Identifying and understanding ecological preferences for habitat or prey

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Abstract

Patchy, non-random associations of species with habitats and of consumers with particular types of food are commonly found in the ecological literature. In many cases, these patterns are reported to show some preference by an animal making choices about its environment. Generally, however, what is reported is simply the pattern of association and the process that gives rise to this pattern is not further examined. Nevertheless, there are numerous concepts that need to be considered simply to demonstrate the pattern, including the spatial and temporal scales at which the observations are made. When animals make choices between two objects, it is difficult to separate out potential negative, neutral or positive responses to either or both of the objects, without well thought-out manipulative experiments. Apparent preference for food may be influenced by "catchability" or "acceptability" of the prey and/or the past history of the consumer and the experiments to separate these effects are naturally complex. Many experiments examining preferences are beset by problems of non-independence and lack of appropriate controls, which makes them difficult to interpret. This review introduces some of the logical, conceptual, experimental and statistical problems that beset many studies of preference and proposes important steps that must be considered in further studies to unravel this fascinating topic. © 2004 Elsevier B.V. All rights reserved.

Keywords: Choice of prey; Experimental analysis; Habitat; Preference

1. Introduction

There have been numerous descriptions of non-random associations of species with particular features of habitat (e.g. invertebrates in streams: Orth and Maughan, 1983;

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Phillips, 2003; marmots: Allainé et al., 1994; insects: Doak, 2000; fish: Holbrook et al., 1990; marine invertebrates: Gray, 1974; Chapman, 1994; koalas: Phillips et al., 2000). In many such studies, discovering patterns of spatial distribution in relation to habitats has been described as preference by the organisms. For example, Phillips et al. (2000) concluded that koalas prefer to use swamp mahogany and drooping red gum more than other trees because they appear to spend more time in them. Similarly, Phillips (2003) equated differences in density of macroinvertebrates in different habitats in streams with "preferences".

In the same way, many descriptions of diets of predators (or, for that matter, grazers) discuss preferences for one type of prey over others when the apparently preferred prey are more numerous in the diet than expected by chance (Paine, 1969).

In both situations, there are serious problems with interpretations when the only evidence about preference is some non-random association. In a thoughtful essay by Singer (2000), the existence of a preference requires (and implies) an outcome of behaviour by an organism. This point has not been sufficiently widely recognized by users of the term "preference" with the result that it is becoming, if it has not already become, useless in descriptions of ecological patterns and processes.

This limited review considers the nature of inferences about preferences and some problems caused when preferences are inferred without appropriate evidence. We also consider the sorts of evidence needed to identify patterns of association with habitat or items of prey, alternative explanations for apparent preferences when there are such associations and some of the issues of experimental design in studies to demonstrate behavioural preferences.

Some of the material has been discussed before—by us and others (Rapport and Turner, 1970; Peterson and Renaud, 1989; Underwood, 1997; Crowe and Underwood, 1999; Singer, 2000; Chapman, 2000; Olabarria et al., 2002). By focussing on specific issues in relation to analyses of preference, we draw attention to the problems and some of the methods needed to resolve them, in the hope that clarity will be greater in future discussions of the ecology of choices of habitat or food.

2. The central problem: association is *not* cause

The issue of greatest importance in discussions of associations between organisms and habitat is a logical one. It does not matter for the purposes of this paper whether the association is at some relatively large, perhaps biogeographical, scale, e.g. a species only found on large, offshore islands and not on a local mainland shows an association with habitat at that scale. Alternatively, a species may be only present (or much more abundant) in some feature of a habitat, such as in crevices on a rocky shore. There is, again, a non-chance association, but at a smaller scale. As a third case, hermit-crabs have often been demonstrated to be more likely to be in particular sorts of shells (Conover, 1978; Bertness, 1980, 1981; Hazlett, 1981), thus showing an association with habitat of a very different sort. Each case would usually be described using different terminology; the second case would generally be thought of as an association with microhabitat. In order to discuss the issues that are common across these examples, here we consider all such

associations to be with "habitat", implying some spatial or other difference from other potential habitats.

Of course, it is rare for ecological patterns of association to be found at only one scale (Wiens, 1976). All scales of observation are in hierarchies set by processes at different scales (Allen and Starr, 1982). The three examples considered above are no exception. A species of hermit-crab found only on large islands off the coast of Africa, living during low tide in intertidal crevices and in only the shells of one of several local species of trochid snail would clearly demonstrate associations with habitat at, at least, three different scales in an hierarchy.

The association described with particular types of shells does not, however, allow an inference that the crabs *prefer* these over other potential homes. That is not demonstrable just from the association. Many other possibilities exist to explain *why* crabs are in shells of only one species of snail. The central point of confusion is therefore that the unarguable (if quantified properly) *observation* of association is mixed inextricably with the proposed *explanatory model* or *theory* (see Underwood, 1990, 1997). Where it is not clearly identified that the interpretation is simply an unjustified assertion, the model that the association is explained by preference on the part of crabs becomes the received wisdom and, eventually, an unquestioned paradigm.

At this point, it is worth considering why it matters that observations and explanations are kept separate. Apart from deeper issues to do with scientific approaches to understanding (Popper, 1968; Chalmers, 1979; Simberloff, 1980), there are serious practical consequences. Caughley and Gunn (1996) provided an excellent example from management of conservation of a rare and endangered species. The Lord Howe Island woodhen (*Tricholimnas sylvestris*) is a rail, confined in distribution to one small island (Lord Howe) off the eastern Australian coast. From about 1887, it had been recorded that there were very few birds and these were only breeding, with little success, on one mountain-top. Numerous suggestions had been made about the problems preventing more successful reproduction in the birds' preferred habitat, e.g. changes in habitat, shortage of food, etc. In fact, the species would probably have gone extinct had it not been realized that the birds were *not* in suitable habitat. From this change of thinking, the model was proposed that introduced pigs, which were not able to persist on that particular mountain-top, were responsible for killing young birds elsewhere on the island. From this, it was hypothesized (Miller and Mullette, 1985) that removal of pigs elsewhere would lead to an expansion of range and increase in numbers of birds. The islanders successfully tested this prediction by eradicating pigs, which led to dramatic occupation by woodhens of numerous other parts of the island (including lowland areas), a much larger population and successful breeding in many different habitats (Caughley and Gunn, 1996). Thus, by assuming that the association of the birds with the mountain-top meant that this was where they "preferred" to be, quite the wrong ecological processes were proposed to be operating. Clearly, in this case, the birds were confined to an unsuitable area not by some preference, but because everywhere else was inimical—even though better habitat for reproductive success. By confusing the observations (where the birds were found) with an explanation (why they were there), the long-term conservation of the species was hampered. When other explanations (i.e. not preference) were considered, progress was rapid and effective.

Note also that associations of species with particular habitats tend to gloss over an overwhelmingly common observation in ecology—"most species are absent from most places for most of the time" (Begon et al., 1990). Explaining an association as a preference begs the question of what is *not* being preferred, which is usually a larger list of things.

Similar sorts of confusion can set in when the diets of consumers are analysed. For example, if a predatory species is observed to consume large numbers of one species of prey (A) compared with numbers of other species in its diet, this might be interpreted as a preference. Obviously, however, this may simply reflect the fact that species A are more numerous than other species in the habitat used by the predator, which could simply be eating prey at random, without any choice or preference (Crawley, 1984). To avoid such simplistic misinterpretations, it has often been proposed that preference is demonstrated by a difference in the relative proportions of prey in the diet compared with the relative proportions available (e.g. Hassell and Southwood, 1978; Crawley, 1984). This has also been called "electivity" by Ivlev (1961) and this is probably a more suitable term because it does not so forcefully imply behavioural choices by the predator (see later consideration of how relative proportions available can differ from those consumed without there being any choice or preference by the predator).

Again, demonstrating the existence of a preference for particular food requires more than observation, enumeration of the components of diet and quantification of what is on the menu.

2.1. Problems associated with the scales of observation

Observing an association with some particular habitat or some non-random composition of diet is very dependent on influences of the spatial and/or temporal scale(s) over which observations are made.

As a very simple example, consider the analysis of association of small animals with some relatively small habitat, such as cracks and crevices (e.g. Raffaelli and Hughes, 1978; Chapman, 1994) or particular species of seaweed (Buschmann, 1990; Viejo, 1999). The habitat—cracks or plants—is quite small. If the sampling-unit (quadrat, core, photograph, whatever) is relatively large, each unit will probably (usually) contain several units of habitat. Numbers of the species being sampled will vary from one sampling-unit to another, but there will be no data to test hypotheses about the association with cracks or plants. Clearly, the spatial scale of the sampling-unit must be chosen to match the scale at which it has been proposed that there is some association with habitat. Thus, there must be sampling-units that do contain pieces of habitat and units that do not contain the specified habitat, so that any association of numbers of animals and habitat can, theoretically, be found (depending, of course, on the hypotheses about the nature of association, relevant statistical procedures and sufficient intensity of sampling).

Because associations with habitats are often (if not always) hierarchical, there can be quite different results from sampling-units of different sizes in the same area. Consider a species that is strongly associated with seaward faces of ripples on the floor of an estuary, but shows a general decrease in density as you move towards the sea (Fig. 1). Sampling with a small core or quadrat will demonstrate marked variation in association with the structure of ripples. Sampling with a larger core or quadrat will show a marked trend
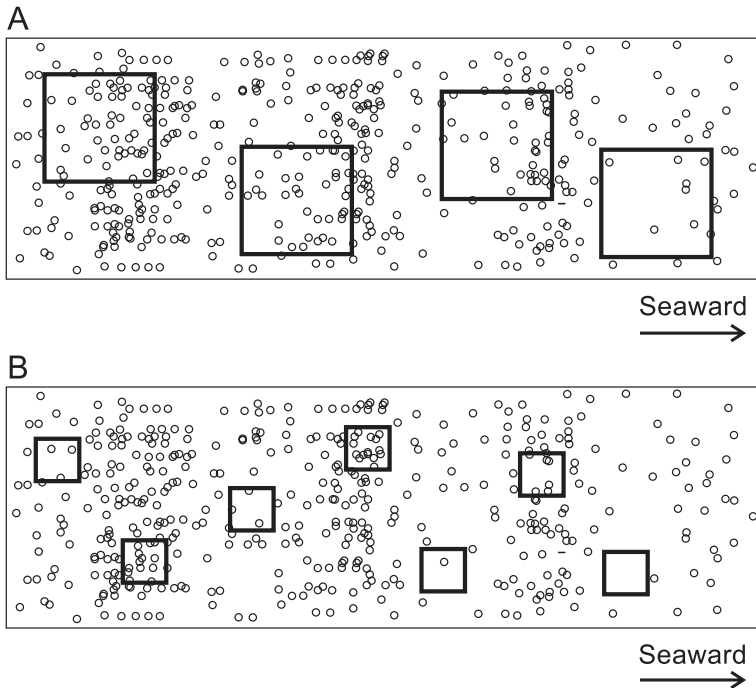
Fig. 1. Illustrating a species that shows a small-scale pattern of dispersion, with larger numbers on the seaward faces of ripples, superimposed on a larger pattern of decreasing abundances as one moves seaward. (A) Sampling-units that are larger than the extent of the microhabitat (the two faces of a ripple) will not measure the pattern of dispersion at this scale, but will measure the trend seaward. (B) Sampling-units small enough to measure abundances in the landward and seaward faces of ripples separately will identify differences in abundances between these habitats, in addition to the seaward trend.

towards the sea, but no pattern with respect to topography of the estuarine floor. This does not matter if the object of the exercise (the hypothesis) is clear; appropriately sized units would be chosen. In more exploratory or preliminary studies, however, observations should probably be made at several scales of sampling-units, to prevent important patterns being obscured.

Temporal scales of sampling are also important influences on the capacity of any study to identify associations of organisms with one or more habitats. For example, Buschmann (1990) described an increase during the night in density of amphipods, *Hyale* spp., on a red alga, which was considered to be a preferred food of the amphipods. During the day, densities were smaller—the amphipods were elsewhere apparently to avoid predators. The shelter from predation provided by the food-plant was insufficient to maintain the association when predators were active.

The ''scale'' of observation also influences interpretations of studies of food consumed by grazers or predators. Averaging the diets of several individuals makes it impossible to understand their diet when individuals are, in fact, eating different things. If, for example, some animals eat only barnacles and others eat only mussels, no individual is consuming

the average diet—a mixture of the two types of prey. Testing hypotheses about diets by keeping track of the individuals has been beneficial. Kitting (1980) recorded feeding by individual limpets, *Notoacmea scutum* in areas where food was predominantly two species of encrusting algae. By measuring grazing in areas with different proportions of the two foods (and bare rock with no macro-algae) and by manipulating the availability of one or other species of food, Kitting (1980) was able to demonstrate that the limpets chose food-types in such a way that individuals maintained a mixed diet of 60% *Petrocelis* and 40% *Hildenbrandia*. Where the availability of one or other was small, the limpets spent more time feeding on it than where that alga was relatively more abundant. In this case, the average diet of individuals has real meaning—the limpets were mostly consuming the same mix of foods. The average availability of foods across different areas would, however, have obscured the complex individual behaviours resulting in the diet of each limpet.

In a different situation, West (1986) recorded what items of prey were being eaten by individual whelks (*Nucella emarginata*) foraging on rocky shores. She noted the prey of each marked snail and found that different individuals had different patterns of preference. If the observations had been simply averaged for each time of observation, it is probable that the preferences would have been missed.

It is also worth noting that temporal patterns of change in preferences by individual consumers can be related to changes in patterns of availability of their prey. The analysis of patterns of consumption of food has led to the development of well-established theories about optimal foraging to maximize the rate of energy consumed, while minimizing the expenditure of energy to consume the food (Pyke et al., 1977; Hughes, 1980). The theory assumes, among other things, that individuals will alter their preferences through time in response to relative differences in availability of different prey, weather and other factors that alter rates of consumption (and impose different risks of harm occurring during foraging, e.g. Burrows and Hughes, 1989). Tests of predictions from this model about consumption are often dependent on having data about diets that are not averaged over long periods of time, so that predicted changes in preference can actually be observed.

For example, there have been several demonstrations of the phenomenon of "switching" between two species of prey (Murdoch, 1969; Murdoch and Marks, 1973). This occurs when a predator consumes disproportionately large amounts of a more abundant (or more frequently encountered) species of prey, but changes (switches) its preference to the other species when it becomes relatively more abundant (Murdoch, 1969). This changing of preference can be made more complex in some marine habitats by the fact that some invertebrate predators require repeated attempts at learning how to handle a new item of prey (e.g. Hughes and Dunkin, 1984a,b). Morgan (1972a,b) demonstrated that *Nucella lapillus* had to learn how to eat mussels and apparently forgot how to do this when mussels were not available and the whelks instead consumed barnacles—which they did not have to drill into to gain food.

Because preferences can alter in complex ways and are dependent on numerous changeable features in an animal's habitat, it is very important that observations are made over the appropriate time-scales. It is therefore crucial that the time-courses are very carefully specified as part of the hypotheses being tested.

One final consideration about time-scales is that patterns of association with habitat or food may be due to a preference that is no longer actually operating. The cause of the preference may have disappeared and the animals may no longer be displaying the behaviour that caused the observed association. As an example, consider the settlement of larvae of a barnacle that are positively influenced by the presence of cues produced by conspecific adults (e.g. Knight-Jones and Stevenson, 1950; Meadows and Campbell, 1972; Crisp, 1974, 1976; Raimondi, 1990). As a result of the preference by the larvae, they settle in larger numbers in patches of habitat where there are adults than in patches without adults. If the adults are then consumed by predators that ignore the small barnacles, there is now a pattern of non-random association with patches of habitat which was caused by a preference. There is, however, no continuing evidence of this cause.

This situation could well prevail in other conditions which involve continuously exercised behaviour, but no longer involve the original preference that created a pattern of association. As an hypothetical example, juvenile snails may choose to stay in certain crevices because they contain adults of their own species. They may be attracted to these crevices from elsewhere or, while moving around, be more inclined to stay in crevices with adults than those without adults. As the snails grow, the adults gradually die and disappear. After a while, there would be a clear pattern of association of the juveniles with only some of the available crevices, provided only that the snails do not move far while foraging and tend to return to their original crevice (for example, as shown for various snails by Levings and Garrity, 1983; Moran, 1985; Fairweather, 1988). The pattern of association with particular crevices is a result of an historic preference, but is not maintained by the behavioural process that caused it. Models for the causes of the observed distribution should include the possibility of it being due to the "ghost of preference past" (to paraphrase Connell, 1980). Hypotheses derived from this model of previous preference clearly cannot be tested under the conditions currently prevailing in the area of study.

Again, the possible relationship between processes of preference that establish or cause an observed pattern is likely to be different from those processes, including quite different behavioural preferences, that maintain an observed pattern. This aspect of time-scale (original cause versus current processes) must be borne in mind during any analysis of preferences as explanations for associations with habitat or prey.

2.2. Alternative explanations for associations with habitat or prey

There have been numerous documented cases of associations between organisms and their habitats that are nothing to do with preferences and often nothing to do with current patterns of behaviour. As an example, consider the intertidal limpet *Patelloida latistrigata*, which is numerous among the barnacles *Tesseropora rosea* along the coast-line of New South Wales (Australia). The limpets are not able to survive in more open areas of rock, largely because they are out-competed for food by other grazers (Creese, 1982). This case is therefore similar to that described earlier for the Lord Howe Island woodhen. The association with barnacles would not seem to be as a result of preference–active choice of this habitat over others. Rather, it appears that they cannot survive elsewhere. This case does, however, have an added twist that the limpets are very rapidly consumed by whelks, *Morula marginalba*, which forage among the barnacles (Underwood et al., 1983). In fact,

the whelks prefer to eat these limpets over other prey (see the experimental data in Fairweather and Underwood, 1983). The whelks can consume the limpets very quickly because they do not have to drill through their shells to gain access (Fairweather and Underwood, 1983). Thus, the limpets live in a habitat where they are very likely to be found and eaten by their predators, so it would seem a strange place to prefer!

There could also be situations where animals move around a landscape, but spend disproportionately more time in one habitat (A) than another (B) compared to the relative areas of each habitat. At first sight, such a case may easily be interpreted as a preference and, certainly, it is a result of behaviour by the animal. It may, however, be the case that dispersal through habitat A is slower simply because it has complex topography or more difficult substratum over which to move (as suggested by Underwood and Chapman, 1989; Chapman and Underwood, 1994). Bovjberg (1984) showed that *Nerita* spp. showed increased kineses in the dark and suggested that that behaviour would cause them to accumulate in crevices, even if these were encountered at random, although he did not have data to illustrate directly an absence of preference. In all of these studies, an absence of preference is implied, but has not yet been unambiguously identified. In addition, aphids have been shown to accumulate in patches, which are not considered the preferred feeding habitat, because of interactions with clusters of conspecifics, which inhibit movement (Turchin and Kareiva, 1989). Similarly, female butterflies may leave preferred habitat (with more host plants) because of "harassment" by males (Turchin, 1991).

For the analysis of apparent preferences by predators, several processes can cause patterns of consumption that are not random (i.e. by random encounter of prey according to their availability). These processes are not preferences, but are consequences of features of the predator–prey interaction. The most obvious process is that predators usually need different periods to consume prey of different types (or sizes). For example, the handling-time (from encounter with an item of prey until feeding ceases) of the whelk *M. marginalba* varied from an average of 4–9 h to eat a limpet, *P. latistrigata*, to an average 44.3 h to eat a tube-worm, *Galeoloria caespitosa* (Fairweather and Underwood, 1983).

As discussed in detail by Rapport and Turner (1970) and by Peterson and Bradley (1978), this will lead to a non-random composition of diet compared with random expectation from availabilities of different prey in the field. Consider a predator such as *M. marginalba* choosing prey at random from two species, each available in equal numbers in an area of shore. If the two types of prey take 1 and 5 h to eat, respectively, it is easy to show that, after 3 h, only one in every eight predators will be seen to be consuming the species with the shorter handling-time. Sampling 3 h after predators start to eat would demonstrate a non-random "choice" of prey, apparently a preference for the species with the longer handling-time. This is obviously a trivial quantitative example, but it illustrates the point that no preference or active choice was being shown. Differences in time taken to handle prey explain the electivity demonstrated by the predators. Real examples were presented by Peterson and Bradley (1978) and Fairweather and Underwood (1983).

A similar result will occur when the "catchability" (Rapport and Turner, 1970) is considered. These authors defined catchability as the time needed to find or catch an item of prey, plus the time taken to consume it (the handling-time). If two items of prey are in equal numbers in a habitat and have equal handling-times, but differ in the time it takes for a predator to catch them, there will be apparent preferences in diet.

The diets of predators are often documented by observing what they are eating at some interval rather than continuously. This is commonly the case for intertidal animals because of the rise and fall of the tide. In very many cases, diets are recorded by analysis of what is in the guts of a sample of consumers. Differences in handling-time (processing-time in the gut) among types of prey will inevitably result in apparent preference for some types of prey. Corrections may be possible for differences in catchabilities, handling-times, etc. (Rapport and Turner, 1970; Peterson and Bradley, 1978; Fairweather and Underwood, 1983). The only really sensible way to test hypotheses about dietary preferences is to couple observations on availability of different types of food, catchabilities and handling-times with experiments on actual choices made by consumers when confronted with controlled arrays of types of food (see later discussion).

The examples considered here demonstrate that positive associations with habitats or items of food are not uniquely interpretable as preferences. Until alternative explanatory models, such as, but not limited to, those considered above, have been demonstrated experimentally to be false and hypotheses about preferences supported by appropriate experimental evidence, it is unwise to invoke preferences by the organisms.

3. Experimental analyses to demonstrate a preference

Demonstrating that a particular association between an organism and a habitat or components of its diet is a result of preference requires very careful experimental analysis. Some of the relevant hypotheses and components of experimental designs to allow logically valid tests will be considered here. Other aspects of this topic were reviewed by Peterson and Renaud (1989).

Consider first that observations suggest that a predator is consuming prey in amounts inconsistent with random choice. In the simplest case of two species of prey, data about diet suggest that species A and B are consumed in the ratio of 3:2, despite being equally abundant. It then is straightforward to propose that this observed pattern can be explained by preference for A over B. An experimental test of this proposition is to confront the predators with a mix of the two species to record their behavioural choices (e.g. Murdoch, 1969; Louda, 1979). It is tempting to propose the null hypothesis that, in the absence of a preference, the predators will eat equal numbers of the two species of prey (H_o: proportion of A eaten, p_A = proportion of B eaten, $p_B = 0.5$).

As discussed by previous authors (Rapport and Turner, 1970; Peterson and Bradley, 1978; Liszka and Underwood, 1990), this is incorrect because it ignores any differences in catchability, handling-time, likelihood of seeking new prey after consuming an item (which may differ from species to species of prey because they provide different amounts of energy or gut-fullness). Instead, it is appropriate to use the following experimental procedure. First, give a sample of predators only one type of prey (species A) and record the number of these consumed over some period of time under experimental conditions. Then, supply an independent sample of predators with the other species, on its own. Suppose that a total of N_A and N_B, respectively, of each type of prey are eaten, per capita of predator, when there is no choice available. These numbers will differ in response to any

differences in the time it takes the predators to start to look for, find, consume and rest after consumption of prey of each type.

Then, the experimental choice can be provided by putting a sample of the predators with an equal number of the two types of prey. If predators do not exercise any preference, they will consume prey at the same rates as when the prey were on their own. Preference would be demonstrated by an excess consumption of one species and a reduced consumption of the other compared with what happened when there was no choice. The null hypothesis (predicted when there is no preference being exercised) is that the proportion of A eaten will be $N_A/(N_A + N_B)$ and, formally:

$$H_o : \; p_A = N_A/(N_A + N_B); \; p_B = N_B/(N_A + N_B)$$

The observed numbers of A or B consumed can then be tested for departures from this null hypothesis, which would indicate a preference.

Refinements of this experiment would include replacing items of prey that are eaten, to ensure that their relative availabilities are not changing during the experiment. The situation is more complex where the numbers of prey are not equal at the beginning of the experiment, as often happens in the field. Under these circumstances, the above procedure can be used only if it is possible to create the three experimental treatments: A alone at their natural density, B alone at their different natural density and the mixture with each species at its natural density. This will be very difficult where numbers of prey are very large.

Extensions of the above scenario involve "cafeteria experiments" where consumers are confronted with an array of (>2) types of food. The appropriate null hypothesis is then constructed from a set of experiments with consumption of each component of diet measured separately (e.g. Hay and Fenical, 1988; Duffy and Hay, 1991).

It is worth noting one complexity inherent in this design. The data used to construct the null hypotheses come from the experimental confrontation of each type of food on its own. These (N_A and N_B) are usually going to be estimated from quite small samples, i.e. the number of independent, replicated consumers for which consumptions are measured will generally be quite small. Where there are many types of food, these sizes of samples will probably be extremely small. This means, in practice, that the consumption of each item of prey, on its own, will be estimated with considerable sampling error. Where these errors are relatively large, appropriate statistical tests must take them into account. So, for example, using the expected proportions of different prey, as described earlier, calculated from the mean number of each item consumed, suggests analysis using, for example, chi-squared tests. The proportions of A and B consumed when the prey are presented together are tested against the proportions (p_A and p_B) predicted from what happens when they are presented separately. Such tests are inappropriate and tend to overestimate the likelihood of finding preferences when there is none.

The problem was explained by Liszka and Underwood (1990) in the context of preferences by hermit-crabs for different types of shells. This has the same logical requirement of experimental design as in the case of consumption of different types of food. Liszka and Underwood (1990) demonstrated how to construct the appropriate null hypotheses for choice of shell for crabs presented with two possible choices. Using maximal log-likelihood calculation of the probabilities involved, it was straightforward

to construct valid tests of the data that incorporated imprecision in estimates used to generate null hypotheses. The algebra is not reproduced here. Liszka and Underwood (1990) only considered the simplest case of two types of shell presented in equal numbers. Extension to situations where numbers of each type are not equal and where there are more than two possible choices has recently been determined (Underwood and Clarke, unpublished).

The analysis of association of numbers of a species with some component of habitat has similar inherent problems. Consider the simple case of an animal in an area which consists of two different types of habitat, say shallow pools and open surfaces on an intertidal rocky shore. The association of densities with one or other of these habitats requires demonstration that the animals are found in one habitat more than expected by chance from its availability in the area. Availability would, in this case, be measured as the proportion of the available surface of rock that is in (p_P) or out of (p_O) pools, respectively. The null hypothesis of no pattern of association is then that the proportion of animals in pools (p_P) or out of pools (p_O) will each equal the corresponding availability of the two habitats. Departure from this null hypothesis will indicate an association with one or other of the two available habitats.

Again, where $þ_P$ and $þ_O$ are estimated with relatively small samples, they will have non-trivial errors which must be taken into account in any statistical analyses. Sometimes, instead, the availability of habitats and the numbers of animals in each component are measured in numerous patches and results can be analysed by regressions, using the data from each patch as an independent estimate (e.g. Underwood, 1976).

In this type of study, the demonstration of preference between habitats cannot use the protocol described above for consumption of different components of diet. If animals are presented with only one habitat, they must all be on (or in) it.

Other types of experiment will be helpful, in which animals are provided with an array of habitats and the numbers moving into each are compared with what is expected when there is no choice. This was described in full by Olabarria et al. (2002) for small gastropods living in sediment or on the plants in dense mats of the intertidal alga, *Corallina* sp. Snails were introduced into experimental arenas with three possible habitats: sediment, *Corallina* with sediment and *Corallina* without sediment. Preference was determined by recording how many animals moved to each habitat from the one in which they were placed at the start. These arenas consisted of one-third of the area having each habitat and, in different treatments, snails started in each of the three possibilities (Fig. 2). The null expectation, if there was no preference, was determined in arenas in which all three thirds consisted of the same habitat, but animals were only introduced to one-third. The proportions of animals moving to another part of the arena occupied by the same habitat allowed estimation of the outcomes of movement when there was no choice (Fig. 2). These data, for each habitat, allowed comparison with the results of movements of the snails to areas of different habitat (Olabarria et al., 2002).

3.1. Experimental analyses of the nature of a preference

It is not possible here to consider every aspect of the nature of preferences, the features of habitat or prey that cause animals to choose them and the cues used to find (or, possibly
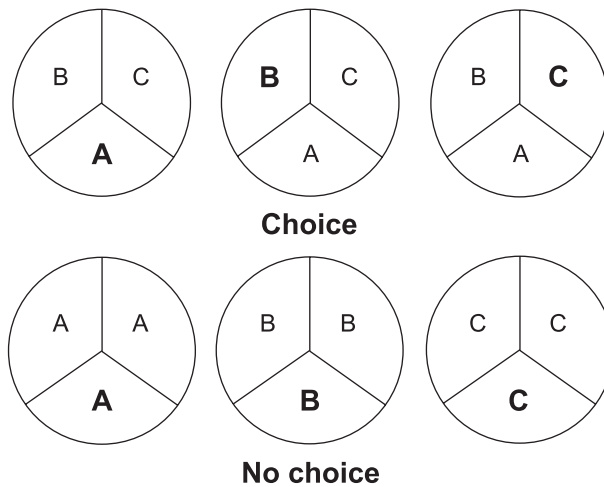
Fig. 2. Experimental treatments to test for choice among three habitats (A, B and C). Animals are placed in each type of habitat (marked in bold) in situations where they are given no choice of other habitat (No choice) and those in which they are offered all three habitats (Choice). Preference is tested by comparing the proportions that move from the initial habitat into alternative habitats, compared to the proportions that move from one patch of habitat into another patch of the same sort. Full details are in the work of Olabarria et al. (2002).

in the case of habitats, to remain in) the preferred one(s). It is, however, worth noting the implications for experimental analysis of some aspects of behavioural preferences.

First, preference may be positive or negative. This is very obvious in analyses of diet– items may be consumed preferentially because they are desirable/palatable or because the alternatives are undesirable/unpalatable. When not given a choice, animals may consume one type of food, even if it is potentially toxic, but avoid it when other types of food are available. The others are preferred—but only because the one not consumed is rejected.

This has led to confusion in terms describing the interaction between consumers and food when it consists of properties of the consumer (preferences) and of the food (acceptability). This led Singer (1986, 2000) to propose three components of choice of food: preference to describe choices made by consumers, acceptability to describe properties of the food and electivity to describe properties of the interaction between the consumer and its food. Direct analogies are possible for choices of habitat, which are illustrated below, modified from Singer's (2000) examples of consumer/diet interactions.

Suppose an animal moving around will stay for equal periods in either habitat A (say, crevices on a rocky shore) or B (small pools). The animal responds similarly to encountering either habitat—it shows no preference. If the two habitats are not available in equal amounts, the animals will spend more time in whichever is most numerous or occupies the most space. Electivity is influenced by availability—the interaction between the animals and the distribution of the habitats; there is no preference nor difference in acceptability.

In the case of items of food, rates of encounter by predators will be influenced by the dispersion of prey. Even if two different items of prey have the same acceptability, elicit no preferences and occur in the same mean density, if one is more aggregated than the other, it

will be encountered less often by randomly searching predators. Thus, apparent preference will be influenced by dispersion (and therefore catchability) of prey, even when there is no preference (Mackay and Singer, 1982). Again, this is a property of the interaction between predator and prey.

If, in contrast, properties of habitats change, electivities will change without there necessarily being a change in preference (Singer, 2000). Suppose periods of hot weather make pools become physiologically more stressful during low tides. As a result, animals in pools are more likely to leave during a subsequent high tide than is the case for animals in crevices. As they move around, the animals continue to show no preference between the two habitats and will enter and stay in either one with equal probability. The association with habitat will, however, definitely show a non-random, positive association with crevices (or, equally, negative association with pools). Because individuals spend more time in crevices, at any period of observation, more will be in crevices than expected by chance from their availability, but not due to any change in their choice of which habitat to enter.

When animals, such as hermit-crabs, learn (Hazlett, 1981) to acquire and occupy one type of shell (habitat) more quickly than another, the decreased handling-time will cause a change in apparent preference. Suppose that two types of shell are equally likely to be encountered by a crab and are equally acceptable in terms of the habitat they provide, but one (A) is more difficult to manoeuvre than the other (B). If crabs learn how to speed up handling of A, there will be a decrease in electivity, even though neither preference by the crab, nor acceptability of the habitat have altered.

Patterns of association can be non-random because of features of the animal/habitat or consumer/diet interaction, by properties of the habitat (or diet) or by behavioural properties of the consumer (and presumably by combinations of these) without preferences being exercised. All of these components must be considered when preferences have been demonstrated and experimental analyses of the processes causing preference are attempted. As an example, if an intertidal animal has been demonstrated to prefer rock-pools over open surfaces, this may be because of positive properties of pools (e.g. they contain food) or negative properties of open surfaces (e.g. the animals on open surfaces are more likely to be eaten by predators during high tide or suffer from desiccation during low tide) or some combination of both. This results in there usually being three general models (+ve properties of one habitat, -ve properties of the other, both) to account for preferences, requiring manipulative experiments to separate them.

How such experiments should be designed is entirely dependent on how well hypotheses can be constructed that predict differentiable outcomes from each model. Suppose, for example, habitat A is preferred because it has the positive attribute that it provides better or greater amounts of food than does habitat B. It can be predicted that small animals taken at random from either habitat and transplanted to and maintained in habitat A will grow faster than those so tested in B (e.g. Olabarria and Chapman, 2001). For larger animals, it might be predicted that sizes of gonads or numbers of eggs produced might be larger in animals in A than in B.

In contrast, it may be proposed that habitat B has the negative property that there is greater stress due to desiccation than in habitat A. This stress may cause decreased amount of time feeding, or decreased efficiency of using the energy gained from feeding. This

model therefore also predicts decreased growth of juveniles transplanted to B and less reproductive activity of animals transplanted to B.

In this scenario, it is impossible to eliminate any of the three classes of general model by experimental tests of the hypotheses about differences in growth or reproduction.

Note that measuring the supplies of food or the amount of stress due to desiccation in the two habitats only partially helps identify the causal processes. The model that there is greater desiccatory stress in habitat B than in A and that this explains the preference for A over B makes it *necessary* that stress will be correctly predicted to be greater in B than in A. Supporting this by rejecting the one-tailed null hypothesis of equal stress (or that stress is greater in A than B) is, however, *insufficient* to use desiccation as the reason animals prefer A over B. Any other difference between A and B may be the cause. Clearly, the model about desiccation can be falsified if it turns out that there is no difference in desiccation between A and B because the necessary prediction is not correct. Experimental analysis is still worthwhile, but caution must be very evident in interpretations where the model is not falsified. As a final caution for this example, the model can only be falsified if the appropriate conditions actually prevail during the experiment. One troublesome feature of virtually all ecological processes is their complexity. So, animals may learn to prefer habitat A over habitat B because of occasional bouts of stress due to desiccation in B. Such stress as a negative feature of B therefore explains the preference but may not be operating during the experiments. Thus, the model is correct, but its hypothesis is wrong because it is tested under inappropriate conditions.

All valid interpretation of experimental tests of hypotheses requires that the conditions required by the hypothesis are actually met (see the references in the work of Underwood (1990)).

In this example, it is necessary to be able to manipulate the food (i.e. to change it to be like that in B) in habitat A to provide a direct test of food as the potential cause of preference for habitat A. Similarly, it is necessary to be able to increase the availability of food (or otherwise alter food) in habitat B to be like that in A. Animals confined to areas of A manipulated to be like B should then grow (or show reproductive activity) like those kept in unmanipulated areas of B. At the same time, animals kept in to unmanipulated areas of habitat A should grow faster (or have larger gonads, etc.) and should be similar to those confined to manipulated areas of habitat B. If this sort of manipulation can be done, it will provide unambiguous tests of hypotheses derived from the model about food as a positive attribute causing preference for habitat A over habitat B. Similar experiments need to be designed to test the model that a negative influence of habitat B causes preference for A.

If such direct manipulations of features of habitat are possible, experimental analyses will be fairly straightforward. Otherwise, this area of explanatory ecology is like a logical minefield!

Field tests of behavioural responses to habitat fall into two types: "passive" and "active" (see Crowe and Underwood, 1998). "Passive" experiments involve altering habitat and waiting for target species to respond to them. They have been used to determine preferences among microhabitats for freshwater fish (Fausch, 1993) and to distinguish among predation, preference for habitat and availability of food as processes affecting abundance of mobile macrofauna in seagrass (Bell and Westoby, 1986; Connolly,

1994). These experiments do not suffer from artefacts caused by the handling of animals, but controls for disturbance to the habitat itself must be incorporated (for example, no undisturbed plots were monitored by Bell and Westoby (1986)). Since no animals are marked, the experimenter cannot be certain of the size of the population of experimental animals and thus cannot separate selection of habitat from differential mortality unless treatments excluding predators and other sources of mortality are incorporated.

"Active" experiments are those in which animals are marked and placed in experimentally altered habitats so that their responses can be monitored. Interpretation of such experiments depends critically on controls to determine the influence of aspects of the transplantation procedure on the behaviour of the animals (see next section). If done well, however, these experiments provide strong evidence that behaviour influences patterns of distribution (Underwood and Chapman, 1989; Chapman and Underwood, 1994; Crowe and Underwood, 1998). Analogous experiments in which the microhabitat was removed (with appropriate controls) and the target species left in place have been reported by Underwood and Verstegen (1988) and Underwood and Barrett (1990).

For some aspects of dietary preference, it has been relatively easy to manipulate negative aspects of some components of diet. For example, chemical composition of seaweeds has been successfully manipulated to demonstrate that unpalatability (chemical defence as a negative property) alters preferences by consumers (extensively reviewed by Hay and Fenical, 1988). Aspects of alteration of diet will not be considered further here, except to note that acceptability has positive and negative elements exactly like those described for analyses of properties of habitats.

3.2. Using experimental transplantation in analyses of preference for habitat

Many models of preferences among habitat require experimental transplantation of animals to allow tests of hypotheses. Despite much previous discussion of transplantation, it is still the case that experiments are done without adequate consideration of the need to be very clear about the hypotheses and very careful about the provision of appropriate controls. The nature of and need for the controls in any experimental transplantation have been fully described and explained by Chapman (1986, 2000) and Underwood (1988). They are briefly summarized here to explain problems of interpretation of statistical analyses.

Consider an example of predation by whelks that feed on barnacles and tube-worms. In some patches of habitat (A), barnacles and worms are scattered in similar ways, in similar densities, but whelks mostly eat barnacles. In contrast, in other areas (B), the densities and dispersions of the two types of prey are similar (and like those in A patches), but whelks mostly eat worms. Thus, there are different, non-random patterns of electivity in the two types of patches. Assume that it is also known that whelks rarely move from one patch to another.

Among other possibilities, it can be proposed that these patterns are explained by different attractiveness of prey in the two types of patch–whelks are equally likely to encounter a barnacle or a worm in either patch. In A patches, barnacles are more "acceptable" or "attractive" to eat. So, on encountering a barnacle, a whelk is, say, twice as likely to start to eat it as in the case when it encounters a worm. In B patches,

worms are more acceptable, so a whelk encountering a worm is twice as likely to eat it than is a whelk that encounters a barnacle. The observed patterns of diet are caused by properties of prey.

An alternative explanation is that whelks have, perhaps due to different availability of prey in the past, learned to consume prey with different probabilities. In A patches, whelks are more likely to attack a barnacle than a worm because that is what they have learned to do. In contrast, in B patches, whelks have acquired a greater likelihood of attacking a worm. The observed patterns of consumption of prey are caused by properties of the whelks in the different types of patch.

A third possibility is that prey differ in acceptability (barnacles are more likely to be eaten than are worms in A patches, vice versa in B patches) *and* the whelks are more prone to attack barnacles in A patches (and worms in B patches) because of learned behaviour.

To distinguish among these three possibilities, experiments will need to estimate rates of encountering prey (all three models assume that barnacles and worms are equally likely to be found in either type of patch). It is also appropriate to consider the experiments described earlier to measure the rates of attack and handling-times when predators from each type of patch are presented with only barnacles, only worms or both species, from each type of patch. Here, however, just consider the following hypotheses which are derived from the explanatory models considered above.

If the observed patterns of consumption are due to attractability of prey differing between types of patch (i.e. are properties of the patches), it is predicted that whelks from A taken to B will change diets to become similar to those in B. Whelks taken from B to A will no longer be like whelks remaining in B, but will become similar to those naturally found in A (see Fig. 3A). If the observed patterns are due to the second model (a property of the whelks), it is predictable that whelks taken from A to B patches will continue to have a diet like those in A and will therefore differ from whelks in B patches (i.e. they will eat more barnacles than worms in B patches, like those in A patches). Whelks transplanted from B to A will show the opposite pattern–remaining like those in B and differing from whelks that have remained in A (Fig. 3B). The third model predicts that each set of transplanted whelks will change, but they will not become similar to those in the patches to which they were transplanted (Fig. 3C).

So, groups of whelks are marked without disturbance in several A patches and in several B patches. Some whelks are then taken from A patches to B patches where they replace whelks transplanted reciprocally to A patches. This maintains the numbers of whelks in each patch. There are numerous things to think about the design of this experiment. For example, are there any consequences of introducing strange whelks to a patch so that the original whelks change their behaviour? If this is possible, it would be better not to mix the two types of whelks in the same patches. Although such issues would need to be carefully considered, they will not be here.

Notice, however, that in an experimental A patch, there are undisturbed A whelks and transplanted B whelks. The first hypothesis predicts that these will show the same pattern of foraging, because it is a property of the patch. The transplanted whelks must change to differ from undisturbed whelks in experimental B patches. The comparisons to be made are inevitably confounded by the necessity to disturb the transplanted whelks. They have been handled, moved to a new patch of habitat and moved to a
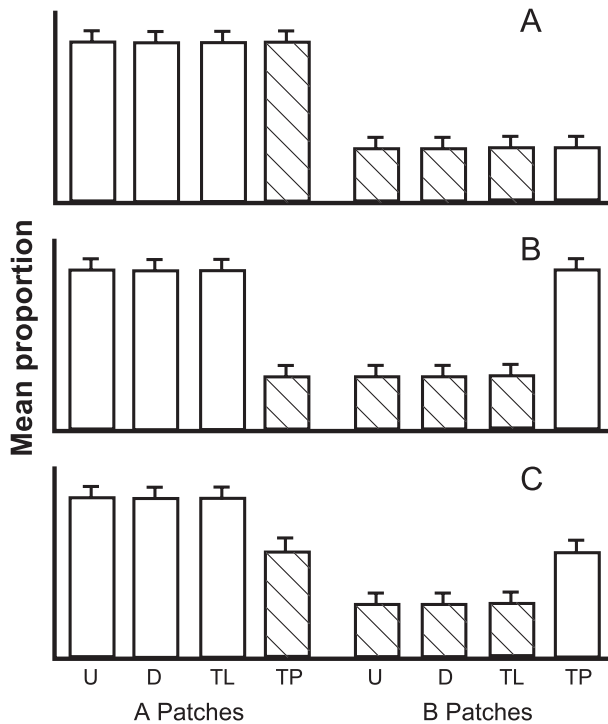
Fig. 3. Hypotheses from three models to explain patterns in diets of whelks in patches of Types A and B (see text). Data are mean (S.E.; *n* replicate patches) proportion of barnacles in the diet. (A) From Model 1, prey have different acceptabilities in A and B patches; (B) from Model 2; predators from A and B patches have different preferences; (C) from Model 3; prey have different acceptabilities and predators have different preferences. For further explanation see the text. U—undisturbed whelks in home patches; D—disturbed whelks in home patches; TL—whelks translocated between A patches or between B patches; TP—whelks transplanted from A to B patches or from B to A patches.

different type of patch. Any of these could cause them to change their behaviour from that shown by undisturbed whelks. The manipulation, rather than the new type of habitat, could make these transplanted whelks start behaving differently and confound the experiment, but without shedding any light on what part of the procedure might be improved to do better experiments. Worse, it is possible that artefacts of the manipulation cause the whelks to show the predicted pattern of diet, but not due to the properties of their new patch but due to the way they have been messed about to get them there.

For a complete discussion of these issues, see Chapman (1986, 2000). Further controls are needed. Whelks in some A patches (and in some B patches) must be handled and moved as required for transplanted animals, but then returned to the same A patches (or B patches). These animals have been disturbed and moved, but have not been taken to a different type of patch nor to an unfamiliar patch. If disturbance itself does not cause any change in behaviour, these animals must continue to act as do the undisturbed controls. If

they change behaviour, it will be clear that there are problems for any interpretation of the experiment and different experimental procedures will be needed to allow valid tests of hypotheses.

Another control is needed to examine any effects of *translocation* of animals to a novel patch, regardless of the prey that may be in it. Whelks moved to a different area of shore may respond by altering their behaviour and feeding differently. To detect this artefact, it is necessary to disturb animals and take them from some A patches (or B patches), but to put them into different A patches (or B patches, respectively). If these animals are influenced by the novelty of the patch (as opposed to attractability of prey), they will forage differently from undisturbed animals in A (or B) patches and indicate an artefact in the experiment (see Chapman, 1999 for an example of changes in behaviour caused by translocation).

So there will be eight groups of whelks. In each A patch, there are undisturbed whelks and disturbed whelks from that patch. There are also whelks translocated from another (or other) A patch(es) and whelks transplanted from B patches. In each B patch, there are undisturbed whelks and disturbed whelks from that patch, whelks translocated from other B patches and whelks transplanted from A. It is now possible to identify what will happen if there are no artefacts and whelks behave as predicted in the hypotheses derived from each model (Fig. 3).

Examination of Fig. 3 indicates a further issue of logic in the statistical analysis of data from such an experiment. There are two ways to approach this. If analysis of variance is used, one factor is Treatment, a fixed factor, with four levels: Undisturbed, Disturbed, Translocated, Transplanted; see Underwood (1997) for fixed versus random factors in ecological experiments. A second factor is Destinations of whelks, the type of patch to which transplants are taken (also fixed, with two levels, A and B patches). Replicate patches are used for each combination of patch and treatment and data are the proportions of barnacles eaten by each group of whelks in each experimental patch.

In this arrangement, Destination A consists of undisturbed, disturbed and translocated groups of whelks originally and finally in A patches, plus whelks transplanted from B to A patches. The analysis of variance would then produce different patterns of significance for Models 1, 2 and 3. Under Model 1, the analysis should show a significant difference between Destinations, but no effect of Treatments. Whelks finishing up in A patches should all have the same diets; whelks in B patches should be similar (Fig. 3A).

Under Model 2, the hypothesis is that there will be an interaction. Whelks transplanted from B to A will not be like those in A. Those taken to B will not be like those in B. The difference between transplant and other treatments will not be the same for the two destinations.

If, however, the analysis is considered from the point of view of the origin of the whelks rather than their destinations, the analyses will show reverse patterns of significance for Models 1 and 2. Under Model 1, there will now be an interaction. Origin A includes undisturbed, disturbed and translocated animals originally in A patches and whelks transplanted from A to B. Model 1 predicts that the transplants will change and be different from all whelks remaining in A. The difference is not the same for that between whelks transplanted to A and those remaining in B (Fig. 3B). In contrast, Model 2 now predicts no interaction, but a significant difference between Origins of whelks.

Model 3 predicts an interaction whether or not considered from the point of view of Destinations or Origins. The patterns of differences among treatments within A and B patches and between A and B patches will, however, be different from that in the interaction due to Model 2 (by Destination) or Model 1 (by Origin). This is not considered in detail here, but is clear from Fig. 3.

So, great care is needed to ensure that analyses are properly constructed or interpretations will become illogical. Explicit statement of the hypotheses is the only way to prevent problems.

3.3. Non-independence in experiments about preferences

Many statistical procedures for analyses of behavioural experiments, including analyses of preferences for habitat or food, require that data are independently sampled. Some examples of consequences to analyses of variance were described in full by Underwood (1997). Where assumptions of independence of data are important (which is the case for many kinds of statistical procedures), experiments must be designed very carefully to ensure that the data comply. This is a complex topic and only an illustration of the issues will be attempted here. It is easier to consider how non-independence may come about as a result of the way an experiment is designed than to provide "rules" about how to avoid the problem. Note, however, that many aspects of an organism's behaviour can involve responses to other individuals. Where such responses cause animals to move, feed, grow, etc., in similar ways, measurements of these processes will often be positively correlated and therefore cannot be independent. Where the behaviour of one individual influences another so that its response is negatively correlated, the data cannot be independent.

Consider a hypothetical example of a predatory animal being experimentally tested for any choice between two types of prey in a Y-maze (Shaw, 1991; Avila, 1998) or similar choice chamber (Pratt, 1974). Prey of each type are placed at opposite ends of the maze/chamber; a specimen of the predator is placed at the intersect. In the case of marine predators, water is then allowed to flow past the prey to the predator. For terrestrial species, similar experiments are done using flows of air (Coull and Chase, 1980).

If there is no bias in the apparatus and no preference by the predator, there should be an equal chance that it moves to encounter either type of prey. After the initial predator has moved far enough to display clearly to which side it has moved, it is replaced by another individual. The outcome of a set of such individuals can then be tested for any departure from an equal proportion moving to each type of prey (e.g. Pratt, 1974).

Any bias for either side of the apparatus (e.g. due to some systematic difference in the flow of water) could be examined by running the apparatus with both inputs containing the same type of prey. Alternatively, it could be eliminated from influencing the outcome by running equal numbers of replicate trials with each type of prey arranged in each of the two sides.

There are at least three different ways that such an apparatus may create non-independence in the data, i.e. in the number of individual replicate predators that move to each of the sides of the chamber. First is the use of the same individual predator in more than one trial. An individual may have an internal tendency to move to the left or right when reaching a junction, regardless of the type of prey present. If an individual has a

tendency to go to the left, it will continue to do so in subsequent trials. This may not matter if the prey are alternated in type in the two sides.

If, however, an individual predator has a particular preference for one type of prey that is not shared, on average, by other individuals, repeatedly using it in trials guarantees that the observation gained from any second or subsequent trial is not independent of the first observation. It could only be appropriate to re-use the same individual where the hypothesis being examined involves comparisons of particular individuals and, for each animal examined, the data consist of the proportion of times it moved to one type of prey. Where the object of the exercise is to test the null hypothesis that the proportion of times predators moves towards one type of prey is equal to the proportion of times they move towards the other type, there must be independent trials and therefore independent individual predators.

The second way that data can be non-independent could be due to the repeated use of the stimuli—the items of prey used. It is possible that, by chance, an individual of one type of prey (A) has some unusual feature or chemistry that triggers an unusual positive response by predators. If the same individual is re-used, each new individual predator will respond to it, creating an excess of positive responses to A compared with B. If, however, new items of A were used in each trial (with each new predatory individual) this bias would not occur.

A third way in which data might become non-independent can occur even if new, independent predatory individuals and new, independent items of prey are used. If, in the previous example, an individual predator leaves any kind of trail or other chemical trace of its movement through the apparatus, subsequent replicates may well follow the same path. There are, for example, many examples of gastropods following each other along mucous trails (reviewed by Chelazzi et al., 1988). Unless the apparatus is rigorously cleaned before each trial, data from subsequent individuals will be influenced by (correlated with) previous individuals. This could prevent any preference from being found. For example, if the two types of prey are being alternated between left and right branches, but predators tend to follow previous trails, they will encounter each type of prey with similar frequencies—even if they really have a preference for one type.

The only way to prevent non-independence of data in these sorts of trials is to ensure that types of non-independence are anticipated and every possible precaution taken to avoid them. Never re-using the same individuals in behavioural studies is a good general rule. If hypotheses require successive measurements on the same individual, ensure that the data are formulated so that one measure only comes from each individual (e.g. the mean or variance of measures or the average difference between measures). Whenever there are more degrees of freedom in statistical analyses than there are separate individuals being measured, there will be non-independence in the data.

In many experiments, creating independence of data can create considerable work. Consider an analysis of potential preferences if it has been hypothesized that grazers do not choose equally among, say, four species of plants. The null hypothesis of equal choice and consumption could be tested by putting equal amounts of the four algae at different points in an area of habitat and counting how many grazers, e.g. urchins, are subsequently seen feeding on each (Paine and Vadas, 1969; Vadas, 1977; Schiel, 1982).

This seems straightforward and the data could be analysed by χ^2 or equivalent tests. There is, however, a serious potential problem of non-independence. Suppose that the urchins respond to the presence of damaged plants by detecting released chemicals and then using these as directional cues. This would cause aggregation of individuals where plants are already being consumed (Dean et al., 1984; Vadas et al., 1986; and as shown in the laboratory by Mann et al., 1984). If, entirely by chance, the first urchin starts to feed on one type of alga, the ensuing release of chemicals and aggregatory behaviour will cause several other urchins to find that food and to eat it. Observation will then show more urchins than by chance—even though no preference for any type of alga is being shown by the animals (Schiel, 1982).

It is quite difficult to prevent this sort of thing in a field experiment. The experimental pieces of food would have to be far enough apart to prevent influences from one unit to another. The urchins would have to be far enough away from the experimental units to have to find them by non-cued movement and then to be able to make a choice to feed or to move elsewhere. These requirements necessitate considerable knowledge of the distances over which potential cues might elicit a response. They also require removal of urchins that are initially too close to the sites where experimental pieces of food are placed. Alternatively, it requires several independent trials so that each initiation of feeding is independent of any others. Then, if the first urchins start feeding at random on the algae, there should be an equal chance, over several trials, of aggregations forming on each type of food.

Experiments to test hypotheses about choices of habitat are equally fraught with problems of non-independence. Consider a simple case of settlement of larval barnacles. These are known to have a remarkable repertoire of behavioural responses to physical (Crisp, 1974) and chemical (e.g. Strathmann et al., 1981; Johnson and Strathmann, 1989) cues when searching for a place to attach, settle and metamorphose. As an illustration of the potential problem, consider an experiment to test the hypothesis that larvae will settle in small pits (3 mm diameter, 3 mm deep) more than on rough patches (of similar surface-area) of substratum. The experimental test consists of providing larvae with a substratum in which replicate pits and rough patches are interspersed, perhaps about 10 mm apart. A number of larvae are introduced into seawater over the substratum and the numbers settling on/in each type of habitat subsequently counted.

Two problems may now beset the interpretation of the experimental data. First, consider what happens if larvae really do prefer the pits, but there are many larvae relative to the space in pits. They will settle into pits, but, as the space in pits fills up, may settle in the unpreferred rough patches. Unless observations are made about the order of site of settlement (which would show an excess over chance of early arrivals in pits), the data would suggest that no preference was being exercised. The hypothesis might have included a defined short period before settlers were examined, which would have allowed counts to be made before all the pits filled up and patches began to be occupied. In this case, unless the hypothesis defines time of settlement of each individual or order of habitat in which individuals settle as the data to collect, interpretation of data will be very difficult. The data are non-independent because, once larvae have filled one habitat, subsequent settlers are no longer presented with the choice specified in the hypothesis. If settlers were

removed as soon as they had settled, all subsequent arrivals could still choose where to settle, if that is what they actually do (except for chemical cues—see below).

The second problem is that many species of invertebrates show aggregative behaviour when settling (Knight-Jones and Stevenson, 1950; Connell, 1961; Barnett and Crisp, 1979; Schmidt, 1982). So, once the first larva has settled, it may produce chemical cues that are attractive to other larvae. In such a case, if the first larva settles at random in either a pit or a rough patch, subsequent arrivals responding to it could well produce data that show a clear preference for whichever habitat had been randomly chosen. Introducing larvae one at a time does not prevent this sort of behavioural non-independence.

The only absolutely safe procedure would be to introduce one larva into the experimental area and to note where it settles. Then the apparatus must be cleaned to create all empty space (the first problem) and to remove any chemical cues (the second issue). This will be an enormous amount of work to get adequate independent replication. The only alternative is to design the experiment to take into account problems of filling the habitats, problems of aggregative cues, etc. The data collected can be chosen to solve the first problem, for example, by hypothesizing about the order in which habitats receive larvae rather than the numbers of larvae settled in each habitat. The second problem might be overcome by letting larvae settle non-independently and running numerous independent trials. For each run of the experiment, the data would be the single value of proportion settled in one habitat (e.g. the pits). If there is no preference, there should be equal numbers of trials where this proportion is greater than 0.5 and where it is less than 0.5. If there is a preference for pits, there will be an excess number of trials where the data are greater than 0.5 (and, if there is a preference against pits, a greater number where the proportion in pits is less than 0.5).

Achieving non-independence of data in studies of behaviour requires considerable ingenuity and effort because so much of the behaviour, even of quite simple invertebrates, is in response to distant cues, other individuals, etc. These always have the capacity to create non-independence among individuals. The only really sure way to proceed is to be aware of the problems for any chosen statistical procedures and to find published examples where the issues have been considered and overcome for the particular sort of study in which you are involved.

4. Conclusion

This brief overview identifies some of the very numerous pitfalls involved in analyses of preferences for habitat or diet. These are still common types of ecological study, so it is worth raising, yet again, the major issues. As demonstrated here, these range across the entire gamut of components of a study. There are conceptual and logical issues about separating associations from real preferences; the former do not unambiguously imply the latter. There are major issues about ensuring that sampling to demonstrate an association is relevant and well-designed. In the case of associations with habitat, there are problems for analyses where estimation of available habitat is imprecise. There are numerous pitfalls in the design of experiments about behavioural choices among potential types of food—because of catchabilities, acceptabilities and handling-times differing. Avoiding non-

independence among apparently replicate trials in experiments takes ingenuity and, often, large amounts of work.

Two encouraging conclusions do emerge from this consideration of some of the issues. First, all of the problems are due to the biology of the organisms, the habitats, the diets. This is greatly encouraging because to overcome the problems requires deep thought about the natural history of the organisms and their needs. This is usually the major source of curiosity that caused the researcher to be involved in the study in the first place. It also provides comfort for those of us who are concerned that numerical and statistical issues might overwhelm the study (and, for that matter, that all ingenuity in biology and ecology is apparently focussed on scales of organization smaller than the individual animals, for example, on molecular approaches to problem-solving!).

Second, despite, the range of problems in such research, there are still numerous situations where an understanding of habitats and diets is crucial. These situations include issues of environmental impacts (which can change availability or suitability of habitats and food-sources), conservation and management of biodiversity (which absolutely require conservation and management of habitats and food-resources) and restoration of habitat (which can only proceed if appropriate habitat is being restored).

Identifying and solving the problems will continue to provide intellectual challenges for ecologists for a long time into the future. This review was written in support of the fascination and the challenges and can be considered successful if it provokes more thought and discussion of cases that have been successful and rigorous.

Acknowledgements

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