Environmental Pollution (Series A) **25** (1981) 123–133

TADPOLES AS INDICATORS OF HARMFUL LEVELS OF POLLUTION IN THE FIELD

A. S. COOKE*

Institute of Terrestrial Ecology, Monks Wood Experimental Station, Abbots Ripton, Huntingdon PE17 2LS, Great Britain

ABSTRACT

Many different chemicals are applied to arable farmland and some are known to occur in associated freshwater ditches and ponds, perhaps in concentrations that are ecologically harmful. A technique using caged tadpoles (Rana temporaria) *has been devised in order to help to assess which chemicals might be potential freshwater pollutants as a result of run-off or spray drift. This bioassay would be applicable to other freshwater situations. Mortality, growth, rate of metamorphosis and occurrence of deformities are monitored at regular intervals.*

A trial is described in which the incidence of deformities was unusually high in cages beside potato fields after application of oxamyl, a carbamate nematicide and insecticide. Monitoring deformities can prove especially informative. Background studies on tadpole deformities are outlined in this paper. Not only can some pollutants cause morphological abnormalities directly, but it appears that tadpole cultures are frequently susceptible to producing a certain type of deformity, and exposure to environmental stress can increase its incidence.

INTRODUCTION

When treated with pollutants in laboratory tests, anuran embryos (pre-hatching stages) or tadpoles (larval stages) can show a variety of sublethal responses such as changes in growth or development rates or pigmentation or a proliferation of morphological deformities (see Cooke, 1970, 1972, 1973*a*; Nishiuchi & Yoshida,

* Present address: Nature Conservancy Council, Godwin House, George Street, Huntingdon, Great Britain.

Environ. Pollut. Ser. A. 0143-1471/81/0025-0123/$02·50
Printed in Great Britain

1972; Bancroft & Prahlad, 1973; Hasegawa, 1973; Cooke & Zoro, 1975; Anderson & Prahlad, 1976; Dial, 1976; Greenhouse, 1976*a*, *b*, *c*; Marchal-Segault, 1976; Birge *et al.*, 1977; Jordan *et al.*, 1977; Dutta & Mohanty-Hejmadi, 1978; Miller & Landesman, 1978). This paper discusses the monitoring of tadpole performance in the field in order to derive information on potentially harmful pollutants in freshwater. For such work, tadpoles have many advantages. They can be collected in large numbers. As regards sensitivity to environmental pollutants, they are fairly representative of freshwater life (see, for example, Lüdemann & Neumann, 1960) and their degree of sensitivity can be altered by varying maintenance techniques (see later). Tadpoles are the equivalent of the later embryonic stages in higher vertebrates and display considerable physiological, anatomical and histological changes during development. With rapid morphological changes taking place, there are ample opportunities for interference or malfunction in the various processes involved, and tadpoles are very prone to become deformed.

Arable farmland is one situation in which tadpole studies can be worth while. A broad spectrum of chemicals is applied to farmland and pesticide residues have frequently been detected in run-off from fields (Nicholson *et al.*, 1962; Bradley *et al.*, 1972; Muir & Baker, 1976; White *et al.*, 1976; Kadoum & Mock, 1978). Unfortunately, for many pesticides no satisfactory analytical technique is available for biological samples. For the remainder, analysis can be costly, and simply knowing residue levels in the water or mud of ditches often does not convey much information of ecological significance. Probably most chemicals that can be detected in such situations are present in concentrations that are ecologically harmless. Monitoring tadpole performance can be a useful technique for the initial stage in an investigation of side effects in freshwater systems that are exposed to many potential pollutants, prior to more detailed studies with certain chemicals. The method involves relating performance to where and when chemicals are applied. If performance remains normal when a pesticide is applied to an adjacent field, then the application of that chemical can be deemed to be harmless to freshwater organisms such as tadpoles. Perhaps residues could have been traced by conventional analysis in the freshwater environment but, by observing the tadpoles, any residues present are known to have been below the level having any gross effect. If, however, a high incidence of deformities occurs or other unusual events are noted after a pesticide application, then this particular chemical warrants more detailed examination. Such observations with tadpoles do not prove that a chemical is harmful but, from a wide variety of chemicals being used, they can indicate which might be harmful and, equally important, can rule out many others as potential pollutants.

As a background, it has been necessary to consider whether to use free or captive tadpoles and also to make a general study of tadpole deformities to aid interpretation of events in field trials.

GENERAL COMMENTS ON TADPOLE DEFORMITIES

As noted by Birge *et al.* (1975, 1977), a high incidence of deformities just after hatching is often associated with poor hatching success. Spawn clumps in the field in which $<50\%$ of the eggs hatch usually produce a high proportion of abnormal tadpoles.

Generally there are two main periods during the larval phase when deformities are produced. Development stages referred to in this paper are those described by Witschi (1956). From hatching up to stage 26 (hind limb paddles) the most common deformity is simple curvature in the horizontal or vertical plane (Fig. 1). Bubbles in

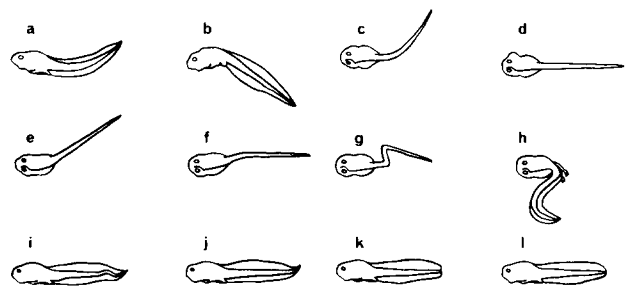

Fig. 1. Examples of common tadpole deformities. a: Upcurved; b: downcurved; c: lateral curve in tail; d: bubble in gill chamber; e: lateral deflection at base of tail; f: slight lateral kink at base of tail; g: severe lateral kink in tail; h: severe lateral kink at base of tail; i: vertical kinking at tip of tail; j: tail tip upcurved; k: 'damage' to tip of tail; l: 'damaged' tail when healed.

the gill chamber also occur during this period. After stage 26, these deformities are rare, affected tadpoles either having died or recovered. By stage 27 (large paddles) a lateral kink is the most common deformity (Fig. 1). Usually there is some recovery from this deformity by stage 30 (just prior to front limb emergence). These points can be illustrated by observations made on a culture in 1977 (Fig. 2). A spawn clump with an unusually low hatch (about 20 %) was collected from the field. Tadpoles were first examined at stage 22 when there were 218 normal and 46 deformed animals (17 % of the survivors). Of the abnormal tadpoles, 44 (96 %) showed simple curvature. The deformed tadpoles and 80 normal tadpoles were reared as two samples. Deformed tadpoles suffered high mortality (as is frequently found with abnormalities) but nearly all the survivors had recovered from their curved state by stage 26. At that time, however, 15 % developed lateral kinks; 13 % of the normals also developed lateral kinks despite being maintained under apparently ideal conditions and having initially shown no signs of any deformity. Here kinking may be a latent effect; for some environmental or genetic reason, certain tadpoles may be destined to have kinks, but these do not develop until the appropriate stage is attained. Alternatively, this particular sample could have been extremely sensitive to producing kinks and the slightest stress during rearing may have been sufficient to induce this deformity.

Sometimes different abnormalities predominate in different stock cultures (groups collected from different sites maintained under good conditions). Thus, in 1972, there was a high incidence of straight hind legs amongst Monks Wood stock tadpoles (Cooke, 1973*a*) and in 1977 there was a high level of tail deformities, especially tail tip deflections and kinks (Fig. 1) which were very rare in previous years. When tadpoles are susceptible to developing a particular deformity, exposure

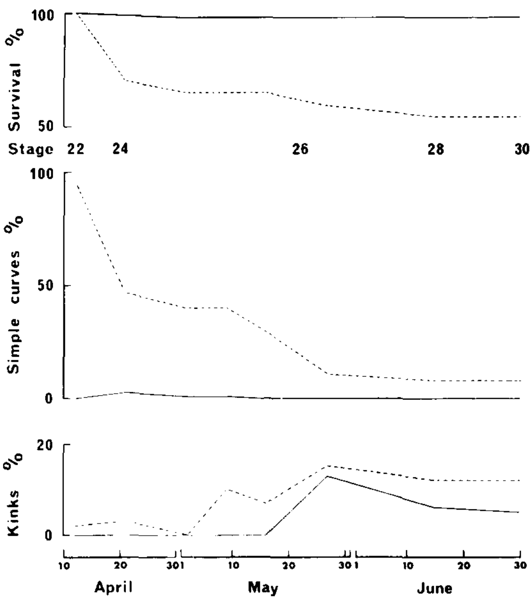

Fig. 2. Survival, development and deformities of tadpoles from a single spawn clump that suffered low hatching success. Tadpoles were reared as two samples: normal at hatch (solid line) and grossly deformed at hatch (broken line).

to a pollutant may increase the incidence of abnormalities (such as kinking, see Cooke, 1973*a*). Exposure to some other forms of stress (e.g. freezing) may have the same result. Thus, in a laboratory experiment, six spawn clumps were sub-divided, part of each clump being frozen for one day and part being maintained as a control. As usual, simple curvature was the dominant deformity at hatching, the incidence ranging from 3 % to 8 % for the controls. For the frozen samples, the incidence of deformities was raised by an average of 5 % compared with their respective controls (paired t test, $t_5 = 3{\cdot}54$, $p < 0{\cdot}05$). So here environmental stress appears to act indirectly, increasing the incidence of a common deformity, rather than directly to produce its own characteristic deformity by some specific biochemical or physiological mechanism.

Physiological state affects the incidence of deformities. For instance, DDT treatment causes a snout deformity in older tadpoles (stage 27 onwards), and it was found that increasing the rearing density of tadpoles decreased the growth rate and led to a marked rise in snout deformities when tadpoles were subsequently treated with DDT (Cooke, 1979). Large tadpoles reared at a low density were very resistant to DDT. Tadpoles in culture groups with a slow development rate and slow growth are more liable to have a high incidence of deformities. In 1976, tadpole cultures from ten different situations were reared under identical conditions at Monks Wood. In the five cultures with the most rapid development, no deformities were noted at metamorphosis but abnormalities, including lateral kinks, were present in the five slowest developing cultures. Similarly, within a single stock culture, tadpoles that were slow to metamorphose were more likely to be deformed.

Much of this information is relevant to selecting tadpoles for field trials. In the absence of chemicals at levels sufficient to induce deformities directly, it is useful to operate with a culture that is somewhat susceptible to a particular deformity and to having the incidence increased by environmental stress. It is a disadvantage to use tadpoles that are physiologically very fit, since they will be relatively unresponsive to pollutants. On the other hand, it would be unwise to use physiologically poor specimens since few might survive. Choice of tadpoles might depend on variables, such as duration of trial or the severity of environmental pollution. It is advisable to avoid tadpoles that have hatched from spawn heavily infested with *Saprolegnia* fungus. Often such tadpoles show an unusually high incidence of deformities and suffer a high death rate; *B. bufo* tadpoles appear especially prone to die around stage 26. Damage to the tip of the tail (Fig. 1) or to the fin is occasionally encountered in situations where it is most unlikely to have been caused by predators. I have seen this in *Rana temporaria*, *B. bufo* and *B. calamita* tadpoles, which had been exposed to toxic chemicals or *Saprolegnia* infestation. The defect may be a symptom of environmental stress. The tail usually heals eventually (Fig. 1) but does not regain its normal shape.

At a critical period in development, a teratogen exerts its influence and causes a particular deformity, as Greenhouse (1976*a*, *b*, *c*) has demonstrated for anuran embryos exposed to substituted hydrazines and aromatic amines. Exposure at other times may result in some mortality but does not lead to the formation of the characteristic deformity. 'Natural' stress during different critical periods of embryonic development may at least partially explain why stock cultures of tadpoles tend to be somewhat susceptible to forming one type of deformity in one year and a different type the following year. The development stage of the tadpoles can influence deformities observed. Thus, DDT induces different deformities at different stages and the extent of mortality also depends on development stage (Cooke, 1972). Starting an experiment in the field with normal tadpoles that have reached stage 27 is most unlikely to result in a high incidence of abnormals via an indirect effect of pollutant stress, since such tadpoles have passed the stages at which the common field deformities occur.

Rana temporaria tadpoles are preferable to *B. bufo* tadpoles because they are larger, easier to handle and observe and are more sensitive to certain pollutants (Cooke, 1972). Similarly, *Rana catesbeiana* tadpoles appear more sensitive than *B. bufo japonicus* tadpoles to chemical pollutants (Nishiuchi & Yoshida, 1972). Elsewhere in the world other species would doubtless prove suitable.

TECHNIQUE USING CAGED TADPOLES

In this area of eastern England, anuran populations are exceedingly rare in arable ditches and similar sites (Cooke & Ferguson, 1974, 1976). Even where they do occur, *Rana* tadpoles become progressively more difficult to catch as they develop, due to mortality, dispersal and increased mobility. For the same reasons, releasing tadpoles into ditches has not been worth while. Estimating mortality of free tadpoles involves dyeing (Cooke, 1978) or other marking and is relatively time-consuming. The most satisfactory technique has been to keep tadpoles in cages (Cooke, 1973*b*, 1977), because then the same individuals can be repeatedly examined with ease, and their mortality determined by counting the survivors. Tadpoles are maintained under (virtually) predator-free conditions. In the wild state deformed tadpoles are probably more likely to be taken by predators than are normal healthy tadpoles and are therefore relatively less likely to be observed. In a cage, however, a deformed tadpole has a greatly enhanced chance of survival. Care should be taken to prevent predators entering the cages; dytiscid larvae or notonectids can quickly eliminate a group of tadpoles.

There are two main problems that can arise when using caged tadpoles in trials on agricultural land. The 'tadpole season' often does not extend far into the summer, when many chemicals are applied. Prolonging the development of tadpoles in order to have animals for experiments when, say, cereal aphicides are sprayed, may be unwise if the resulting tadpoles are of grossly inferior fitness (see above). Also, in the spring it can be difficult finding a sufficient depth of water to accommodate cages in suitable field ditches (see Cooke & Ferguson, 1976). When crops are being drilled in the spring, farmers generally attempt to keep water levels as low as possible.

Birge *et al.* (1975) adopted a different approach, collecting water samples from the field and conducting toxicity tests in the laboratory with anuran spawn, determining hatching success and incidence of abnormalities amongst survivors. This technique has also been used at Monks Wood in support of the main trials. It enables water actually flowing from field drains to be tested.

Caged tadpoles have been used successfully to study the effects of applications of individual chemicals directly into water bodies (Cooke, 1973*b*, 1977) and during these trials activity has been monitored. It has not, however, proved necessary to monitor activity in a routine manner when tadpoles have been caged in ditches on arable land to screen a range of pesticides. Effects on activity tend to be of three types. First, organochlorines such as DDT affect the nervous system, causing hyperactivity characterised by spasmodic movements (Cooke, 1970, 1972). This can be detected readily without recourse to some type of activity index. Secondly, other chemicals, such as demeton-*S*-methyl, cause tadpoles to become sluggish (unpublished observations). Such behaviour is more difficult to detect in the field, but prolonged exposure is invariably accompanied by decreased growth and increased mortality which are easier to monitor. Thirdly, many morphological abnormalities impair movement, but as all deformities are registered there is no point in noting those which also result in a change in swimming behaviour.

In trials conducted in this area, tadpoles were generally found to survive well when caged in agricultural ditches, and growth and development were similar to those of samples caged in non-agricultural sites. However, where the water was very clear and could be seen to contain little animal or plant life, growth was poor, presumably because of inadequate food. This applied to both agricultural and non-agricultural locations. Growth was almost invariably good in any eutrophic ditch with substantial algal growth.

Cages used in the trials measured 30 × 30 × 30 cm. They were usually mounted with about 10 cm protruding above the water surface; it was found essential to allow well grown *Rana temporaria* tadpoles access to the surface. Initially, forty tadpoles were introduced into each cage. Mortality, median development stage, numbers and types of deformities were recorded at about 10-day intervals or more frequently if a chemical of particular interest was being applied to the ditch or to an adjacent field. Two samples were taken during each trial, usually reducing numbers to thirty and then twenty per cage; sampled tadpoles were individually weighed, classed according to development stage, killed and preserved. As soon as one tadpole in a cage had acquired front legs (stage 31), all were returned to the laboratory for examination. Animals will drown if left in cages beyond this stage.

As an example of a trial, an experiment undertaken in 1977 is briefly described. The study area of 76 ha was situated on rich peatland in Cambridgeshire on the southern edge of the Fenland. The drainage system of the area was independent from that of the surrounding land; most of the fields were equipped with drainage tiles, the remainder being sufficiently well drained without tile systems. To illustrate how performance could vary between different situations, observations on tadpoles in four of the cages are summarised in Fig. 3.

Cage 1 was maintained between fields of barley and potatoes. Mortality occurred throughout the trial. Development and growth were slow, with many deformities, mainly involving vertical curvature and tail tip defects. Cage 3 was between barley

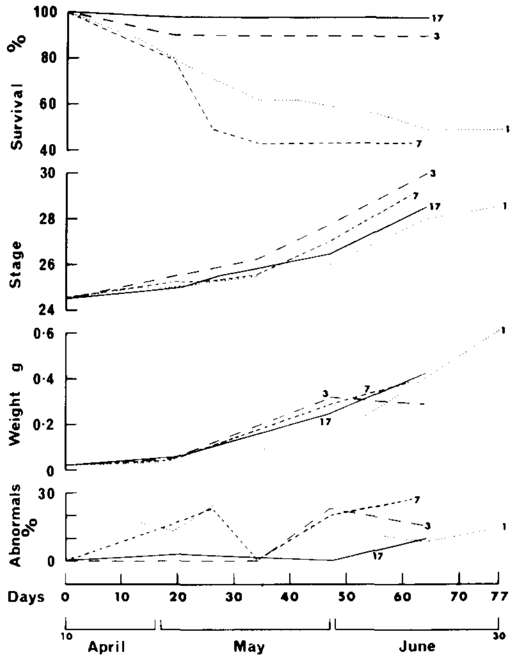

Fig. 3. Examples of tadpole performance in different cages during a single trial in 1977. Cage 1: In a ditch between fields of barley and potatoes. Cage 3: Between barley and beet. Cage 7: Between beet and potatoes. Cage 17: Control (cage in Woodwalton Fen National Nature Reserve).

and sugar beet. Survival was good, development was rapid and growth was satisfactory. Abnormalities did not appear until late in the experiment, when some tadpoles developed lateral kinks. Cage 7 was between sugar beet and potatoes. Mortality was considerable during the first month, but thereafter no more tadpoles died. Development and growth were normal. Incidence of deformity showed two peaks—lateral curves of the body predominated after about four weeks and tail tip defects were numerous amongst older tadpoles. Cage 17 was kept as a control in a pond in a nearby nature reserve. Survival was good and rates of development and growth were about average. A few lateral kinks were noted late in the experiment.

The three principal crops grown in the study area were potatoes, barley and sugar beet, and of the sixteen tadpole cultures maintained in the main trial, eight were adjacent to each of these crops. Therefore, for each crop, comparing the performance of tadpoles in the eight cages next to a crop with those in the eight cages away from the crop could indicate whether harmful effects might have resulted from the pesticide regime for that particular crop. Performance did not tend to be poor beside beet or barley, but tadpoles next to potato fields suffered significantly higher incidences of vertical curvature deformities (Mann Whitney test, $U_{8,8} = 5, p < 0{\cdot}01$)

or tail tip deformities ($U_{8,8} = 7{\cdot}5, p < 0{\cdot}01$) as depicted in Fig. 4. If these deformities resulted from pollution then the compound responsible was likely to have been oxamyl. No other pesticide was applied to the potato fields until the end of May, and oxamyl was used only on potatoes, being drilled from the 13th to the 26th of April.

Tadpoles were treated with oxamyl in the laboratory to determine its effects. Several types of deformity were observed. For instance, exposure for 1 h to a nominal concentration of 100 ppm oxamyl (the granules containing 10 % active ingredient did not dissolve completely) resulted in 90 % of tadpoles with vertical curvature deformities, similar to those seen in the field. After being kept in clean water for another day, 44 % of these had tail deformities such as kinks and deflections. These deformities seemed to be the result of abnormal posturing, rather than permanent skeletal defects. In the field, vertical curvature deformities also

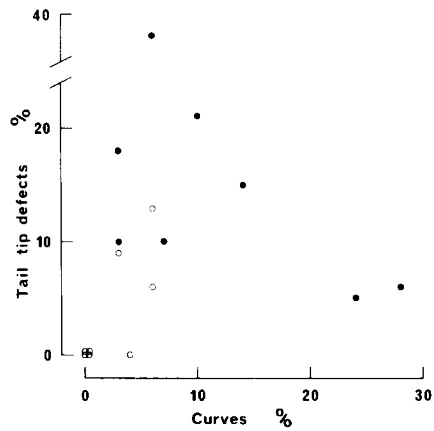

Fig. 4. Scattergram showing the maximum incidences of tail tip defects and vertical curvature deformities in cages beside potato fields (●) and away from potato fields (○).

often appear to be of muscular origin; tadpoles thus afflicted can straighten themselves, but are curved when resting. Lateral deformities—such as kinks at the base of the tail—cannot, however, be corrected by the tadpole's movements.

Treatments with oxamyl in the laboratory did not produce tail tip deformities. This group of deformities embraced a range of kinks or deflections in the very tip of the 'spine' of the tail (Fig. 1), usually later during the larval phase when the hind legs were developing. These deformities had, prior to 1977, never been observed in large numbers but, during that year, occasionally occurred in control and stock cultures as well as in the field cages. Thus, tadpoles used in the trial in 1977 were probably especially prone to produce this type of deformity and, as suggested previously, exposure to pollution or other forms of stress might increase the incidence of such deformities. Lateral kinking was also relatively common in 1977 amongst older tadpoles in control and stock cultures and was especially high amongst some groups caged in the field.

The evidence did not prove conclusively that oxamyl caused harmful effects on tadpoles in the freshwater system studied. It did, however, indicate that of nineteen pesticides applied to the three main crops whilst the cages were in place, oxamyl warranted further consideration.

ACKNOWLEDGEMENTS

I thank the following for help and advice: K. Cook, Dr J. Dempster, Mrs S. Freestone, Mrs M. Haas, Dr I. Newton, Dr D. Osborn and Dr H. R. A. Scorgie.

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