USE OF LIFE TABLE DATA IN TOLERANCE EXPERIMENTS

by

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Résumé

Utilisation de données tabulaires sur les cycles biologiques dans des expériences de tolérance.

Dans le problème de la conservation des espèces, il est important d'évaluer leur tolérance à des conditions d'environnement suboptimales ou à des polluants, aussi bien en terme de reproduction que de soma. Des données démographiques sur les cycles biologiques, intégrées au moyen de calculs, puis disposées **en** tableaux, fournissent des informations précises telles que e_x (espérance de vie moyenne), $\mathbf{R_o}$ (taux net de reproduction par durée de cycle individuel) et, la **plus** importante **:** r_{max} (taux réel de croissance naturelle par individu et **par** jour). Les effets de concentrations croissantes de DDT sur les paramètres utilisés pour **les** tableaux des cycles (Hummon, 1974), ont fait l'objet d'une étude plus poussée sur *Lepidodermella squammata,* Gastrotriche commun d'eau douce. Ces animaux, parthénogénétiques obligatoires, ont été élevés individuellement dans des lames creuses, depuis leur éclosion. Les données furent établies par intervalles d'une demi-journée pour plus de 50 individus, chacun des tests comprenant aussi une série de contrôle.

Des résultats précédents ont montré que les paramètres e_x, R_o et r_{max} évoluent tous en raison inverse des concentrations en DDT et que, même à 0,05 ppm de DDT, il apparaît un désavantage plus marqué, de 10 pour cent, pour le r_{max}. La valeur statistique des résultats des données tabulaires a été établie en séparant les données en deux moitiés et en calculant deux nouveaux cycles tabulaires pour chaque ensemble de conditions. Cette technique augmente par 1 facteur à 3 les estimations faites sur les valeurs des cycles mis en tableau pour chacun des ensembles. Une analyse de variance d'un modèle I à une entrée est alors appliquée aux résultats — en retranchant selon la séquence les données pour des ensembles de tests se rapprochant plus ou moins des conditions de contrôle — jusqu'à ce qu'aucune différence significative ($P > 0,10$) n'apparaisse parmi les valeurs des ensembles de données restantes. Dans ce cas, pour chacun des trois paramètres du cycle considéré — e_x, $\mathbf{R_o}$ et r_{max} — il existe des différences notables entre les données du contrôle et les tests de conditions à 0,05, 0,5 et 5 ppm en DDT, mais il n'y en **a** pas entre celles du contrôle et celles des tests à 0,05 ppm en DDT.

Introduction

Niche is perhaps most appropriately considered to be a property of demes or reproductive populations of a species rather than a property of an ecosystem. The niche of a population has been defined as an n-dimensional hypervolume, formed by the linear ordering of tolerance limits for all environmental parameters which are necessary for the perpetuation of that population (Hutchinson, 1957). One can think of the niche in this sense as a reproductive hypervolume. However, one can also think of a

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somatic hypervolume, whose limits must be as great or greater than those of the reproductive hypervolume. It is not unusual, in fact, to find reproductively sterile individuals or groups of individuals living on the fringes of a species' distribution, individuals who would be occurring within the somatic but without the reproductive hypervolume of the species. Even within the centre of a species' distribution it is common experience that a slight perturbation of environmental conditions is more likely to affect the reproductive capabilities of an organism than to cause its death.

The usual method of assessing tolerance limits of a population to suboptimal environmental conditions or pollutants is by means of somatic lethal limits. But, how much more relevant it is to assess tolerance limits in terms of reproductive lethal limits, as was done fully twenty years ago by Birch (1953) for the effect of temperature, moisture and food on reproductive rates of a grain beetle. More recently, life table data have been used to evaluate the effects of DDT (Hummon, 1974) and mine acid effluents (Faucon and Hummon, 1976) on longevity and reproductive rates of a common freshwater Gastrotricha species, *Lepidodermella squammata* (Dujardin, 1841). Inasmuch as demographic life history data tend to integrate the n-dimensions of the niche hypervolume into single parameter units—such as e_x (mean life expectancy), R_o (net reproductive rate per individual lifetime) and, most importantly, r_{max} (intrinsic rate of natural increase per individual day)—effects of gradient alterations on a single parameter can be analysed against the somatic and reproductive hypervolume with a great deal more sensitivity than using traditional measures of somatic lethal limits.

The present paper will consider methods, advantages and drawbacks of life table analyses of reproductive lethal limits. And, using the data of Hummon (1974), a means of testing levels of significance between relevant values will be proposed.

Methods and materials

Several things are prerequisite to success in this means of assessing tolerance limits. First and most obvious, females of the test organism must be able to be cultured one by one through an entire life cycle in the laboratory. Organisms with short life cycles are preferable to those with long life cycles and, other things being equal, those with a demonstrably important role in the functioning of an ecosystem are preferable to those whose role is less apparent. Next, the organism must have recognizable beginning and end points to its life cycle. The time of hatching or birth is an acceptable beginning point, though the time of egg laying, fertilization or initiation of embryonic development is preferable. The end point is generally not so much of a problem, except that some reliable criterion of death must be established in order for there to be consistency within mortality data. And next, the number and beginning times must be discernible for offspring of each female. It is most convenient to construct a life table based on females alone, but where dioecious organisms are concerned, this carries with it the assumption that offspring of both sexes are produced in equal numbers and also the necessity of determining whether, in fact, the assumption is valid. Care must be taken that one or more sexually mature males be available from the onset through the entire period of female reproductive maturity, since the combination of an immature male and a mature female retards reproductive effort and gives misleading results. Application of these techniques to monoecious cross-fertilizing organisms is generally free of complications, except in instances where sexual maturity is non-simultaneous. In the latter case, organisms should be treated as if they were dioecious. The problems of sexes and the timing of maturation are absent from the relatively few monoecious self-fertilizing organisms or those which reproduce by most forms of parthenogenesis, as all of these individuals can be considered in the life table, all being female. Finally, the time interval over which life table data are to be collected must be adjusted to the speed of events occurring within the life history itself.

Our own work has been with the minute (150 µm long) but widely distributed freshwater gastrotrich, *Lepidodermella squammata* (Dujardin, 1841). This organism is easily cultured, has a short life cycle, is an obligate parthenogen, possesses relative genetic constancy and has an important role in ecosystem energetics, since it is a detrital and bacteriophagous secondary herbivore.

The procedures used by Hummon (1974) are briefly outlined below. A commercial culture of L. *squammata* was obtained from Connecticut Valley Biological Supply (Southampton, Massachusetts), in order that both this and future work could be based on the same culture stocks. Animals were cultured individually in depressions of multidepression slides, transfers being made of newly hatched juveniles by means of a mouth pipette. All animals cultured under a given set of conditions were housed together in an evaporation resistant box and all such boxes were kept in a constant temperature chamber. Depressions were stocked with food as their animals were being prepared for culture, using a dilute baker's yeast suspension made up in the appropriate control or test medium. Test media included .05, .5, 5 and 50 ppm DDT in final solution. Life history data were obtained at 0.5 day intervals for more than 50 individuals each per set of control and test conditions. Particular attention was paid to times at which eggs were laid, number of eggs laid, times at which juveniles were hatched and transferred, and times of deaths.

TABLE 1

Selection pressures on the gastrotrich *Lepidodermella squammata* associated with increasing levels of DDT and derived from life table parameter values for e_x, R_0 and r_{max} based on complete sets of cultured individuals (data from Hummon, 1974).

Conditions	e_x	W_{e_x}	s_{e_x} %	R_o	W_{R_o}	s_{R_o} %	r_m	W_{r_m}	s_{r_m} %
Control	22.1	.97	3	4.36	1.00	0	.36	1.00	0
DDT, .05 ppm	22.8	1.00	0	4.01	.92	8	.32	.90	10
DDT, .5 ppm	17.4	.76	24	3.34	.77	23	.23	.64	36
DDT, 5 ppm	7.3	.32	68	.83	.19	81	—.03	—.09	109
DDT, 50 ppm	1.2	.05	95	.00	.00	100			

e_x Mean life expectancy in days from time egg is laid
R_o Net reproductive rate per individual lifetime
r_m Intrinsic rate of natural increase per individual day
W Fitness relative to maximum e_x, R_o or r_m observed, respectively,
s. p. 100 Selection coefficient [(1 - W)100] expressed as percent dibility associated with conditions under which a population lived.

After shifting the mean duration of embryonic development to a point prior to hatching of the juveniles, day zero was taken to be the beginning of the embryonic period. From mortality data (d_x = no. dying during age class from an original cohort of 1,000) obtained under control and test conditions, a series of survivorship columns (l_x = no. alive at beginning of age class from an original cohort of 1,000) were calculated, following the methods of Birch (1948) and Andrewartha and Birch (1954). These values were modified to provide a series of age distributions (L_x = no. alive at midpoint of age class) and summed from oldest to youngest age class to give the mean life expectancy in number of animal days yet to be lived by a newly laid egg (max. e_x, here termed simply $e_{x,} = \sum(t)L_x$, where t is 0.5, the length of each age class). This is the first of our life table parameters. Another series of columns were then set up based on age specific fecundity rates (m_x = no. eggs laid during age class per individual alive at end of age class), which were modified by the age distributions to show the contributions of age class fecundity rates and which were summed to provide the net reproductive rate per individual lifetime ($R_o = \sum L_x m_x$), our second life table parameter. And, by setting $\sum e^{-rx} L_x m_x = 1$ and solving for r (r_m or r_{max} = intrinsic rate of natural increase per individual day), we have our third and final life table parameter.

Table 1 reviews the results of Hummon (1974), presenting values of e_x, R_o and r_{max} for control and test conditions and analysing differences between values for each life table parameter in terms of relative fitnesses (W) and selection coefficients (s). For each of the life table parameters, selective pressures can be seen to increase with increasing levels of DDT, the one exception being in e_x values where maximal life expectancy is slightly higher under conditions of .05 ppm DDT than under control conditions.

The question that presents itself is at what point in considering each of these life table parameters are differences between the values statistically significant. In answer to this question, we have here split the individuals forming a complete life table population into two halves, one half comprised of individuals showing high Reproductive Indices and one half comprised of individuals showing low Reproductive Indices. In cases where an odd number of individuals formed the original life table population, that individual showing the median Reproductive Index is omitted. The Reproductive Index [RI = Σ(I/time *i*th egg laid - time parental egg laid)] was formulated by Faucon and Hummon (1976) as a means of identifying individuals whose life history characteristics, early and numerous reproductive efforts, would contribute to a high intrinsic rate of natural increase. From each of the resulting split populations new life tables are drawn up and e,, Ro and r_{max} values calculated. Use of this technique increases from one to three the estimates of life table parameter values for each of several sets of conditions. A One Way, Model I Analysis of Variance is then conducted on the results—sequentially removing data for sets of conditions least to most like those of the control conditions—until no significant difference ($P > .10$) occurs among values of the remaining data sets. The .10 level of significance was chosen prior to any calculations and is justified over the more usual .05 level because of the extreme conditions imposed by splitting the complete life table population into halves which exhibit maximal differences from one another.

RESULTS

Fig. 1 shows the graphic relationship between estimates of life table parameter values, based on life tables calculated from complete and split sets of individuals for control and test conditions. Estimates based on complete life table populations (horizontal bars) always lie between the extremes (vertical bars) derived from split life table populations. Only two anomalies appear, one being the asymmetric differences between estimates of R_o for the control condition based on split as opposed to complete life tables and the other being the minute range of differences between estimates of R_o for the .05 DDT condition based on split life tables.

In Table 2, the results of Analyses of Variance are set forth. It can be seen that in each case, sequential removal of data from 5 and .5 ppm DDT conditions is necessary before significant differences can no longer be determined at the .10 level among the remaining data sets. Thus, the null hypothesis must be accepted that there is no statistically discernible difference only between populations cultured under control conditions and conditions of .05 ppm DDT with respect to e_x, R_o or r_{max} parameters. Of course, the most important of these is the r_{max} parameter, since it tends to integrate both of the others into a single parameter.

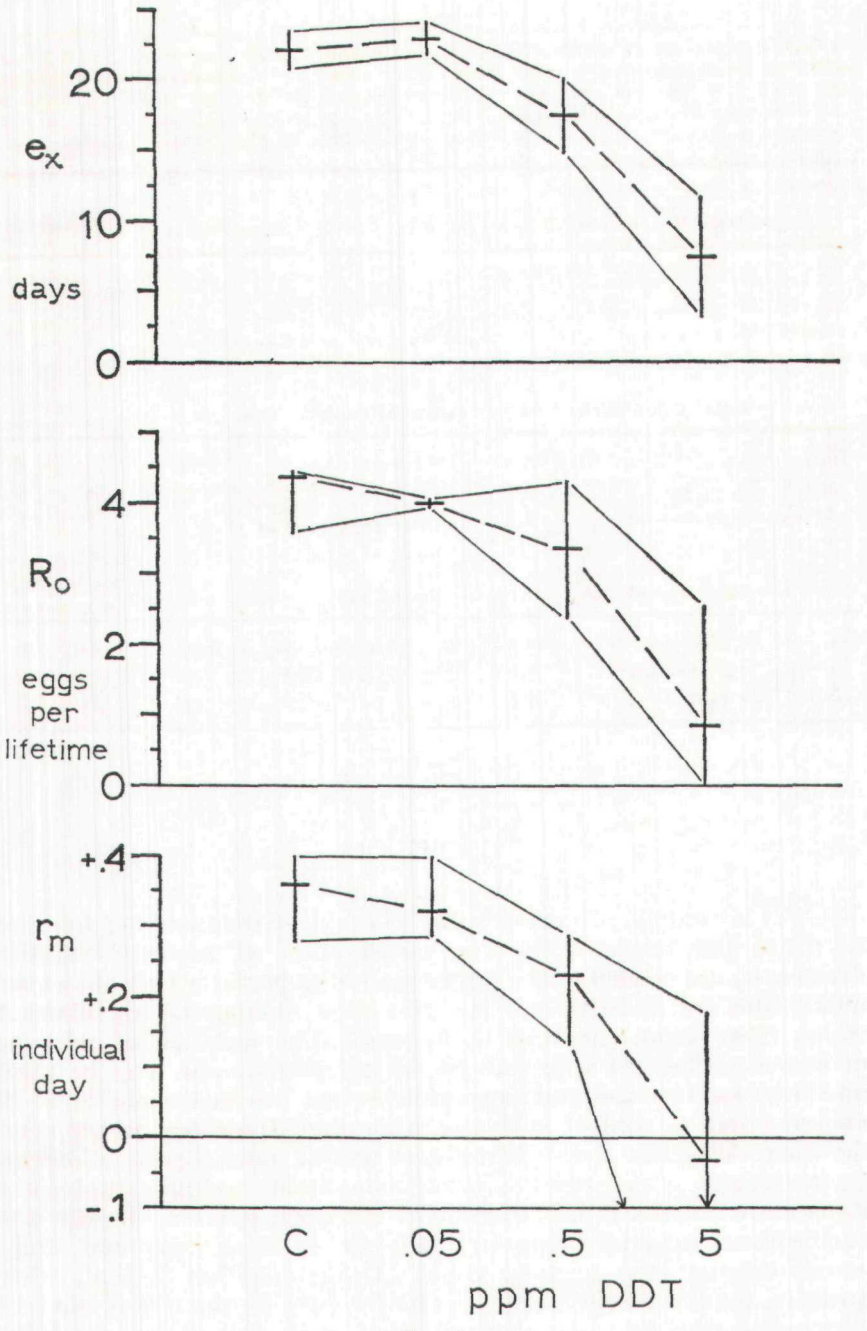

FIG. 1.

Graphie relationship between data values of three life table parameters—e_x, R_o and r_{max}—for *Lepidodermella squammata* cultured under control, .05, .5 and 5 ppm DDT in final solution.

Horizontal bars (connected by dashed lines) represent life table parameter values based on complete sets of cultured individuals. Vertical bars (upper and lower ends connected respectively by solid lines) represent the range of life table parameter values resulting from a split of cultured individuals into two halves, an upper half comprising data from individuals showing high Reproductive Indices and a lower half comprising data from individuals showing low Reproductive Indices.

TABLE 2

Output from a series of One Way, Model I Analysis of Variance (ANOVA) tests on three estimates of life table parameter values for *Lepidodermelia squammata* under each of several sets of conditions—sequentially removing data from sets of conditions least to most like those of the control conditions—until no significant difference (P > .10) occurs among values of the remaining data sets.

Data sets included in ANOVA	df	Calculated F value	Table F value	Probability of occurrence
		e_x days		
Control, .05,. 5, 5 ppm DDT	3,8	F = 21.7	$F_{.001} = 15.8$	.001 > P
Control, .05, .5 ppm DDT	2,6	F = 7.79	$F_{.025} = 7.26$	.025 > P
Control, .05 ppm DDT	1,4	F = .547	$F_{.50} = .549$	P > .50
		R_o eggs per lifetime		
Data sets included in ANOVA	df	Calculated F value	Table F value	Probability of occurrence
Control, .05,. 5, 5 ppm DDT	3,8	F =8.38	$F_{.01} = 7.59$	.01 > P
Control, .05, .5 ppm DDT	2,6	F = 9.33	$F_{.025} = 7.26$	.025 > P
Control, .05 ppm DDT	1,4	F = .208	$F_{.50} = .549$	P > .50
		r_m individual day		
Data sets included in ANOVA	df	Calculated F value	Table F value	Probability of occurrence
Control, .05, .5,5 ppm DDT	3,8	F = 13.6	$F_{.005} = 9.60$	.005 > P
Control, .05,5 ppm DDT	2,6	F = 3.55	$F_{.10} = 3.46$	.10 > P
Control, .05 ppm DDT	1,4	F = .041	$F_{.75} = .117$	P > .75

DISCUSSION

The advantage of demographic life table assessments of tolerance limits is the relevance to the perpetuation of populations; their drawbacks are related to the time required to perform both the experiments and calculations and the restricted number of organisms to which these techniques can be applied. The question of relevance of any experimental approach to natural populations may be raised and the case for laboratory experimentation lies in the ability of the experimenter to control variables other than those few under consideration. Whereas direct field assessment of natural populations and their changes under conditions of suboptimal environmental conditions or pollutants is an objective of research, it often is quite difficult without adjunct laboratory data to establish clear and simple causal relationships in field work. Our contention is that, where possible, the laboratory analysis is better done by the use of life table data on somatic and reproductive lethal limits rather than by means of the more traditional LD_{50} or LC_{50} somatic lethal limits.

In this paper, we have provided a means whereby differences in values of the life table parameters e_x, R_o and r_{max} related to differing experimental conditions, can be assessed statistically. In the case of *Lepidodermelia squammata*, we have demonstrated that statistically significant alterations of longevity and reproductive rate occur with concentrations of DDT at 5 ppm and above.

Summary

In view of the perpetuation of species, it is relevant to assess tolerance to suboptimal environmental conditions or pollutants in reproductive as well as somatic terms. Demographic life history data, integrated by means of life table calculations, provide sensitive measures of such information as e_x (mean life expectancy), R_0 (net reproductive rate per individual life time) and, most importantly, r_{max} (intrinsic rate of natural increase per individual day). Effects of increasing concentrations of DDT on life table parameters (Hummon, 1974) are further analysed for the common freshwater gastrotrich *Lepidodermella squammata.* These obligate parthenogens had been cultured individually in slide depressions, each juvenile being transferred to its own depression upon hatching. Life history data were collected at 0.5 day intervals for more than 50 individuals, each under control and test conditions.

Previous results showed that e_x, R_0 and r_{max} all decreased with increasing concentrations of DDT and that even at .05 ppm DDT there was a 10 p. 100 selective disadvantage in r_{max}, The question of assigning statistical significance to the results of life table data has now been resolved by splitting the data into two halves and calculating two new life tables for each set of conditions. This technique increases from one to three the estimates of life table parameter values for each of several sets of conditions. A One Way, Model I Analysis of Variance is then conducted on the results—sequentially removing data for sets of conditions least to most like those of the control conditions—until no significant difference ($P > .10$) occurs among values of the remaining data sets. In this case, for each of the three life table parameters considered—e_x R_o and r_{max}—there were significant differences among data from control, .05, .5 and 5 ppm DDT conditions and among data from control, .05 and .5 ppm DDT conditions, but not among data from control and .05 ppm DDT conditions.

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