seabird Group, got into top gear. Five times during each winter, on set weekends, the volunteers walk a total of 2000 km of coastline to check for stricken birds.

Their findings, the number of birds found, their species, sex and extent of oil pollution (where that is the cause of their deaths) will be meticulously recorded on a postcard and sent to the RSPB headquarters in Sandy, Bedfordshire.

In this way the Society has, since 1971, been able to build up a picture of the oil pollution problem as it affects birds in the UK, and its variations both regional and seasonal.

The surveys have shown that the mortality rate is higher during January and February when the birds are at their most vulnerable because of the bitter cold and also that the two worst regional blackspots are the North East of England and the East coast of Scotland. More encouragingly, they have also revealed that fewer birds are now dying as a result of oil pollution than in the late 1960s.

In January, unless a major oil spill occurs, probably 1000 seabirds will be found during the volunteers' weekend walkabout on UK beaches. Between a quarter and a half of these will have died from the affects of oil pollution.

Every year in February the survey goes international and watchers in Britain work in the knowledge that their counterparts in West Germany, Denmark, Holland, Belgium and France will help to build up an overall European picture.

Britain, with considerably more volunteers than anyone else, has a midway placing in the five-country pollution table—between Denmark and Germany who have the worst problems, and the west coast of France which suffers least.

While there has been a recent reduction in the oiled-bird mortality rate in the seas of Northern Europe, the RSPB volunteers continue to maintain their ever-vigilant watch for beach pollution. The beached bird survey organiser, Miss Clare Lloyd said "As old pollution problems disappear, new ones often emerge. For this reason we are keeping a close eye on all the North Sea oil drilling operations."

A Cytochemical and a Biochemical Index of Stress in *Mytilus edulis* L.

The stability of the lysosomes and the latency of lysosomal enzymes of *Mytilus* are responsive to temperature and nutritive stressors. An empirical biochemical index of the molar concentration ratio of taurine:glycine in the mussel's tissue is also sensitive to stress. Changes in both of these indices correlate with the amount of energy available to the mussel for growth and are useful indicators of the degree of stress experienced by *Mytilus* in the natural environment.

In any attempt to establish useful indices of the biological effects of environmental changes, including pollution, at least two important criteria must be considered. These are, firstly, that a measurable change in a biological process (the response) occurs that bears a quantitative relationship with the altered environmental factor (the stimulus) and secondly, that the response can be shown to have a detrimental effect on growth, reproduction or survival. In this context an index is a variable which estimates the response of the individual animal to an environmental stimulus. The numerical value of the index must be a function of the stress acting on the animal and must be sensitive over the range of values for the stimulus that are anticipated or are relevant in the particular case. The second criterion is equally important. A change in the environment, whether natural or brought about by man, cannot be considered a deterioration unless the resulting change in the biological material is shown to be detrimental to the chances of survival of the individual or the population.

Indices of biological response may, of course, be sought at any level of complexity from the cell to the

ecosystem. In this paper we describe two indices of stress in *Mytilus*, one based on cytochemical and the other on biochemical observations. In both cases the index is responsive to environmental changes within the normal ecological range of the species. In order to meet the requirements of the second criterion discussed above, these indices have both been related in laboratory experiments to a physiological index, the 'scope for growth'. This index, which we have discussed elsewhere (Bayne, 1975a; Bayne, Widdows & Thompson, 1976; Widdows, 1976) is a measure of the energy available to the individual for growth and the production of gametes after the demands of respiratory metabolism have been met. It provides an estimate not only of physiological condition, but also of the ecological consequences of stress, since a reduced scope for growth and its derivative, growth efficiency, will have obvious detrimental effects for the individual. We have also demonstrated that a decline in the scope for growth is reflected in a reduced fecundity and viability of spawned eggs (Bayne, 1975b). The scope for growth therefore provides a physiological and an ecological correlate for the biochemical and cytochemical indices of stress.

Lysosomes and Lysosomal Enzymes

Lysosomes have been implicated in many physiological processes in bivalve molluscs, including intra-cellular digestion, resorption, storage, excretion and control of cellular economy (Owen, 1972). Lysosomal hydrolytic enzymes are of central importance in these processes and associated with their function is the property known as structure-linked latency. This phenomenon appears to be related to the structural binding of the hydrolases to the interior of the lysosomal membrane and to the lipoprotein matrix of the lysosome (Koenig, 1969). The selective impermeability of the lysosomal membrane to substrates may also be involved (Verity, 1973).

Reduced stability of lysosomes has been associated with a variety of conditions such as inflammatory responses of synovial lining cells in rheumatoid arthritis (Chayen & Bitensky, 1971), lysosomal sequestration of metals (Goldfischer, 1965; Moore & Stebbing, 1976) and physical exertion, hyperthermia, gravitational and emotional stress in rats (Gabrielescu, 1970). These conditions labilize the lysosomes resulting in reduced latency and increased levels of free hydrolase activity (Fig. 1). The induction of free enzyme activity as opposed to latent or bound activity can be measured cytochemically.

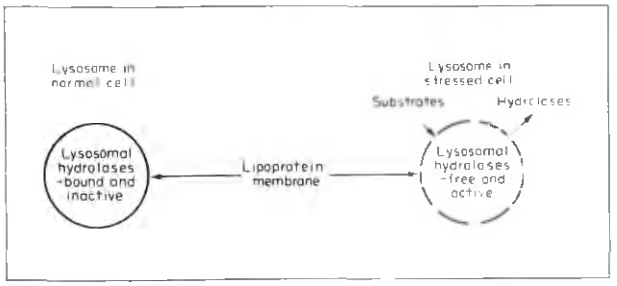

Fig. 1 Diagrammatic representation of the alterations in structure-linked latency of lysosomal hydrolases induced by stress.

TABLE 1
Lysosomal latency and the scope for growth in *Mytilus edulis*.

Experimental condition	Labilization period of glucosaminidase as % of control ± SE	Scope for growth (calories/day)
10°C/Fed	100 ± 5	+20
10°C/Starved	100 ± 5	—20
18°C/Fed	75 ± 5	0
18°C/Starved	30 ± 18	—79
25‰/Fed	75 ± 5	—18
15‰/Fed	40 ± 20	—40

A cytochemical staining method for the measurement of lysosomal stability in the digestive cells of *Mytilus edulis* has been developed, based on the length of incubation (the labilization period) at pH 4.5 that is required to give maximum staining intensity for the enzymes β-glucuronidase and *N*-acetyl-β-glucosaminidase (Moore, 1976). The method employs naphthol AS-BI glucuronide and glucosaminide as substrates respectively and collagen-derived polypeptides (Sigma P5115 and P5163) to stabilize the cytochemical reaction in cryostat sections.

Measurements of lysosomal stability in the digestive cells of *Mytilus* from a population in the Lynher river (Plymouth) over a period of eight months indicated consistent values for the labilization period, ranging from 16–20 min. Moore (1976) recorded a reduced latency for *N*-acetyl-β-glucosaminidase in mussels subjected to a thermal shock ($\Delta C = 13$–14°C). This reduction in labilization period was shown (Moore, 1976) to be reversible by treatment with a hormone known to stabilize lysosomal membranes (Weissmann, 1969).

The results of further experiments are shown in Table 1. Mussels were maintained for 60 days at 10 (= ambient temperature) and 18°C, at 33, 25 and 15‰ salinity, and either fed with cells of *Phaeodactylum* or starved. The mussels at 10°C with food represent the control condition and their mean labilization period (18 min) is taken as 100%. The labilization periods of the other conditions are presented as percentages of the control. Starvation at 10°C did not induce a significant decrease in latency, in spite of the negative scope for growth in these mussels, which signifies the utilization of body reserves for maintenance metabolism. This may indicate that lysosomal regulation of auto-digestive processes was being maintained under these conditions. In all other experimental treatments there was significant reduction in the labilization period and over all treatments there was a significant correlation between scope for growth and

TABLE 2
Changes in taurine and glycine concentrations and the scope for growth in *Mytilus edulis*.

Sampling time (days)	Experimental condition	Taurine (μmoles/g wet wt, ± SE)	Glycine (μmoles/g wet wt, ± SE)	Taurine / Glycine (mean ± SE)	Scope for growth (calories/day)
14	12°C/fed	31.27 ± 3.07	16.99 ± 0.84	1.85 ± 0.18	+22
	12°C/Starved	34.09 ± 1.35	17.85 ± 1.66	1.94 ± 0.12	—18
	19°C/Fed	34.17 ± 0.77	13.03 ± 0.78	2.65 ± 0.13	+17
	19°C/Starved	34.44 ± 3.34	13.27 ± 1.29	2.66 ± 0.34	—45
	22°C/Fed	36.26 ± 1.57	12.72 ± 0.33	2.86 ± 0.16	0
	22°C/Starved	33.98 ± 0.56	12.56 ± 0.70	2.74 ± 0.16	—68
28	12°C/Fed	30.06 ± 2.51	13.00 ± 1.56	2.35 ± 0.19	+22
	12°C/Starved	29.35 ± 1.88	13.30 ± 1.96	2.27 ± 0.23	—18
	19°C/Fed	33.71 ± 2.94	8.83 ± 0.88	3.90 ± 0.21	+17
	19°C/Starved	34.84 ± 0.77	10.99 ± 0.96	3.24 ± 0.21	—45
	22°C/Fed	36.14 ± 1.74	11.45 ± 1.46	3.40 ± 0.55	0
	22°C/Starved	30.62 ± 2.17	6.17 ± 1.15	5.30 ± 0.73	—68

latency (coefficient of correlation $r = 0.84$ for 5 degrees of freedom; $p < 0.05 > 0.01$).

Degenerative processes in animal tissues often involve autolysis by lysosomal hydrolases (Miller & Wolfe, 1968) while autophagy (Ericsson, 1969) involves the controlled digestion of cytoplasmic components within autolysosomes (Moore & Halton, 1973,1976). Autophagy can therefore provide the organism with a physiological survival mechanism, as a controlled utilization of stored energy reserves. or it may represent a pathological response to a stressor, or both of these (Ericsson, 1969). The results presented here indicate that decreased stability of lysosomes is related to the level of autolytic activity in the digestive cells of *Mytilus,* which are known to act as stores for reserves, which are mobilized at times of stress (Bayne, Thompson & Widdows, 1976). Our results also suggest that quantitative cytochemistry of lysosomal hydrolases can provide a suitable method for the investigation of catabolic changes at the cellular and sub-cellular level. Lysosomal hydrolases are associated with stress-induced structural and autolytic changes in the digestive cells of a number of gastropods (Moore, 1971; Moore & Halton, 1973) and a similar response has been quantified cytochemically in the hydroid *Campanularia flexuosa,* where metal-induced degeneration is related to an increase in free activity of lysosomal *N*-acetyl-β-glucosaminidase (Moore & Stebbing, 1976). The quantification of lysosomal latency clearly has potential as an index of pollution effect.

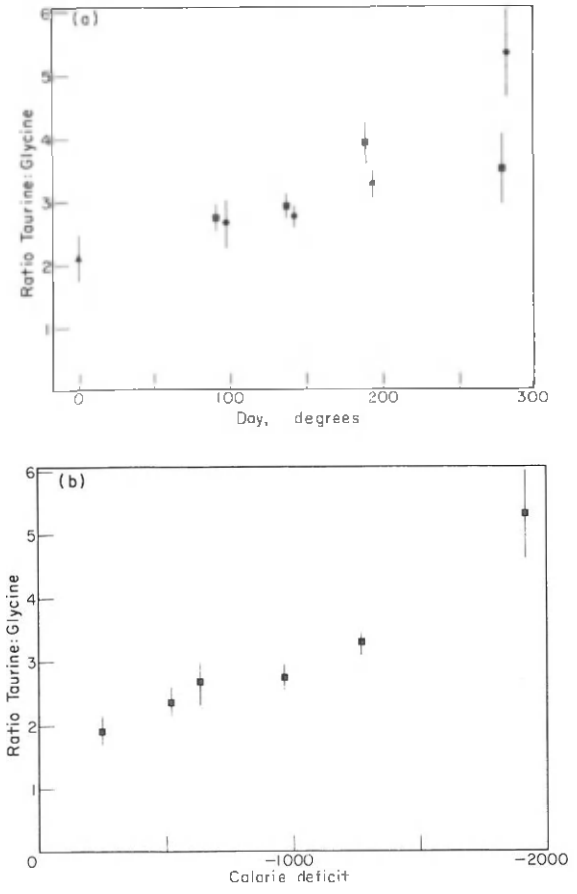

Fig. 2 A Changes in the taurine:glycine ratio (mean ± SE) in tissues of starved (•) and of fed (■) *Mytilus edulis* related to day degrees. B The taurine:glycine ration (mean ± SE) in tissues of starved *Mytilus* related to total calorie deficit calculated as scope for growth × days.

The Concentration Ratio of Taurine:Glycine

In comparing populations of the hard clam *Mercenaria mercenaria* from polluted and clean habitats of Narragansett Bay, Rhode Island, Jeffries (1972) described a stress syndrome in the polluted stock in terms of a series of morphological, cytological and biochemical observations. Prominent amongst these were changes in the concentrations of some of the free amino acids of the gill and mantle tissues; taurine and glycine increased and decreased, respectively, in response to environmental or laboratory stress. Jeffries therefore proposed the tissue molar ratio of taurine to glycine as a convenient index of stress. This proposal was tested in our laboratory using *Mytilus edulis.*

Mussels were collected from the Lynher population and allowed to acclimate over 14 days at field-ambient temperature (12°C), with cells of *Phaedoctylum* as food; this served as the control condition. Five other conditions were then established as follows: 1. Ambient temperature without food; 2. 19°C, with food; 3. 19°C, without food; 4. 22°C, with food; 5. 22°C, without food. Five individuals were removed from each of the six conditions after a further 14 days and 28 days, their tissues removed (the digestive glands discarded), damp-dried on filter paper, weighed and then frozen in liquid nitrogen and stored at −18°C. The amino acids were extracted by homogenisation in 70% ethanol and assayed on a commercial amino acid analyser (LKB model 4010). Animals were also collected from the field population at 6–8 week intervals and treated as above to obtain a seasonal picture of the taurine and glycine concentrations.

The taurine concentrations in the field samples showed no consistent changes over a ten-month period (June 1975 to April 1976) and had a mean value of 31.62 ± (S.E.) 1.26 μmol/g wet wt ($n = 25$). In contrast, glycine concentrations declined from 22.09 ± 0.85 in June to 13.39 ± 1.51 in November, with a seasonal mean of 18.16 ± 1.13 μmol/g wet wt ($n = 25$). The seasonal mean for the taurine:glycine ratio was therefore 1.86 ± 0.11 which was similar to the value of 1.85 ± 0.18 for the control animals after 2 weeks in the experiment (Table 2; 12°C/fed). The taurine concentration in the tissues did not change in response to temperature or ration stress and was little affected by the length of time the animals were kept under laboratory conditions. The concentration of glycine, however, declined in response to temperature increase but not in response to the lack of food. Glycine concentrations were lower after four weeks than after two weeks (Table 2), and when the taurine:glycine ratio was plotted against the product of the temperature increment and time (i.e. temperature above ambient in °C × days; Fig. 2a) an approximately linear relationship is suggested, both for fed and starved animals.

In attempting to relate the taurine:glycine ratio to the scope for growth, the problem arises that whereas, after 14 days, the physiological processes that integrate into the scope for growth (rates of feeding and oxygen consumption, and assimilation efficiency) were in steady-state, glycine concentrations in the tissues were not. Also ration, which has a marked effect on the scope for growth, appears to have little effect on the concentration of glycine. We have therefore calculated a calorie

deficit for individuals that were in negative energy balance at two and at four weeks and have plotted these values against the taurine:glycine ratio in Fig. 2b. There is a linear relationship between the two, but no such relationship is apparent between the amino acid ratio and scope for growth for the individuals in positive energy balance.

The taurine:glycine ratio appears to offer some promise as an empirical index of stress, therefore, at least when the stressor is temperature. Our unpublished observations suggest also that this ratio is responsive to salinity change although the relationship is complicated due to the involvement of the amino acids in isosmotic intracellular regulation. But more research is required before any general conclusions can be drawn as to the usefulness of this index in environmental studies and, particularly, more information is required on the underlying causes of the observed changes in glycine concentrations.

Conclusions

Both of the indices discussed in this paper are responsive to environmental stressors, particularly temperature and they can each, to different degrees, be correlated with a general index of physiological condition, the scope for growth. To that extent they meet, at least in part, the main criteria suggested earlier as necessary for the derivation of indices of stress. They also demonstrate a third important consideration in studies of this kind, namely the need for basic biological understanding of the processes underlying the responses that are being considered. In both cases we can recognise an emergent property of the individual organism under stress, namely the requirement to utilize its reserves of energy for metabolic maintenance. The known functions of lysosomal hydrolases relate directly to this requirement for controlled autolysis and the stress index that emerges bears a logical correspondence with a significant property at the level of the 'whole organism'. Extrapolations from knowledge of lysosomal latency to inferences about environmental health are therefore feasible, especially in view of the other known functions of lysosomes in storage and excretion of material, including pollutants. However, there is no such equivalence between the changes in the taurine:glycine ratio and the scope for growth and although a statistical relationship between the two is suggested, without more fundamental biological understanding the potential of this index in pollution studies is limited. A proper framework of scientific theory is essential for realising the maximum gain from the use of indices of condition in environmental research.

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