ES 667

Investigating Potential for Effects of Environmental Endocrine Disrupters on Wild Populations of Amphibians in UK and Japan: Status of Historical Databases and Review of Methods

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(Received January 9, 2007; accepted February 6, 2007)

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Key words: amphibian, population, endocrine disrupter, pesticide

Concern over global declines among amphibians has resulted in increased interest in the effects of environmental contaminants on amphibian populations, and more recently, this has stimulated research on the potential adverse effects of environmental endocrine disrupters in amphibans. Laboratory studies of the effects of single chemicals on endocrine-relevant endpoints in amphibian, mainly anuran, models are valuable in characterizing sensitivity at the individual level and may yield useful bioassays for screening chemicals for endocrine toxicity (for example, thyroid disrupting activity). Nevertheless, in the UK and Japan as in many other countries, it has yet to be demonstrated unequivocally that the exposure of native amphibians to endocrine disrupting environmental contaminants results in adverse effects at the population level. Assessing the potential of such effects is likely to require an ecoepidemiological approach to investigate associations between predicted or actual exposure of amphibians to (endocrine disrupting) environmental contaminants and biologically meaningful responses at the population level. In turn, this demands recent but relatively long-term population trend data. We review two potential sources of such data for widespread UK anurans that could be used in such investigations: records for common frogs and common toads in several databases maintained by the Biological Records Centre (UK Government Centre for Ecology and Hydrology), and adult toad count data from 'Toads on Roads' schemes registered with the UK wildlife charity 'Froglife'. There were little abundance data in the BRC databases that could be used for this purpose, while count data from the Toads on Roads schemes is potentially confounded by the effects of local topology on the detection probabilities and operation of nonchemical anthropogenic stressors. For Japan, local and regional surveys of amphibians and national ecological censuses gathering amphibian data were reviewed to compile survey methodologies and these were compared with methods used in the UK and other countries. Substantial consensus exists in amphibian survey methodologies and this should be exploited in the initiation of coordinated monitoring programs for widespread and common anuran amphibians in Japan and the UK to generate long-term robust and standardized population trend data. Such data would support comparative ecoepidemiological assessments of the impact of environmental endocrine disrupters in these two cooperating countries.

1. Introduction

Over the last 30 years or more, concern has escalated over global declines in amphibian populations.[1] Although earlier reports were typically anecdotal and limited in time-scale, geographical range or taxonomic breadth, more recent metaanalyses have confirmed marked declines in amphibian populations in North America and Europe.[2] A number of factors may have contributed to this phenomenon to varying degrees, including habitat destruction and modification,[3–5] climate change,[6,7] increased UV exposure,[8] introduction of non-indigenous species,[9] the spread of virulent pathogens,[10] and adverse effects of chemical contaminants such as pesticides.[11] In some cases, amphibian population declines or extirpations have been shown to reflect the interaction of multiple factors. For example, declines in toad populations in the Pacific Northwest USA appear to result from a complex cascade of causality, triggered by global climate changes, but effected through the impact of pathogenic fungi.[12] A recent global assessment of the status of amphibian populations indicates that the primary contributing factors to population declines vary among different regions of the world.[13]

Many temperate amphibian species occur in habitats affected by agricultural activity,[14–16] and the potential adverse effects of crop protection products on amphibian populations have often been cited. However, amphibians likely experience a combination of environmental stresses arising from agricultural activity, through drainage of wetlands, rerouting of water sources for irrigation, eutrophication of surface waters through fertilizer use[17] and through reduced habitat connectivity,[15] apart from any adverse effects of pesticides and herbicides. DeSolla *et al*. reported reduced hatching success of eggs from anuran and urodeles species exposed *in situ* at agricultural sites in British Columbia, Canada, relative to reference sites, and this difference correlated with basic water quality parameters.[18]

There have been some reports of local extirpation of amphibian populations due to direct toxicity of insecticide application.[19] Although there is a fairly extensive literature regarding the toxicity of pesticides to amphibians in laboratory conditions,[20] amphibians inhabiting agricultural landscapes may be exposed to a variety of environmental contaminants, such as fertilizers (including sewage sludge), insecticides, herbicides, and veterinary pharmaceuticals in feedlot effluent, which could exert toxic effects on larval or adult survival, development, reproduction or immune function.[21] The toxicity of nitrogen fertilizers (nitrates, urea) and their by products (nitrite) has been demonstrated for larvae of a number of amphibian species, with evidence for significant variation in sensitivity among species.[22–25] Although it is unclear whether environmental concentrations of nitrogenous fertilizers ever reach concentrations sufficiently high to adversely affect amphibian populations through direct toxicity, the indirect effects of eutrophication through nitrate contamination of water bodies in the agricultural landscape have the potential to affect aquatic organisms including amphibians.[26] Evidence suggests that ionic composition is a significant factor for relative suitability of water bodies for amphibian species[27] and ionic changes associated with the eutrophication of surface waters may have affected species distributions and abundance in Britain.[28]

Our understanding of the current effects of environmental contaminants on wild populations of amphibia in the UK may lag behind that in the USA, where local extinctions[29] and concern over increased incidence of limb malformations[30] have led to increased activity in this field. For example, the US Geological Survey coordinates both the Amphibian Research and Monitoring Initiative (ARMI) and the North American Amphibian Monitoring Program (NAAMP, also called the 'frog calling survey'), which is a collaborative national program, supported by state natural resource agencies, which uses standardized call survey methods to gather population data on vocal amphibians.[31]

Historical records indicate that declines in British amphibian populations occurred in the middle of the last century, continuing through the 1960s,[32,33] consistent with evidence from the metaanalysis of amphibian population trends in Europe. Declines in Britain during this period have been primarily attributed to changes in land use patterns, in particular althrough agricultural intensification, although the common frog (*Rana temporaria*) also appeared to disappear from London parks in the 1960s.[34] After a period of apparent stabilization in the 1970s,[35] Hilton-Brown and Oldham reported further declines in British toads in central and southern England during the 1980s[5] and more recently, Carrier and Beebee have analyzed qualitative data on frog and toad populations in Britain. Questionnaires were distributed to professional and amateur herpetological recorders and respondents reported an increase, a decrease or a lack of change in populations for which there were monitoring data for at least 5 years in the period 1985–2000. Data from 277 sites across England Scotland Wales indicated declines in common toad poplulations in central, eastern and southeastern regions, which was not reflected in other regions or by common frog populations in the same regions.[36] This pattern is the reverse of that observed during earlier decades,[34] and the reasons for this apparent toad-specific decline in central and southeastern regions in recent years are at present unclear.

Several investigators have suggested that exposure to pesticides may have been a contributing factor in the declines in British amphibian populations,[34,37] particularly in the 1960s when their use in agriculture increased markedly. In response to this concern, Cooke did much work in the 1970s on the effects of pesticides on native amphibian larvae in laboratory and field studies.[37–42] These studies indicated the potential adverse effects of chemicals such as dieldrin and DDT on amphibian larvae inhabiting water bodies in agricultural areas, and more generally the potential of using amphibian larvae as monitors of environmental contamination. However, information on the effects of more modern-use crop-protection products on temperate amphibians is little, and the susceptibility of this group to the endocrine disrupting activity of some of these chemicals remains unclear.

The possibility that endocrine disrupting contaminants (EDCs) adversely affect amphibian populations is often listed as a contributing factor in amphibian declines. In part, this may reflect the general concern that pesticides and other environmental contaminants may exert adverse effects on amphibians through endocrine toxicity at lower concentrations than those at which the systemic toxicity of pesticides has been reported. Moreover, several reports on apparent alterations in gonadal differentiation (*e.g.*, change in sex ratio, hermaphroditism) resulting from larval exposure to known or suspected endocrine disrupters have stimulated interest in this area.[43–45] Consequently, a literature base concerning the effects of endocrine disrupters on the reproductive axis in amphibian models is being developed in response to these substance-specific issues, while research on the effects of thyroid active chemicals has been stimulated by the likely utility of an amphibian larval metamorphosis assay for the detection of thyroid-function disrupting chemicals.[46,47] Additionally, amphibians, in particular anuran amphibians (frogs and toads) inhabit a variety of lentic aquatic environments (ponds of varying sizes, lakes, ditches and smaller ephemeral standing water bodies), often closely associated with human environmental impact, both urban and agricultural. These species can therefore be used as potential indicators for monitoring the EDC pollution of aquatic environments other than those readily monitored with existing fish models (i.e., rivers receiving industrial or municipal waste water outflows).

What is not forthcoming, however, is information on whether endocrine disruption is actually a phenomenon of concern in wild populations of amphibians. Observations of high incidences of intersex and elevated vitellogenin concentrations in fish in British rivers have stimulated much research aimed at understanding both the mechanism and population/community level effects of endocrine disruption of fish exposed to estrogenic sewage effluents. This research has been complemented with monitoring and a demonstration program to investigate methods of further minimizing the effects of human and pharmaceutical estrogens on wild fish populations in UK receiving waters. Although the potential of environmental endocrine disrupters to adversely affect wild amphibian populations exists, and the tools and endpoints are becoming available for assessing this potential, the question still remains whether there is a problem to be addressed.

Consequently a population level approach to assessing anthropogenic effects on amphibian populations the UK and Japan is important in placing the potential role of environmental endocrine disrupters in a broader context. Amphibians are widespread throughout urban and agricultural landscapes in Britain, and distribution data have been collected in recent decades by volunteer and professional herpetologists alike. Nevertheless, we remain ignorant as to whether British amphibian populations are being affected by chemical pollutants resulting from human activity. Similarly, although much research on the mechanisms of action of endocrine disrupters in amphibians is being conducted in Japan, largely supported by the Japanese Ministry of Environment, to date this has not been complemented with dedicated studies on the health and status of wild populations of amphibians in the context of potential exposure to environmental endocrine disrupters. We aim to resolve this situation by exchange of information and, where possible and appropriate, methodologies by which the effects of environmental contaminants in general, and endocrine disrupters in particular, on native amphibians in these two countries can be assessed. Given the of evidence of large temporal fluctuations in natural amphibian populations, assessing the impact of environmental endocrine disrupters on amphibians at the population level will require relatively long-term population trend data. Consequently any current assessment will depend on the availability of historical data and ongoing assessment will require a robust baseline dataset and the establishment of routine monitoring programs using common and appropriate methodologies.

2. Availability of Historical Amphibian Abundance and Distribution Data in UK

As part of a project funded by the Department of Environment, Food and Rural Affairs of the UK government to assess the potential effects of environmental endocrine disrupters on wild populations of native amphibians in Britain, two principal sources of historical amphibian population data have been assessed; data maintained by the Biological Records Centre, available through the National Biodiversity Network; and annual count data from Toads on Roads schemes around the UK, accessed by the amphibian conservation organization Froglife.

2.1 *Biological Records Centre (BRC)*

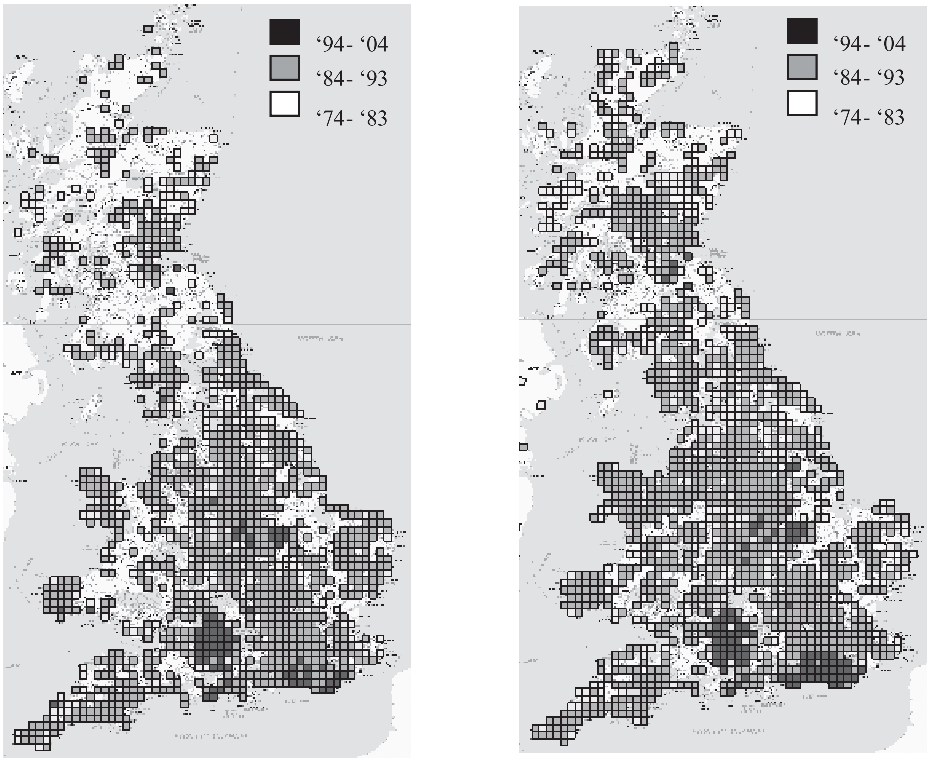
Distribution and abundance data on a large number of native British species of plants and animals are maintained by the Biological Records Centre (BRC; http://www.brc.ac.uk/) and Centre for Ecology and Hydrology (http://www.ceh.ac.uk/). Databases can be queried through the National Biodiversity Network (NBN) gateway, and the curator of amphibian databases (Henry Arnold, Centre for Ecology and Hydrology) was contacted to secure full access of all relevant amphibian datasets held by BRC, which included the following.

- BRC Reptile and Amphibian Dataset (Reptile and amphibian records extracted from the BRC Herptile database)
- Leicestershire Amphibian and Reptile records (Historic records from 1960 to 2000 maintained by the Leicestershire Environmental Resources Centre)
- Lothian Wildlife Information Centre Secret Garden Survey
- Staffordshire Wildlife Trust Nature Reserves Inventory

- Amphibian Records of Wiltshire 1900–2003 (Records held by the Wiltshire and Swindon Biological Records Centre)

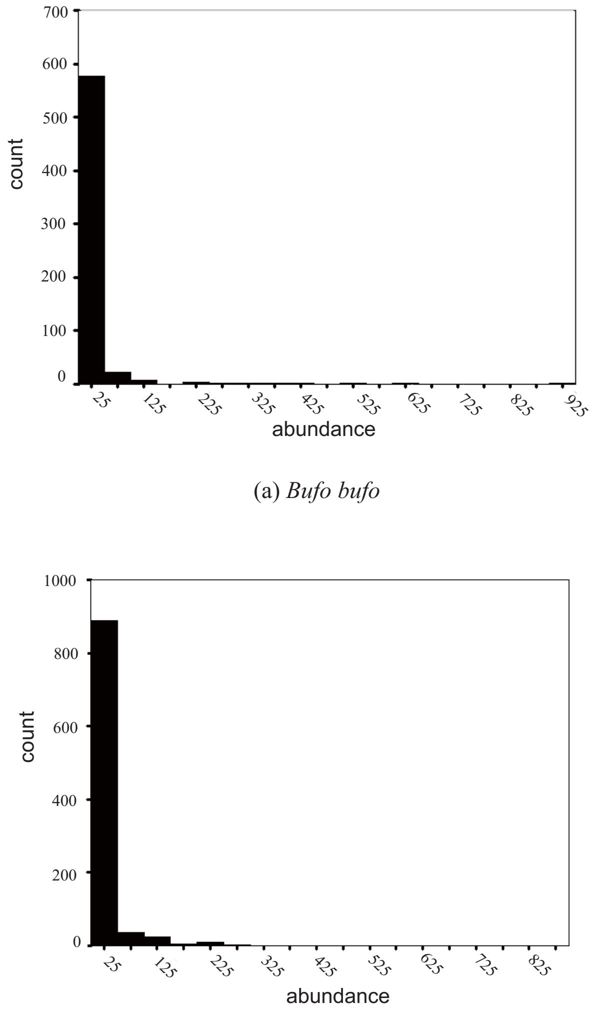
Distribution maps for both species generated through the NBN Gateway indicated an artefactual reduction in the recorded distribution from 1994 onwards, owing to little recording and data collation after the publication of the Atlas of UK Amphibian Distributions[48,49] (Fig. 1).

Raw data from these databases relating to *R. temporaria* (common frog) and *Bufo bufo* (common toad) were downloaded, and inspection of these records indicated that there was negligible abundance data available within them. Of the five datasets with amphibian data held by the BRC, only one (BRC Herptile database) features abundance records for either species. In this dataset, 617 of 7,118 records (8.7%) for *B. bufo*, and 968 of 14,289 records (6.8%) for *R. temporaria* gave information on abundance. However, a large number of these abundance records are single sightings (352 for Bufo and 430 for Rana; see Fig. 2 for the histogram of abundance records for these species from this database). The abundance data represents a large number of sites with a limited number of records for each site, and there is no apparent overall



(a) *Bufo bufo* (b) *Rana temporaria*

Fig. 1. Ten-kilometer distribution maps for periods 1974–1983 (white pixels), 1984–1993 (light grey pixels), and 1994–2004 (dark grey pixels) for (a) common toad (*Bufo bufo*) and (b) common frog (*Rana temporaria*) in Great Britain. The maps were extracted from databases maintained by the Biological Record Centre, Centre for Ecology and Hydrology and maps generated in the National Biodiversity Network Gateway.



(a) *Bufo bufo*

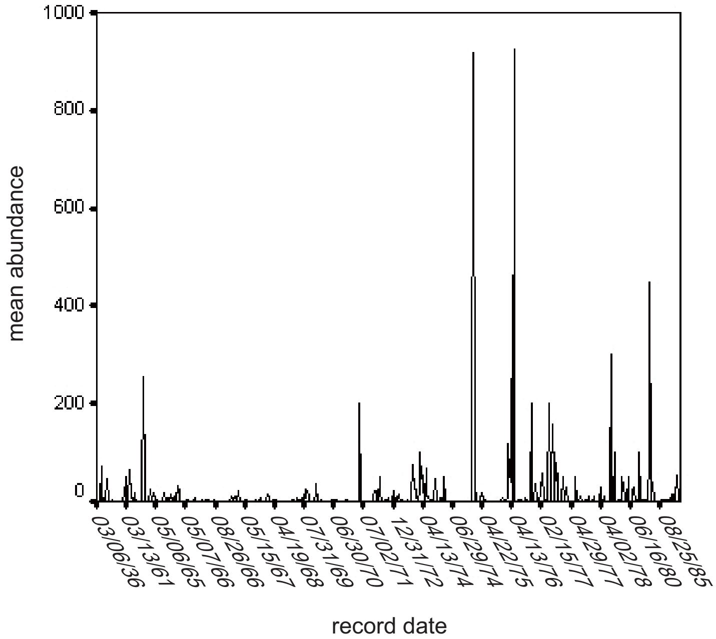
(b) *Rana temporaria*

Fig. 2. Histograms of abundance counts for (a) *Bufo bufo* and (b) *Rana temporaria* in BRC Herptile database, accessed courtesy of Biological Records Centre and Centre for Ecology and Hydrology.

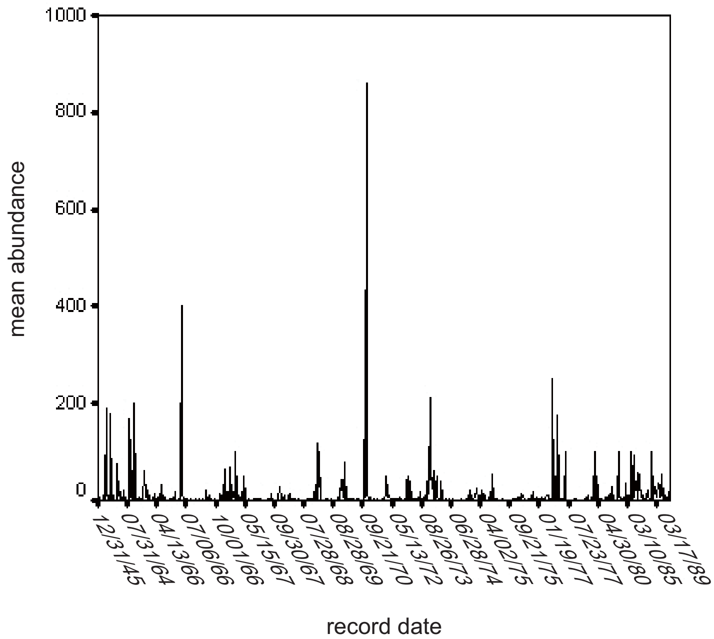
trend in the abundance over the time span of the entire database for either species (Fig. 3). There were a small number of sites featured in this database with long temporal runs of abundance records: two sites with runs spanning over 10 years for *B. bufo* and two sites with runs spanning over 5 years for *R. temporaria*. Consequently, this database does not provide long-term abundance data for sufficient sites with varying potential exposure to environmental contaminants to be useful in studies of the impact of environmental contaminants on these native species.

2.2 *Toads on Roads data*

Since the 1980s, in many parts of the UK, local groups of volunteers have assisted toads crossing busy roads during spring breeding migrations. Due to their high site fidelity and synchronized migration to breeding sites from over wintering



(a) *Bufo bufo*



(b) *Rana temporaria*

Fig. 3. Mean abundance scores by year for (a) *Bufo bufo* and (b) *Rana temporaria* in BRC Herptile database.

habitat, toads are generally affected by road mortality to a greater extent than frogs or newts. Where established breeding migration routes cross busy roads, mortalities can be very high; movement is generally in late February to early March and occurs mainly between 6 and 11 pm, when weekday commuting and evening traffic is high.

Since 1989, 'Froglife' (http://www.froglife.org/), a wildlife charity focusing on amphibian conservation, has coordinated toad crossing schemes, and have encouraged volunteers to record basic data about the numbers of toads assisted across the road, numbers killed, and weather and traffic information among others, throughout the period when toads are crossing roads. This data has been stored in various formats as paper records by Froglife, and copies of all records were kindly provided by Froglife. This data has been stored electronically in a Microsoft Access data-base containing 223 sites, and yearly count/mortality data for a subset of these sites has been collated in an excel spreadsheet. For the 220 sites that could be located by grid reference or address, x-y coordinates on the British national grid were generated to enable mapping in ArcMAP (Fig. 4). Of these sites, 68 have count data for more than one year, 52 have count data for more than 3 years, and nine sites have count data stretching over more than 10 years. The longest period over which data have been collected for any given site is 18 years.

For the 68 sites with usable yearly count data, several indices of data 'quality' were generated. 'Span' was the total number of years over which yearly count records had been recorded and reported for a site, i.e., the number of years between the first record and the last record. A population trend index was generated, which represented the sum of year-on-year proportional changes, calculated for each year in which counts, x, were recorded as

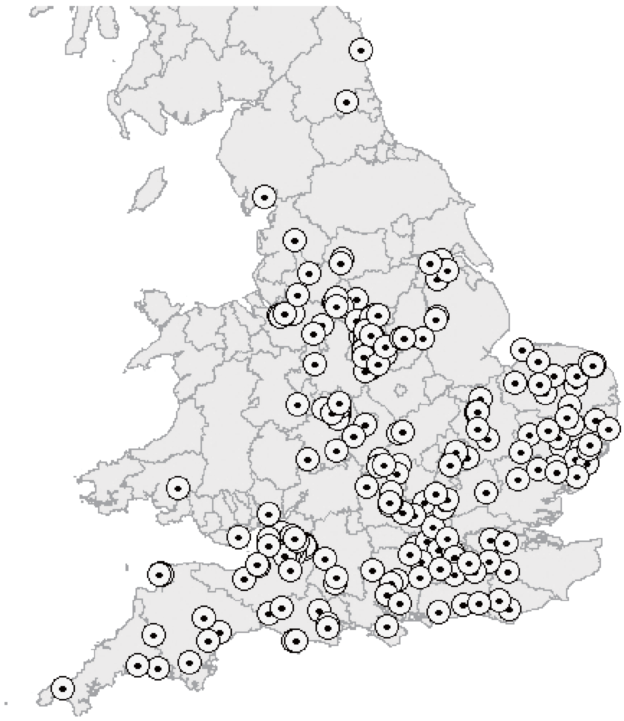


Fig. 4. Location of 220 'Toads on Roads' schemes registered with Froglife in England and Wales. The basemap indicates Great Britain vice counties.

Increasing years: $$\frac{(C^{x}/C^{x-n})-1}{n} \quad (1)$$

Decreasing years: $$\frac{(C^{x-n}/C^{x})-1}{n} \quad (2)$$

where C is the adult toad count for year x, and n is the number of years between consecutive count records. The population change index for each site is the sum of positive population changes (i.e., increases) minus the sum of negative population changes (i.e., decreases). Selected sites with varying population trends are shown in Fig. 5, where trends in yearly count data can be compared with the index of change calculated as explained above.

Figure 6 shows the location of the 52 sites in the Toads on Roads Database for which the 'span' index was greater than 3. Site markers are color coded to indicate population trend, with red markers indicating sites in decline, yellow markers indicating stable sites and green markers indicating sites with increasing trends in total toad crossing counts. This map integrates information on pesticide usage (kg/hectare) between February and May (during toad spawning and larval development) by river catchment, retrieved from the Environment Agency of England and Wales' database for Prediction of Pesticide Pollution in the Environment (POPPIE) database.

This comparison was performed as a crude initial step in testing the hypothesis that trends in toad populations, as estimated from road crossing count data, are not correlated with surface water pesticide concentrations. No clear spatial correlation is evident between the distribution of sites with declining trends in toad counts and pesticide usage at the catchment level, or with the aggregated predicted surface water concentration of commonly used groups of pesticides (*e.g.*, conazoles, pyrethroids, carbamates, triazoles) by catchment, as retrieved from the POPPIE database (data not shown). This is not surprising, as there are significant data gaps in any extrapolation from predicted surface water concentration (in rivers) to actual concentrations in relatively small static water bodies favored by toads for spawning, which in many cases feature no direct surface water inflow from rivers draining agricultural areas. Moreover, the use of these two sources of data (for toad populations and exposure to pesticides) is further confounded by considerable uncertainty regarding the accuracy of road crossing counts as an estimate of adult toad population number at any given site. As noted by Cooke *et al.* (2004) road mortalities can provide a useful means of estimating trends in a local toad population that crosses a road(s) to reach a spawning site. Mortality rate is likely to reflect traffic intensity, killing width of tires, velocity of crossing toads and crossing angle. If independent factors such as traffic intensity and tire width are constant, increasing number of road kills over time may actually indicate an increasing toad population, which if road mortality continues may eventually plateau and then decline if mortalities become unsustainable by the population.(50)

We have used the total counts of live (i.e., rescued) and dead (road kill) toads at Toads on Roads sites to estimate population trends at each site. The value of unadjusted count data as an estimate of the true population number depends on the probability of the detection of adult toads at a given survey site (*e.g.*, toad crossing site). This relationship can be summarized in the simple equation:

$$C = pN, \quad (3)$$

where C is the counts, N is the true population number and p is the detection frequency.(51)

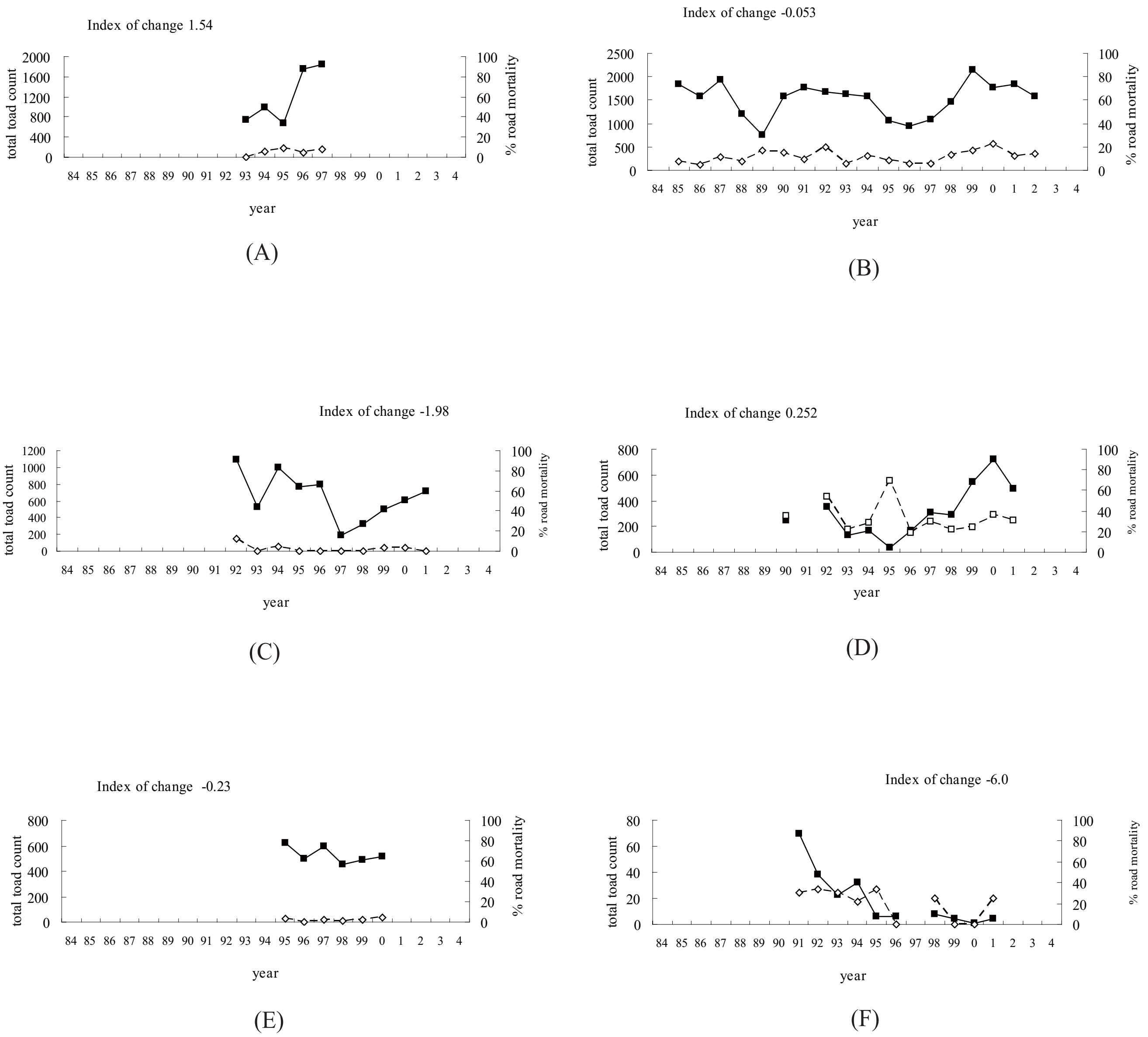


Fig. 5. Population trends for six 'Toads on Roads' sites in England, constructed from annual road crossing data submitted to Froglife. Solid lines represent total toad numbers recorded each year (live and dead) and broken lines represent percentage mortality (number of dead toads/total number of toads). The legend indicates the population trend 'index of change' calculated as described in the text, and used to generate color coding of site markers in Fig. 5.

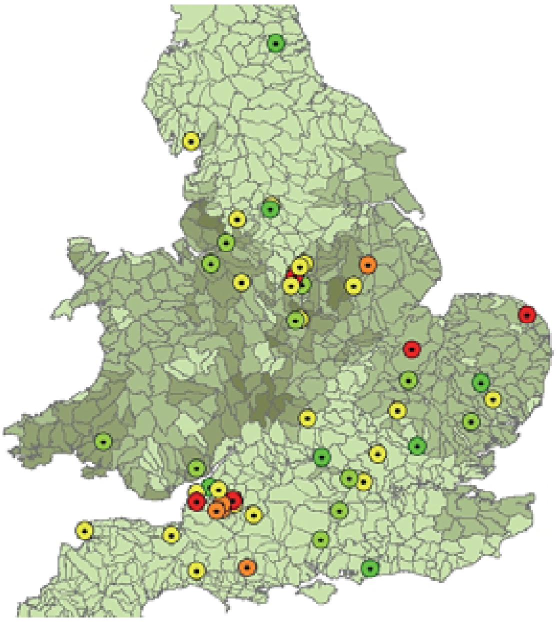


Fig. 6. Location of 52 'Toads on Roads' crossing sites with count data for more than 3 years (not necessarily consecutive). Site markers are color coded according to the index of change for that site. Green markers indicate sites with positive index of change reflecting an overall increasing trend in total (live plus dead) count data over the years recorded. Red markers indicate sites with negative index of change reflecting a decreasing trend in count data, and yellow markers indicate sites with index of change close to zero, indicating little overall change in yearly counts over the recording period. The basemap was generated in ArcMAP using pesticide use intensity data for 1998 from February to May from the POPPIE database maintained by the Environment Agency of England and Wales. Darker colors indicate high total pesticide use intensity (kg/hectare), lighter colors indicate low pesticide use intensity.

From eq. (3), it follows that where detection probability is near 1, C can be used as reasonably accurate surrogate for N. However, p may vary significantly between survey sites.[51] For example, it can be expected that where an adult toad population translocates from a discrete area of suitable terrestrial habitat (*e.g.*, a woodland) across a single road to a single discrete water body for spawning, detection frequency will be high, particularly if the road crossing is patrolled, most toads are rescued and mortalities are recovered and counted each night (precluding problems with scavenging and carcass removal). Toad crossing sites vary considerably in their topology — at some sites, road crossing counts may represent only an unknown fraction of the total local adult toad population. Consequently, the comparison of population trends constructed from unadjusted count data between sites with varying and unknown detection probabilities is subject to significant uncertainties.

Moreover, putting these uncertainties aside, what count data from Toads on Roads sites tell us may vary between sites depending on the degree of and change in the impact of road mortality, and on the local topology of the toad crossing site. Declines at some sites may represent the direct impact of unsustainable road mortalities, while at other sites declines may reflect the operation of other environmental stressors such as habitat modification or deleterious effects of environmental contaminants including endocrine disrupters. As an example of this, Fig. 6 shows a cluster of toad crossing sites in Avon centered around Bristol with predominantly declining trends in unadjusted road crossing counts over quite long periods (mean span for data sets, 10 years). Inspection of notes on record sheets for these sites and maps of the local area clearly suggests that changes in local habitat availability and local traffic patterns are the most likely causes of most of these declines.

3. Existing Ecological Information and Methods of Field Survey on Amphibians in Japan

As noted above, it will be useful to compare amphibian population status — either by change in distribution or through temporal analysis of abundance data — and potential exposure to environmental contaminants, including endocrine disrupters, in the UK and Japan. Differences between UK and Japanese amphibian species in terms of sensitivity to endocrine disrupters and life histories, as well as differences in agricultural practice including pesticide use, may contribute to different responses of amphibian populations between these countries. As a start towards the harmonization of methods for future comparative research, we have reviewed available reports of amphibian population surveys in Japan to compile commonly used methods.[52–54]

3.1 *Field survey methods used by Japanese researchers*

In the breeding period, direct observation (sighting or capture, observation of carcasses such as those from road mortality), and presence of calling amphibians were the routinely used survey methods of assessing the presence of adult frogs. Observation and counting of egg mass and larvae were also useful methods during the reproductive and postreproductive periods. The survey parameters typically recorded were as follows: species and number of amphibians, sighting frequency, number of pairing, audible calling, sex ratio, snout-vent length, weight, developmental stage of ovary, number and location of egg masses, number of eggs in one spawning, fertility ratio and start/peak date of breeding. Because the breeding migration and spawning activities of amphibians are affected by environmental conditions, weather, air temperature, water temperature, rainfall, vegetation, land improvement of a waterfront area, and water quality were also often recorded.

Concerning the survey of larval amphibians, enclosure with a box quadrat or capturing with a gill net, a Tamo net, or a fixed net or throwing a net were methods used for estimating larval population sizes. Quantitative data typically recorded were the number of larvae, and in some instances length/wet weight. The length of a waterfront surveyed is an important parameter to record as shoreline accessibility has a direct impact on detection frequency.

Telemetry, the use of a magnetic tip, and the route census of migration before and after the breeding season were useful methods of collecting ecological information on amphibians outside of the breeding season. In the winter season, digging the ground and checking the bottom of branches have been used to assess the presence of hibernating amphibians, and in these instances snout-vent length, ground temperature, air temperature, underground depth and status of the individual (*e.g.*, live/dead) were recorded. For purposes of addressing population dynamic issues, information on age structure requires more intensive surveys involving mark-recapture methods with the measurement of body size and in some cases use of toe clipping for the study of bone growth rings to estimate age.

3.2 *Local survey projects*

We collected information on amphibian survey projects organized by local government and other organizations that were focused on the local scale, and which frequently employed residents (i.e., amateurs) in data collection. A summary of the methods used in these surveys is shown in Table 1. Sighting of adults, egg mass and larvae and checking for frog calling were the principal methods used in these sur-

Table 1
Summary of methods usedin local amphibian surveys in Japan.

	Sendai City[55]	Tsukuba City[56]	Tsuchiura City[57]	Tochigi Pref.[58]	Hino City[59]	Shizuoka City[60]	Osaka Pref.[61]	Hyogo Pref.[62]	Kurashiki City[63]	Kochi Pref.[64]
Researcher	Elementary and Junior high school students Civil researchers, Residents	No information	Civil researcher	Residents	23 people in total including residents and students of Tokyo University of Agriculture and Technology	Civil researchers and residents (Hearing)	Member of NPO Senior Nature College and residents	Residents	Chiefly elementary students and their family	Researchers and residents
Subject species	*Buergeria buergeri* *Bufo japonicus formosus* (2 species in 2 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rana porosa porosa* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Rana catesbeiana* *Rana rugosa* *Bufo japonicus formosus* (8 species in 4 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Rana porosa porosa* (5 species in 3 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rana porosa porosa* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Rana rugosa* *Rana catesbeiana* *Buergeria buergeri* *Bufo japonicus formosus* (8 species in 4 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rana porosa porosa* *Rana catesbeiana* *Rana rugosa* *Bufo japonicus formosus* (6 species in 4 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rhacophorus arboreus* *Buergeria buergeri* *Rana rugosa* *Rana limnocharis* *Rana catesbeiana* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Rana tagoi tagoi* *Rana nigromaculata* *Bufo japonicus* (11 species in 4 families in total)	*Hyla japonica* *Rhacophorus schlegelii* *Rhacophorus arboreus* *Buergeria buergeri* *Rana rugosa* *Rana limnocharis* *Rana catesbeiana* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Rana tagoi tagoi* *Rana sakuraii* *Rana nigromaculata* *Rana porosa* *Bufo japonicus japonicus* *Bufo japonicus formosus* *Bufo torrenticola* (16 species in 4 families in total)	*Rhacophorus arboreus* Others (1 species in 1 family in total)	*Hyla japonica* Others (1 species in 1 family in total)	*Hyla japonica* *Rhacophorus schlegelii* *Buergeria buergeri* *Rana rugosa* *Rana limnocharis* *Rana tagoi tagoi* *Rana nigromaculata* *Rana catesbeiana* *Rana* sp. *(Rana japonica/ Rana ornativentris)* *Bufo japonicus japonicus* (11 species in 4 families in total)
Subject area	throughout Sendai City	throughout Tsukuba City (Selected 593 points)	30 points selected in Tsuchiura City	throughout Tochigi Pref.	throughout Hino City (Divided into 9 areas)	throughout Shizuoka City	throughout Osaka Pref. (excluding surveys in restricted, dangerous, coastal and inland areas and survey with boats)	throughout Hyogo Pref. (information from other prefectures is also covered)	throughout Kurashiki City (in the unit of school district)	throughout Kochi Pref.
Survey method	visual check/capture and identification observation of egg mass check for audible calling check for dead animals	visual check for adult, juveniles, larvae, egg mass, check for audible calling	visual check for adult, juveniles, larvae, egg mass, check for audible calling visual check for adult, juveniles, larvae and egg mass (*Rana* sp.)	capture and identification	check for audible calling	visual check for adult, larvae, (location recorded photo optional)	adult (check for audible calling/ visual check/ capture and identification) larvae (visual check/ capture and identification) egg mass (visual check and identification)	visual check for adult, larvae, egg mass check for audible calling	visual check for adult, dead animal, egg mass check for audible calling	visual check for adult, egg mass
Recording method	input to survey sheet and internet	not known	not known	input to survey sheet and internet	not known	sighting data transmission sheet	survey sheet and recorder	input via internet	not known	record sheet

veys for assessing presence/absence data and these methods generated local distribution maps. In some survey projects, the approximate number of amphibians was recorded and habitat suitability was assessed. Some survey projects were conducted as a contribution to environmental education for students and children. Most local research projects disclosed presence/absence data on amphibians, together with other animals, plants and geographical information in databases made available through dedicated web sites with search function. Some projects also employed geographical information system methods to disclose distribution maps based on the data collected. Most survey projects covered only 2 to 3 years, and only a few projects continue to collect data.

3.3 *National environmental census projects relevant to amphibians*

Nationwide surveys of habitats suitable for amphibians have been conducted as part of three national environmental censuses in Japan: the 'Green Census' supported by the Ministry of the Environment; the 'Riverfront Census' supported by the Ministry of Land, Infrastructure and Transportation; and 'Survey on Wildlife in Rice Fields' supported by a joint project by the Ministry of the Environment and the Ministry of Land, Infrastructure and Transportation. The survey methods used in each of these censuses are summarized in Table 2. The main survey methods in all census projects were visual sighting and detection of amphibian calling. As census surveys, these activities provided snapshots of habitat quality and the distribution of wildlife including amphibians and consequently did not generate quantitative population data on amphibian populations over consecutive years.

Table 2
Summary of methods used for gathering information on presence of amphibian species in nationwide ecological survey activities in Japan.

	Green Census[65]	Census of Riverfront[66]	Survey on Creatures in Rice Fields[67]
Researchers	Survey of all species: those with expertise Survey on Creatures in Neighborhood: residents	Local bureau of land improvement, local organizations and those with academic experience	Basic survey: local researchers Common survey: residents including elementary and junior high school students
Subject species	Survey of all species: all species Survey on Creatures in Neighborhood: *Bufo japonicus* *Rana catesbeiana* *Buergeria buergeri* newts	All species	All species of anuran
Subject area	Throughout Japan	River channel and area within 200 to 500 m along river	Path between rice fields, embankment of canals and farm road
Recording method	Check sheet, topographic chart	Check sheet, topographic chart	Check sheet, photograph sheet, topographic chart
Check method	Adult, juveniles, larvae: visual check and capture for identification check for audible calling	Visual check (with or without photograph and capture for identification, field sign (including calling), others	Visual check, capture and photographing of adults (if it is difficult to identify the species on-site, the photographs are used)

4. Discussion and Conclusions

The assessment of the effects of environmental contaminants, including endocrine disrupters, on amphibian populations will require a multilevel approach. This will likely combine laboratory studies of single chemicals or simple mixtures, with biological and chemical screening of relevant environmental extracts, field-to-lab studies with native species, and *in situ* studies of amphibian survival growth, development and reproduction. Although the demonstration of the effects of chemicals at the individual level in laboratory models such as *Xenopus* will obviously play an important role in building lines of evidence, in isolation they can at most signal a potential for the adverse effects of these chemicals in wild populations. In the UK and Japan, is it not currently known whether amphibian populations are experiencing health problems that could be attributed to exposure to EDCs. In this respect, endocrine disrupter research on amphibians differs from that in fish, where observation of a population-level feminization triggered a research program to better understand the mechanism.[68,69]

To this end, it is important to establish whether populations of native UK and Japanese amphibians are currently undergoing declines in distribution and or abundance that could be correlated with anthropogenic pressures. Historical reports indicate declines in most UK amphibians during the latter part of the last century, largely associated with agricultural intensification and associated changes in land use. Although dedicated action plans for species with restricted geographical ranges and or highly specialized habitat requirements have been largely successful and well integrated with national biodiversity action plans, it is less clear whether more recent and undefined environmental stressors have impacted our more widespread and common species, for example the common toad, over which there is growing concern.

Given the need for relatively long-term data by which to assess trends in amphibian populations, in the short term, the only practical approach available is an ecoepidemiological one, which relies heavily on the quality of amphibian population data and the resolution of data that could be used to predict exposure of populations to environmental toxicants. An assessment of databases available for UK widespread anuran amphibians (*e.g.*, BRC Herptiles database) indicates that long runs of robust abundance data are not widely available.

In light of the concern over the status of the common toad in Britain, we have also assessed the potential of road crossing/casualty data, collected by Toads on Roads scheme volunteers, for the generation of yearly estimates of adult breeding toads at perennial breeding sites. Only a small subset of such schemes have gathered sufficient data in the past for the construction of population trends, and many of those sites are either no longer operating or no longer collecting count data. Overall, there is a high level of variability in the type and quality of data collected among sites, which further confounds uncertainty as to the comparability of count data between sites with different local topology, traffic and habitat.

Few national resources of amphibian population data exist in Japan. A number of local or regional surveys have been conducted in recent years and have been reviewed; however, these surveys have been conducted essentially as censuses for the inventory of biodiversity and the generation of distribution maps. Equally, amphibians have been included as target species in several national censuses of biodiversity, riverine wildlife, and wildlife inhabiting rice fields.

Given the data resources available in the UK and Japan for assessing recent trends in amphibian populations, attempting to dissect out population level effects of environmental contaminants such as EDCs from among other anthropogenic and natural stressors will be a challenging process. Generating more comprehensive and robust population data with which to support this process will require renewed effort and ideally the adoption of a coordinated monitoring strategy, as evident in the USA currently.

According to information gathered from surveys conducted in Japan together with those in the UK, a summary of amphibian field survey methods common between the two countries is summarized in Table 3. Clearly, the methods used in our two countries are largely the same (with the possible exception of collection of count data in Toad Crossings). Consequently, common methods of population monitoring of amphibians can be used, and should form the basis for coordinated programs in the UK and Japan. In turn, such programs could enable a comparative approach to assessing the effects of endocrine disrupters on temperate island nations with high population densities and well-established agricultural landscapes but different amphibian fauna.

As an example of the potential value of this approach, Table 4 shows the list of the Japanese anuran amphibian species recorded in the local surveys summarized in Table 1 and the two common and widespread British anurans, along with general breeding habitat preferences. British anurans rarely breed in lotic environments, preferring static water bodies such as lakes (primarily for toads), ponds, ditches and marshes (primarily for frogs). Consequently, when attempting to compare population trends with potential exposure to agricultural pesticides (such as in Fig. 6) there is a mismatch between the habitat in which British amphibians may be exposed to aquatic contamination, and the aquatic habitats for which modelling data (such as data the POPPIE database provides) are available. In contrast, several native Japanese species spawn in streams and rivers and analysis of population trends for the more widely distributed species (*e.g.*, *Rana rugosa*) across landscapes with differing levels of agricultural activity and pesticide use could be informative. Equally, the arable-dominated agricultural landscape in Britain is well drained and generally inimical for British amphibians outside of gardens and woodland refugia. In contrast, many of the Japanese anurans listed in Table 4 frequently inhabit and spawn in ditches and marshes associated with rice paddies. Consequently, for these species it may prove easier to locate well-established breeding populations in areas where potential exposure to agrochemicals is high along with locally matched reference populations with lower exposure.

Future monitoring of widespread anuran amphibian species in the UK and Japan to support an ongoing assessment of the impact of environmental contaminants, including EDCs, should ideally be integrated with existing conservation practices and initiatives. In the UK, concern over the conservation status of British amphibians and reptiles has led to the initiation of a national recording scheme organized by the Herpetological Conservation trust. This scheme, the National Amphibian and Reptile Recording Scheme (NARRS), aims to coordinate recording activity across Great Britain to generate more consistent data on population and conservation status of UK amphibians and reptiles. NARRS comprises ongoing national surveys of sand lizard, smooth snake, natterjack toad and adder as well as newly initiated schemes to monitor widespread amphibians. With respect to the latter, the current plans are to limit recording to presence/absence to manage demands on volunteers, and in recognition of the uncertainties associated with estimating population trends from unadjusted count data. However, if appropriate methods of generating population trends on an ongoing basis can be routinely adopted by volunteers in only a subset of current contributors, then such monitoring could complement and extend existing historical data and provide highly valuable information for assessing effects of environmental contaminants in the future. We hope that such a strategy can be adopted and coordinated in the UK and Japan.

Table 3
Consensus field survey methods of amphibian population surveys in Japan and UK.[35,52–54]

Amphibian survey method	
Survey during breeding season	Observation (nighttime): To carry out a systematic nocturnal survey, walk slowly around the edge of the pond training the torch beam into the water, counting the number of animals of each species.
: several counts are advisable to generate a robust estimate of abundance. : to estimate whether the recorded counts are maximum numbers, measure the temperature and humidity at night.	Netting: To sample the pond edge systematically, aim to make one net sweep at a regular interval or each vegetation type of the shoreline around the entire perimeter. Record the percentage length of the pond perimeter covered by the diameter of your net and the total number of sweeps made. Unlikely to be reliable for *Bufo bufo* (UK) that are typically present in highly uneven distributions and often shoal in deeper water inaccessible for netting
	Trapping: The trap will sit on the pond surface at a regular interval of the shoreline around the entire perimeter. Record the number of traps used, the number of hours they were in position, the number of traps per length of the pond edge and the approximate temperature during the period. Mostly suitable for urodeles (newts and salamanders).
	Spawn: Spawn clumps may be gently separated from each other and counted. If the spawn is congealed into an inseparable mass, record the approximate dimensions of the total area covered by the mat. The numbers of spawn clumps can be used as an estimate of the numbers of breeding adult pairs for *Rana temporaria* (UK), but is not reliable for estimating the breeding populations of *Bufo bufo* where spawn strings are difficult to distinguish and often highly inaccessible. Suitable vegetation needs to be checked for eggs of urodeles.
	Mortality on roads: Use road mortality as estimate of breeding population and the starting date of migration for breeding. May be useful for estimating adult populations of *Bufo bufo* (UK) — unclear whether this method could be adopted for some species in Japan.
	Calling: Identify the species with calling and estimate their number. Not typically used for survey of anurans in UK (Rana and Bufo) although for vocal species in Japan may be more useful. Can provide indication of abundance although not quantitative (*e.g.*, as used in NAAMP program in USA).
Survey during non-breeding season	Metamorphosed juveniles: To search for evidence that successful metamorphic emergence is occurring, check terrestrial habitat surrounding breeding sites for emerging metamorphs. Can be affected by vegetation type, ambient temperature and weather conditions at time of search. Observation of metamorphs can be aided by providing additional refugia (*e.g.*, logs, sacking) and this could be standardized cover boards.
	Terrestrial survey: The availability and quality of suitable aquatic and terrestrial habitats should be recorded. Some standardized schemes for the characterization of habitat quality have been established as part of amphibian monitoring schemes, e.g., North American ARMI monitoring program, UK NARRS. Significant land use changes should be recorded on an ongoing basis.

Table 4
Preferred breeding habitats of common Japanese and British anuran amphibians. The Japanese species listed are those featured in the local amphibian surveys, summarized in Table 1.

Species	(Breeding) Habitat		
	Lotic	Lentic	Rice fields
Japan			
Hyla japonica	✗	✓	✓
Rhacophorus schlegelii	✗	✓	✓
Rhacophorus arboreus	✗	✓	✓
Buergeria buergeri	✓	✗	✗
Rana rugosa	o	✓	✓
Rana limnocharis	✗	✓	✓
Rana japonica	✗	✓	✓
Rana ornativentris	✗	✓	✗
Rana tagoi tagoi	✓	✗	✗
Rana nigromaculata	✗	✓	✓
Rana porosa	✗	✓	✓
Bufo japonicus japonicus	✗	✓	✗
United Kingdom			
Bufo bufo	✗	✓	nr
Rana temporaria	✗	✓	nr

o **:** *Rana rugosa* inhabits predominantly slow moving brooks/rivulets; nr : not relevant. Information on habitat/breeding habitat preferences for these species was obtained courtesy of the following web site (http://www3.ocn.ne.jp/~herpsgh/amphibians.html).

Acknowledgments

We would like to thank Julian Howard of Froglife (UK) for providing Toads on Roads records and Geoff Brighty and Tatiana Boucard of the Environment Agency of England and Wales for access to and provision of pesticide usage data for 1998, retrieved from the POPPIE database. Work conducted by DBP and SL was funded by the UK Government Department of Environment Food and Rural Affairs (DEFRA) under the 'Endocrine Disrupters in the Aquatic Environment' program, contract no. CPEC 18.

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