The use of serological techniques for gut-content analysis in Cephalopods

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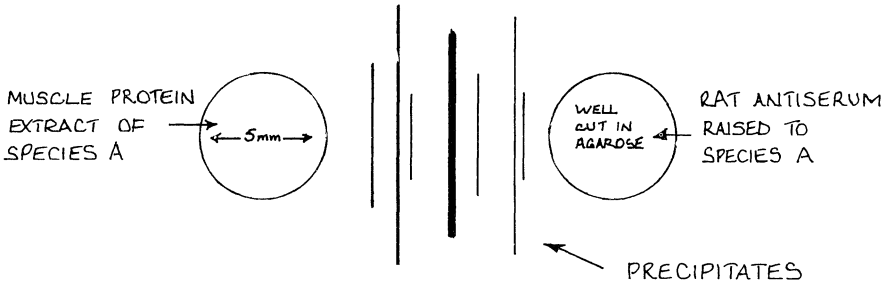
Cephalopods are important both to commercial fisheries and as a segment of the marine food chain supporting marine vertebrates, in particular whales, seals and some birds (Clarke, 1977, 1983). There is however, little information on their food intake and trophic relationships.

Conventional methods of gut content analysis can be applied to cephalopods such as squid but may be unreliable because, in general, recognisable fragments of prey are not ingested. In recent years serological analysis has been employed to unravel the complex food webs of benthic communities (Young et al., 1964; Young, 1973; Feller et al., 1979; Feller & Gallagher, 1982). The techniques involve the use of anti-sera, containing antibodies to putative prey muscle proteins, which have been raised in a taxonomically unrelated species (such as rats or rabbits). These antisera can be used to specifically identify the protein origin of food fragments in the gut of the predator.

Mammalian antibodies form insoluble complexes with their

homologous antigens; in the work described here these antigens are the muscle proteins of a crab species to which the antiserum has been raised. When placed in wells in a supporting medium, such as agarose, and allowed to diffuse together these complexes can be seen as precipitation lines between the two wells (Fig. 1). This passive method of contact between antigens and an antiserum is purely qualitative and only gives information concerning the number of antibody/antigen reactions that have occurred. In the case shown in Fig. 1 this number is 7.



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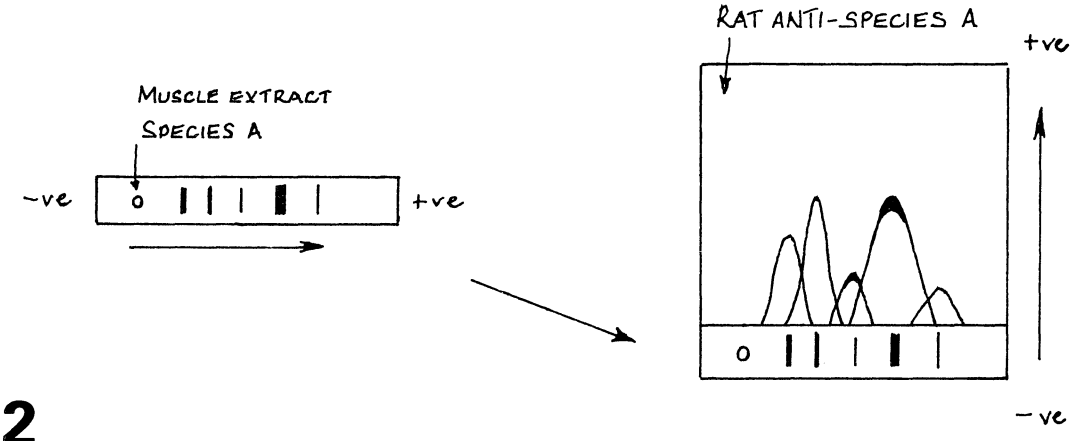
In the application of serological analysis to the gut contents of Eledone cirrhosa we have increased the sensitivity of the technique by using crossed immunoelectrophoresis (CIE). The soluble material from a homogenate of muscle proteins from a potential prey species (antigens) are separated electrophoretically in agarose (along the horizontal axis of the final plate, fig. 2). The agarose strip containing the separated antigens is moved to a glass plate and a new gel containing homologous antiserum is cast on the same plate. The antigens are then electrophoresed a second time, this time at right angles into the antiserum-containing gel (along the vertical axis of the final plate, fig. 2). The immune precipitates apppear as peaks (Fig. 2). Each prey species examined gives a characteristic pattern



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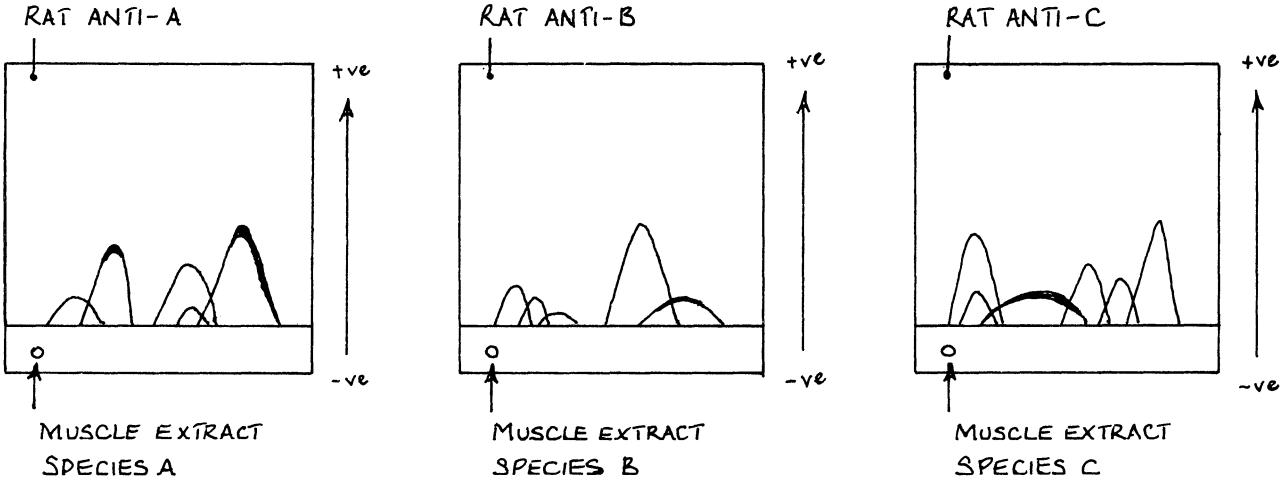
of immune precipitates, which we call a signature, when electrophoresed into its homologous antiserum (Fig. 3).



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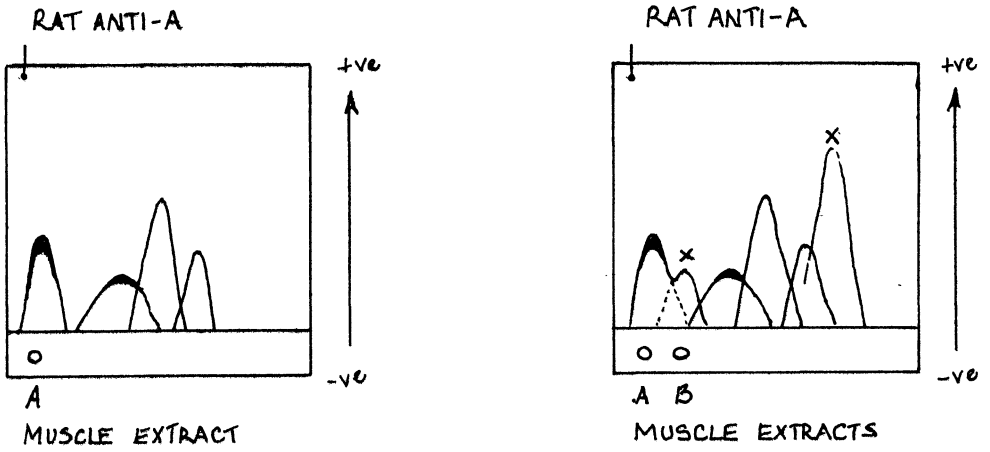
One of the major problems encountered with serological techniques is the cross-reaction of antisera with prey species other than the one to which they have been raised. These are said to result from interaction with heterologous antigens. When dealing with taxonomically related prey species it is inevitable that some proteins will be

antigenically similar. The presence of cross-reactions considerably reduces the confidence with which an antiserum can be used to identify gut contents.

One way of reducing this problem is to identify precisely those proteins which are common to several prey species and by using CIE this is possible. By applying homologous and heterologous antigen mixtures on a tandem arrangement during the first electrophoretic separation, it is possible to directly compare the profile of precipitation peaks that each mixture forms during the second-stage electrophoresis (Fig. 4). Cross-reactions appear as new isolated peaks or peaks that are wholly or partially linked to peaks that normally arise between the homologous antigens and their antiserum.



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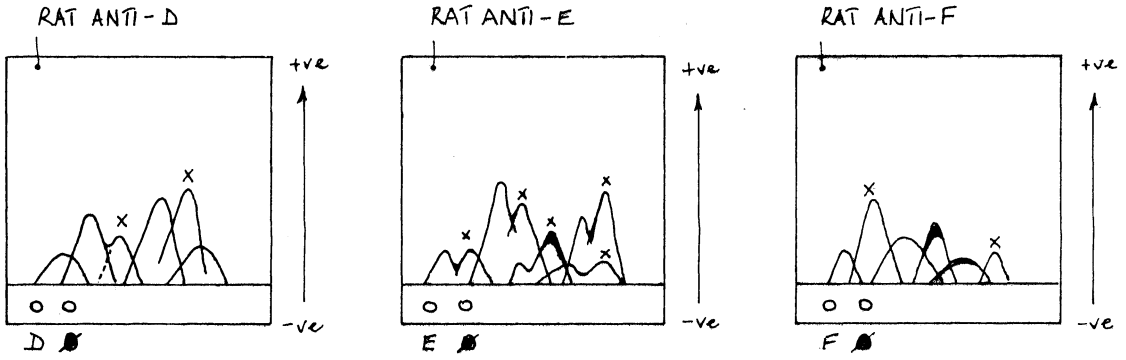
X = CROSS-REACTIONS

If the peaks are totally linked (Fig. 5) then the protein in species A and B can be considered to be antigenically identical. This situation is rare between different species. More common is the occurrence of partially linked peaks which show that the

cross-reacting protein shares certain structural similarities with the protein to which it is joined and so is recognised by the same antiserum.

In contrast to the passive diffusion methods employed in most serological analyses, cross-reactions can actually be used to identify unknown gut contents by comparing them in tandem CIE experiments, with control muscle extracts as shown in Fig. 5. When nearly all the cross-reacting peaks arising from the unknown sample (● in Fig. 5) are linked in reactions of complete identity with those of one of the control extracts a near-perfect match is achieved and the origin of the meal can be confidently identified. In Fig. 5 the meal eaten by the octopus can be recognised as species E. It is possible that the same technique can be used to reliably identify the sources of a mixed meal in the same way.



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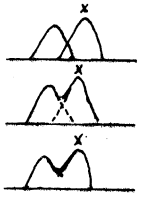
D:E:F = MUSCLE EXTRACTS FROM THESE SPECIES
● = UNKNOWN GUT CONTENTS OF AN OCTOPUS
X = CROSS-REACTIONS

FROM THE MANY REACTIONS OF COMPLETE IDENTITY THE UNKNOWN GUT CONTENTS (○) ARE PROBABLY SPECIES E.



= NON IDENTITY
= PARTIAL IDENTITY
= COMPLETE IDENTITY

So, by using CIE it is possible to utilise cross-reactions, previously considered to be the major draw-back of serological analysis to increase the certainty of accurate gut contents identification. We are presently developing techniques to screen gut contents of large field samples of Eledone and hope to extend this area of research to squid species. Further details of these techniques are soon to be published in Journal of Experimental Marine Biology and Ecology.

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