THE ECOCLOGY OF VECTOR SNAIL HABITATS AND MOSQUITO BREEDING PLACES

The experimental approach to basic problems

by

R. C. Muirhead-Thomson, D.Sc.

CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>2</td>
</tr>
<tr>
<td>General considerations</td>
<td>3</td>
</tr>
<tr>
<td>Temperature</td>
<td>8</td>
</tr>
<tr>
<td>Dissolved oxygen</td>
<td>12</td>
</tr>
<tr>
<td>Water movement</td>
<td>15</td>
</tr>
<tr>
<td>Organic matter and pollution</td>
<td>16</td>
</tr>
<tr>
<td>Salinity</td>
<td>19</td>
</tr>
<tr>
<td>Sampling methods</td>
<td>21</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>23</td>
</tr>
<tr>
<td>Conclusions</td>
<td>25</td>
</tr>
<tr>
<td>Summary</td>
<td>26</td>
</tr>
<tr>
<td>References</td>
<td></td>
</tr>
</tbody>
</table>
INTRODUCTION

In the last few years there have been considerable advances in the control of malaria and of the vector anopheline mosquitos, advances which in many cases mark the final phase of a campaign which has been pursued vigorously for many years in one form or another. Bilharziasis on the other hand is proving extremely difficult to control, and is a problem which far from being kept in check, is being intensified in many areas by drainage and irrigation schemes. Recent authoritative surveys of the bilharziasis problem (WHO 1957; Standen, 1957; American Journal of Tropical Medicine and Hygiene, 1955; Watson, 1957) have all expressed concern about the lack of progress in control, and they have been unanimous in deploiring the shortage of information about the fresh-water snails which form the intermediate hosts of Schistosoma. The need for more critical knowledge about these snails is particularly urgent in view of the fact that present methods for the control or eradication of Schistosoma are mainly based on the control or eradication of the snails themselves. As Standen (1957) says: "The outstanding gap in our knowledge of the transmission of schistosomiasis centres round the lack of information concerning the ecology and taxonomy of the molluscan vectors."

A study group of the World Health Organization has recently - 1957 - surveyed the available knowledge about the ecology of these vector snails, and a comparison has been drawn between this scattered and scanty knowledge on the one hand, and the vast amount of information about mosquito ecology on the other hand. That report has provided the incentive to compile this present review, and to investigate in more detail the basic problems and methods of study employed in the two fields. The fact that the review is based on practical experience of mosquito ecology, but only on theoretical knowledge of snail ecology, is a weakness which will expose it to criticism and enable specialists in snail ecology to affirm with some justification that it is very easy for outsiders to underestimate the difficulties of handling and studying snails in the laboratory and in the field. However this is a risk which must be faced by anyone attempting to bridge the gap between two specialized fields, and it is felt that the attempt will be justified if it helps to show up old problems in a new light.
GENERAL CONSIDERATIONS

At the present moment control of the anopheline vectors of malaria is based mainly on the destruction of the adult mosquito by means of house treatment with residual insecticides, but for many years malaria control was based on destruction of larvae in the breeding place, on naturalistic methods of making the breeding place unsuitable, or on the complete elimination of the breeding places themselves. In the 20-year period before the introduction and widespread application of DDT and other residual insecticides, a vast amount of work was done in many countries on the ecology of anopheline breeding places. Many of the problems which those workers dealt with, with considerable success in many cases, are basically similar to those which now confront the snail ecologist. Anopheles mosquitoes breed in many different kinds of water, rice fields, swamps, borrow pits, ditches, and temporary pools, but different species of Anopheles show marked preferences for particular types of water body. In Assam, Anopheles minimus breeds mainly along the grassy edges of streams and perennial rivers, and is rarely found in the stagnant water of rice fields and weedy ponds and tanks. At the other extreme, Anopheles gambiae in Africa prefers small temporary pools and puddles exposed to the sun, and is rarely found in the permanent water of ponds and swamps.

These peculiarities of distribution and selection of habitat are also familiar to malacologists, some species of freshwater snails being restricted to certain types of water collection and absent from a wide range of other available water. Within the chosen habitat itself the snails may be limited to certain restricted foci. In some cases these peculiarities of distribution may be fortuitous, but there are many well established instances which indicate a marked preference for a certain type of habitat, as shown for example by the peculiar distribution of Bulinus truncatus in Central and South Iraq (Watson, 1950), by the distribution of Bulinus(Physopsis) globosus and Biomphalaria pfeifferi in Sierra Leone (Gordon et al., 1935), and by the marked preference of Bulinus senegalensis in the Gambia for certain types of laterite pool (Smithers, 1956). Writing about conditions in Brazil, Dobrovolny & Barbosa (1952) report as follows: "It is interesting that Tropicorbis and Australorbis rarely if ever inhabit the same waters. As far as can be ascertained there is not a single proven instance where
populations of both genera have been found together in the same area of a stream although the reasons why they do not live together are not apparent."

In the case of mosquitoes these peculiarities of breeding site selection are determined by two distinct factors, namely the selective behaviour of the female mosquito looking for a place to lay its eggs, and the reactions of the larvae to the aquatic environment in which they find themselves. The choice or suitability of the breeding place is not determined entirely by the marked selective powers of the female mosquito, because after the eggs have been laid subsequent alterations in the nature of the breeding site, caused for example by flood, pollution or drying out, may render the habitat unsuitable for development of the larvae. In the case of vector snails the problem is simplified by the fact that with few exceptions - such as the amphibious Oncomelania - the snail spends its entire life in the watery habitat or in the mud left at the bottom when the water dries up.

In this age of progressive specialization it is extremely difficult for workers in the field, especially in the tropics, to keep up to date with developments outside their own restricted sphere. This difficulty is particularly regrettable in ecological studies involving basic relations of a living organism to specific types of environment. But many of the problems now facing malacologists have been studied intensively by malarologists and entomologists for a number of years, and at this early stage in the study of snail ecology the methods used and the results obtained in the study of Anopheles could be studied with advantage. The standard textbooks on Ecology and Limnology are unduly concerned with conditions in temperate climates, and the limited amount of hydrobiological work that has been done in the tropics has dealt mainly with lakes and with other large collections of water of interest in fish culture (e.g. Talling, 1957). In the case of the smaller water collections, often those of particular interest as habitats of snails and anopheline mosquito larvae, a great deal of our available knowledge has been gained in the course of work on mosquitoes.

This work on mosquitoes is of interest to the malacologist not only on account of the information which has been gained about the chemical and physical environment, but also
because of the great development in the experimental approach to fresh water ecology. In the early studies on mosquito ecology the chemical and physical factors in different types of water were measured, and an attempt was made to correlate these measurable differences with the different preferences shown by different mosquitoes. Fundamentally similar methods have been employed and are still being employed in the study of snail habitats (Andrade et al., 1955; Azavedo et al., 1955, 1957; Harry et al., 1956, 1957).

From about 1935 onwards, however, research on anopheline mosquito breeding places revealed an increasing use of the experimental approach in which an attempt was made to study the effect of these different chemical and physical factors one at a time. The normal breeding place of a particular mosquito was altered in different ways so that the influence of single factors, such as light and shade, water movement, presence or absence of vegetation, salinity, mechanical obstruction and so on could be studied. At the same time parallel experiments under controlled conditions in the laboratory were designed to amplify or clarify these field tests. Examples of work on those lines will be described under the appropriate headings below.

The advent of DDT and the residual insecticides produced an abrupt change of interest from the ecology of mosquito breeding places to research and control of the adult anopheline mosquito. With the emphasis on adult control by treatment of houses and habitations with insecticide, interest in the breeding habits of mosquitoes rapidly dwindled. In the last two or three years, however, there has been a recrudescence in studies on larval habitats, particularly in those areas where the use of residual insecticides in houses may not be sufficient by itself to interrupt transmission by unusually efficient vector species. Some of the work that is going on now in East Africa on the ecology of Anopheles gambiae breeding places may indirectly help to throw some light on the ecology of vector snails.

A study of the lists of references in recent papers on vector snails reveals only too clearly that many workers in this field are unaware of the significant and illuminating developments in the closely allied field of mosquito ecology. There is no real reason for them to be discouraged by the admittedly enormous bulk of literature in the latter field, as the whole subject of anopheline ecology in the tropics has been
reviewed by the present author (Mairhead-Thomson, 1951). This review covers publications up to 1950 which includes the period of most productive work on anopheline breeding places. Significant, and relevant, developments since that time will be referred to below.

Outside the field of mosquito ecology there is a great deal of scattered information about the ecology of tropical waters in general. A great deal of the relevant information has been included in the bibliography of the above review, but there is no doubt that it is far from complete, and there has been no opportunity of bringing that information right up to date. Again it appears, to judge from the lists of references attached to publications on snail ecology, that developments in that wider field have not in general been available to workers on snail ecology in the tropics. A few significant developments will be referred to below, but there are undoubtedly many others which the present author himself has overlooked.

In tropical Africa for example there are now several extremely active fresh-water biological centres of research, such as that at Jinja on Lake Victoria, and the University of Khartoum on the Nile. Although, as mentioned above much of their work is concerned with large water bodies, they do provide centres of technical knowledge about so many aspects of the chemistry, physics and biology of tropical waters that malacologists and entomologists alike would benefit by maintaining close contact with these organizations.

When discussing the basic problems in the ecology of snail habitats and mosquito breeding places there is one important feature in which the malacologists are working at a greater disadvantage, namely that the taxonomy of Planorbis snails is still in an unsatisfactory condition; many of the important vector snails show such a wide variation that confusion of identity can easily arise, even by experienced workers. Mosquito ecologists have been extremely fortunate in this respect. The taxonomy of mosquitoes, particularly those which are vectors of disease, has been dealt with so thoroughly that with few exceptions there is seldom any difficulty in identifying the different species of Anopheles in larval and even in egg form. Where larval characters are likely to be confused, identity can usually be confirmed readily by breeding out samples of larvae and
identifying the adults which emerge. Where larval and adult characters of sub-specific
groups are not clearly differentiated, as with the *Anopheles maculipennis* group in
Europe, the egg characters have proved a valuable guide to the type of larval habitat
used by different species. In one extreme case where closely allied forms appear to be
indistinguishable morphologically in adult, larval and egg stage - such as is the case
with salt-water *Anopheles gambiae* and typical *A. gambiae* on the East African coast - a
simple laboratory test involving sudden changes in salinity has provided a clear-cut
physiological method of identifying larval populations.

In the case of vector snails there is no doubt that in some areas critical work on
ecology will tend to be hampered by confusion of identity. There is nothing more
discouraging or confusing to the ecologist than to have the identity of his material
queried after publication, particularly when the question of vector snails and non-vector
snails is concerned. The case of *Bulinus forskalii* Ehrenberg is one in point (Schwetz,
1956). Wright (1956) has pointed out that although this species is found in the Gambia,
the vectors identified as this species by McCullough & Duke (1947) have proved to be
*B. senegalensis*. In the same way it appears that the report of *B. forskalii* being a
vector in Mauritius is based on inaccurate information.

As mentioned above, the different factors which make up the composition of the
mosquito breeding place influence not only the aquatic stages, egg, larva and pupa, but
also the behaviour of the female mosquito looking for a place to lay its eggs. In the
same way the study of the snail environment has a double significance in that the various
factors may influence not only the snail itself and its spawn, but also affect, sometimes
in quite a different way, the activity and behaviour of the *Schistosome miracidia*, the
degree to which they penetrate the vector snail, the development of the sporocyst in the
snail host, and the rate or periodicity with which cercariae are finally shed into the
water. In addition, the reactions of infected snails differ in many ways from those
of normal healthy snails in being for example more susceptible to high temperatures and
to molluscicides. These factors combine to make the ecology of vector snail habitats
a much more complex study, but at the same time a much more fascinating one.
A comprehensive account of the ecology of snail habitats and mosquito breeding places, particularly with regard to the experimental approach, is beyond the scope of this brief review, but a few examples of the methods used and the results obtained may help to reveal the advantages of closer liaison.

TEMPERATURE

Water temperature is undoubtedly one of the most important features of the physical environment, and as it is also one of the simplest to measure it has received a great deal of attention both in the field of mosquito ecology and of snail ecology.

In the literature on vector snail ecology in the tropics there has been a great deal of random measurement of temperature and it is not always certain that the maxima and minima reported have in fact been measured at the time of maximum and minimum temperature respectively. The general impression is that even though some species tend to be found in cooler waters than others, temperature itself, even in tropical waters, is seldom a limiting factor.

In studies on mosquito breeding places maximum and minimum thermometers have been fixed on a floating bamboo framework, with the bulbs, shaded from direct sunlight, just below the surface of the water. In snail studies Hoffman & Zakhary (1954) have used a bimetallic seven-day recording thermograph in a water-tight container suspended from anchored floats and submerged 15 inches below the surface of the water. In mosquito studies a recording mercury-in-steel distance thermograph has been used in some cases. Experience suggests that all automatic recording methods should be checked or supplemented from time to time by direct measurements at and approaching the period of maximum water temperature, and that wide differences may be recorded in the same water collection depending on whether the temperature is measured just below the surface or at deeper levels.

In general the maxima recorded in snail habitats have seldom exceeded 35-36°C which is not considered high enough to have any injurious effect on the snails themselves. However, there are a few observations which indicate the need for more critical studies. In his work on the amphibious Oncomelania, Ritchie (1955) recorded water temperatures of
40°C in paddy fields in the early stages of cultivation before the rice plants had grown sufficiently to afford shade. He suggests that these maxima may have played a part in restricting the snail to limited parts of the rice field such as the irrigation and drainage outlets where the flow of water reduced the temperature. The fact that some species of snail are markedly more susceptible to the effects of high temperature than others was clearly indicated by Gordon et al. (1934) who found that after a two-hour exposure to 40°C all their Biomphalaria pfeifferi died, while 19 out of 20 Bulinus globosus still survived. A similar exposure of Australorbis to 40°C for two hours produces a high mortality during the period of exposure (de Witt, 1955). While these comparatively short exposures to very high temperatures may provide clear-cut and dramatic effects, the influence of longer exposures to sublethal temperatures may prove equally important in the natural habitat, contributing to snail mortality and behaviour in a slower but possibly equally effective manner.

In the field of mosquito ecology the methods used in studying the reactions of Anopheles minimus and other species to temperature are particularly instructive. This species breeds, along with several others, along the grassy edges of streams, rivers and open drains. It is seldom found in collections of stagnant shallow water, rice fields in particular. Temperature measurements during the hot rainy season in Assam showed that the maximum water temperature in rice fields and shallow borrow pits regularly exceeded 40°C, and that on occasions temperatures of 42°C were recorded. In streams and rivers the maximum at this time seldom exceeded 34°C. The thermal death point of the larvae of the different anopheline species found in these different habitats was tested by exposing them to a gradual increase in temperature - at the rate of about 1°C each five minutes - until a point was reached when the five-minute exposure was sufficient to kill all the larvae. The results were as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Thermal death point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles minimus</td>
<td>Streams</td>
<td>41°C</td>
</tr>
<tr>
<td>A. insulæflorum</td>
<td></td>
<td>40°C</td>
</tr>
<tr>
<td>A. hyrcanus</td>
<td>Ponds and rice fields</td>
<td>44°C</td>
</tr>
<tr>
<td>A. barbirostris</td>
<td></td>
<td>44°C</td>
</tr>
<tr>
<td>A. vagus</td>
<td>Shallow bare-edged sunlit puddles</td>
<td>45°C</td>
</tr>
</tbody>
</table>
Anopheles minimus, by associating with running water, avoids exposure to the high temperatures regularly recorded in still shallow water, temperatures which would quickly prove fatal. But there is evidence that longer exposures to temperatures below the thermal death point may still have an adverse effect, and it was observed that when the temperature of a perennial river in which A. minimus was breeding regularly reached a maximum of 38°C on several consecutive days, it was followed by a sharp fall in the number of larvae. In this connexion the maximum temperatures reached in running water habitats may be of more biological significance than the same maxima recorded in still shallow water. In running water, temperatures at or near the maximum are maintained for a period of several hours, whereas in shallow stagnant water the maximum is usually reached as a short sharp peak. In shallow still water the highest temperatures occur at the surface, and a few inches below the surface the temperature may be several degrees lower. In such habitats the extreme effect of high temperature can be avoided by submerging, a point which has to be considered in the ecology of both snails and mosquito larvae. In running water, effective mixing ensures that there is no thermal stratification, the maxima recorded at the surface indicating the conditions throughout the bulk of the water.

The existence of thermal stratification in large water bodies such as lakes has long been recognized. The occurrence of the same phenomenon in small collections of water at certain times of the day has received less attention, (Vaas & Sachlan, 1955). The work of Onabamiro (1952, 1954) on temperature differences at different layers in small ponds in Nigeria is of particular interest to ecologists, especially as an attempt has been made to relate these differences to the daily migration of Thermocyclops, the intermediate host of Dracunculus medinensis, from one water layer to another.

Work on mosquito ecology has shown that the aquatic stages, egg, larva and pupa, differ in their reactions to high temperature, and that young larvae for example are more resistant than older larvae. It is possible that corresponding differences exist between different growth stages of snails. In all this work the use of standard methods of exposure and mortality measurement would be a great advantage in snail ecology, and might enable more accurate comparisons to be made between different species and different stages, as well as between healthy and infected snails.
An accurate knowledge of the temperature range encountered in normal habitats at different seasons of the year is of great importance in the study of both snails and mosquito larvae. The exact effect of these seasonal temperature changes on growth and other activities can seldom be judged satisfactorily in the field, and it is necessary to fall back on the support of controlled laboratory experiments. In the case of mosquito eggs and pupae – non-feeding stages – an accurate curve can be plotted showing the direct relation between increasing temperature and increased development. Curves plotted in this way showing the development of eggs and pupae of *Anopheles minimus* (Muirhead-Thomson, 1951) and *Anopheles sergenti* (Saliternik, 1956) at different constant temperatures show a striking resemblance to curves plotted by Gordon et al. (1934) showing the duration of the developmental cycle of *Schistosoma mansoni* in *Biomphalaria pfeifferi*, and that of *S. haematobium* in *Physopsis globosa*.

In the case of mosquito larvae, factors besides temperature influence the growth rate. In addition, the larvae of many anophelines do not thrive so well in the laboratory as in their natural habitat, and it is not always possible to assume that the results obtained in the laboratory necessarily apply in the field. These difficulties probably apply equally well to snails kept in aquaria.

In experimental work on the effect of temperature it has long been the custom to expose the animal to a series of constant temperatures. In this way consistent results can be obtained, and experiments can readily be repeated elsewhere. Nevertheless, water temperatures in snail habitats and mosquito breeding places are seldom constant for any length of time, and one cannot assume that the growth and behaviour of the animal at a certain temperature will be the same whether that temperature is a constant one or the mean of alternating temperatures. Although the use of constant temperature experiments might appear at first sight to be more accurate in controlled scientific experiments, results of more direct application to the field would probably be obtained if more attention were paid to the use of controlled alternating temperatures, say 15°C to 20°C, 15°C to 25°C, 20°C to 25°C and so on. The use of the constant temperature
experiment also fails to take into account the effect of temperature changes within the optimum range. The spawning activity of snail vectors for example is inhibited by high temperature, but stimulated by a change to cooler water (Gordon et al., 1934). There may be other activities affected by sudden changes such as warming and cooling.

As mentioned above, the vector snail ecologist has also to consider the effect of temperature on the activities and penetration of miracidia, the growth of sporocysts, the discharge of cercariae, and the efficacy of molluscicides (Standen, 1951, 1952; Kunty, 1946; Hoffman & Kakhary, 1951, 1954). No attempt will be made to deal with this aspect of vector ecology in this review, but it is obvious that many of the points considered above will have a direct bearing on this other aspect of vector snail ecology.

**DISSOLVED OXYGEN**

Work on mosquito ecology in the tropics has provided a great deal of information about the range of oxygen concentration in various types of water body, such as ponds, streams, and rivers. Workers in that field have shown that in stagnant tropical ponds with abundant plant growth there is a considerable degree of supersaturation on sunny days with the oxygen content frequently as high as 150 per cent. saturated. Even higher figures - over 200 per cent. saturated - have been recorded in snail habitats in East Africa (van Someren, 1946). At the low end of the scale, work on mosquito ecology has shown that oxygen may be absent, or only present in very low concentration, in many types of tropical water. The importance of low oxygen tensions, usually combined with high concentrations of carbon dioxide, was early recognized by fresh water biological work in South America (Carter & Beadle, 1930; Carter, 1934). A similar low oxygen tension has been recorded in many of the inland pans of South Africa (Hutchinson et al., 1927) and in the papyrus swamps of East Africa (Carter, 1955), a finding which may be of significance in the ecology of African snail vectors of *Schistosoma*.

In mosquito ecology dissolved oxygen by itself has not been revealed as a factor of great importance, and has not been the subject of much experimental work. However, it is a factor which has to be taken into account in the experimental pollution of anopheles breeding places, and in that capacity will be discussed later.
In the study of Planorbis snails - which can utilize dissolved oxygen - this factor is naturally of greater importance in the environment. Some genera of aquatic snails such as Pila are found in papyrus swamps and other waters with low values for dissolved oxygen, but most fresh-water snails require water with a high oxygen content. The observations made by Wright (1956) are particularly instructive in that he has drawn attention to the fact that oxygen tension is liable to show wide variations in different parts of the same habitat at the same time. For example, there is a microhabitat of high oxygen tension on the underside of water lilies, a favourite place for snails. A similar localized zone of high oxygen tension may also exist at the roots of rice and other growing plants, and this may play a part in the preference shown by some snails, such as Bulinus forskalii, for the lower parts of grass and rice roots. In the case of B. forskalii the habitat may play a significant part in the degree to which the snail is exposed to miracidial infection in nature. Miracidia are normally negatively geotropic and positively phototropic, and the combined effect of these reactions is to direct the miracidium to the surface of the water, away from the deeper preferred sites of B. forskalii. Although this species can be infected with Schistosoma haematobium under laboratory conditions, it has not been found naturally infected.

Wright's demonstration of the important part played by the microhabitat has a significant bearing on mosquito ecology as well as on snail habitats. Work on mosquito breeding places has made us familiar with the idea of the physical microhabitat, and the fact that in any one breeding place local variations in light and shade, in mechanical obstruction, and in water movement may play a vital part in determining the concentration sites of larvae within the habitat, or determine the exact location selected by the female mosquito for laying its eggs. For example, larvae associated with streams and running water are in fact restricted to those parts of the habitat, such as the grassy fringe, where the water movement may be practically nil. The snail ecologists have now introduced us to the idea that other factors, dissolved oxygen at least, may also show wide differences in different parts of the same habitat, and that there is at least a correlation between these differences and the nature of the preferred concentration sites of the snails. These local variations may play an additional role in that infected snails (Australorbis) are more susceptible to oxygen lack than uninfected ones.
Macan (1949) has shown that the same idea of the chemical microhabitat also applies to another important constituent of the snail's environment, namely the calcium concentration. He has shown that single measurements of figures for calcium concentration and for alkalinity may be quite misleading in that both figures may vary considerably (1) in different parts of heavily overgrown ponds or tarns, (2) from one inflowing stream to another, and (3) at different times according to the rainfall.

Several studies have been carried out on the oxygen requirements of aquatic snails, *Australorbis* in particular, and on the way in which respiration is affected by such factors as molluscicides, infection with intermediate stages of *Schistosoma*, and salinity (von Brand et al. 1948, 1949; Edwards, 1951). A method of testing the reactions of various aquatic invertebrates - including pond snails - to different pressures of dissolved oxygen has been described by Munro Fox (1954) who has shown that with *Planorbis corneus* and *Lymnea* the reactions of young snails to certain oxygen tensions may differ from those of the adult snails. The basic technique of maintaining regular oxygen tensions for long periods by bubbling controllable mixtures of oxygen and nitrogen through the water has been further developed by Downing & Merkens (1957) and Alabaster et al. (1957) in their work on the respiration and survival of fresh-water fish when exposed to constant and controllable tensions of oxygen and carbon dioxide for long periods. It seems that these methods could be tried out with advantage on the intermediate snail hosts of *Schistosoma* in warm countries.

It is possible that some workers in snail ecology may be unaware of the fact that the question of oxygen content of various natural waters, solubility of oxygen under different conditions, and improved methods for measuring dissolved oxygen are the subject of continual study by workers on fresh-water ecology and on water pollution (Truesdale & Knowles, 1956; D.S.I.R., 1957). Two recent reports - Truesdale et al. 1955; Mortimer, 1956 - are particularly valuable for the information they provide about revised figures for the saturation concentration of oxygen in fresh water and in sea water at temperatures of 0°C to 37°C, and for aids in calculating, from the observed oxygen concentration, the percentage saturation according to temperature and altitude.
WATER MOVEMENT

A number of observations have been made on the reactions of snails to water movement, and to the part played by that factor in the selection and suitability of the habitat. For example, Helmy (1953) has attributed the presence of Biomphalaria boissyi in the Nile delta and in the Sudan, and its absence from the whole of Upper Egypt, to the sensitivity of the snail to water movement - the slope of the Nile between Cairo and Khartoum being too great to allow breeding foci. A simple observation with swirling water in a vessel showed that Bulinus truncatus will remain attached under conditions in which Biomphalaria is washed out. In Iraq however, Watson (1950) observed that Bulinus truncatus is sensitive to water movement and is confined to stagnant water in which the rate of flow is very low.

From observation alone it is not always possible to say that the absence of particular snails from running water habitats is due directly to water movement. As mentioned above, many streams have a microhabitat of perfectly still water at the edges, particularly where there is fringing vegetation, and as a result reading of current velocity may give misleading results about conditions at the edge or even at the bottom of the stream.

In the course of work on mosquito ecology the question of water movement has been studied in some detail, and the experimental approach to this factor may be of interest to snail ecologists. Single larvae of different anophelines from different habitats were induced to attach themselves to a pin fixed in the centre of an experimental channel in the laboratory. The flow was increased slowly from zero until a point was reached when larvae could no longer hold on. In this way it was found that the larvae of the stream breeders, such as Anopheles minimus, A. maculatus and A. aconitus were not measurably more resistant to flow than the larvae of swamp and rice field breeders such as Anopheles hyrcanus and A. barbirostris. However, the stream breeding larvae are well adapted in other ways to avoid the danger of increased water flow in that they are strongly attracted towards the shade provided by the vegetation fringing the stream. This reaction automatically takes them into a zone of still or very slowly flowing water, and is intensified by any increase in water movement.
As mentioned above the concept of the microhabitat is important when studying the immediate environment of the snail or the mosquito larva. As far as snail vectors are concerned there are clearly great opportunities for more exact studies on the range of water movement to which snails are normally exposed, and for more exact comparisons, under both laboratory and field conditions, of the reactions to measured flow of different species, different stages of the same species, and of infected and uninfected snails.

Before the era of residual insecticides one method of controlling mosquito breeding in water courses was by artificial flushing at intervals. This method was used against species which normally breed along stream edges, as well as against those which breed in relict pools in drying stream or river beds. While remarkably effective in most cases it was found that one species, the African Anopheles gambiae, reacted in an unforeseen way in that it could avoid the immediate effect of flushing by rapidly submerging and staying submerged for a period of two or three hours until the flood subsided. At the same time the regular flushing, which was carried out in the dry season, encouraged the growth of vegetation along the arid stream banks, and provided new and better protected breeding places. It seems likely that problems of a similar nature will be encountered in snail ecology in relation to stream control, canalization, damming and irrigation.

ORGANIC MATTER AND POLLUTION

The organic content of the habitat and the degree of pollution are factors of great importance in the lives of vector snails and anopheline mosquitoes alike. Most anophelines breed in fairly clean unpolluted water and are discouraged by excessive pollution. With the vector snails some degree of organic pollution appears to be favourable up to a certain point, but beyond that becomes unfavourable. There are also specific preferences; Biomphalaria pfeifferi for example tolerating a greater degree of pollution than Bulinus (Blair, 1956).

Workers on mosquito ecology have provided a great deal of information about the quantity and quality of organic matter in different types of natural habitat in the tropics, and about the range encountered in each type of breeding place. Their conclusions reached about the value of the different types of chemical estimation have a distinct bearing on the nature of vector snail habitats.
The experimental approach to this question has been particularly well developed in mosquito ecology by polluting breeding places, both streams and ponds, with cut vegetation and following the subsequent changes in water composition by means of standard methods of water and sewage analysis. Parallel with these progressive chemical changes and subsequent re-purification, observations have been made on the reactions of larvae to the polluted water in the breeding place, and to the effects of pollution on the egg-laying activities of the female mosquito. The course of pollution was followed mainly by the figures for dissolved oxygen, free and saline ammonia, albuminoid ammonia, and oxygen absorbed from permanganate, among others. The oxygen absorbed from permanganate was found to be the best single indicator of organic content and degree of pollution, and the one which is simplest to carry out in the laboratory.

In the case of anopheline mosquitoes, increasing organic content proved to be a limiting factor through its influence on oviposition. Larvae themselves were unaffected by degrees of pollution up to 20 or 30 times greater than that which deterred the female from laying its eggs. The effect of this pollution and subsequent repurification on the oxygen content of the habitat is equally striking, but these changes are probably of more consequence to the reactions of vector snails than to mosquitoes or their larvae.

Work on both snail ecology and mosquito ecology has revealed the importance of distinguishing the different types of organic pollution, whether of animal or vegetable origin, pollution due to factory effluents, and so on. Anopheles gambiae for example may tolerate a considerable degree of faecal pollution in the shallow sunlit pools in which it breeds, but it is very sensitive to organic pollution caused by decaying vegetation.

The amount of organic matter in the water is a particularly valuable index of conditions in the comparatively small water bodies which form the habitats of many snail vectors and many anopheline mosquitoes. In these shallow habitats there is a close association between the organic matter in the water and the organic content of the underlying soil - an important microhabitat of many vector snails. The organic content
of the subaqueous soil in turn has a profound influence on the nature and distribution of the vegetation in and around the habitat.

The influence of changes in organic matter within a snail habitat is well brought out in van Someren's (1946) observations on *Limnea* (Radix) caulliaudi, the intermediate snail host of liver fluke in East Africa, and a species which has a very similar habitat preference to Biomphalaria pfeifferi. In one of the habitats stagnation of the water was followed by an increasing accumulation of organic trash and by lowered oxygen tension, as a result of which both species of snail were killed off.

In the field of mosquito ecology a somewhat similar situation arises in the case of *Anopheles culicifacies* in South India which breeds, among other places, in freshly dug borrow pits (Russell & Rao, 1942). As the pits grow older there is a progressive decline in the density of larvae, a decline closely associated in some way with the total organic matter (formed mainly by plankton and amorphous matter), and quite unrelated to many other factors such as pH, carbon dioxide, oxygen, alkalinity, hardness etc, which were measured at the same time. Experiments of this nature would be particularly illuminating in the study of snail habitats, especially if they were designed to distinguish the effects of organic accumulation alone from that of the oxygen depletion accompanying pollution.

A close association is frequently found between increased incidence of vector snails and increasing irrigation and water control. In this connexion it is worth noting that the more storage of water by impounding above a dam may have a marked effect on the composition of the water. Observations on the White Nile above the Gebel Auliya dam (Brook & Raoska, 1954) have shown that as the river water slackens to produce lake-like conditions above the dam there is a numerical increase - up to 100-fold - in the plankton. Near the dam itself the vast development of plankton produces an increased turbidity of the water, and also an increase in dissolved oxygen concentration associated with the rise in phytoplankton.

High densities of vector snails are frequently found where water movement is reduced by artificial dams and causeways, and it would be interesting to see whether the
biological changes associated with impounded river water in the Nile are in fact duplicated in a small scale in the streams and watercourses which form favourite habitats of many vector snails.

SALINITY

In most inland habitats of vector snails and anopheline mosquitoes - with a few notable exceptions - salinity is a factor of negligible importance; but in deltaic and estuarine areas, where there is flooding by tidal rivers, and irrigation by tidal flow, salinity may prove to be a limiting factor. For example, salinity plays an important part in the ecology of Bulinus truncatus in Iraq where evaporation during the hot weather may raise the salinity of some swamps to a lethal point (Watson, 1950, 1953, 1957). The exact lethal point according to salt concentration and degree of exposure has not been worked out, but field observations indicate that Bulinus is not normally found where the salinity exceeds 550-600 parts per 100,000.

The salinity of mosquito breeding places has received a great deal of attention by malarologists, particularly in view of the fact that several efficient vector species can breed in the brackish water of coastal swamps. One of these species, Anopheles sundalcus, has been the subject of particularly intensive research due to the fact that since about 1930 it has extended its range from one of its permanent foci in the Sunderbunds at the head of the Bay of Bengal and invaded various parts of the east coast of India. Work on that species, and also on the salt water anophelines of Africa (Anopheles gambiae melas on the west coast and the salt-water form of A. gambiae on the east coast) has dealt with problems fundamentally similar to those encountered in the study of salinity in relation to snail ecology.

In the experimental line, the reactions of fresh-water and brackish-water mosquito larvae to sudden changes in salinity under controlled conditions have revealed physiological differences which may be of great diagnostic value. For example, on the east African coast typical fresh-water Anopheles gambiae and the local salt-water form are indistinguishable morphologically in the adult, larval and egg form, but the reactions of larvae to changes in salinity are sharply defined. When larvae taken from
a coastal breeding place, or bred out from eggs in the laboratory, are transferred to
75 per cent. pure sea water (23.8 g NaCl per litre) all the salt-water larvae survive at
least six hours, while all the larvae of typical A. gambiae are dead within two hours.
In the present state of taxonomy of the vector snails it is possible that simple
laboratory tests of this kind might help to differentiate closely related species in
which morphological features are variable or unreliable.

In this connexion the observations of Edwards (1951) are of particular interest in
demonstrating the effect that salinity has on the respiration of snails. Working with Australorbis - a New World intermediate host of Schistosoma mansoni - he found that
immersion of the snails in 0.3 to 1.0 per cent. NaCl causes an immediate increase in
oxygen consumption. In 0.3 to 0.5 per cent. NaCl the rate returns to normal within a
few hours. But concentrations above 0.5 per cent NaCl cause gross disturbance in
respiration within a few hours, and death within a few days. The author says: "It would
be of some interest to relate these results with the distribution of the snails and the
influence of such factors as backwash of the sea into the streams from which the snails
are obtained. One might expect to find specific and racial differences in the snails in
their distribution in relation to salinity."

Other studies on salt-water Anopheles gambiae have dealt with a problem very
similar to that of Bulinus truncatus in Iraq. Observations carried out on a brackish
pond which formed an ideal breeding place for this mosquito showed that when the pond
dried up slowly with the onset of the dry weather, there was a corresponding increase in
the salinity, which however had no harmful effect on breeding until a figure of about
83 per cent. pure sea water was reached. At this point, larvae suddenly became very
scarce, and were completely absent by the time the salinity had become the same as that
of undiluted sea water.

Among other more general points revealed by work on mosquito ecology is the
suggestion that the combination of salinity and high organic content may be a better
indication of optimum conditions than salinity alone. In addition salinity figures
based on chloride estimations alone may prove to be misleading, especially in the case
of estuarine areas where dilution of sea water with land drainage alters the proportions
of the contained salts, since river water contains more sulfate than chloride and more calcium than magnesium. The chemical and physical conditions likely to be encountered in different kinds of inland saline water collections are again very different from those of sea or estuarine waters, and have been described by Beadle (1932, 1943).

Snail studies directed towards the wider field of the transmission of Schistosoma have also to take into account the fact that salinity may be a factor of some importance to the intermediate stages of the parasite. Standen (1951) for example, has shown that salinity inhibits hatching of the ova of Schistosoma mansoni in direct proportion to the concentration. Salinities as low as 0.05 per cent. have some inhibitory effect, while at salinities of 0.6 per cent. and above hatching normally ceases.

**SAMPLING METHODS**

When we consider the problem of sampling populations of snails and of mosquito larvae there are obviously a great many features in common. In both fields several different methods are used, each of which has its advantages and its disadvantages. For estimating larval density such indices as 'larvae per 100 dips', 'larvae per square yard or metre', 'larvae per linear yard or metre' (stream edges) and 'larvae per man hour' have been used according to the type of breeding place and abundance and distribution of the larvae. In the same way snail counts have been based on unit time, unit area or number per dip, as well as on the use of artificial concentration sites in the form of palm leaf traps.

In both fields there are real difficulties in the way of arriving at an efficient standardized technique. Many of the methods used are sufficient for most practical purposes, particularly when larvae or snails are abundant, but they tend to reveal serious defects at low densities. In the field of malaria control by means of residual insecticides it has become increasingly important to improve methods of larval sampling at low densities in order to indicate what progress is being made towards mosquito eradication in a particular area. Trapido's work on this problem in Sardinia (1951) revealed the severe limitations of standard sampling methods at these low densities, and also disclosed how biased such results could be due to the fact that differences in larval behaviour enabled some species to escape detection more effectively than others.
The problem of sampling at low densities applies equally well to vector snails in which the distribution within the habitat may be very patchy, and where even low numbers of snails may be of great importance either because of their great biological potential or as foci of infection. The question is also of importance in appraising the results of molluscicide treatment (Williams et al. 1957).

A more critical approach to the question of larval sampling has been made by Wilson & Msangi (1955) with particular reference to the technique worked out by Christie (1954). This sampling method, which applies to small collections of water and to breeding places of Anopheles gambiae in particular, involves complete evacuation of all the water in the habitat and straining out the larvae through a series of graded sieves. In this way an accurate idea is obtained of the total larvae in that particular breeding place, and this figure can then be compared with previous estimates of the larval population based on dipping and other sampling methods. The results of this technique again show clearly what a high proportion of the larvae may escape detection by even the most thorough dipping.

It appears that whatever sampling method is used some attempt should be made to estimate its efficacy either by liberating known numbers of snails or mosquito larvae and recapturing them by the same sampling technique, or by repeated sampling in the same habitat. Advances on these lines have been made by Olivier & Schneidermann (1956) in which snails are collected from marked areas of the habitat for a measured length of time. The number is recorded and the snails are replaced in the habitat by scattering throughout the collecting area. The same procedure is repeated in the same area on subsequent days, and in this way it is possible to work out for each collector the percentage error at different snail population densities, and to compute the number of counts necessary to reach a desired level of accuracy.

A technique of marking, releasing, and recapturing snails has been developed by Pimental et al. (1957) with special reference to the study of the daily movements or peregrinations of snails. Apart from its value in behaviour studies, it seems a promising method of estimating snail populations and assessing the value of sampling
techniques. Although little work of this kind has been done with mosquito larvae - possibly because of the relatively brief duration of their aquatic existence - the marking and recapture technique has been developed to a high degree in the study of tse-tse fly populations (Jackson, summarized by Buxton, 1956). The experience gained by tse-tse workers should prove of value to those interested in developing this line of investigation in vector snail ecology.

MISCELLANEOUS

The existence of predators which might play a part in controlling the numbers of larvae in mosquito breeding places has long been recognized, and the part played by fish in particular in the natural and artificial control of mosquito breeding has received a great deal of attention. Very few controlled experiments have been carried out however on the role of the smaller predators such as insect larvae and aquatic bugs. This question has been tackled in a more critical manner by Christie (1956) in work on Anopheles gambiae in East Africa. The method is to set up a comparison experiment in which one breeding pool is left untouched while another has the predatory fauna strained out with an 80-mesh sieve, and the breeding place restocked with newly hatched A. gambiae larvae. In this way the larval survival can be studied under the following conditions: (a) in the presence of other naturally occurring fauna; (b) in the presence of some of the naturally occurring fauna, and (c) in the absence of naturally occurring fauna. These experiments showed convincingly how the absence of predators greatly increased the survival rate of the larvae.

Vector snails are subject to attack by a wide range of predators and parasites (WHO 1957; Michelson, 1957) whose relative importance is difficult to estimate. Although these predators appear to cover a much wider faunal range than those affecting mosquito larvae, the experimental method of investigation by exclusion of different predators from mosquito breeding places, might well be applied with modification to allied problems in snail habitats.

The experimental study of predators and their influence on mosquito larvae and on snails is still in its infancy, and it appears that little progress can be expected
without a keener appreciation of the different ways in which different predators may produce a controlling effect on the population. For example, some may act as 'density dependent factors', killing a higher proportion of the population when the population density is high. Others may act as 'density independent factors', in which the proportion of the population killed is independent of population density. In other cases the proportion of the population killed by a particular predator may decrease with increase in population density, and still other relationships may exist (Varley, 1953, 1957; Macfadyen, 1957).

One outstanding feature common to the ecology of many snails and mosquito larvae is the marked selection shown by different species, and the fact that many apparently suitable water bodies are not utilized as habitats. Many years ago Lang (quoted in Macan, 1949) transferred eggs and adults of Limnaea stagnalis and L. peregra to fish ponds where neither species occurred. The adults survived and apparently thrived, but eggs did not develop. Owing to the selective behaviour of the female mosquito in choosing a site to lay its eggs, mosquito larvae do not normally find themselves in completely atypical habitats. Nevertheless, it would be interesting to know what would happen to larvae in such a case. This simple type of experiment could be extended with advantage in both fields.

Ecological studies on snail habitats and mosquito breeding places usually centre round one particular animal or group of animals. Ecologists however cannot afford to ignore developments in allied fields, or to remain aloof from the possible repercussions of their specialized activities. This need for a wider outlook is well illustrated by the following examples. When DDT aerial spraying was carried out against tse-tse fly in the game reserves in Zululand, it produced, as might be expected, a high mortality among many other insects including those in pools and ponds. But the treatment also produced a quite unprecedented increase in the abundance of snails in these habitats (Omer-Cooper, 1949). In recent years there has been an increasing amount of scientific research on fish cultivation in the inland waters of East Africa. (Colonial Research, 1955-56, East African Fisheries Research Organization, Annual Report.) This work has disclosed the important part played by snails in the economy of lakes, and the many way...
in which their activities are bound up with those of fish. Many African waters are deficient in sulfate and snails, by secreting sulfuric acid add this essential plant nutrient to the water. It is also stated that snails feed on a variety of tough plant tissues and therefore play an important part in accelerating decomposition. Snail faeces also provide material for increasing plankton growth and for rendering water suitable for fish cultivation. On the other hand, some fish feed mainly on snails, but although these fish have been introduced - along with Tilapia - into dams, they evidently cannot reduce the snail population sufficiently to play a part in Bilharzia control.

CONCLUSION

A few years ago progress in the study of snail ecology was appraised by Macan (1949) as follows: "It is generally true that the study of the ecology of fresh-water molluscs has reached a stage where distribution in the field has been correlated with certain attributes of the habitat, but not the stage of experimental investigation of the nature of this correlation." Although there have been considerable advances since that time, especially in the study of the Oriental Oncomelania and the New World Australorbis and Tropicorbis, this assessment still applies equally well today.

The main object of this brief review has been to underline the fact that at this comparatively early stage in the scientific study of vector snail ecology, workers in that field could study with advantage the experimental methods used and the results obtained in the longer established field of mosquito ecology. At the same time it is felt that mosquito ecologists in turn could benefit by a keener appreciation of the problems being encountered in the study and control of vector snails. In this introductory review it has been possible to deal with only a small proportion of the total contributions in this restricted field of ecology. In addition there are almost certainly many pertinent references which have been unavoidably overlooked. But perhaps sufficient ground has been covered to emphasize the need for much closer liaison on research problems of mutual interest, not only with regard to snail vectors and mosquito breeding places, but also in the wider field of the ecology of tropical waters in general.
The emphasis in this review has been on a few physical and chemical factors of the environment, these factors being selected because they lend themselves to measurement and to the design and control of experiments in the laboratory. However, this can only be regarded as the first stage in experimental ecology, a stage which must lead inevitably to a study of the more complex components of the environment, and a much closer study of large-scale modifications which are brought about mainly by man's activities. As Varley (1957) says: "If ecology is to play a part in the world and in nature conservation, ecologists must not hold themselves aloof. They must instead learn to use and understand experimental methods which provide the most powerful tools in scientific analysis, and use them to investigate the basic principles of ecology which operate on animals and plants everywhere."

SUMMARY

The ecology of fresh-water snails - in particular those which act as intermediate hosts of the schistosomes which cause bilharziasis in man - has been reviewed in the light of the much more extensive knowledge about the breeding places of anopheline mosquitoes. Many of the problems involving the study of snail habitats and anopheline mosquito breeding places have a great deal in common, and best lend themselves to interpretation, both in the field and in the laboratory, by experimental ecological methods. At the present moment studies on those lines are much more advanced in the field of mosquito ecology than in the field of snail ecology, and it is felt that there would be considerable advantages to be gained by a closer liaison between the two isolated groups of workers. An attempt has been made to show that workers in both these fields would benefit from improved facilities for keeping in touch with general developments in the wider field of fresh-water ecology.

I am greatly indebted to Dr W. Alves, Director of the Malaria and Bilharziasis Research Laboratory, Salisbury, Southern Rhodesia, and to Dr C. A. Wright of the Natural History Museum, South Kensington, London, for reading through the original typescript and for making many helpful comments and criticisms.
REFERENCES

Alabaster, J. S., Herbert, D. W. M., & Hemens, J. (1957) The survival of rainbow trout (Salmo gairdnerii Richardson) and perch (Perca fluviatilis L) at various concentrations of dissolved oxygen and carbon dioxide. Ann. appl. Biol. 45, 1, 177-188


Carter, G. S. (1934) Results of the Cambridge expedition to British Guiana 1933. The fresh waters of the rain forest area of British Guiana. *J. Linn. Soc. (Lond.)*, (Zool), **32**, 147


Department of Scientific & Industrial Research (1957) Water pollution research. London, H.M.S.O., p. 74


East African Fisheries Research Organization. Annual Reports (1952-56) Nairobi
Edwards, G. A. (1951) Influence of infestation and other factors upon the respiration of the snail *Australorbis glabratus*. Publ. Avulsas do Instituto Ageu Magalhaes, 1, 2, 9-26


Khalil, M. (1930) The role of the Nile in the dissemination of snail intermediate hosts of *schistosoma* in Egypt. *J. Egypt. med. Ass.* 12, 137

Kunty, R. E. (1946) Effect of light and temperature on shedding of *Schistosoma mansoni* cercariae. Naval Medical Research Institute, Bethesda


References


van Someren, V. D. (1946) The habits and tolerance ranges of Limnaea (Radix) caillaudi, the intermediate snail host of liver fluke in East Africa. J. Anim. Sci. 15, 170


Wright, C. A. (1956c) Some factors affecting the susceptibility of snails to infection with miracidia of schistosoma sp. *Bull. Soc. Path. exot.* 49, 6, 1211-1220