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Time-to-event analyses of ecotoxicity data

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Intensity and duration of exposure dictate the effect of a toxicant. Consequently, any assessment of ecological risk that does not include a sound understanding of both concentration and duration effects is compromised. This being the case, it is surprising that the predominant approach in ecotoxicology (concentration-effect modeling) inefficiently includes exposure duration. Ecological risk assessment can be enhanced with time-to-event models that can easily include concentration, exposure duration, and other important covariates. Time-to-event methods are described and linkage made to relevant ecological techniques, i.e. life table analyses and genetic selection models.

Keywords: statistics; survival time; toxicity; ecological risk assessment.

Introduction

Both intensity (dose or concentration) and exposure duration determine a toxicant's effect. If either is increased, the probability of a toxicant molecule interacting adversely with an active receptor site increases. It follows that any assessment of ecological risk is compromised without full consideration of toxicant concentration and exposure duration. To this end, concentration-effect methods have been applied for different durations notionally reflecting 'acute' or 'chronic' time scales. This provides a gross understanding of changes in effect at different durations. Alternatively, a measure of effect based on concentration-effect methods such as the LC50 ('lethal concentration' of the test chemical for 50% of the organisms tested) may be calculated for a series of times and an empirical relationship generated. One data set may be used to estimate LC50 values at different times during an exposure; however, data quality is often compromised at some time intervals. There may be no mortality at low concentrations early in the exposure and complete mortality at higher concentrations toward the end of the experiment. This can be avoided by using different concentration ranges in separate tests varying in duration. This second approach avoids diminished data quality but requires more time, expense and organisms.

Widespread use of these approaches to include duration and intensity of exposure stems more from our bias toward concentration-effect methods than from objective method selection. Time-to-event (survival time, failure time, time-to-failure, resistance time, time-response) methods are more appropriate but remain under-utilized (Sprague, 1969; Dixon and Newman, 1991; Newman and Aplin, 1992; Newman, 1995). With time-to-event techniques, the times to respond (e.g. durations of exposure prior to dying) are noted for all individuals instead of the proportion of all exposed individuals responding by the end of the exposure period. Time-to-event models incorporating

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concentration (time-concentration-effect models) can greatly enhance assessment of ecological risk. Further, statistical power is generally higher because the time-to-event design generates more data than the concentration-effect design (Gaddum, 1953; Finney, 1964). This increase in power makes it easier to study and accurately model covariate effects, thereby achieving a more comprehensive understanding for effect prediction. The objective of this paper is to briefly present the advantages of these alternative methods for ecological risk assessment by demonstrating their utility for modelling sublethal and lethal effects, and their convenient linkage to ecologically meaningful models.

Methods

Time-to-event approach

Time-to-event methods are described in numerous books (Kalbfleisch and Prentice, 1980; Miller, 1981; Lawless, 1982; Fleming and Harrington, 1991; Cox and Oakes, 1994). General description as pertinent to ecotoxicology is provided here and elsewhere (Dixon and Newman, 1991; Newman and Aplin, 1992; Newman, 1995).

In a concentration-effect test, a series of tanks receives different concentrations of toxicant and the proportion of exposed individuals surviving in each tank is noted at a predetermined time such as 96 h. If seven exposure concentrations are used, seven proportions would be generated, although 0 and 100% mortalities at some concentrations could compromise the information content of a subset of these proportions. Using a time-to-event approach, the exposed individuals held at the seven concentrations would be monitored through time and their times-to-death recorded. If twenty individuals were used for each concentration, 140 pieces of data (7 concentrations $\times$ 20 times-to-death per concentration) would be generated. However, some individuals may survive to the end of the exposure period and the only information available regarding their response is survival beyond a certain time: survivors would be right censored. Regardless, much more information is obtained from the test if times-to-death are noted (140 versus 7 data points). Further, covariates associated with individuals, such as weight or sex, can easily be incorporated into the data structure.

These data can be used in nonparametric, semiparametric or fully parametric time-to-event models, and to test for significance of covariates (Dixon and Newman, 1991; Newman, 1995; Newman and Dixon, 1996). Only the fully parametric methods are described here. Data are fit to an accelerated failure time model (Equation 1) with maximum likelihood methods.

$$\ln t_i = f(x_i) + \epsilon_i \tag{1}$$

where t_i = time-to-event for individual i, $f(x_i)$ = a function relating quality x of individual i to $\ln t$, and ϵ = error term. The $f(\,)$ can be a linear or some other function of the covariate(s), e.g. $f(x) = \mu + \beta$[toxicant concentration] or $f(x) = \mu + \beta \ln$[toxicant concentration]. The covariate(s) acts on ln time-to-death or 'accelerates' the time-to-death. The error (ϵ) can be described by either a gamma, logistic, normal, Weibull, or another distribution. Use of maximum likelihood methods accommodates censored observations.

Hazard (probability of dying during a time interval) may remain constant

(exponential function) or change during exposure. Hazards can change with time yet remain proportional among groups of interest (Weibull function). With models using the gamma, logistic and normal functions, hazards change over time and do not remain proportional. Consequently, the accelerated failure model can also be described as a proportional hazard model under the assumption of a Weibull or exponential function,

$$h(t_r x_i) = e^{f(x_i)} h_0(t) \tag{2}$$

where $h(\,)$ = the hazard or proneness to die, and $h_0(\,)$ = the hazard for a reference class. A reference class may be the control animals or an arbitrary group. The advantage of a proportional hazard model is that the relative hazards of various classes (e.g. smokers versus nonsmokers) or effects of continuous covariates on hazard remain constant with time. Hazards can be expressed as relative risks regardless of exposure duration. For example, risk of dying for animals held at one concentration can be expressed relative to the risk of dying for control animals, e.g. risk of dying is increased 1000 times relative to controls by exposure to a certain toxicant concentration.

Fitting time-to-event data

Data consist of times-to-event and any relevant covariates for all individuals. Only a minimum survival time (duration of the experiment) is available for survivors. Covariates can be continuous variables such as weight, or class variables such as sex. These data would be fit by maximum likelihood methods using candidate distributions for ϵ and functions of the covariates. The general appropriateness of a particular distribution may be evaluated using a series of linearizing functions of the proportion dying versus exposure duration (Dixon and Newman, 1991). Candidate models can also be compared using the log likelihood statistics generated by most computer packages. However, the log likelihood statistics are not used directly if models differ in number of estimated parameters. For example, a model assuming a Weibull distribution for ϵ has one more parameter (a shape parameter) than a model assuming an exponential distribution. Akaike's information criterion (AIC) can be used to compare candidate models varying in complexity because it adjusts the log likelihood statistic for the number of estimated parameters. That model with the smallest AIC provides the best fit.

$$\mathrm{AIC} = -2(\log \textit{likelihood}) + 2P \tag{3}$$

However, the AIC only measures relative fit and none of the candidate models may be appropriate. Plots of the original data against model predictions ensures that an appropriate model is selected.

Example data sets

Time-to-stupefaction: forty zebrafish (*Brachydanio rerio*) were exposed to 49.9 mg per L of benzocaine over time (seconds) until equilibrium loss was noted for each fish. Exposures were conducted until all fish were stupefied, i.e. there were no censored observations. This simple data set is used to illustrate time-to-event modelling including error distribution selection.

Time-to-death (toxicant concentration as covariate): a model from the reanalysis (Newman *et al.*, 1994) of time-to-death data for various-sized mosquitofish (*Gambusia holbrooki*) exposed to different concentrations of sodium chloride (Newman and Aplin,

1992) illustrates the richness of information obtainable from time-concentration-effect modelling. A quantity of mosquitofish (401) ranging in weight 0.024–1.489 g wet weight were exposed to six sodium chloride concentrations ranging 10.3–20.1 g NaCl per L. Times-to-death were noted every 8 h for 96 h with some fish surviving exposure.

Time-to-maturity under thermal stress: data from Mulvey *et al.* (1994) were reanalysed to demonstrate how sublethal effects information directly applicable to demographic analysis can be generated with time-to-event methods. Mosquitofish neonates were exposed to normal (25 °C) and high (32 °C) temperatures, and times-to-maturity noted daily for ten weeks. Only time to reach sexual maturity for females is discussed.

Time-to-death (relative fitness): Newman (1995) reanalysed mosquitofish mortality data from Diamond *et al.* (1989) using a proportional hazard model (Weibull distribution). Every 3 h for 10 d, times-to-death were noted for 711 fish exposed to approximately 1 mg per L of inorganic mercury. Covariates included fish sex, wet weight and genotype at the glucosephosphate isomerase-2 (GPI-2) locus. Results were expressed as relative risk and relative genetic fitness under the assumption of proportional hazards.

Results

Time-to-stupefaction

This data set had no censored observations. The cumulative distribution for proportion of the population succumbing (Fig. 1, top panel) is a sigmoidal curve that can potentially be linearized with several candidate transformations. Log normal (probit metameter), log logistic (logit metameter), or Weibull (Weibull metameter) distributions for ϵ were explored (Fig. 1, bottom panel). Both the log normal and log logistic models fit these data adequately as suggested by the linearization of data and random distribution of transformed data about the lines. The Weibull model seemed less appropriate because there was the slight pattern to data points about the line. The AIC was also used to more accurately assess the relative fits for various distributions for ϵ and $f(x)$ for the covariate, wet weight. Preliminary model exploration indicated that the ln of weight provided better fit than weight. Therefore, the accelerated failure time model was the following,

$$\ln t_i = \mu + \beta(\ln \mathit{weight}_i) + \epsilon_i \tag{4}$$

or, after transformation to arithmetic units of time,

$$t_i = e^{\mu} e^{\beta(\ln \mathit{weight}_i)} e^{\epsilon_i} \tag{5}$$

where μ = intercept, and β = coefficient for the effect of ln(weight) on ln time-to-stupefaction. The ϵ is quantified with a scale parameter (σ) and W (described below). This model (Equation 4) was fit with a maximum likelihood method (SAS Institute, Inc., 1988) assuming exponential (two estimated parameters), Weibull (three estimated parameters), log normal (three estimated parameters), log logistic (three estimated parameters), or gamma (four estimated parameters) models. AIC values for the exponential (93.2), Weibull (65.5), log normal (61.1), log logistic (62.3), and gamma (63.1) indicated that the log normal function best fit these data, although the log logistic also had a low AIC value. The model for predicting time-to-stupefaction was,

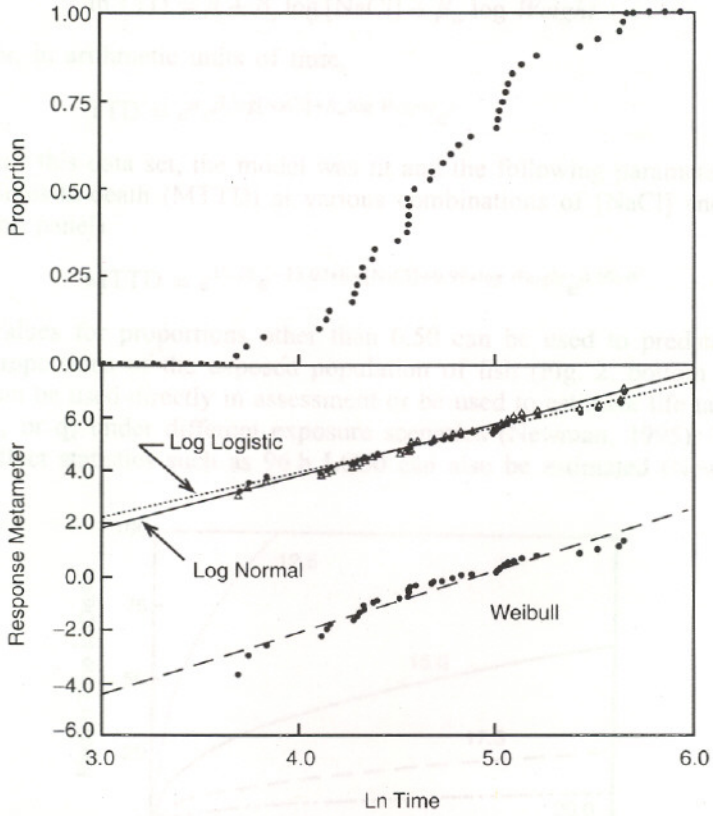

Fig. 1. Time-to-stupefaction data for zebrafish exposed to benzocaine. The cumulative proportion of the exposed fish stupefied with duration of exposure (top panel) is a sigmoidal curve. The sigmoidal curve may be linearized assuming a log normal (probit metameter vs ln time, solid line and Δ), log logistic (logit metameter vs ln time, dashed line and ●), or Weibull (Weibull metameter vs ln time) model (bottom panel).

$$t_i = e^{6.46} e^{1.21(\ln\, weight)} e^{0.48 * W} \tag{6}$$

where W = effect metameter for a specified proportion, e.g. 0.50 for the specified distribution (Newman, 1995). The median time-to-stupefaction (0.50) for a log normal model would have a $W = 0$ so $e^{0.48 * W}$ becomes 1 in Equation 6. The predicted median time-to-stupefaction (MTTS) for a 0.25 g zebrafish would be $e^{6.46} e^{1.21(ln\ 0.25)}$ or 119 s, an estimate consistent with the raw data depicted in Fig. 1 (top panel).

Time-to-death (toxicant concentration as covariate)

This data set has two continuous covariates (NaCl concentration and fish wet weight). Using the AIC as described above, the best fit was obtained by log transforming the covariates (log [NaCl], log (weight)) and assuming a log normal distribution for ϵ (Newman and Aplin, 1994; Newman, 1995). The accelerated failure time model was,

$$\ln \mathrm{TTD} = \mu + \beta_s \log [\mathrm{NaCl}] + \beta_w \log\ Weight + \epsilon \tag{7}$$

or, in arithmetic units of time,

$$\mathrm{TTD} = e^{\mu} e^{\beta_s \log[\mathrm{NaCl}] + \beta_w \log\ Weight} e^{\epsilon} \tag{8}$$

For this data set, the model was fit and the following parameters used to predict median times-to-death (MTTD) at various combinations of [NaCl] and fish wet weight (Fig. 2, top panel).

$$\mathrm{MTTD} = e^{19.28} e^{-13.02 * \log[\mathrm{NaCl}] + 0.99 * \log\ Weight} e^{0.50 * W} \tag{9}$$

Values for proportions other than 0.50 can be used to predict times-to-death for other proportions of the exposed population of fish (Fig. 2, bottom panel). Such information can be used directly in assessment or be used to estimate life table parameters such as l_x, d_x or q_x under different exposure scenarios (Newman, 1995). Traditional concentration-effect statistics such as 96 h LC50 can also be estimated (Newman, 1995).

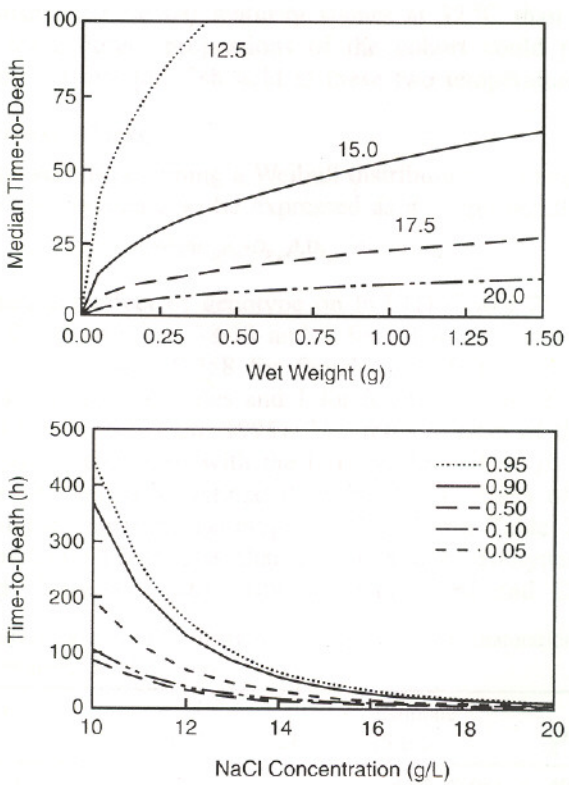

Fig. 2. Prediction of time-to-death for mosquitofish as a function of sodium chloride concentration and fish weight. Median time-to-death is predicted as a function of salt concentration (12.5 to 20.0 NaCl g per L) and fish weight (top panel). Proportions dying other than 0.50 (median) can be predicted using the appropriate constants in Equation 9 (bottom panel).

Time-to-maturity under thermal stress

Other life history events such as time to reach sexual maturation can be analysed with these methods and provide useful information for demographic analysis of stressor effects on populations. Analysis of data from Mulvey *et al.* (1994) clearly demonstrates this point (Table 1). These data were best fit with a log logistic model ($W = 0$ for $P = 0.50$). Note that the covariate is a discrete class variable, not a continuous variable such as weight or salt concentration. With class variables, one class is arbitrarily selected as the reference class and assigned a dummy value of 0 (e.g. 25 °C in Table 1). The remaining class(es) (e.g. 32 °C in Table 1) is assigned a dummy value of 1. Although not shown in the previous examples, a χ^2 statistic is used to determine if a covariate has a significant effect on ln time-to-event. There is a highly significant influence of treatment on time-to-maturity for female mosquitofish (Table 1). The median times-to-maturity (MTTM) at 25 °C and 32 °C are predicted from the following models.

$$\text{MTTM}(32\ ^\circ\text{C}) = e^{4.07}e^{-0.70*1}e^{0.23*0} = 29\text{d} \tag{10}$$

$$\text{MTTM}(25\ ^\circ\text{C}) = e^{4.07}e^{0*0}e^{0.23*0} = 58\text{d} \tag{11}$$

Female mosquitofish reach sexual maturity sooner at 32 °C than at 25 °C. Solving the above equations for different proportions of the cohort could provide additional life history information for mosquitofish held at these two temperatures.

Time-to-death (relative fitness)

Time-to-death data were fit assuming a Weibull distribution; consequently, the accelerated failure time model below can also be expressed as a proportional hazard model.

$$\text{Ln TTD} = e^{4.134}e^{3.157(Weight)}e^{\beta_g*\text{D}_g}e^{\beta_s\text{D}_s}e^{-0.36651*0.514} \tag{12}$$

where β_g = estimate for effect of genotype on ln TTD (Table 2), D_g = dummy variable having values of 0 for genotype 38/38 and 1 for all other genotypes, β_s = estimate of effect of fish size on ln TTD (0.358 for females and 0 for males), and D_s = dummy variable having values of 0 for males and 1 for females. W for $P = 0.50$ of the Weibull distribution is −0.36651 (Newman, 1995). The relative risks of the different genotypes (class variables) can be estimated with the term, $e^{-\beta g/\sigma}$ where β_gs are listed in Table 2 for the six genotypes and σ is estimated to be 0.514. Risks of dying are expressed relative to that of the reference genotype (38/38). From Table 2, it is clear that the reference genotype has a higher risk than the other five genotypes. These relative risks can be converted easily to relative fitnesses (w) (Hartl and Clark, 1989) for each

Table 1. Effect of thermal conditions on rate of sexual maturation of female mosquitofish data from Mulvey *et al.* (1994)

Variable	Label	df	Estimate (S.E.)	χ^2	Probability*
Intercept (μ)		1	4.07 (0.06)	4990	0.0001
Treatment (β)		1		77	0.0001
	32 °C	1	−0.70 (0.08)	77	0.0001
	25 °C	0	0		
Scale (σ)		1	0.23 (0.03)		

*Probability of obtaining a χ^2 statistic of this magnitude by chance alone.

genotype by normalizing each risk to that of the most robust genotype (66/100) (Newman, 1995). These relative fitnesses are calculated in the right hand column of Table 2.

Conclusion

Emphasis on the concentration-effect approach compromises ecological risk assessment because inclusion of exposure duration is ineffective. More appropriate time-to-event methods remain underutilized in ecotoxicology today. When used in ecotoxicology, time-to-event analyses seldom extend beyond the Litchfield method (Litchfield, 1949) for estimation of LT50 ('lethal time' for 50% of the organisms tested at a particular concentration). Notable exceptions are Steadman *et al.* (1991), Roy and Campbell (1995), Schlueter *et al.* (1995), and Sun *et al.* (1995). Methods are described here that allow more accurate analysis of effect by incorporating both exposure concentration and duration. Covariates are easily included in time-to-event models; consequently, the ability to predict effects on field populations is enhanced.

A central concern in risk assessment is population viability yet concentration-effect data are not directly relevant to estimation of population viability. The few studies of population viability that applied demographic methods (life table analysis) clearly demonstrated the value of producing appropriate data, i.e. vital rates (Daniels and Allan, 1981; Allan and Daniels, 1982; Caswell, 1996; Sibly, 1996). Indeed, Caswell (1996) detailed a demographic bioassay that allows projection of population condition under toxicant stress. As demonstrated here, the implementation of time-to-event methods allows generation of data directly applicable to such analysis. Two data sets were analysed with these methods and generated essential pieces of information for demographic analysis: time-to-maturity and time-to-death models. As evidenced by calculation of relative fitnesses from a mosquitofish data set, prediction of genetic changes in stressed populations may also be enhanced with these methods. For these reasons, more widespread use of time-to-event methods is advocated to enhance ecological risk assessment.

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Table 2. Conversion of relative risks to relative fitness for mosquitofish GPI-2 genotypes modified from Newman (1995)

Mosquitofish GPI-2 genotype	Estimated β_g (S.E.)	Estimated Relative risk	Estimated Relative fitness (w)
100/100	0.370 (0.117)	$e^{-0.370/0.514} = 0.487$	0.402/0.487 = 0.82
66/100	0.468 (0.117)	$e^{-0.468/0.514} = 0.402$	0.402/0.402 = 1.00
38/100	0.362 (0.123)	$e^{-0.362/0.514} = 0.494$	0.402/0.494 = 0.81
66/66	0.389 (0.125)	$e^{-0.389/0.514} = 0.469$	0.402/0.469 = 0.86
38/66	0.339 (0.141)	$e^{-0.339/0.514} = 0.517$	0.402/0.517 = 0.78
38/38	0	$e^{-0/0.514} = 1.000$	0.402/1.000 = 0.40

share the mosquitofish time-to-maturity data. The authors would also like to thank Drs M. Mulvey and C. Strojan for comments on an earlier version of the manuscript. Ms R. Jagoe drafted the figures.

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