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Dellar, M.E.; Boerlijst, S.P.; Holmes, D.S.T.

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RESEARCH ARTICLE

Improving estimations of life history parameters of small animals in mesocosm experiments: A case study on mosquitoes

Martha Dellar[1,2] | Sam P. Boerlijst[1] | David Holmes[3]

[1]Institute of Environmental Sciences, University of Leiden, Leiden, The Netherlands

[2]Deltares, Utrecht, The Netherlands

[3]Mathematical Institute, University of Leiden, Leiden, The Netherlands

Correspondence
Martha Dellar
Email: m.e.dellar@cml.leidenuniv.nl

Abstract

1. Mesocosm experiments enable researchers to study animal dynamics, but determining accurate estimates of survival and development rates of different life stages can be difficult, especially as the subjects may be hard to sample and mortality rates can be high. We propose a new methodology for estimating such parameters.

2. We used an experimental set-up with 48 aquatic mesocosms, each with 20 first instar mosquito larvae and under 1 of 12 treatments with varying temperatures and nutrient concentrations. We took daily subsamples of the aquatic life stages as well as counting the emerging adults. We developed a method to estimate the survival and development probabilities at each life stage, based on optimising a matrix population model. We used two different approaches, one assuming the difference between predictions and observations was normally distributed, and the other using a combination of a normal and a multinomial distribution. For each approach, the resulting optimisation problem had around 100 parameters, making conventional gradient descent ineffective with our limited number of data points. We solved this by computing the formal derivatives of our matrix model.

3. Both approaches proved effective in predicting mosquito populations over time, also when compared against a separate validation dataset, and the two approaches produced similar results. They also both predicted similar trends in the survival and development probabilities for each life stage, although there were some differences in the actual values. The approach which only used the normal distribution was considerably more computationally efficient than the mixed distribution approach.

4. This is an effective approach for determining the survival and development rates of small animals in mesocosm experiments. We have not found any other reliable methodology for estimating these parameters, especially not from incomplete data or when there are many different experimental treatments. This methodology enables researchers to gain a much more detailed understanding

of the life cycles of small animals, potentially leading to advances in a wide range of areas, for example in mosquito-borne disease risk or in considering the effects of biodiversity loss or climate change on different species.

1 | INTRODUCTION

Controlled mesocosm experiments enable researchers to understand the mechanisms driving ecological processes. They provide an opportunity to focus on certain aspects of a system without additional complicating factors. They have been described as a middle ground between a highly controlled but ultimately unrealistic laboratory study, and a highly realistic but high variance and difficult-to-interpret field study (Semlitsch & Boone, 2009). Experimental studies on aquatic animals are often conducted in aquatic mesocosms, usually a pond or container filled with water and a community comprised of selected species, subjected to natural conditions. Such experiments have enabled researchers to study a huge variety of factors affecting the dynamics of insects, fish and other small species, such as predation, responses to different chemicals, competition effects or climatic effects (e.g. Chase & Shulman, 2009; Ng'habi et al., 2018; Schrama et al., 2018). These types of studies are often used when considering wider issues. For example, models of mosquito-borne disease risk generally use estimations from such experiments (e.g. Beck-Johnson et al., 2017; Ellis et al., 2011). It is therefore vitally important that the parameters taken from these studies are as precise as possible. Similarly, mesocosm data have been used to parametrise models on biodiversity monitoring (Pfrender et al., 2017), effects of pesticides on wildlife (Kattwinkel et al., 2016) and the ecological effects of climate change (Fordham, 2015), all areas of concern where accurate estimations are highly important.

To achieve a full understanding of aquatic animal development, it is helpful to be able to monitor their different aquatic life stages. For example, mosquitoes lay eggs (oviposit) in water, which hatch into first instar larvae (L1), then develop through the L2, L3 and L4 stages before becoming pupae and then finally emerging as adults. If we want to fully understand mosquito dynamics and how these are affected by various external factors, it is helpful to know the development and survival rates for each aquatic stage. These can be very difficult to measure in a mesocosm experiment, since we are dealing with very small, highly mobile organisms in a relatively large area, which tend to dive while feeding or if they are disturbed (Merritt et al., 1992; Sih, 1986; Workman & Walton, 2003). Detection may be further complicated by the presence of vegetation or particulates in the water.

Previous studies have dealt with this issue in different ways. Some only count adult mosquitoes and measure the time from oviposition or hatching to emergence, without monitoring the intervening stages (Chase & Knight, 2003; Schuler & Relyea, 2018). Others remove all or some of the mosquitoes after a set amount of time to see what life stage they are at. In some cases these are then returned to the mesocosm (Duchet et al., 2017; Petranka & Doyle, 2010) and in others not (Buxton et al., 2020; Silberbush et al., 2005). If they are returned then the sampling process may affect their survival and have consequences for the validity of the experimental results, but if they are not returned there is no chance to collect information on their subsequent development. Knight et al. (2004) avoids this issue entirely by regularly visually inspecting each mesocosm and counting the number of mosquitoes at each life stage, without physically interacting with them. While this would seem to give the most complete results, it is rather time-consuming and prone to errors. It is also unlikely to be effective in mesocosms where the water is not very clear or where there are obstructions (e.g. vegetation), especially since larvae might naturally move across the water column to feed (Merritt et al., 1992).

Regardless of the counting method used, it is then necessary to estimate the survival and development probabilities from the resulting data. This is straightforward with all aquatic life stages grouped together (e.g. Schrama et al., 2018), but becomes more complicated when calculating estimates for multiple distinct aquatic life stages. Knight et al. (2004) also looked at mosquitoes and had the advantage of complete counts at each life stage on each day, which is not possible for many experiments. However, they assumed that all individuals develop at an average rate and to compute this one needs to know the number of individuals that passed through each life stage. If all individuals survive the experiment then this is straightforward, but if there is high mortality, as was the case in some of their experiments, then it is very important to understand at which life stage(s) this mortality occurred, which is not deducible from the data. This demonstrates that estimating these parameters is a difficult problem even when complete data are available. Grant et al. (2020) estimate the survival probabilities of monarch butterflies. While this is not a mesocosm experiment, it involves similar considerations. They sample a small proportion of the population and use a Bayesian state-space model to estimate the survival probabilities of different development stages. This appears to work well, but is dependent on the developmental rate of the different life stages being known and used as a model input. In addition, we expect that the optimisation routines they use would fail to converge if they were interested in a larger number of output parameters—for example, if one wanted to understand the effects of multiple experimental treatments. In

de Valpine and Knape (2015) and Knape and de Valpine (2016), they use a Monte Carlo algorithm to deduce Bayesian estimates of development and mortality rates in stage-structured cohort models for grasshopper and brine shrimp populations. This is a promising method, allowing for variance in the development and mortality of individuals and also for unobserved life stages. As long as the number of parameters to be estimated is not too large it should work well, although it is extremely computationally intensive. However, if there are a large number of parameters (e.g. if there are many different experimental treatments) then their algorithm is unlikely to converge. We have not found any reliable method in the literature for estimating both survival and development rates of intermediate life stages based on incomplete data, especially when a significant number of treatments are considered or when there is significant mortality. A more sophisticated statistical technique is required to accurately estimate these life history parameters.

We propose a new methodology for determining the development and survival rates of small animals at different life stages from subsampled data in mesocosm experiments. By small we indicate that they are elusive due to their size, although behaviour or high mobility could also result in suboptimal detection rates and this method would be applicable. Our method involves sampling a portion of the mesocosm population in a way which minimises disruption to the organisms involved. We then develop a matrix population model, where for given survival, development and detection probabilities, it predicts the number of animals at a given life stage observed each day. Finding the probabilities that maximise the likelihood of the observed values is then a problem in numerical optimisation.

We use mosquitoes as a case study, but the methodology is applicable to any mesocosm experiments studying the dynamics of small, difficult to count animals with multiple life stages, such as insects, fish or amphibians.

2 | METHODS—EXPERIMENTAL SET-UP

2.1 | Study site

Two mesocosm experiments were carried out under field-like conditions at the Living Lab field station of Leiden University, The Netherlands. The main experiment took place in May–June 2020 and provided data for model parameterisation. The second experiment in August–October 2020 provided data for model validation. Both experiments explore the separate and interactive effects of temperature and eutrophication on the Northern house mosquito *Culex pipiens* s.l. life-history traits, as part of a series of experiments on food availability.

2.2 | Treatments

The experiments were carried out in 65-litre black polyethylene tubs filled with 30 litres of dechlorinated tap water, which was left for 48 hours prior to the experiment. To buffer temperature fluctuations, each mesocosm was placed in a second, fully buried identical tub, thus providing an air-filled layer of insulation (Krol et al., 2019). This served to prevent absorption of external heat and dissipation of internal heat. Each mesocosm was spiked with a standardised concentration of algae and bacteria, acquired by filtering water from a neighbouring lake through a 53 μm plankton net bucket, so that each litre of water in the mesocosms contained a concentration of bacteria equal to that found in a litre of water in the lake. To simulate the different levels of eutrophication covering the oligotrophic–hypertrophic range, cow manure (2.4% N; 1.5% P_2O_5; 3.1% K_2O) was added. This served to mimic oligotrophic, low eutrophic, highly eutrophic and hypertrophic water bodies (0 mgL^{-1}, 10 mg/L, 20 mg/L and 100 mg/L N-total respectively) within the main experiment and oligotrophic, highly eutrophic and hypertrophic water bodies (0 mg/L, 20 mg/L and 100 mg/L N-total respectively) within the validation experiment (Loeb & Verdonschot, 2009). The temperature was regulated by 200 W aquarium heaters (HSaqua) controlled by a timer calibrated to the natural 14-hour daylength. The temperature within the main experiment was kept constant at 20, 25 and 30 °C, whereas the temperature within the validation experiment was kept at 20 °C. All treatment combinations were allocated in a full-factorial random block design and replicated four times, they are summarised in Table 1 and a full description is included in the supplementary materials. For the validation experiment, treatments 1, 7 and 10 were used. Each mesocosm was covered with an emergence trap (Cadmus et al., 2016) to prevent natural colonisation. The mesocosms were thereafter left to acclimate for 2 weeks so that the bacterial communities could stabilise.

2.3 | Rearing and allocation of larvae

Culex pipiens s.l. egg rafts were collected during the 4 days prior to the start of the experiments from naturally colonised black plastic buckets filled with 6 litres of 100 mg N-total (hypertrophic) ditch water. The larvae were subsequently allowed to hatch in a white plastic bucket containing 10 litres of lake water where they were kept at ambient temperature until the start of the experiment.

At the start of the experiment, 2 weeks after acclimatisation, all water within the mesocosms was filtered with a 300um sieve. This served to remove any hatched macro-invertebrates and to prevent the sieves from clogging during the identification of mosquito life

TABLE 1 Overview of the main experiment treatments

Treatment	1	2	3	4	5	6	7	8	9	10	11	12
N total (mg/L)	0	0	0	10	10	10	20	20	20	100	100	100
Temperature (°C)	20	25	30	20	25	30	20	25	30	20	25	30

stages during the experiment. Twenty first instar larvae were thereafter randomly selected and added to each mesocosm. The tubs were covered with emergence traps (Cadmus et al., 2016) to prevent (a) colonisation by natural populations, (b) predators from entering and (c) emerged adults from escaping. The low larval density served to exclude potential effects of density dependence (Alcalay et al., 2018). The water level was kept stable by daily replenishing the evaporated volume after the measurements were taken.

2.4 | Measurements and life stage identification

The temperature in each mesocosm was recorded every 15 minutes for the duration of the experiment by a temperature logger (iButton DS1921G#F5D). Larval development was measured 5 days a week by stirring clockwise once with a 40 mm wide 200um sieve to create a current which prevents the larvae from diving. The sieve was subsequently used to collect the larvae by fully submerging the sieve and moving anti-clockwise twice. All collected larvae were morphologically identified to developmental stage by using the size of the head capsule as a morphological indicator (Becker et al., 2010). The identifications were compared daily with a previously reared reference collection of *Cx. pipiens* developmental stages to ensure consistency. The procedure was repeated up to five times until at least five larvae were sampled. At the end of the experiment, the contents of all mesocosms was filtered through a 250um plankton net and all remaining larvae and pupae were counted and identified to developmental stage.

3 | METHODS—MODELLING AND STATISTICAL ANALYSIS

3.1 | Model outline

An example of the results measured in a single mesocosm is shown in Table 2. It is unknown what total number of larvae and pupae were present in the mesocosms on days 1 to 8 since not all were sampled and there will have been some mortality, either due to natural causes or cannibalism.

We model the progression of the mosquitoes through each life stage using a matrix population model. The population at each life stage on day i is represented by a vector P_i:

$$P_i = \begin{bmatrix} P_i^{L2} \\ P_i^{L3} \\ P_i^{L4} \\ P_i^{P} \\ P_i^{A} \end{bmatrix}.$$

In particular, $P_o = [20, 0, 0, 0, 0]$, as we always start the experiment with 20 L2 larvae (while L1 larvae are initially placed in the mesocosms, it is

TABLE 2 Example of data from a single mesocosm (taken from the main experiment, mesocosm 9, treatment 2). No data were collected on day 2. Bold numbers (i.e. 'Day 9' and 'Adults') indicate certainty about the count. Italic numbers indicate a subsample of the total population

Day	L2 larvae	L3 larvae	L4 larvae	Pupae	Adults
1	*2*	*1*	*2*	*0*	**0**
3	*2*	*4*	*1*	*0*	**0**
4	*0*	*5*	*3*	*0*	**0**
5	*0*	*0*	*2*	*9*	**0**
6	*0*	*0*	*0*	*11*	**1**
7	*0*	*0*	*0*	*2*	**12**
8	*0*	*0*	*0*	*0*	**13**
9	**0**	**0**	**0**	**0**	**14**

assumed that they all survive and develop into L2 by the time the experiment begins, since this only takes around 2 days (Loetti et al., 2011), there was no predation and they had sufficient food).

The probabilities of surviving a given day for a particular life stage are given by s^{L2}, s^{L3}, s^{L4} and s^{P}. The probabilities of developing from one life stage to the next on a given day are given by d^{23}, d^{34}, d^{4P} and d^{PA}. These can be combined to produce an expression for the population P_{i+1} on day $(i + 1)$ from P_i, where for each life stage the value is the survivors from the previous day, plus those which have developed from the previous life stage, minus those which have progressed to the subsequent life stage ('*' indicates elementwise multiplication):

$$P_{i+1} = \begin{bmatrix} P_{i+1}^{L2} \\ P_{i+1}^{L3} \\ P_{i+1}^{L4} \\ P_{i+1}^{P} \\ P_{i+1}^{A} \end{bmatrix} = P_i \begin{bmatrix} s^{L2} \\ s^{L3} \\ s^{L4} \\ s^{P} \\ 1 \end{bmatrix} + P_i \begin{bmatrix} 0 \\ s^{L2} \\ s^{L3} \\ s^{L4} \\ s^{P} \end{bmatrix} * \begin{bmatrix} 0 \\ d^{23} \\ d^{34} \\ d^{4P} \\ d^{PA} \end{bmatrix} - P_i \begin{bmatrix} s^{L2} \\ s^{L3} \\ s^{L4} \\ s^{P} \\ 0 \end{bmatrix} * \begin{bmatrix} d^{23} \\ d^{34} \\ d^{4P} \\ d^{PA} \\ 0 \end{bmatrix}.$$

This can be simplified to the following

$$P_{i+1} = \begin{bmatrix} s^{L2}(1-d^{23}) & 0 & 0 & 0 & 0 \\ s^{L2}d^{23} & s^{L3}(1-d^{34}) & 0 & 0 & 0 \\ 0 & s^{L3}d^{34} & s^{L4}(1-d^{4P}) & 0 & 0 \\ 0 & 0 & s^{L4}d^{4P} & s^{P}(1-d^{PA}) & 0 \\ 0 & 0 & 0 & s^{P}d^{PA} & 1 \end{bmatrix} P_i.$$

This model produces predictions for the population at each life stage on each day, for a given set of survival and development probabilities.

3.2 | Simple (all-normal) approach

Larvae and pupae are small and highly mobile, meaning we must account for their detection probabilities before trying to optimise our

survival and development probabilities. We assume that, in sampling from the mesocosm, there is only a moderate probability (say c^{L2}) that we will capture and count any given L2 larva, and we similarly define c^{L3}, c^{L4} and c^P. We assume the detection probability of an adult (c^A) is one (adults are easy to count), and that these probabilities are constant across the different mesocosms and treatments, so that we only have to introduce four new parameters into our optimisation problem (which already has 2x48 parameters, the development and survival probabilities for each of 4 life stages and 12 treatments). We multiply our predicted mosquito populations at each life stage by our detection probabilities, that is,

$$\text{Predicted detected population on day } i = \begin{bmatrix} c^{L2} \\ c^{L3} \\ c^{L4} \\ c^P \\ 1 \end{bmatrix} * \begin{bmatrix} P_i^{L2} \\ P_i^{L3} \\ P_i^{L4} \\ P_i^P \\ P_i^A \end{bmatrix}.$$

Given values for our 100 parameters (96 development and survival probabilities and 4 detection probabilities), we can make predictions for the number of mosquitos detected at each life stage and each day of each treatment. Our optimisation problem is then to find the values for these parameters which minimises the sum of the squared differences between the predicted and observed populations. This corresponds to the maximum likelihood estimator for the parameters, assuming that the output errors of our model are normally distributed as functions of their input error. The errors will not be perfectly normally distributed and it may be that a different distribution would be more appropriate, but this involves a relatively simple calculation and provides a useful baseline for more complex approaches. In Section 3.3 we describe a more realistic model of the error.

At this point we have a standard optimisation problem: find the values of the parameters which minimise the total error. However, with 100 parameters and limited data points, standard algorithms have little chance of success. The best use gradient descent, but simply estimating the gradient of the error function at a single point requires 101 evaluations of the model, and does not give very good results because of the complexity of the functions coming from a matrix model run for around 20 days. As a result, standard numerical techniques were unable to solve this optimisation problem.

The key realisation that transforms this approach into a practical technique is that the error function we are trying to minimise is just a polynomial function in the input variables, as it is created solely through addition, multiplication and subtraction. This means that it is formally differentiable (as opposed to merely numerically differentiable, which in this case would be around 100 times slower (as there are 100 parameters)). It is not a small polynomial; it is of degree 44 in 100 variables; but this is not beyond the capabilities of symbolic algebra packages. The Python SymPy package (Meurer et al., 2017) was able to explicitly write down and evaluate the polynomial in under 5 minutes; computing all 100 derivatives took another 3 hours. With the help of these derivatives we were able

to optimise our model via gradient descent (using the L-BFGS-B algorithm within the Python SciPy module (Virtanen et al., 2001)) in 42 minutes. All this was performed on a standard laptop (6 cores, 12 logical processors, 32GB RAM).

3.3 | A more realistic approach (mixed distribution)

Our previously stated assumption that model error is normal may not be true in all cases. It would be more realistic to find those parameter estimates which maximise the likelihood of seeing the observed data assuming that mosquitos (larvae and pupae) are sampled with replacement from the total population, that is, assuming the error follows a multinomial distribution. Counting of adult mosquitoes was always reliable, so assuming a normal error there is reasonable. The same applies to the final day's results, as full counts rather than subsampling were performed. Therefore, this approach uses a multinomial distribution to calculate the error in larvae and pupae counts with the exception of the final day (italic values in Table 2), and a normal distribution for the adult counts and the final day (bold values in Table 2).

We again need to account for the difficulty of detecting larvae and pupae. We are now trying to use a method which is as realistic as possible, and so we are not going to use the simple detection probabilities which were used in the all-normal approach. Mosquitoes were sampled until at least five were found, which only really gives us information about the relative numbers of mosquitoes, rather than the absolute numbers. Therefore, a realistic approach is to use relative frequencies. We define the frequency of sampling a pupa (r^P) as one, and estimate the relative frequencies of sampling larvae (r^{L2}, r^{L3}, r^{L4}) relative to this. Since the adult count data are analysed separately from the larvae and pupae data and the adult counts are considered reliable, we do not have to adjust these data to account for the probability of detection. Predicted larval counts ($P_i^{L2}, P_i^{L3}, P_i^{L4}$) are multiplied by their respective relative frequencies (r^{L2}, r^{L3}, r^{L4}) before calculating the log likelihoods.

Note that because we use relative rather than absolute frequencies and treat adult predictions differently to larvae and pupae predictions, it is not possible to produce predicted *detected* populations, only predicted populations. This is because we have no way of knowing the larvae and pupae numbers relative to the adult numbers.

The values to be optimised were the 96 treatment-specific *s* and *d* values, the three relative frequencies for detection (r^{L2}, r^{L3}, r^{L4}) and the standard deviation (σ) for the normal distribution. Log likelihoods were calculated for each replicate and combined to produce a total log likelihood (see section 3.4 for details). This total was made negative and then minimised using the L-BFGS-B algorithm, to find the values of *s, d, r* and σ which maximised the log likelihood. To calculate the log likelihoods, the following formulae were used:

Normal distribution (adults and final day):

$$\text{Loglikehood} = \frac{1}{2}\log 2\pi\sigma^2 - \frac{1}{2\sigma^2}\left(O_j - P_j\right)^2, \quad j \in \{L2, L3, L4, P, A\}.$$

Multinomial distribution (L2, L3, L4 and pupae for all days except final):

$$\text{Log likelihood} = \log\left(\frac{N!}{O_{L2}!\,O_{L3}!\,O_{L4}!\,O_P!}\prod_{j\in\{L2L3L4P\}}P_j^{O_j}\right),$$

where $O_{L2}, O_{L3}, O_{L4}, O_P, O_A$ are the observed values for each life stage from a given day in a given replicate; $P_{L2}, P_{L3}, P_{L4}, P_P, P_A$ are the equivalent predicted values; and $N = O_{L2} + O_{L3} + O_{L4} + O_P$.

While the predicted values (P_j) are polynomials, the total log likelihood is clearly not, and indeed SymPy was not able to formally compute its derivatives. To solve this, we observed that the log likelihoods above are formed by products, sums and composites of logarithms and polynomials. We could calculate the derivatives of the different parts separately. The derivatives of the P_j could be symbolically computed using SymPy (as in the all-normal approach). Formal derivatives of the log likelihood could then be computed by repeated applications of the chain rule for derivatives of composites. This was just enough to allow our optimisation to run; it required considerably more computing resources than the all-normal approach, taking in total around 21 hours on a high-performance computing cluster.

This approach will still not perfectly model the error distribution, but we have made an effort to make it as representative as possible of our sampling procedure. It is probably the best we can do while keeping the computations feasible. Also, multinomial assumptions are often made by researchers in these areas, so it seemed valuable to test the validity of this approach.

3.4 | Practical notes for implementation

Additional notes on how these methods were applied to our specific case are included in the supplementary materials.

3.5 | Comparing results

We compare the number of adults emerging over time for each approach and for the observed data. Because the mixed distribution approach uses relative frequencies rather than detection probabilities, it is not possible to produce predictions which are directly comparable with the observed data for the larval and pupal stages. Instead, for these stages we compare the detected proportions of each life stage present.

3.6 | Validation

Two validation tests were performed, one based on data from the second experiment and the other using simulated data. Using data from a separate experiment tells us if our estimated parameters are generally applicable. Three treatments were included in the validation experimental data, with 0, 20 and 100 mg/L nutrients at 20°C. The optimised survival and development probabilities for these treatments (calculated from data from the main experiment), as well as the optimised detection probabilities and relative frequencies, were used to determine the predicted population using both

approaches. These predictions were then visually compared with the data from the validation experiment.

The second validation test was a simulation analysis. We created 10 sets of biologically realistic survival, development and detection probabilities across 12 hypothetical treatments. This was done by making small changes to the estimates generated by the all-normal approach when applied to the data from the main experiment. These changes were randomly chosen within a range around the original values, with the range defined as ±50% of the distance between the value and either 0 or 1 (whichever was closer). These new sets of probabilities were taken to be 'true' probabilities for the purposes of this analysis. We then created a stochastic agent-based model which, given a starting number of mosquitoes, produced estimates of how the population might change over time, based on given survival and development probabilities (for full details see model code for creating inputs). We used this model to generate 10 sets of 'observed' data (with four replicates per treatment), one for each set of 'true' probabilities. We then applied our methodology using both the all-normal and mixed distribution approaches to these 'observed' datasets to see if we could recover the 'true' (input) survival and development probabilities. We compared the 'true' probabilities with the predictions using a two-way ANOVA (value ~ true/predicted + treatment). This showed us if there was a significant difference between the 'true' and predicted values and also if our predictions were good enough to detect differences between treatments.

4 | RESULTS

4.1 | Predicted populations

Figure 1 shows the predicted cumulative number of adult mosquitoes under each approach, as well as the numbers from each replicate of the experimental study. For all 12 treatments, both predictions fitted the data well. The final numbers of predicted adults fell roughly in the middle of those found in the replicate mesocosms and in most treatments adults began to emerge at around the same time in the observations and predictions. For the treatments with the lowest temperatures (20°C, treatments 1, 4, 7 and 10), the predictions showed a gradual emergence over time, while the observed data showed all adults emerging within a very short period. This sudden emergence coincided with a particularly warm period, which raised the mesocosm temperature above 20°C. Mesocosm temperature was controlled by a heater, but there was no way to cool it down if the ambient temperature rose above the target temperature. The predictions using each approach (all-normal and mixed distribution) tended to be very similar to one another.

Graphs showing the predicted larval and pupal populations can be found in the supplementary materials. Both approaches gave reasonable estimates for the detected proportions of larvae and pupae. For L2 larvae the predictions using the normal approach seemed slightly more representative of the observed data, but for pupae the mixed approach seemed better (at least for some treatments).
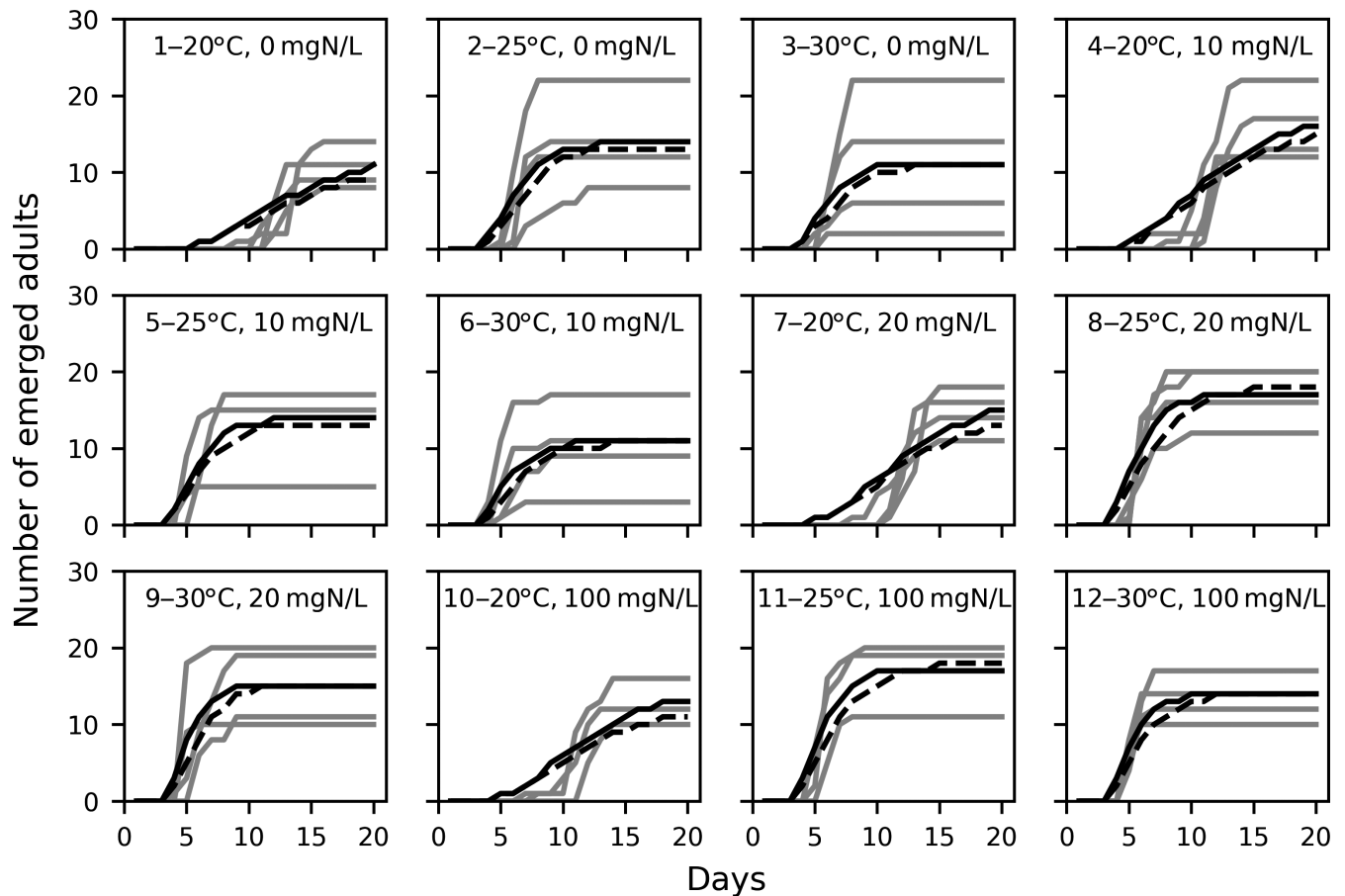
FIGURE 1 Cumulative numbers of emerged adults over the course of the experiment, for each of the 12 temperature and nutrient treatments. The solid black line shows the predictions under the all-normal approach; the dashed black line shows the predictions under the mixed distribution approach; and the grey lines show the measured data from each replicate mesocosm
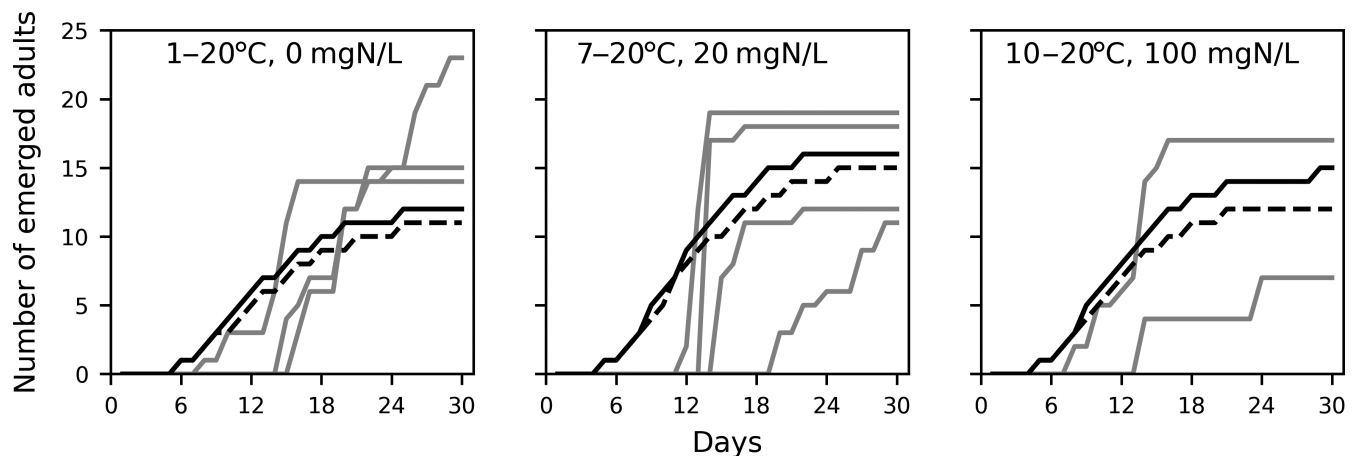

FIGURE 2 Cumulative numbers of emerged adults over the course of the experiment, for each of the validation treatments. The solid black line shows the predictions under the all-normal approach; the dashed black line shows the predictions under the mixed distribution approach; and the grey lines show the measured data from each replicate mesocosm

4.2 | Validation

4.2.1 | Experimental data

The number of emerging adults over time from the validation data was compared with the predictions from the two different approaches. The results are shown in Figure 2. For treatments 7 and 10, the final predicted number of adults was within the range found in the different replicates, while for treatment 1 the predictions were slightly lower than for all the replicates. In all cases, the predictions showed adults emerging earlier than happened in reality, particularly for treatment 7.

Equivalent graphs showing the detected proportions of the other life stages can be found in the supplementary materials. In general, predictions from both approaches are reasonably representative of the observed data. Both approaches suggest that L2 and L3 larvae develop slightly faster than they did in reality, but this seems to correct itself by the time they become L4 and pupae. For all life stages, neither of the two approaches appears to produce significantly better predictions than the other.

4.2.2 | Simulation analysis

For each approach (all-normal and mixed distribution) we derived sets of 'true' and predicted probabilities, each with one 'true' and one predicted value for each of the 12 treatments. There were 80 of these probability sets (2 probability types (development and survival) x 4 life stages x 10 sets of 'true' values). We performed a two-way ANOVA for each of these 80 sets of probabilities. For the all-normal approach, there was no significant difference (p < 0.05) between the true and predicted values in 72.5% of cases and a significant difference between treatments in 50.0% of cases. For the mixed distribution approach these were 71.3% and 55.0% respectively. In cases where there was a significant difference between the true and predicted values, there was still a significant difference between treatments 86.3% and 69.6% of the time for the all-normal and mixed distribution approaches respectively.

4.3 | Development and survival probabilities

We also compared the survival and development probabilities for each life stage across the different treatments and between the two approaches. The survival probability is the probability of surviving from 1 day to the next. The development probability is the probability of developing to the next life stage on any given day. The results for L3 larvae are shown as an example in Figure 3. Results for the other life stages can be found in the supplementary materials. In general, the two approaches found similar trends in the effects of temperature and nutrient concentration, although there was some variation in the values. For example, both approaches agreed that development probabilities generally increase with temperature, but the mixed distribution approach tended to predict higher development probabilities than the all-normal approach for the L2 and L3 life stages and lower development probabilities for the L4 and pupae stages. Both approaches agreed that pupae have very high survival rates, no matter what the conditions, but the mixed distribution approach predicted substantially lower survival probabilities for L4 larvae under the highest temperature treatment than the all-normal approach.

4.4 | Detection probabilities and relative frequencies

The detection probabilities and relative frequencies are shown in Table 3. These are not directly comparable, since they represent different quantities and are used in different ways. The detection probabilities represent the chance of sampling a mosquito at a given life stage, while the relative frequencies are a combination of the chances of detection and the total number present. The frequency for pupae was set to one and the others were calculated relative to this. Both the detection probabilities and the relative frequencies show that as mosquitoes get larger, they are easier to sample.

5 | DISCUSSION

Accurately estimating the life history parameters of aquatic animals such as mosquitoes can be of great importance (Moller-Jacobs et al., 2014; Tomé et al., 2014). We proposed two methods for determining the survival and development rates of the different life stages of aquatic animals. One was a simple model based on the normal distribution and the other assumed the data were a combination of normally and multinomially distributed and were designed to better represent the sampling procedure. Both methods produced reasonable results, with predicted populations closely fitting the observed data, both in the main and the validation experiment. The predictions showed a gradual adult emergence over time, while the observed data showed all adults emerging within a very short period. The least good fit was found in the lowest temperature treatments. The all-normal approach and the mixed distribution approach give very similar predictions and show similar trends in the predicted survival and development rates. Variation between predictions from the two methods is very small compared with the observed variation.

5.1 | Predicting adult emergence

The predicted adult emergence using each approach (all-normal and mixed distribution) tended to be very similar to one another, although it was more gradual than in reality. This was most noticeable in the lowest temperature treatments (20°C). Our model assumes that mosquitoes develop independently from one another, which will tend to favour gradual rather than sudden emergence. However, an external factor, such as a particularly sunny day, could cause many mosquitoes to emerge at once. This seems to be the case here, as the time of the sudden emergence in the lowest temperature treatments coincided with a particularly warm period, which raised the mesocosm temperature above 20°C.

When the predicted adult emergence was compared against a separate validation dataset, we again saw this gradual rather than sudden emergence in the predictions. These were also using the 20°C temperature treatment and so may have been subject to similar difficulties in keeping the temperature from rising too high, especially since this experiment was performed in late summer. In two of the three validation treatments the final predicted number of emerged adults was within the range found in the different replicates (Figure 2), indicating that the model produces reasonable estimates. For treatment 1 the predictions were slightly lower than all the replicates. This is because in the original experiment the
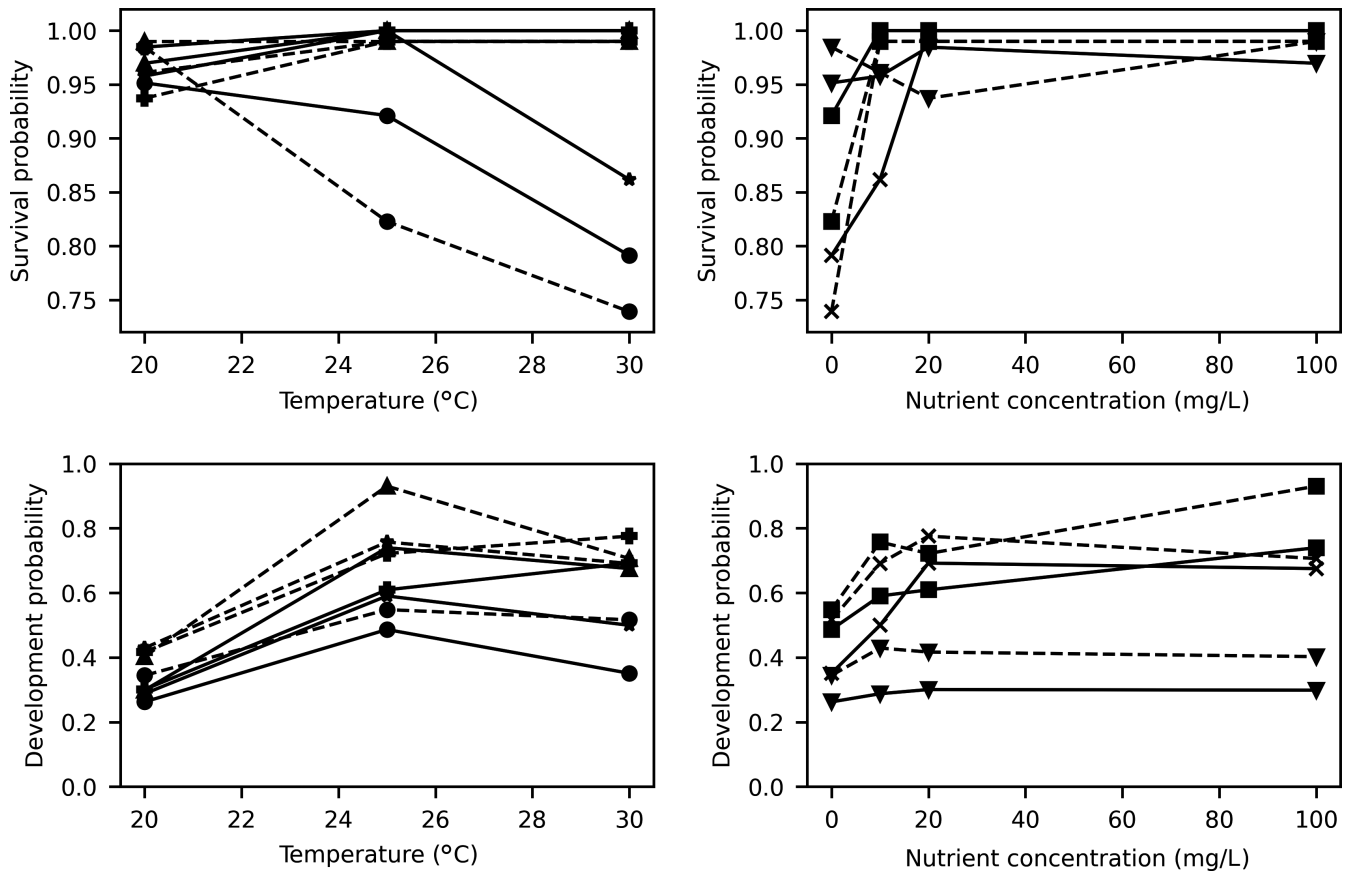
FIGURE 3 Survival (top) and development (bottom) probabilities for L3 larvae for different temperature (left) and nutrient (right) treatments. The symbols ●, ★, ✚ and ▲ represent the nutrient treatments 0, 10, 20 and 100 mg/L respectively. The symbols ▼, ■ and ✖ represent the temperature treatments 20, 25 and 30°C respectively. The solid lines show the probabilities under the all-normal approach; the dashed lines show the probabilities under the mixed distribution approach

TABLE 3 Detection probabilities for each life stage associated with the all-normal approach; and relative frequencies of each life stage associated with the multinomial part of the mixed distribution approach

Life stage	Detection probabilities	Relative frequencies
L2	0.21	0.17
L3	0.33	0.37
L4	0.64	0.81
Pupae	0.78	1

different replicates for treatment 1 generally had lower numbers of emerged adults than in the validation experiment. This may be a case of overfitting, but it should be noted that in all treatments there is a lot of variation between the different replicates. It is hard to tell from this amount of data whether this discrepancy is due to overfitting or is simply to be expected given the amount of variation involved. Of all the 12 treatments, 1, 7 and 10 were among those with the least good fit. These were the treatments used in the validation experiment and the results were still satisfactory, suggesting that the model is doing a reasonable job of predicting emerging adults.

5.2 | Predicting larval and pupal populations

While the predicted detected larval and pupal populations were mostly very similar, there were noticeable differences for the lowest temperature treatment. Here, the mixed distribution approach tended to predict lower proportions of L2 and L3 larvae, which is consistent with the finding that this approach tended to predict higher development probabilities for the early life stages. The same pattern was found when comparing predictions with the validation dataset. As previously discussed, there were some difficulties with maintaining the correct temperature for this treatment which may have affected the results. For other treatments, the predictions were very much in line with the observed data. Looking at the survival and development rates, we found that the two approaches showed similar trends in the effects of temperature and nutrient concentration, although there was some variation in the values. Comparing the proportions of the different larval and pupal populations, it appears that when there is a difference in the results of the two approaches, the normal approach is more effective for the earlier life stages, while the mixed approach is more effective for the later life stages. The earlier life stages were found to be harder to detect than the later stages. It is likely that when making predictions of organisms with

a relatively high detection probability, there are benefits to using a statistical methodology that is representative of the sampling procedure (i.e. our mixed distribution approach). However, when the organisms have a low detection probability, it is better to use a more general statistical methodology (i.e. our all-normal approach).

5.3 | Simulation analysis

The simulation analysis showed that for both approaches, our methodology usually produces no significant difference between predicted and true survival and development probabilities. It also is often able to identify significant differences between experimental treatments. We did not expect it to always find differences between treatments, since sometimes the true values are very close together and our method is not precise enough with this amount of data to distinguish between them. It should also be noted that the 'observed' data for this analysis were generated using a stochastic agent-based model which is very different from the matrix population model used in our optimisation procedure. That our methodology was nevertheless able to derive good approximations for the 'true' values demonstrates its efficacy.

5.4 | Comparing approaches

The multinomial distribution was chosen as it well represented the sampling procedure used, particularly when combined with the use of relative frequencies. On the other hand, the multinomial distribution assumes that all the variation is due to the sampling process rather than due to any other factors. The wide discrepancies between different replicates suggest that there were other factors in play. By using the normal distribution, we assumed some degree of variation which can be due to a wide range of factors, such as environmental disruptions, over-simplicity of the matrix model or the sampling process. Then again, a disadvantage of the normal distribution is that it puts some probability mass on fractional and negative values of mosquitoes, although it is relatively simple to correct for this when calculating predictions. There are arguments for and against both approaches, but given that they produced very similar results, none of the drawbacks seem to have had a significantly adverse effect and both approaches appear to be suitable.

From a practical point of view, the all-normal approach is considerably more computationally efficient. The mixed distribution approach required the use of a computing cluster (around 21 hours) while the all-normal approach could be performed on a conventional laptop (less than 4 hours). Modelling all parameters using a normal distribution proved to be sufficient in this case, however, this may not always be so, and we recommend that other researchers wanting to use this method also consider distributions which are representative of their sampling procedure, particularly if detection probabilities are high.

Considering alternative methods, Grant et al. (2020) used a Bayesian state-space model to estimate the survival probabilities of the different life stages of monarch butterflies. They assume that developmental rates for each life stage are known and use them as an input in their model. Also, their data are sampled in the wild and do not involve different experimental treatments, meaning that they have far fewer outputs relative to their input data than in our case. This leads to a much simpler optimisation problem which can be solved numerically without the differentiation techniques that we use. Their method has the advantage that it does not require a known initial starting population, as ours does, and so can be used outside of controlled experimental conditions. Their method is also likely considerably less computationally intensive than our own, and so is a good option where developmental rates are known. The stage-structured cohort models used in de Valpine and Knape (2015) and Knape and de Valpine (2016) are the closest method we have found to our own. Their model is more complex than ours and considers the mortality and development of individuals, rather than assuming that all members of a life stage are homogeneous. In cases where their algorithm converges then it is a very good option, however, as the number of parameters to be estimated increases, the likelihood of achieving convergence diminishes. Our method can handle large numbers of parameters because we define the gradient function ourselves rather than relying on standard algorithms, but this is highly unlikely to be possible for Knape and de Valpine's method due to the complexity of their model.

5.5 | How this may be used

Our methodology enables researchers to determine the survival and development rates of the different life stages of small animals in mesocosm experiments, being effective with incomplete data, in the presence of high mortality and when there are many different experimental treatments. We have not found any other effective methodology for estimating these parameters. The method of Knight et al. (2004) only works for complete datasets with low mortality, the method of Grant et al. (2020) does not allow for the estimation of development rates, only survival, and the method of de Valpine and Knape (2015) and Knape and de Valpine (2016) does not allow for a large number of unknown parameters. Our methodology provides a valuable way of analysing development and survival in stressor-based experiments, such as dose–response experiments, where increased mortality is expected (Damgaard et al., 2002), while minimising bias from handling. This methodology enables researchers to gain a much more detailed understanding of the life cycles of various small animals, potentially leading to advances in a wide range of areas.

Unequal stressor-responses across developmental stages provide insight into how interventions to manage disease risk and/or mosquito populations should be timed. They also demonstrate how stressors can change age structures within a population, which could subsequently affect interactions both within and between species, for example via cannibalism (Koenraadt & Takken, 2003; Kweka et al., 2012; Mastrantonio et al., 2018). In this study, we have

shown that mosquitoes at different life stages respond differently to different temperature and nutrient treatments. This could help to improve future mosquito control efforts and help with the development of more effective intervention strategies to curb the spread of mosquito-borne diseases. In particular, our results indicate that while higher temperatures increase development rates, they also reduce survival for the larval stages. This is consistent with previous experimental results (Alcalay et al., 2018; Loetti et al., 2011; Oda et al., 1999; Ruybal et al., 2016). It suggests that a period of hot weather could lead to more adult mosquitoes in the short term as they develop faster, but fewer in the medium term as those which were in the earliest life stages at the beginning of the hot period are less likely to survive to maturity. Such insights could contribute to the development of carefully timed public awareness campaigns. Other topics which could benefit from a more detailed understanding of the life cycle parameters of small animals are the responses of such animals to chemicals like fertilisers and insecticides (Kattwinkel et al., 2016; Tomé et al., 2014), the effects of climate change (Fordham, 2015) and biodiversity monitoring (Pfrender et al., 2017). This methodology is also applicable in studies outside aquatic mesocosms. For example, the work of Grant et al. (2020) shows that monarch butterfly populations may be modelled with a similar approach, although our method does require them to be in a controlled environment with a known starting population.

6 | CONCLUSIONS

This research has determined that statistically optimising survival, development and detection parameters based on data from replicate mesocosms is an effective way of estimating the survival and development rates of mosquitoes in mesocosm experiments. This methodology can potentially also be applied to mesocosm experiments involving other animals with multiple developmental stages which are difficult to count, and in contrast to other methods, it is applicable to situations where data are incomplete or where there is high mortality. Researchers can use this method to accurately estimate life history parameters which were not previously available. Such parameters can be of use in a variety of areas, such as disease control (in the case of mosquitoes) or helping us to better understand the effects of climate change on a wide range of species.

AUTHORS' CONTRIBUTIONS
M.D. and D.H. designed the methodology and wrote the code to implement it; S.P.B. planned the experiment and collected the data; M.D. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA AVAILABILITY STATEMENT
All data and code are available on the Dryad Digital Repository and Zenodo digital repositories (Dellar et al., 2022a, 2022b). Dryad Digital Repository https://doi.org/10.5061/dryad.18931zcxw; Zenodo https://doi.org/10.5281/zenodo.5148154

ORCID
Martha Dellar https://orcid.org/0000-0003-1044-4265
Sam P. Boerlijst https://orcid.org/0000-0001-8957-186X
David Holmes https://orcid.org/0000-0002-6081-2516

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