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MULTIPLE DISCRIMINANT ANALYSIS OF MACROBENTHIC INFAUNAL ASSEMBLAGES

PAUL K. S. SHIN[1]
Zoology Department, University College, Galway, Ireland

Abstract: Multiple discriminant analysis is applied, in a general benthic survey, to correlate the animal associations with some measurements of physical and chemical variables. Four major macrobenthic assemblages are identified in the study area. In addition, species characterizing the various faunal groups are defined. The delineation of such animal assemblages is mainly due to the relative organic carbon content and the fine silt-clay fraction (positive graphic skewness) of the sediment, which is largely deposited in the sampling area by a river drainage. The application of multiple discriminant analysis to demonstrate such correlative relationships between biotic and abiotic variables in benthic studies is discussed.

INTRODUCTION

Marine benthic surveys usually generate a large bulk of station–species records. Such records, together with measurements of some environmental conditions, are the only data available and thus must be relied upon to elucidate any structure of, or causal relationships within, benthic ecosystems. Various mathematical treatments have been employed to reduce large amounts of ecological data to manageable proportions and to search for patterns in sets of multi-species records. Multivariate analyses, such as classification and ordination methods, have been widely applied in marine benthic studies (e.g. Field, 1970; Day *et al.*, 1971; Williams & Stephenson, 1973; Christie, 1976). Classification sets out to identify discrete patterns (clusters) of co-occurrence within the station–species data whereas in ordination, samples are ordered along a set of co-ordinate axes, which in an ecological context, usually corresponds to gradients of environmental conditions (Whittaker, 1967; Clifford & Stephenson, 1975).

Multiple discriminant analysis (canonical variate analysis) is a statistical method which determines functions whose application to the original data maximizes the observed variations among different groups (Cooley & Lohnes, 1971). Unlike classification and ordination, this method begins with a set of stations which have been already grouped and aims only to search for the relationship between these groups. Since one starts with already defined clusters, multiple discriminant analysis is not a pattern analysis method and has not been widely employed in benthic studies.

[1] Present address: Fisheries Research Station, Aberdeen, Hong Kong.

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Polgar (1975) attempted to explore and develop multiple discriminant analysis as a technique to characterize and compare benthic macrofaunal assemblages. A mapping of the sampling stations into discriminant space may reflect the responses of community structure to environmental variations. His method, however, requires stratified sampling designs with respect to the environmental change or gradient being investigated.

This paper suggests a further application of multiple discriminant analysis of data from a general benthic survey and illustrates the stages involved in employing such an analysis to correlate the animal assemblages with some measured environmental conditions.

MATERIAL AND METHODS

SAMPLING

The survey area, North Bay, is located in the northeastern sector of the inner Galway Bay on the west coast of Ireland. The region to the north of the Bay is drained by the River Corrib, its outflow affecting, to a considerable extent, the salinity and bottom sediment characteristics. A total of 83 stations was sampled on a 0.4 × 0.4 km modular grid pattern within the whole extent of the area studied (Fig. 1). Station depth was recorded by an echo sounder (Ferrograph "Offshore" 500) and was then corrected back to zero chart datum.

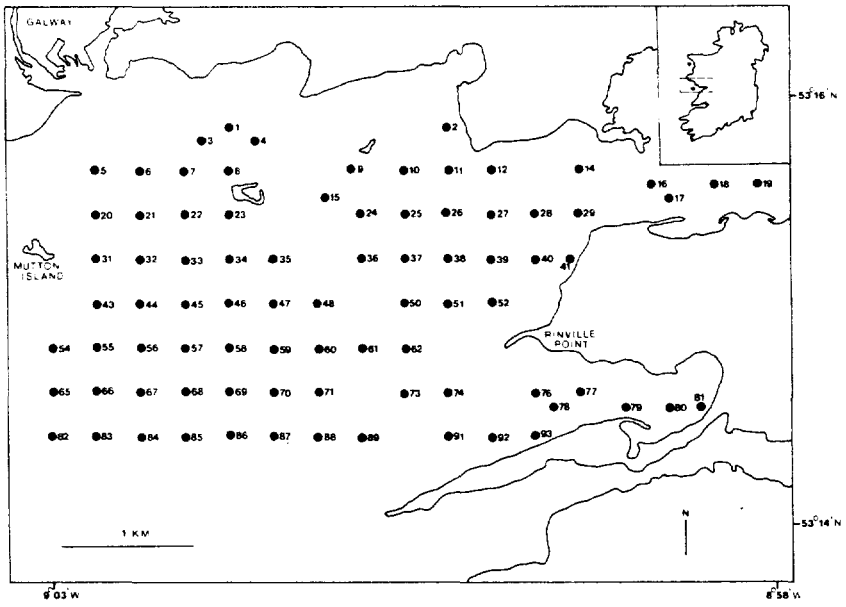

Fig. 1. The location of the sampling stations in North Bay (west coast of Ireland).

Two replicate 0.1-m^2 Van Veen grab samples were taken at each station, one for the faunal study and one for sediment analyses. All faunal samples were washed through a 1-mm^2 sieve and the residue was preserved in 5% borax buffered formalin before being sorted, identified and counted in the laboratory. Granulometric analysis of the sediment samples was carried out according to the steps outlined in Folk (1974). The cumulative size frequency of each sediment sample was plotted and the size distribution parameters, i.e., graphic mean, sorting, skewness, and kurtosis, were calculated (Folk, 1974). In addition, the organic carbon content of the sediment was determined by the chromic acid oxidation method (Holme & McIntyre, 1971) and the heavy metals, zinc, copper, lead, and iron were analysed by atomic absorption following the Telfon bomb digestion method (Bernas, 1968).

DATA ANALYSIS

A total of 258 species was identified in this survey. Owing to programming limitations, species which were only recorded twice were omitted in data analysis procedures. A faunal data set of 83 stations × 192 species was adopted and later transformed logarithmically (ln (species count + 1)) prior to the subsequent analyses. In the case of abiotic variables, e.g., organic carbon and heavy metals (zinc, copper, lead, and iron) the percentage values were transformed into angular values using arcsine $\sqrt{\text{percentages}}$ (Bilyard & Carey, 1979).

A hierarchical, agglomerative classification was carried out to discern the station (site) groupings using the Bray–Curtis similarity index (Bray & Curtis, 1957) with a group average sorting method (Clifford & Stephenson, 1975).

The grouping of the sampling stations which resulted from the classification was assessed by the *F*-test (Sokal & Rohlf, 1969). The *F* value for every species was calculated and listed in order of the magnitude of its contribution to the different station groups. For each species, the geometric mean (mean of the logarithmic abundance) was also computed (Elliott, 1977) and was presented as an aid to assess the group (or groups) of stations defined by that species.

Multiple discriminant analysis (Davies, 1971) was carried out to correlate the station group separation with the physical and chemical environmental variables measured. The results of the four particle size distribution parameters, the organic carbon value, the heavy metals, zinc, copper, lead, and iron, and the station depth were divided into groups according to the site group formation resulting from the classification. Thus this method of analysis elucidates the separation of faunal assemblages in a ten-dimensional environmental space. In addition, the significance of the discriminant functions obtained from the analysis was assessed by the chi-squared test (Bartlett, 1947).

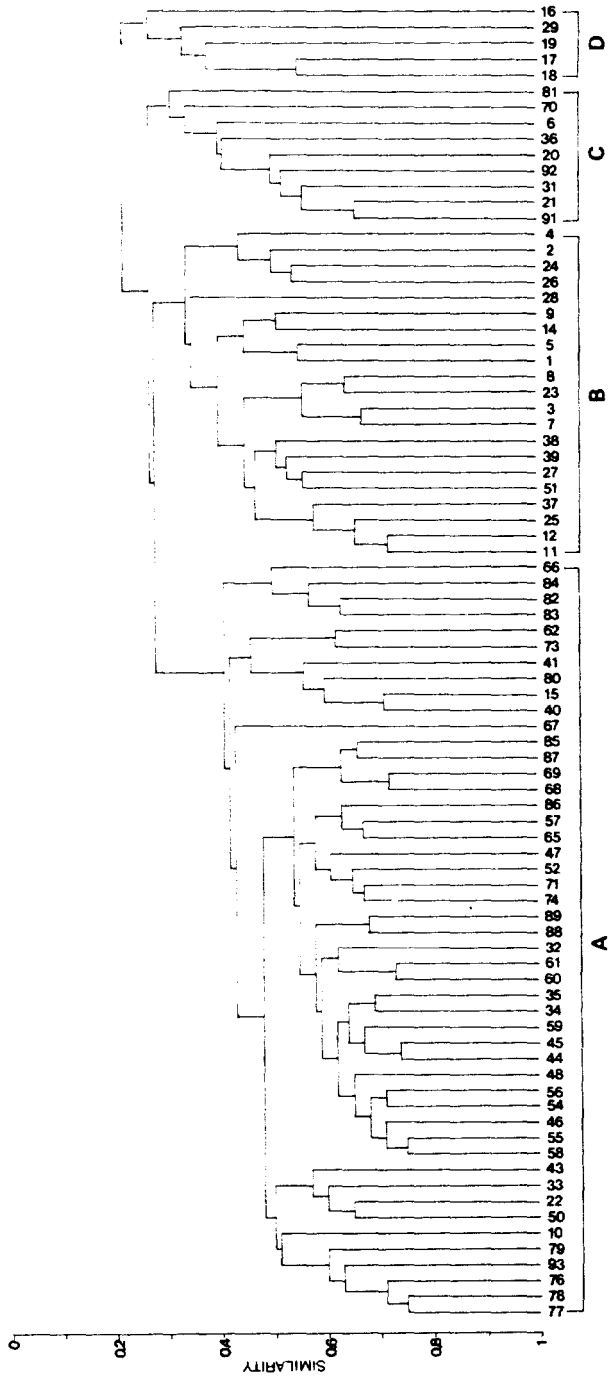

Fig. 2. Dendrogram showing the grouping of stations from the results of the classification method.

site Group B whilst the bivalve *Abra nitida* was numerically dominant in Group C. At site Group D, the geometric mean numbers of the species, which notably included the polychaetes *Streptosyllis websteri*, *Microphthalmus similis*, *Pisione remota*, and *Malacoceros ciliata*, were relatively low in comparison to the other three station group members.

Table II shows the results of the multiple discriminant analysis of the environ-

TABLE I

Results of the *F*-test with species having *F* values at $P \leq 0.001$ significance level.

Species	*F* value (3,79 d.f.)	Geometric mean no. individuals/0.1 m^2			
		Group A	Group B	Group C	Group D
Prionospio malmgreni Claparède	46.13	15.75	0.58	1.71	0.43
Pholoë minuta Malmgren	30.43	9.73	0.20	0.30	1.61
Paradoneis lyra (*sensu* Laubier & Ramos)	28.20	4.75	0.00	0.29	0.00
Mysella bidentata (Montagu)	27.09	13.16	1.25	0.79	0.32
Melinna palmata Grube	25.04	8.83	0.41	0.22	0.67
Scalibregma inflatum Rathke	22.92	4.75	0.13	0.00	0.72
Streptosyllis websteri Southern	22.18	0.02	0.00	0.00	1.05
Lumbrineris gracilis Ehlers	20.65	7.30	0.20	0.17	0.00
Tellina fabula Gmelin	19.66	3.22	26.63	0.26	1.14
Ampharete grubei Malmgren	16.74	7.17	0.80	0.42	0.00
Tanaid spp.	15.66	2.74	0.00	0.00	0.00
Microphthalmus similis Bobretzky	15.42	0.00	0.00	0.00	1.43
Eteone longa/flava (Fabricius)	14.86	3.83	0.80	0.26	1.61
Terebellides stroemi Sars	14.59	2.73	0.00	1.27	0.00
Exogone gemmifera (Pagenstechner)	14.14	1.38	0.03	0.08	0.00
Lagis koreni Malmgren	13.40	4.03	0.47	0.74	0.00
Magelona mirabilis (Johnston)	13.26	0.05	1.64	0.13	0.00
Thyasira flexuosa (Montagu)	12.77	17.66	1.58	4.25	0.00
Abra alba (Wood)	12.46	17.83	2.45	3.50	2.31
Mediomastus fragilis Rasmussen	11.57	6.19	0.03	0.70	1.51
Thracia phaseolina (Lamarck)	10.85	6.38	1.17	0.08	0.32
Ampelisca tenuicornis Lilljeborg	10.75	1.50	0.00	0.08	0.00
Goniada maculata Oersted	10.26	0.73	0.03	0.00	0.00
Notomastus latericeus Sars	9.88	1.79	0.19	0.56	0.00
Pisione remota Southern	9.34	0.00	0.00	0.00	1.37
Glycera convoluta Keferstein	8.55	1.89	0.14	0.68	1.27
Magelona alleni Wilson	8.33	0.97	0.00	0.00	0.00
Aricidea neosuecia Hartman	7.19	2.01	0.23	0.08	0.00
Mya arenaria Linnaeus	6.84	1.69	0.31	0.17	0.25
Lumbrineris latreilli Audouin & M. Edwards	6.67	0.96	0.00	0.00	0.00
Spiophanes bombyx (Claparède)	6.65	3.63	4.57	0.17	0.00
Aonides oxycephala (Sars)	6.42	1.70	0.00	0.00	0.38
Malacoceros ciliata (Keferstein)	6.19	0.00	0.00	0.00	0.25
Nephtys hombergii Audouin & M. Edwards	6.10	8.75	11.46	7.52	0.95
Perioculodes longimanus (Bate & Westwood)	6.10	0.23	0.82	0.00	0.00
Amphiura filiformis (Müller)	6.07	1.09	0.09	0.00	0.00
Abra nitida (Müller)	6.07	2.02	1.44	14.18	0.00

mental conditions recorded in the study. The discriminant functions (DF) I and II contribute 91.80% of the total separation among the groups ($P \leqslant 0.001$). Station positions along these two function axes are plotted in Fig. 4. As most of the stations have positive values on the two axes, attention is focussed on the coefficients of discriminant functions which produce the co-ordinates. A high positive value along the discriminant function I is due to the organic carbon content and, to a lesser extent, the iron concentration and the graphic kurtosis of the sediment samples. A positive value along the discriminant function II is the result of the graphic skewness and also of the mean grain size of the sediment.

The centroid of Group A is well separated from that of Groups B and D along the axis DF I whilst B and D are far apart when their centroids are projected along the axis DF II (Fig. 4). Group C is distinct from Group A along the second discriminant function axis but is separated from Group B along the first one. Groups C and D are outstanding with reference to either axis in this two-dimensional plot. In essence, the separation of Group A from B and D is due to the higher percentage of organic carbon content in the sediment at the Group A stations (Table II). On the other hand, the difference in the degree of skewness of the particle size distribution at the sampling stations results in the delineation of site Groups A and C as well as B and D.

DISCUSSION

BENTHIC ASSEMBLAGES

Four major site groups were delineated in the classification method based on the faunal data. Station Group A is defined by the numerically dominant species such as *Abra alba*, *Thyasira flexuosa*, *Prionospio malmgreni*, and *Mysella bidentata*. In addition, other characterizing species include *Melinna palmata*, *Lumbrineris gracilis*, *Ampharete grubei*, and *Pholoe minuta* (Table I). This assemblage is seen as being an admixture of the classical *Syndosmya* (= *Abra*) *alba* community (Thorson, 1957) and the *Melinna palmata* association of the *Amphiura filiformis* sub-community (viz. Keegan *et al.*, 1976). The *Syndosmya* (= *Abra*) *alba* community occurs in sheltered and estuarine environments on a mixed to muddy bottom rich in organic material. The *Melinna palmata* association of the *Amphiura filiformis* sub-community, as reported by Keegan *et al.* (1976) in Galway Bay (west coast of Ireland), is found on sediment with a silt-clay content ranging from 7 to 15%.

Site Group B in this survey is characterized mainly by *Tellina fabula*, *Nephtys hombergii* and, to a lesser degree, by species such as *Spiophanes bombyx*, *Magelona mirabilis*, and *Perioculodes longimanus*. This association is equivalent to the *Tellina fabula* sub-community described by Sparck (1935) as replacing the *Syndosmya* (= *Abra*) *alba* community on clean sand in shallow water. Most of the stations in this site group were on hard-packed sand.

The equality of the group means of the physical and chemical factors has not been tested in the multiple discriminant analysis applied in this study. Even with mathematical transformations, the data may not be truly normal in distribution and, thus, the equality of the among-group and within-group data matrices is assumed and not tested (viz. Buzas, 1967). Moreover, the Bartlett's test is also sensitive to non-normality (Buzas, 1967) giving more "weight" to those discriminant functions that account for most of the total variability.

A similar analytical method has been applied by Green & Vascotto (1978) to correlate the spatial patterns of freshwater zooplankton with some environmental factors. It should be noted, however, that multiple discriminant analysis, like many other statistical methods, is only applicable to continuous data. Sometimes obviously important factors, such as the presence of pieces of limestone, may influence the fauna but cannot be quantified on a continuous scale.

Many multivariate methods have been employed in elucidating the possible statistical relationships between biotic and abiotic variables on benthic data. For example, ordination techniques such as principal component analysis have been applied to identify the functional components of the benthos (Hughes & Thomas, 1971). Lie (1974), on the other hand, used principal component analysis of a benthic fauna followed by multiple regression to explore the correlation between the extracted components and the environmental variables. Principal component analysis, however, is sensitive to heterogeneity and should only be employed to analyse relatively homogeneous data, i.e., those with few zero records (Hughes *et al.*, 1972). More recently, Poore & Mobley (1980) demonstrated that, with an appropriate method of data reduction, canonical correlation analysis may also be useful in defining the statistical relationships between the environment and the fauna. In this study, the application of multiple discriminant analysis shows that the macrobenthic infauna of the sampling area, which clusters into groups with similar species composition, occupies different positions in the environmental space. Most important of all, the original ten-dimensional space can be reduced to two dimensions representing two ecologically interpretable factors. No data reduction scheme, except for the omission of the rarely recorded species, and no stratified sampling design are required. In addition, the same analysis can also be applied as a significance test to the delineation of benthic assemblages resulted from classification methods (Holland *et al.*, 1977; Mountford *et al.*, 1977). To this end, the proposed method of relating the distribution of species with environmental variables seems applicable to benthic studies, particularly to general survey data.

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1969) and specimens from Irvine Bay, Firth of Clyde, have been used in previous laboratory studies (Pullin *et al.*, 1980).

P_{O_2} measurements in the field and in the laboratory (Pullin *et al.*, 1980) have indicated that the P_{O_2} in the burrow may fall to below 40 Torr during periods when the fish is resting in the terminal chamber. Against this background the present study investigates the respiratory properties of the whole blood of *Cepola* and a theoretical analysis is made of the function of the haemoglobin under environmental conditions.

MATERIAL AND METHODS

C. rubescens were obtained from ≈ 50 m depth by trawling in Irvine Bay, Firth of Clyde, Scotland. Animals were allowed to recover for 4 wk in flowing sea-water aquaria (15 ± 1 °C, 34‰ salinity) containing mud substratum to a depth of ≈ 70 cm. Only animals which had formed burrows and were occupying them were used in the subsequent analysis of whole blood respiratory properties.

BLOOD SAMPLING

Blood samples were taken avoiding contact with atmospheric air, from fish which had been swimming at the mouth of the burrow, directly from the caudal vein into a heparinized syringe (Cortland saline adjusted to 160 mEq $\cdot l^{-1}$ sodium and heparinized at 100 IU $\cdot ml^{-1}$). The blood samples were always dark red in colour and their origin was later verified by dissection. Caudal vein blood was used because it has been shown not to differ significantly from true mixed venous blood in both teleosts and elasmobranchs (Itazawa, 1970; Piiper & Baumgarten-Schumann, 1968). Each fish to be sampled was netted and a blood sample quickly drawn. The sampling procedure was performed within 10 s and no evidence of increased lactate levels due to handling was found. Due to the small size of the animals studied (mean body wt = 83 g) it was only possible to obtain 0.5–0.75 ml of blood from each animal. The blood samples were immediately placed on ice prior to analysis.

OXYGEN DISSOCIATION CURVES

Whole blood oxygen dissociation curves were constructed by equilibration of 50-μl blood samples in tonometers (Radiometer, BMS II) thermostatted at 15 °C, with known gas tensions supplied by gas mixing pumps (301/a-f, Wösthoff, Bochum, F.R.G.). After equilibration (≈ 20 min) a 15-μl sample was taken from the tonometer and the total oxygen content (C_{O_2}) measured using the method of Tucker (1967). Haemoglobin bound oxygen (C_{HbO_2}) was then calculated by subtracting physically dissolved oxygen (P_{O_2} of the tonometer) from the measured total oxygen content. For a given pH value (P_{CO_2}) the oxygen carrying capacity

The effect of pH on oxygen affinity is shown in Fig. 2. The Bohr effect ($\Delta \log P_{50}/\Delta pH$) was -1.19 ($r = -0.99$) for whole blood for a pH range from 7.3 to 8.1. This compares with a value of -0.78 for stripped haemoglobin over a pH range of 7.3 to 7.8 (Pullin *et al.*, 1980). At the mean pH_v, P_{50} was 27 Torr for whole blood compared to 7 Torr for stripped haemoglobin.

The haemoglobin of *Cepola* also exhibits a strong Root effect i.e. oxygen carrying

TABLE I

Blood respiratory properties of *Cepola rubescens* maintained in burrows in the laboratory: n_{50} is calculated Hill coefficient at P_{50}; $(O_2cap)_{max}$ measured at P_{O_2} = 190 Torr and P_{CO_2} = 0.73 Torr.

	Units	Mean	± SD	*n*
Body mass	g	82	17	6
pH_v	–	7.55	0.03	6
In vivo P_{50}	Torr	27	3	4
n_{50}	–	1.56	0.48	4
Bohr factor	$\Delta \log P_{50}/\Delta pH$	−1.15	0.12	4
$(O_2cap)_{max}$	vol.%	3.99	0.57	6
Pv_{CO_2}	Torr	3.18	1.05	6
$[HCO_3^-]$	mmol·l^{-1}	4.18	0.73	6
Buffer value	mmol·l^{-1}·pH^{-1}	5.43	2.03	6

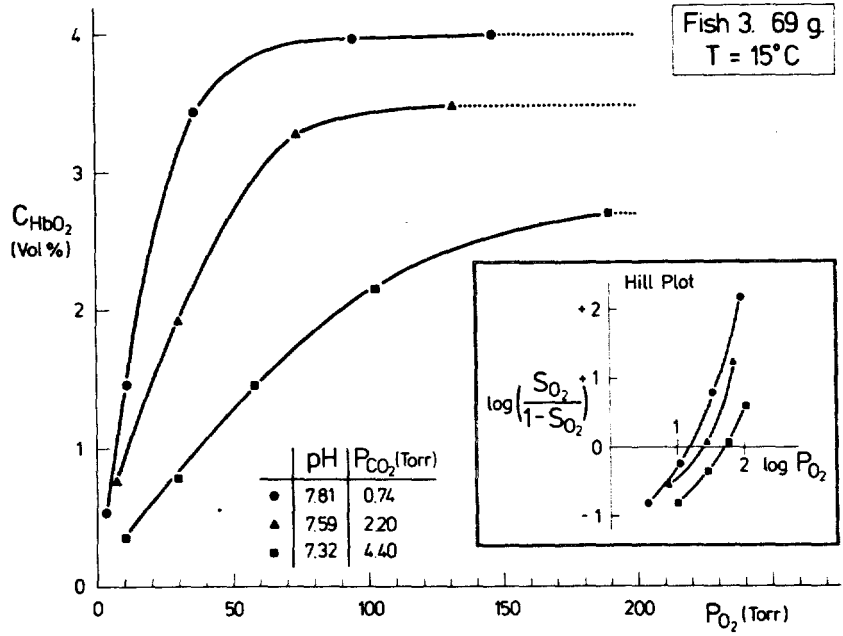

Fig. 1. Whole blood oxygen dissociation curves for a 69-g *Cepola* measured at 15 °C and at three different P_{CO_2}/pH levels: inset shows Hill plot for the three curves.

capacity, O_2cap, decreases with increasing P_{CO_2} and decreasing pH (Fig. 3). Little or no Root effect is seen at pH values >7.9, (defined as $(O_2cap)_{max}$), however, at the mean pH of venous blood the Root effect resulted in a 20% reduction in oxygen carrying capacity from its maximum level. At the lowest pH tested 7.2, oxygen carrying capacity had decreased by 42%.

Discussion

In those organisms which burrow into marine sediments oxygen may become a limiting factor and, therefore, respiratory adaptations may become an important requirement for survival.

Pullin *et al.* (1980) have shown that when *Cepola* spends long periods concealed in its burrow then the burrow water P_{O_2} may be below 40 Torr for several hours. *Cepola* may, therefore, be exposed to hypoxia, hence the ability to change haemoglobin oxygen affinity in response to environmental stress may be advantageous, as shown for the eel (Wood & Johansen, 1972; Weber *et al.*, 1976). This adjustment in oxygen affinity is mediated through the large Bohr effect shown by *Cepola* haemoglobin.

Relatively large Bohr shifts are shown in some of the benthic living flatfish which may also be exposed to hypoxia (Weber & De Wilde, 1975) and a number of burrowing organisms also show large Bohr shifts (Toulmond, 1970; Miller *et al.*, 1977). A direct correlation of burrowing with high Bohr shift must, however, be dependent on the individual species' response to hypoxia in terms of acid-base balance.

Cepola is a relatively inactive quiescent species with a low oxygen consumption (Pullin *et al.*, 1980) and a low oxygen-carrying capacity for blood (≈ 4.0 vol.%). Root (1931) and Putnam & Freel (1978) both found a correlation between low oxygen-carrying capacity in sedentary benthic forms compared to high oxygen capacity in active pelagic forms.

Large Root effects similar to that shown by *Cepola* have been demonstrated in a number of species of marine fish e.g. mackerel and toadfish (Root, 1931), eel (Steen, 1963), and flounder and plaice (Weber & De Wilde, 1975). *Cepola* has a well-developed swim bladder and the Root effect may assist in the maintenance of neutral buoyancy while the fish is in the mouth of the burrow.

The physiological significance of Bohr and Root effects occurring together and the consequences for gas-exchange are, however, not fully understood although correlations have been made between the Bohr effect and environmental stresses (Willmer, 1934; Johansen & Weber, 1976). In an attempt to understand how Bohr and Root effects may interact in gas transport Fig. 4 has been constructed from in vitro oxygen dissociation curves. Three curves are shown each representing a different pH/P_{CO_2} level. The influence of the Bohr and Root effects on oxygen

content of the pigment is shown by Lines A, B and C. Lines A and B represent a hypothetical situation in which only a Root effect (Line A) or a Bohr effect (Line B) are operative. Line C represents the observed in vitro change in P_{50} with pH i.e. both Bohr and Root effects are operative. Four possible levels of arterial

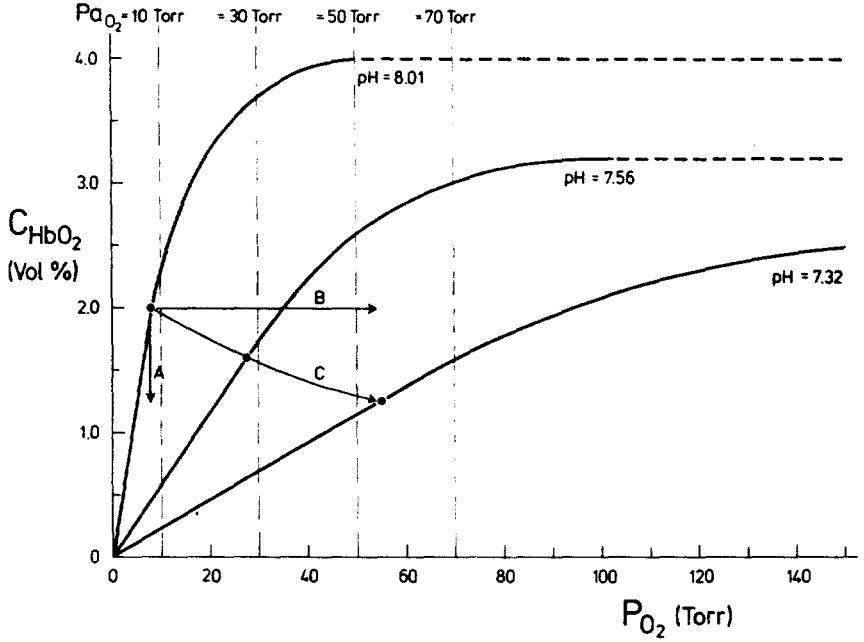

Fig. 4. Relationship between haemoglobin bound oxygen (C_{HbO_2}) and P_{O_2} at three different pH levels for the blood of *Lepola* measured at 15°C: Line A represents the theoretical change in (C_{HbO_2}) with only a Root shift present; Line B represents the theoretical change in P_{50} with only a Bohr shift present; Line C represents the in vitro change in P_{50} with Bohr and Root shifts present; four theoretical levels of Pa_{O_2} are shown.

blood P_{O_2} are shown and for each pH/P_{50} level the $(Ca–Cv)_{O_2}$ difference can be calculated for a given Pv_{O_2} value. A similar calculation can be made for the case where no Root effect is present and, therefore, $(O_2cap)_{max}$ remains the same at all pH levels, whereby P_{50} changes due to the Bohr effect. The results are expressed by plotting the $(Ca–Cv)_{O_2}$ difference against pH for the different arterial and venous blood P_{O_2} levels (Fig. 5A–D).

As the supply of a given oxygen demand is met by the product of cardiac output and the $(Ca–Cv)_{O_2}$ difference, a large $(Ca–Cv)_{O_2}$ difference is more favourable in energetic terms. A certain venous driving pressure for oxygen is, however, necessary to maintain the supply of oxygen at the cellular level. It can be seen in Fig. 5A–D that in all cases, except at high pH values, where the Root effect is absent, the presence of the Root effect appears to be disadvantageous for O_2 transport at a given cardiac

output. This disadvantage may be a by-product of requiring a Root effect to help maintain buoyancy via the swimbladder and thus resulting in an overall energy saving. The Root effect may also function like the Bohr effect in helping to supply rapidly metabolizing tissues which may show pH gradients. Fig. 5 represents the

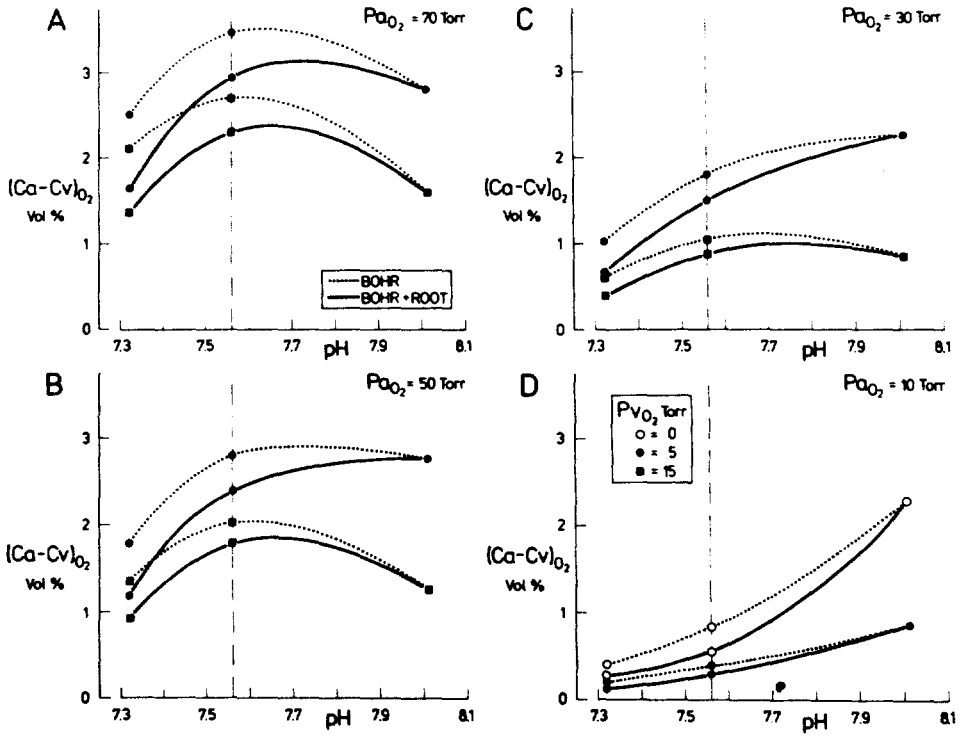

Fig. 5. Calculated effects of change in pH on arterial–venous oxygen content difference, $(Ca–Cv)_{O_2}$, at different values of Pv_{O_2}, for four different levels of Pa_{O_2} (A,B,C,D): physically dissolved oxygen is accounted for; continuous line represents calculations based on Line C (see Fig. 4) with both Bohr and Root effect present; dotted line represents calculations based on Line B (see Fig. 4) with only a Bohr effect present; vertical dashed line indicates the mean measured pH of venous blood.

theoretical levels for Pa_{O_2} and Pv_{O_2}, the actual levels will depend upon environmental conditions, but in general when the oxygen pressure in the inhalant water decreases then both *Pa* and Pv_{O_2} will decrease, but at differing rates. Initially when *Cepola* is swimming at the mouth of the burrow the P_{O_2} of inhalant water will be high, exceeding 100 Torr (Pullin *et al.*, 1980). If arterial oxygen tensions are high around 70 to 50 Torr (Fig. 5A,B) then a blood pH of 7.56 will not be detrimental to the oxygen transport system and when the venous driving pressure is around 15 Torr then this pH will maintain the $(Ca–Cv)_{O_2}$ difference near optimal. Higher pH values are detrimental to oxygen transport due to the shifting of the oxygen

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