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Mortality estimates of stage-structured populations must include uncertainty in stage duration and relative abundance

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I explored mortality estimation for stage-structured populations, building on previous work that applied vertical life-table methods to populations of copepods. A new Bayesian approach for estimating mortality rates accounts for uncertainties in stage duration and number counted by stage, which have not been fully incorporated into previous analyses. This method assumes that mortality is similar among similar life stages. Results using simulated data show that realistic values of the standard deviation of stage duration and number of individuals counted result in reliable mortality estimates, though with wide confidence intervals. This uncertainty obscures variation in estimated mortality between successive stages and can also obscure bias due to violation of underlying assumptions such as that of a stable stage distribution. More importantly, the uncertainty calls into question many previous mortality estimates across pairs of life stages that do not account for these sources of uncertainty. The method was applied to an introduced population of the brackish-water cyclopoid copepod *Limnoithona tetraspina* in the San Francisco Estuary. Despite the uncertainties, results were interpretable: mortality was highest in nauplii and lowest in adults, probably because of high vulnerability of nauplii to invertebrate predators and low vulnerability of adults to fish predation.

KEYWORDS: Bayesian methods; vertical life table; San Francisco estuary; copepod; *Limnoithona tetraspina*

INTRODUCTION

Population processes in nature include reproduction, growth and development and mortality. In copepod populations, birth rates can be estimated to reasonable precision (Hirst and Bunker, 2003). Methods have been developed for determining growth and development with estimates of precision in the laboratory (Klein Breteler *et al.*, 1994; Gould and Kimmerer, 2010) and in the field (Burkill and Kendall, 1982; Kimmerer and McKinnon,

1987; Hirst *et al.*, 2005; Liu and Hopcroft, 2006; Kimmerer *et al.*, 2007).

Mortality rate is the least well-constrained population parameter. Mortality throughout the life cycle of a closed population must balance reproduction plus net change in abundance. Estimated lifetime mortality rates from field studies generally match this expectation (Hirst and Kiørboe, 2002). The distribution of mortality rates among stages would be more useful than lifetime mortality rates for understanding population dynamics and the influence of different mortality factors such as size-selective predation (Ohman and Hirche, 2001). Yet estimating mortality of particular stages in the life cycle from field samples has proved difficult and controversial (Ohman, 2012).

Three general approaches are available for estimating mortality from samples of stage-structured populations. Each requires knowledge of stage durations under field conditions and abundance of each life stage in each sample. Horizontal (life-table) methods can be applied when a population is sampled repeatedly and mortality is estimated from the rate of change of abundance by life stage after accounting for development (Aksnes and Ohman, 1996; Aksnes *et al.*, 1997). Vertical methods apply at a point in time, using relative abundance of successive stages to infer mortality (Mullin and Brooks, 1970). Several methods have used information both from samples taken at time intervals and from relative abundance of life stages within each sample (Wood, 1994; Bi *et al.*, 2011).

The horizontal and vertical methods require an assumption of a stable age distribution (Aksnes and Ohman, 1996; Ohman, 2012, but see Gentleman *et al.*, 2012). All methods require an assumption of balanced immigration and emigration to and from the population. Neither assumption is likely to be met in many real populations (Gentleman *et al.*, 2012). An additional assumption, not often met, is that the sampling design is equally effective for all life stages (Eiane *et al.*, 2002), requiring consideration of the spatial distribution of the population by life stage and variable vulnerability to sampling gear (Ohman, 2012).

Vertical methods are the most commonly applied of the three methods because samples need not be taken at short intervals (e.g. Mullin and Brooks, 1970; Kimmerer and McKinnon, 1987; Aksnes and Ohman, 1996). Generally, vertical methods infer mortality from relative abundance of a series of life stages with known stage durations. Previous studies have examined the effect of inherent variability and violation of the assumption of steady recruitment (Gentleman *et al.*, 2012). However, three additional sources of uncertainty in mortality estimates have not been addressed fully.

First, the problem is mathematically underdetermined: for two successive life stages, there are two unknown mortality rates and one known abundance ratio (Fager, 1973), requiring an additional assumption about the distribution of mortality among stages. This problem has been addressed by assuming similar mortality among successive stages, which differ by only ~15–20% in length (Durbin and Durbin, 1978) and are likely to be similarly vulnerable to predators. The Ratio model (Gentleman *et al.*, 2012) assumes constant mortality between one stage and the next (Mullin and Brooks, 1970). This implies, illogically, that mortality is constant between successive stages but not within a stage. A more realistic assumption is that mortality is constant across a series of stages (Kimmerer and McKinnon, 1987), but this requires a stable stage distribution over a longer period than for the Ratio model. A more elegant alternative is to allow mortality to vary smoothly between successive stages and times (Wood, 1994). Nevertheless, most mortality estimates have been made using the Ratio model (Aksnes and Ohman, 1996; Gentleman *et al.*, 2012).

Second, relative abundance of successive stages is determined from counts of samples or subsamples. Counting error injects uncertainty into the ratios of abundance and therefore the mortality estimates. The effect of counting error on uncertainty has been discussed (Wood, 1994; Aksnes and Ohman, 1996) but not used to produce error estimates in applications of the vertical method (Aksnes and Ohman, 1996; Gentleman *et al.*, 2012).

Third, uncertainty in estimates of stage duration causes uncertainty in mortality estimates. Variability in stage durations used as input to individual-based models produced error in mortality estimated by the Ratio model (Aksnes and Ohman, 1996; Gentleman *et al.*, 2012). This influence was modest, although the variability in development time among individuals can induce bias in estimated mortality (Gentleman *et al.*, 2012). However, stage durations are usually determined from laboratory and field-based measurements in which individual variability is not determined (but see Twombly and Burns, 1996). In general, laboratory estimates of stage duration have been presented as point values, although some reports have provided error estimates (Klein Breteler *et al.*, 1994; Gentleman *et al.*, 2008; Kimmerer and Gould, 2010).

Error from multiple sources can best be incorporated into a nonlinear statistical problem by using Monte Carlo simulations. Bayesian methods (Gelman *et al.*, 2004) are suitable for this purpose because of the ease of including multiple sources of error and obtaining probability distributions of mortality estimates.

In this paper, I developed a Bayesian approach to vertical methods for stage-structured populations. This

approach was applied to simulated data to compare among three alternative estimation models: the Ratio model (Gentleman *et al.*, 2012), the Constant model (Kimmerer and McKinnon, 1987), and a hybrid of these two, the Step model. Then mortality was estimated using the Constant model for a population of copepods in the San Francisco Estuary. All simulations used parameter values appropriate for rapidly developing populations with high mortality, characteristic of tropical to warm temperate regions, but I show below that the results apply to any population meeting the assumptions of the method.

Three important aspects of population dynamics are excluded from this analysis. First, egg mortality is probably one of the largest loss terms for pelagic copepods (Hirst and Kiørboe, 2002; Hirst *et al.*, 2007); although some of the principles demonstrated below apply to egg mortality, I have nothing to add to the general approach. Second, diapause results in distortions or violations of assumptions of the vertical methods, and is not addressed here. Third, I do not address the causes of mortality.

METHOD

Table I lists all symbols used in these models and analyses. Table II provides a summary of simulations used to test the methods. Assume (for the moment) constant recruitment into the first stage of interest (e.g. for copepods, Copepodite 1, C1), constant mortality for a time equal to the duration of all stages of interest, and negligible immigration to and emigration from the population. Model derivations and notation follow previous analyses (Mullin and Brooks, 1970; Aksnes and Ohman, 1996; Hirst *et al.*, 2007; Gentleman *et al.*, 2012). The relationship between abundance and mortality of a single life stage using the "Basic" method (Gentleman *et al.*, 2012) is

$$n_i = R_i \frac{1 - e^{-m_i D_i}}{m_i}$$
$$R_{i+1} = R_i e^{-m_i D_i} \quad (1)$$

where subscripts indicate stage, n_i is abundance, R_i is the rate of recruitment to Stage i, m_i is mortality and D_i is duration. Time and volume units cancel out (see below) but, for discussion purposes, time is in days and abundance in m^{-3}. If mortality approaches zero, Equation (1) collapses to $n_i = R_i D_i$. The duration of the adult stage can be considered infinite for simplicity (Ohman *et al.*, 1996 provide alternatives) so that Equation (1) for adults becomes

$$m_a = \frac{R_a}{n_a} = \frac{R_c e^{-m_c D_c}}{n_a} \quad (2)$$

where subscript a refers to adults and c to copepodites, either in aggregate or the final copepodite stage (C5).

Modeling mortality

I used simulated data (next section) to analyze three alternative models for estimating mortality from data comprising counts and stage durations. The Ratio model assumes mortality is constant across pairs of successive life stages (Mullin and Brooks, 1970; Aksnes and Ohman, 1996, Gentleman *et al.*, 2012). The Constant model assumes constant mortality across several life stages (Kimmerer and McKinnon, 1987). In the Step model, mortality is assumed

Table I: Symbols used in equations or text with definitions and units

Symbol	Definition	Equation	Units
D_i	Stage duration	(1)	days
m_i	Daily mortality rate	(1)	day^{-1}
n_i	Abundance of stage i	(1)	m^{-3}
R_i	Rate of recruitment to stage i	(1)	$m^{-3}\ day^{-1}$
HR	Rate of production of eggs that hatch	(3)	eggs $female^{-1}\ day^{-1}$
N_i	Count	(5)	–
V_i	Effective volume sampled	(5)	m^3
N_{tot}	Total count of all stages (Constant, Step) or two stages (Ratio)	(6)	–
A_i	Intermediate variable: survival from Stage 1 to Stage i	(7)	–
p_j	Proportion of Stage i in total of Stages i and $i+1$	(6)	–
p_i	Proportion of Stages i in the total count	(9)	–
K	Intermediate variable for calculating p_i	(10)	m^{-3}
d_i	Laboratory-determined stage duration	(11)	days
Φ	Ratio of field to laboratory stage durations	(11)	–
CV_D	Coefficient of variation of development time	Text	–
RSD_m	Standard deviation of mortality estimate/true mortality value	Text	–

Subscript i refers to individual stages in a series (nauplii or copepodites), j to individual stages or a pair or group of stages, a to adults, and c to copepodites. Time and volume units are provided but they cancel out of the equations.

Table II: Characteristics of simulations (Sim) 1–3

Sim	Purpose	Estimation models tested	Setup	Total samples	Constant parameters	Variable parameters
1a	Effect of varying N_{tot} and CV_D on RSD_m	Constant, Ratio	Five stages, isochronal; D_i fixed. Sample from multinomial with N_{tot}	84	$D_i = 1$ day $m = 0.1$ day^{-1}	$CV_D = 0.0001, 0.01, 0.1, 0.2$ N_{tot} 21 values log-linear nominally 40–40 000
1b				51	$D_i = 1$ day $m = 0.1$ day^{-1} $N_{tot} = 500$	CV_D 51 values 0–0.5
2	Effect of different mortality patterns combined with CV_D and N_{tot} on estimation error	Constant, Step, Ratio	As in Simulation 1 but with $D_i = 1$, 1, 1.2, 1.5, 2 days	36 × 6 replicates	None	CV_D 0.001, 0.1, 0.2 N_{tot} 200, 500, 1000, 10^9 Mortality schedules: Steady at 0.15 Declining linearly with Stage 0.22–0.1 Irregular 0.20, 0.20, 0.08, 0.08, 0.20 day^{-1}
3	Effect of temporal variation in recruitment on estimation error	Constant, Step, Ratio	As in Simulation 1	100	$D_i = 1$ day $CV_D = 0.1$ Nominal $N_{tot} = 500$ $m = 0.1$ day^{-1}	Recruitment varied daily from 0.4 to 2.4 with a median of 1, by a lagged random walk with first-order partial autocorrelation of 0.29

Symbols as in Table I.

to be constant for the first two or more stages and constant at a potentially different value for the remaining stages.

In Equation (1), n_i and D_i are considered known and R_i and m_i are unknown. Since the number of unknowns exceeds the number of equations, a constraint must be imposed so that the problem can be solved. The Ratio model reduces the number of unknowns by taking a ratio of abundance of each pair of stages (Mullin and Brooks, 1970; Gentleman *et al.*, 2012)

$$\frac{n_i}{n_{i+1}} = \frac{e^{m_j D_i} - 1}{1 - e^{-m_j D_{i+1}}} \tag{4}$$

which can be solved iteratively for m_j, the joint mortality between Stages i and $i + 1$.

Using the actual count, data require several additional steps. The abundance values n_i are based on counts of a subsample or the whole sample from the field.

$$n_i = \frac{\mathcal{N}_i}{V_i} \tag{5}$$

where $\mathcal{N}_i$ is the number counted and V_i the effective volume sampled, i.e. the volume filtered by the net times the fraction subsampled for counting. Combining Equations (4) and (5) and rearranging gives

$$\begin{aligned} p_j &= \frac{\mathcal{N}_i}{\mathcal{N}_i + \mathcal{N}_{i+1}} = \frac{\mathcal{N}_i}{\mathcal{N}_{tot}} \\ &= \frac{e^{m_j D_i} - 1}{e^{m_j D_i} - 1 + \frac{V_{i+1}}{V_i}(1 - e^{-m_j D_{i+1}})} \end{aligned} \tag{6}$$

where p_j is the expected proportion of stage i in the total number counted in both stages and $\mathcal{N}_{tot}$ is the total count. These values are used below in a binomial distribution to estimate the likelihood of the joint mortality m_j. If both stages are counted in the same subsample, the V values cancel out.

For the Constant and Step models Equation (1) can be recast for a series of development stages as follows. If A_i is survival from the beginning of Stage 1 to the beginning of Stage i, then

$$\begin{aligned} A_1 &= 1 \\ A_{i+1} &= e^{-\sum_{j=1}^{i} m_j D_j}, \quad 1 \le i < \mathcal{N}_{stage} \end{aligned} \tag{7}$$

and Equation (1) can be expressed as

$$n_i = R_1 A_i \frac{1 - e^{-m_i D_i}}{m_i} \tag{8}$$

Substituting Equation (5) into Equation (8) and rearranging, the proportion counted in each stage is

$$p_i = \frac{N_i}{\sum_{\text{all } i} N_i} = \frac{R_1 V_i A_i}{N_{\text{tot}}} \frac{1 - e^{-m_i D_i}}{m_i} \quad (9)$$

where p_i is the expected fraction of the total count in life stage i, given the other parameters. The estimation model is fitted to data (below) with the vector of values p_i as the proportions following a multinomial distribution.

In the Constant model, Equation (9) reduces to

$$p_i = \frac{R_1 V_i A_i}{N_{\text{tot}}} \frac{1 - e^{-mD_i}}{m} = k V_i A_i (1 - e^{-mD_i}) \quad (10)$$

where k represents the terms that do not vary by stage and the A_i values vary only with stage duration. Since the values of p_i must sum to 1, k is arbitrary and its components need not be determined.

The Step model applies Equation (9) over two separate ranges of life stages (in the simulations below, C1−3 and C4−5). A further constraint is that the rate of molting out of the first step equals recruitment to the second step [Equation (1)], linking the two sets of equations to yield one set of p_i values that can be analyzed together.

The use of proportions in all three estimation models has not only reduced the number of parameters so mortality can be calculated, but has also eliminated all units. In particular, the products of m_i and each of the D values in Equations (4), (6) and (10) are unitless, so a given proportion of counts could correspond to high mortality in a population that develops rapidly or low mortality in a slowly developing population. Analyses below use specific values of mean stage durations of 1−2 days, and mortality rates are presented in inverse days (day^{-1}). To apply these analyses to populations with, say, 10-fold longer mean stage durations, 10-fold lower mortality rates would yield identical proportions by stage.

Simulated data

Simulations 1−3 (Table II) were constructed for populations with five life stages (e.g. copepodites) with various assumed values of mortality, mean and coefficient of variation of stage duration, total number of copepods counted and (in Simulation 3) time-varying recruitment. In Simulations 1 and 2, counts per stage were generated by sampling from multinomial distributions [Equation (9)] based on the selected parameters; in Simulation 3, they were generated from a cohort model. Parameters included the total number of copepods counted (N_{tot}), mortality by stage, mean stage duration, and recruitment to the first stage in Simulation 3 (Table II). The coefficient of variation of the stage durations (CV_D) was also varied as input to the Bayesian analysis but was not used in the simulations.

Simulation 1 determined how mortality estimated by the Constant and Ratio models varies with N_{tot} and CV_D. Uncertainty in mortality was measured as the standard deviation of mortality divided by its true mean (denoted here as RSD_m, relative standard deviation of mortality). Mean duration of all stages was 1 day and mortality rate was 0.1 day^{-1}. Simulation 1 was run in two segments (Table II): (a) with four values of CV_D and 21 values of N_{tot}; and (b) with $N_{\text{tot}} = 500$ and 51 values of CV_D from ∼0 to 0.5. These values were chosen to extend from ∼0 to beyond the range of carefully determined values of CV_D (Kimmerer and Gould, 2010), and from very small to impracticably large N_{tot}.

Simulation 2 compared output of the three estimation models using simulated count data produced using 36 combinations of mortality, N_{tot}, and CV_D. Mean stage durations were chosen to represent a typical pattern for calanoid copepodites at 1, 1, 1.2, 1.5 and 2 days. Mortality was imposed in three patterns, each having a mean weighted by stage duration of ∼0.15 day^{-1}: steady among stages at 0.15 day^{-1}, declining linearly with stage from 0.22 to 0.1 day^{-1} and irregular at 0.30, 0.30, 0.01, 0.01, 0.20 day^{-1} for Stages 1−5. The irregular sequence was selected to be the most amenable for distinguishing mortality among pairs of stages by the Ratio model. Three values of CV_D and four values of N_{tot} included achievable values and values that eliminated variability (Table II). Six simulations were run for each combination of parameters for a total of 216 runs.

Simulation 3 tested the effect of time-varying recruitment into the first of five stages to compare how the three estimation models performed when the key assumption of constant recruitment was violated. Mortality was held at 0.1 day^{-1}. The recruitment rate was varied daily and mortality was estimated using each of the three models. To establish a realistic pattern of recruitment variation, a synthetic time series was developed with a pattern of variance and autocorrelation similar to that of the daily egg production for *Paracalanus parvus* in Bahia Magdalena, Mexico (Gómez-Gutiérrez *et al.*, 1999, Fig. 4). This time series was used as a multiplier to modify R_1 in a cohort model based on Equation (1) with a 0.1-day time step. The base value of R_1 was set to produce a nominal value of N_{tot} of ∼500. This cohort model was spun up for 20 days, then sampled daily for 100 days.

Fitting a Bayesian model

A Bayesian hierarchical model (Gelman *et al.*, 2004) was set up using Equations (6) and (10). Bayesian models

estimate the probability distributions of parameters from their likelihoods given the data, combined with assumed prior distributions for each parameter. Since prior information is unlikely to exist for mortality, an uninformative prior distribution was used, which was normal with a mean of 0 and a standard deviation of 10 day^{-1}. This prior had no effect on the posterior distributions as shown by testing with alternative standard deviations. Mortality was not constrained to be positive, as negative values can arise if assumptions of the method are not met or if parameters are highly uncertain. Prior distributions for stage durations were normal with means and standard deviations from Simulations 1–3 or from data, with a constraint that they could not go below 0.1 day to prevent unrealistic values that caused instability in model fitting. This constraint on average would remove ~3% of the values at the highest value of CV_D of 0.5, and a negligible fraction at a moderate value of 0.2. The model was coded so that these values were sampled from the prior distributions but not updated by the Bayesian algorithm (using the "cut" function, Lunn *et al.*, 2000).

Data entered into the Bayesian model included the counts by stage, means and standard deviations of stage durations and control parameters. The Bayesian model sampled from the prior distributions for duration of each life stage and applied Markov Chain Monte Carlo simulation to calculate p_j [Equation (6), Ratio model] or p_i [Equation (10), Step or Constant model]. The counts by stage and total counts were used to determine likelihoods for binomial (Ratio model for individual pairs of stages) or multinomial distributions (Constant and Step models), resulting in full statistical distributions of the mortality estimates. Output included estimates with 95% credible intervals for mortality in each pair (Ratio) or group (Step) or all (Constant) stages. Accuracy and precision were compared among the Constant model and means across all stages from the Ratio and Step models weighted by the mean durations of stages (Ratio mean and Step mean, respectively).

Bayesian models were run in WinBUGS (Lunn *et al.*, 2000) using procedures used in estimating stage duration (Kimmerer and Gould, 2010) and growth rate (Gould and Kimmerer, 2010). Each model was run with triplicate Markov chains of length 11 000 after 10-fold thinning to reduce autocorrelation, and the first 1000 samples were discarded to eliminate effects of initial conditions. Output was checked for autocorrelation and consistency of results between the first and second halves of the output, and various diagnostic statistics were examined (Kimmerer and Gould, 2010). Model output comprised posterior distributions for mortality and expected numbers per stage including 95% credible intervals (similar to confidence intervals). The three estimation models were assessed by comparing the statistical distributions of mortality estimates with the simulated values. Expected numbers per stage were compared with simulated or observed (field) counts to check for fit and bias and to further verify the model code. Accuracy of the code and the method were also explored using small-scale simulations and by comparing Bayesian estimates with estimates developed by alternative methods.

A field example

I applied the Constant model to a population of *Limnoithona tetraspina* from the San Francisco Estuary. *Limnoithona tetraspina* was introduced in 1993 (Orsi and Ohtsuka, 1999) and is the most abundant copepod in the brackish region of the estuary during spring–autumn (Bouley and Kimmerer, 2006). It grows and reproduces slowly and appears to be chronically food-limited (Bouley and Kimmerer, 2006; Gould and Kimmerer, 2010). Based on reproductive and development rates, mortality rates were expected to average ~0.05 day^{-1} across all life stages (Gould and Kimmerer, 2010). The key objective was to ascertain the distribution of mortality among nauplii, copepodites and adults, which was expected to provide a clue to the cause(s) of the mortality.

Eighteen samples taken in March–August 2007 (Gould and Kimmerer, 2010) were re-examined and all life stages of *L. tetraspina* were counted in subsamples. Five of these samples were from a salinity of 2 and the remainder from a salinity of 5, near the center of distribution. This population meets the assumptions of vertical methods except for some sampling bias (below). The rate of change of the population during the sample period averaged ~1.5% day^{-1}, close to the zero rate required under the assumption of the method. Immigration was assumed to be negligible because there is no source population, and net losses from the population due to dispersion and advection would appear as mortality.

Mortality was determined separately for nauplii, copepodites and adults. Median total counts per stage were 369 nauplii, 240 copepodites and 213 adults. Laboratory stage durations (Kimmerer and Gould, 2010) were first corrected for differences in temperature between the laboratory and the field, and the corresponding variability was included in CV_D for each sampling date. An additional correction for food-limited development, determined in molt-rate experiments using field-collected samples (Gould and Kimmerer, 2010), took the form of a ratio of field to temperature-corrected laboratory stage durations for all life stages:

$$D_i = \frac{d_i}{\Phi} \tag{11}$$

where d_i is the laboratory-determined duration of Stage i corrected for field temperature and Φ is the ratio of field to laboratory stage durations for a series of stages from molt-rate experiments conducted during most sampling events. Φ values were entered into the Bayesian analysis with standard errors, and the mean and standard deviation of all estimates of Φ were used for the first four sample dates, when molt rates were not measured.

Mortality of nauplii and copepodites was determined as described for simulated data using Equation (10). Preliminary comparison of observed with predicted counts by stage showed that nauplius stages 1–3 were consistently under-represented in the samples, presumably because they were too small to be retained by the 53-μm mesh net, so only Stages 4–6 were included in the analysis to calculate mortality of nauplii. Mortality of adults with sexes combined was determined using Equation (2). Terminal molting of copepodites (R_a) was calculated within the WinBUGS analysis of copepodites from Equation (1) and adult mortality was then calculated in WinBUGS by sampling from a Poisson distribution for N_a.

RESULTS

Mortality estimates determined from Simulations 1–3 (Table II) using all three estimation models had probability distributions indistinguishable from normal distributions. Estimates were generally accurate and unbiased in Simulations 1 and 2. However, estimates were very imprecise in simulations using realistic values of CV of stage duration (CV_D) and total copepods counted (N_{tot}).

In Simulation 1, uncertainty in mortality estimates was acutely sensitive to both CV_D and N_{tot}, and this sensitivity was about twice as high for the Ratio model as for the Constant model (Fig. 1). If variability of stage duration is negligible (Simulation 1a, Fig. 1A and B), the standard deviation of mortality divided by its true value, RSD_m, could be reduced to 10% only by counting ~20 000 copepods in all stages (Ratio model, mean of all stages, Fig. 1A) or ~5000 copepods (Constant model, Fig. 1B). With more realistic counts of 500 copepods, RSD_m was roughly linearly related to CV_D (Simulation 1b, Fig. 1C and D). A realistic CV_D of 10% resulted in RSD_m of ~100% for the Ratio mean and ~50% for the Constant model.

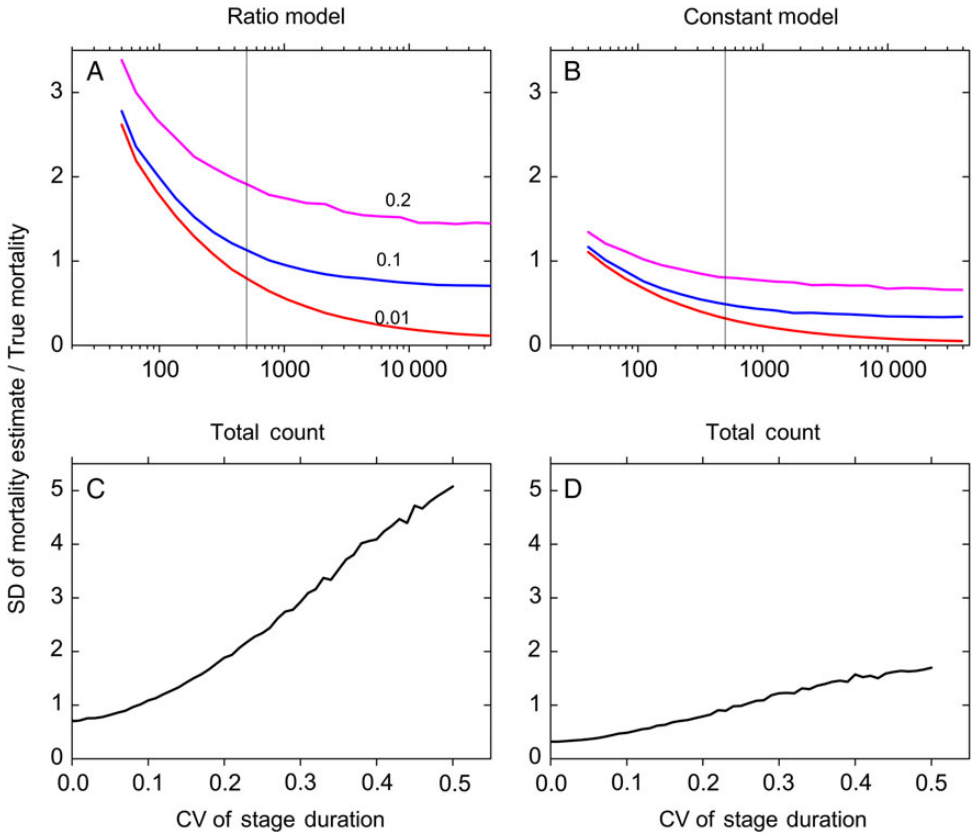

Fig. 1. Simulation 1: RSD_m (Table I) for simulated data with five stages with mean duration of 1 day each and actual mortality of 0.1 day^{-1}. **A** and **B** (Simulation 1a), Response of RSD_m to total number of copepods counted in all stages for three values (numbers in panel) of the CV of stage duration CV_D. **C** and **D** (Simulation 1b), Response of RSD_m to variation in CV_D for a total count of 500 (vertical lines in A and B). A and C were averaged over all four pairs of successive stages by the Ratio model. B and D were determined by the Constant model.

Results of Simulation 2 are most easily compared among the Constant model and the means across stages for the Step and Ratio models. Results differed among these models as shown by the frequency with which credible intervals included the true value (i.e. accuracy) and the frequency with which they excluded zero (a measure of precision) (Fig. 2, Table III). Accuracy but not precision differed among mortality schedules. In the runs with $CV_D = 0$ and $N_{tot} = 10^9$ (Case 1, Invariant, Table III), all three models gave point estimates with negligible variance and all were perfectly accurate for the steady mortality case. In Case 2, with $CV_D = 0$ and $N_{tot} \leq 1000$, some variability occurred particularly for the irregular schedule with the Constant model (61% accurate, Table III). However, the Constant model was far more precise than the other two models.

In the third case, which includes all of the realistic values of CV_D and N_{tot} (Table III), all estimation models were accurate for all mortality schedules, but precision was reduced. As an alternative measure of accuracy, the percent difference between the true mortality value and the mean of all estimates from each estimation model for the steady mortality case was 3% or less, while for the declining mortality schedule it was 11% or less. The irregular mortality schedule resulted in 24% underestimate of mortality on average from the Constant model, 15% overestimate from the Step model and 12% underestimate from the Ratio model.

Standard deviations also showed greater precision in the Constant model: the ratios of standard deviations of the sample means of the Ratio model to those from the Constant model had a mean of 2.3 (range 2.0–2.5; not shown). Similar ratios from the Step model to the Constant model had a mean of 1.7 (1.5–1.9). Among the results for individual pairs of stages from the Ratio model, correlations were strong and negative between mortality estimates of pairs of stages: −0.52 between Stages 1–2 and 2–3, −0.29 between Stages 2–3 and 3–4 and −0.42 between Stages 3–4 and 4–5.

The results for individual pairs of stages under the Ratio model showed very low precision even in Case 2, in which about two-thirds of the credible intervals for individual pairs of stages included zero (Table III). In Case 3, almost all of the credible intervals included zero. Furthermore, the Ratio model could reliably distinguish mortality among life stages in the Declining and Irregular schedules only in Case 1, while credible intervals of all four pairs of stages overlapped in most (Case 2) or all (Case 3) samples (Table IV).

In Simulation 3, variable recruitment degraded mortality estimates by all three estimation models (Fig. 3,

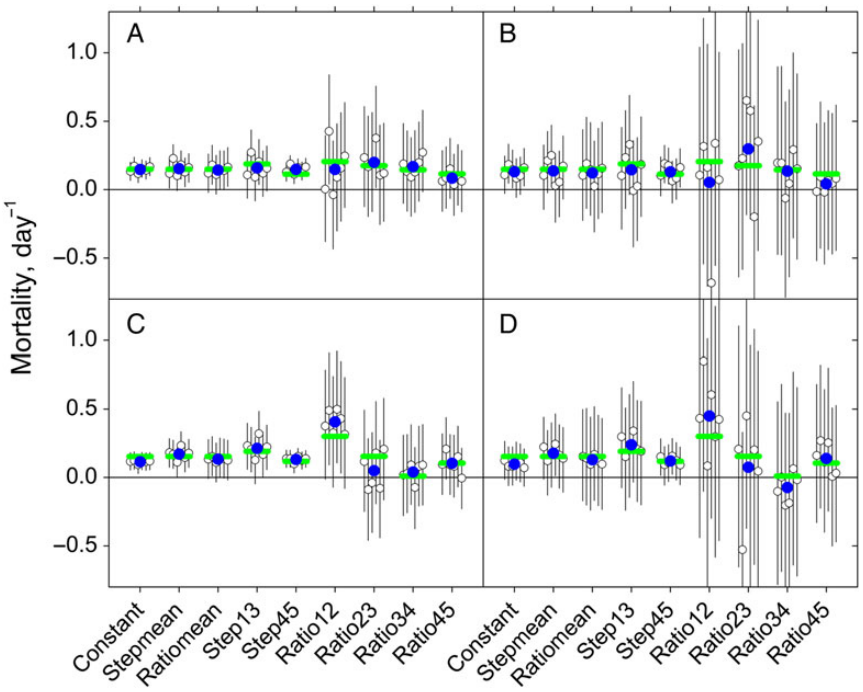

Fig. 2. Simulation 2: Example comparison of mortality estimates by the Constant, Step and Ratio models for a simulated population of five copepodite stages with durations of 1, 1, 1.2, 1.5 and 2 days, showing estimates with 95% credible intervals. Step mean and Ratio mean are duration-weighted means across all stages; labels with numbers are sample data for groups of stages (two groups for Step, four pairs of stages for Ratio). Open circles with error bars are for single samples. Filled circles are means among six replicate samples. Horizontal bars are actual mortality as means weighted by stage durations. **A**, **B**: declining true mortality of 0.22, 0.19, 0.16, 0.13, 0.10 day^{-1}. **C**, **D**: irregular true mortality of 0.30, 0.30, 0.01, 0.01, 0.20 day^{-1}. A, C: $CV_D = 0.1$ and $N_{tot} = 1000$. B, D: $CV_D = 0.2$ and $N_{tot} = 200$.

Table III: Simulation 2: comparison of mortality estimates from 216 simulations

	% Including true value			% Excluding 0		
Estimation model	Steady	Declining	Irregular	Steady	Declining	Irregular
Case 1: Invariant, $CV_D = 0$, $N_{tot} = 10^9$						
Constant	100	0	0	100	100	100
Step mean	100	0	0	100	100	100
Ratio mean	100	0	0	100	100	100
Step individual groups	100	0	0	100	100	100
Ratio individual pairs	100	75	50	100	100	100
Case 2: Low variance, $CV_D = 0$, N_{tot} 200, 500, or 1000						
Constant	94	100	61	94	100	94
Step mean	94	94	89	83	83	89
Ratio mean	100	100	100	72	78	83
Step individual groups	92	75	92	83	83	89
Ratio individual pairs	90	93	90	31	26	35
Case 3: $CV_D = 0.1$ or 0.2, N_{tot} 200, 500, or 1000						
Constant	100	100	96	92	92	71
Step mean	100	98	100	42	48	71
Ratio mean	100	100	100	27	29	17
Step individual groups	100	96	98	53	60	59
Ratio individual pairs	100	100	100	2	3	5

Each value is the percentage of simulated values for which 95% credible intervals included the true value (left three columns) or excluded zero (right three columns), demonstrating accuracy and precision, respectively. Columns separate values according to the three mortality schedules (Table II). Blocks of values are grouped by whether coefficient of variation of stage duration CV_D is zero and total count N_{tot} is effectively infinite.

Table IV: Simulation 2 for ratio model

Mortality schedule	Case 1	Case 2	Case 3
Steady	0	3	0
Declining	100	2	0
Irregular	100	13	0

Percent frequency with which mortality of any two pairs of stages could be distinguished by their 95% credible intervals not overlapping. Data are displayed for each mortality schedule and case (from Table III).

Table V). During times when recruitment was changing rapidly, credible intervals of the mortality estimates by all three models either did not include the actual mortality value of 0.1 day^{-1} or included zero. As in Simulations 1 and 2, uncertainty in mortality estimates was highest for the Ratio model and lowest for the Constant model. Graphical comparison of predicted and observed counts was uninformative about the magnitude and direction of the inaccuracy. Estimated mortality deviated from true mortality by an amount that was strongly and positively related to the rate of change of total abundance over the previous day (Fig. 4 for the Constant model). This positive relationship is expected [Equation (1)] because increasing recruitment rate raises the relative abundance of earlier stages, and therefore the mortality estimate.

Mortality of *L. tetraspina*, determined by the Constant model and Equation (2) for adults, was consistently higher in nauplii than in copepodites, and lowest in adults (Fig. 5). Mortality of nauplii and copepodites, but not adults, increased through the sampling period. No difference was apparent between samples taken at salinities 2 and 5. Median mortality rates were 0.13 day^{-1} for nauplii, 0.05 day^{-1} for copepodites and 0.03 day^{-1} for adults.

DISCUSSION

Estimating mortality is difficult, and it is remarkable that a defense was required against those who claim it to be impossible (Ohman, 2012). Several authors have examined the effects of variability in recruitment, immigration and sampling bias on mortality estimates (Aksnes and Ohman, 1996; Gentleman *et al.*, 2012; Ohman, 2012). Here I have focused on two additional impediments to mortality estimation that have received less attention: uncertainty in estimates of stage duration and variability inherent in count data.

Several key points are highlighted by this analysis. First, the relative abundance of a series of two or more life stages depends on the product of mortality and stage duration and not the absolute value of either. Therefore, the results presented here, though derived for copepods that develop rapidly, apply equally well if development is slow. Second, a high coefficient of variation in the estimate of stage duration CV_D or low total count N_{tot} can produce mortality estimates with very large uncertainty for any estimation model, but particularly for individual pairs of stages with the Ratio model. And third, even considering these sources of error, robust and interpretable mortality estimates remain feasible, but investigators

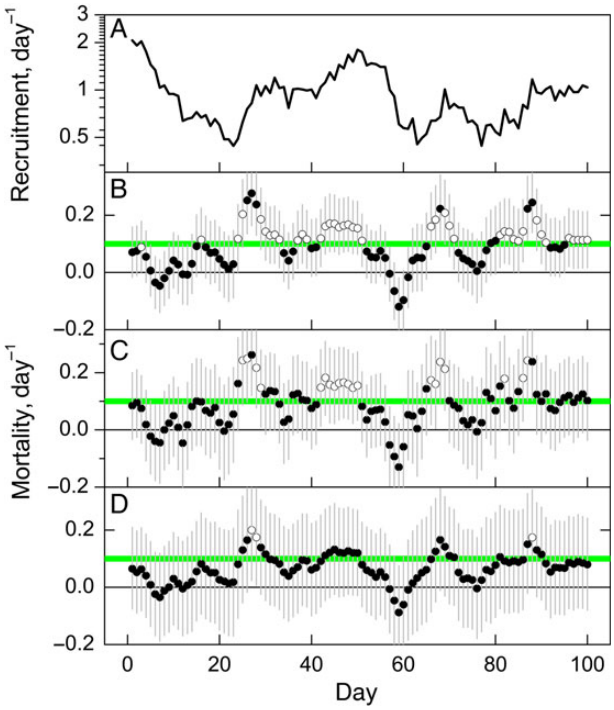

Fig. 3. Simulation 3: Effects of temporally variable recruitment on mortality estimates based on five stages with a mean duration of each stage of 1 day and coefficient of variation 0.1, total count varying with a mean of 431 (range 227–925), and true mortality 0.1 day^{-1}. (**A**) Simulated recruitment to Stage 1 over 100 days. (**B**–**D**) Estimated mortality with 95% credible intervals for each day. Horizontal line, true mortality. Filled circle, 95% credible intervals include zero or exclude true mortality; open circle, 95% credible intervals include true value but not zero. (B) Constant model; (C) Step mean; (D) Ratio mean.

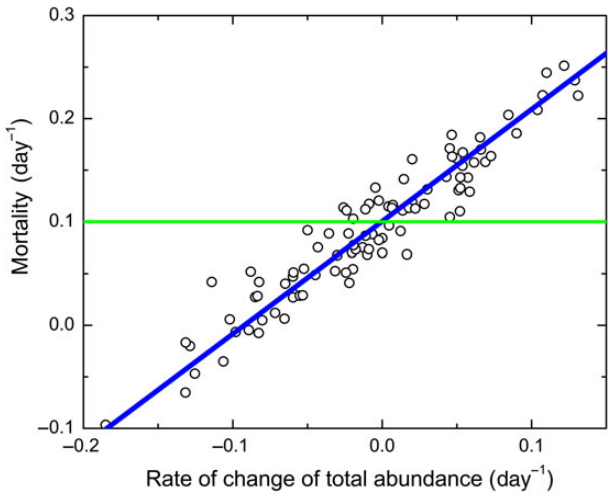

Fig. 4. Simulation 3: Daily mortality estimates from Fig. 3B (Constant model) plotted against the rate of change of total population abundance over the previous day. The horizontal line gives true mortality and the fitted line is from a geometric mean regression.

Table V: Simulation 3: as in Table III for time-varying recruitment

Estimation model	% Including true	% Including 0
Constant	83	53
Step mean	92	78
Ratio mean	99	97
Step individual groups	91	74
Ratio individual pairs	100	100

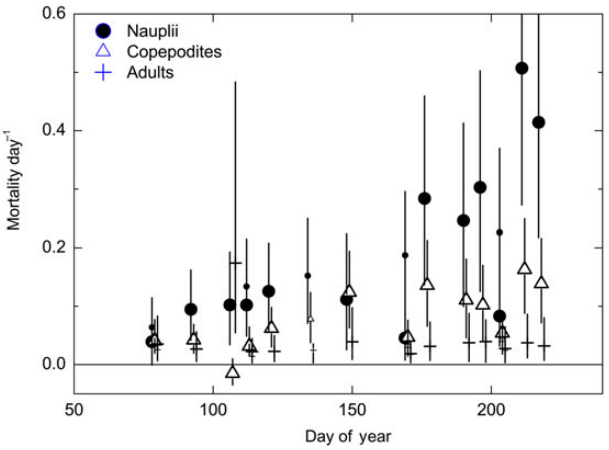

Fig. 5. *Limnoithona tetraspina* from 18 samples taken in spring–summer 2007. Mortality estimates by the Constant model with 95% credible intervals for all three gross life stages. Small symbols are for salinity 2, large symbols salinity 5.

should calculate mortality explicitly taking these two sources of variability into account.

These problems apply equally to estimates made using single samples by the vertical life-table method and to those using more comprehensive approaches such as response-surface methods (Wood, 1994) and inverse methods that apply across samples (Bi *et al.*, 2011). Unfortunately, these uncertainties call into question the reliability of mortality estimates based on single samples using the Ratio model (Figs 1 and 2) unless estimates are refined through sufficient replicate sampling (Hirst *et al.*, 2007; Ohman, 2012).

All three estimation models were accurate with little bias but with low precision (Table III, Fig. 2). Mortality estimates using the Constant model were consistently more precise than those using the Ratio model when the latter results were averaged (with weighting by stage duration) across all pairs of stages (Ratio mean, Tables III and V, Figs 1–3). The Constant model achieves higher precision than the Ratio model by analyzing all data together, providing more degrees of freedom and a wider range of abundance values, both of which increase precision. Under the Ratio model, each pair of stages is treated

as independent, without the requirement that the survivors of Stage $i+1$ become recruits to Stage $i+2$ [Equation (1)]. The Step model gave intermediate results between Constant and Ratio. The relative order of fit of the estimation models was the same even in the irregular mortality case, which had been expected to favor the Ratio model (Tables III and IV Fig. 2). Time-varying recruitment resulted in bias and high uncertainty (Fig. 3, Table V), and none of the estimation models performed well; however, the performance ranking of the models was the same as in the other simulations.

Negative correlations between pairs of mortality estimates by the Ratio model (Simulation 2) are one source of the high uncertainty (Fig. 2). These correlations arose from the count data; for example, if the number counted for Stage 2 was low, and those for Stages 1 and 3 high, relative to their expected values, the calculated mortality would be higher for Stages 1−2 and lower for Stages 2−3. This is an artifact of the Ratio model itself, not the simulations, since count data from samples would suffer from the same problem.

All three models use the product of stage duration and mortality rather than either alone, so that mortality can be considered to scale inversely with stage duration. However, other processes that operate at different time scales from stage duration may be influential when stage durations are long. These include physical processes, such as seasonal variability, mixing and advection, and biological processes, such as feeding by fish schools and diapause. Thus, long stage durations are associated both with low daily mortality rates (Hirst and Kiørboe, 2002) and opportunities for confounding factors to influence the population under study. In these situations, the Ratio model may be preferable to the Constant model because steady conditions are required over only the duration of two stages, not several stages (Gentleman *et al.*, 2012).

Counting error

The strong influence of low counts on the uncertainty inherent in count data has influenced subsampling practices for decades (Lund *et al.*, 1958). Yet little attention has been paid to the uncertainty in mortality estimates due to variation in counts, although consequences have been explored using the Ratio model with data generated by an individual-based model (Fig. 2 in Aksnes and Ohman, 1996). However, that analysis applied to the equation for the Copepodite 5—adult transition (Equation 2), which is less affected by variability from counts and from uncertainty in stage duration than the equation for the Ratio model [Equation (4); compare Equations (7b) and (7a) in Aksnes and Ohman, 1996]. Bi *et al.* (Bi *et al.*, 2011) found bias in mortality as uncertainty in simulated abundance increased but did not propagate the inherent variability of the count data into the mortality estimates.

The relationship between number counted and uncertainty in the result can be used to determine the counts needed for the selected estimation model (Figs 1A and B). For example, a target for the ratio of standard deviation to true value of mortality (RSD_m) of 0.5 would result in a lower 95% confidence limit just above zero. With $CV_D = 0.01$ (effectively zero), this would require a count of ∼1500 for the Ratio (Fig. 1A) and ∼200 for the Constant model (Fig. 1B).

In a real population, the number of animals sampled may vary substantially with life stage, e.g. because of high mortality or greatly varying stage durations. The precision of mortality estimates by any of the estimation models will be affected more by the minimum number counted in a stage than by the mean. Precision can be improved either by increasing the total count or by analyzing rarer stages in a larger subsample. Similarly, different sampling methods may be used for different stages (Ohman, 2012). In either case, these calculations must take the subsample or sample volumes into account [Equations (6) and (10)].

Stage duration

Previous studies (e.g. Aksnes and Ohman, 1996; Gentleman *et al.*, 2012) have examined how variability in stage duration among individuals affects mortality estimates. This source of variability and even bias (Gentleman *et al.*, 2012) is not addressed here because it has been thoroughly explored. Yet the effect of uncertainty in estimates of stage duration on uncertainty in mortality estimates has rarely been examined. This uncertainty includes imprecision in laboratory estimates of stage duration, the correction for temperature and the effect of food limitation in the field. Klein Breteler *et al.* (Klein Breteler *et al.*, 1994) developed methods for estimating uncertainty in laboratory development, but had difficulty determining the joint stage durations of successive stages. A Bayesian approach to estimating stage duration from development experiments determined confidence intervals for each stage by combining the error inherent in the count data with replication error (Kimmerer and Gould, 2010). The distributions of stage durations among individuals are generally skewed (Klein Breteler *et al.*, 1994; Gentleman *et al.*, 2008), but simulated data with skewed individual distributions gave estimates of stage duration with approximately normal distributions (Kimmerer and Gould, 2010).

As with uncertainty in counts, the propagation of errors in stage duration limits precision of mortality estimates. With an unrealistically large count (i.e. no

counting error) and $CV_D = 0.1$, RSD_m for Ratio mean (weighted mean across all stages) is ~0.7 and RSD_m from the Constant model is ~0.3 (Fig. 1A and B). RSD_m by all three estimation models is roughly proportional to CV_D (Fig. 1C and D). If CV_D values are similar among all stages, RSD_m for individual pairs of stages by the Ratio model is about twice as large as that for Ratio mean (Fig. 2). These results contrast with those for similar values of the CV of stage duration among model individuals (Aksnes and Ohman, 1996; Gentleman *et al.*, 2012). Presumably, the variability in mean stage duration in the individual-based models used to produce those results would scale inversely with the square root of the number of individuals modeled. Thus, the error in estimating stage duration may impose a more severe constraint on mortality estimates than previously suggested.

Recent estimates of CV_D in the laboratory have been 2–5% for *L. tetraspina* and 10–15% for *Pseudodiaptomus forbesi*, the difference largely due to differences in replication and the number of copepods counted (Kimmerer and Gould, 2010). Coefficients of variation in estimates of field development are larger than those from the laboratory (Gould and Kimmerer, 2010). In the analysis of the data for *L. tetraspina*, the variability in field development, determined by the molt-rate method on Stages C1–4, was applied to all life stages under the assumption that food limitation affected all life stages and that nauplii and copepodites consume similar food (Vogt *et al.*, 2013). However, durations of later stages may be extended more than those of earlier stages under food limitation (Hart, 1990). Therefore, a better practice would be to determine stage duration in the field separately for nauplii and for copepodites. In particular, any variation in the degree of food limitation among stages would have to be considered. The effect of food limitation in the field [Φ in Equation (11)] can be applied across several stages. This adjustment reduces both the magnitude and the precision of each mortality estimate.

Recommendations

Previous reports on mortality estimation have recommended careful consideration of sampling strategy, accounting for temporal variability in recruitment and for immigration (Gentleman *et al.*, 2012; Ohman, 2012). These need not be repeated here. The key recommendation is for the researcher to be aware of the pitfalls in mortality estimation, including those described here, and determine how best to overcome them.

Mortality estimates for a series of stages with similar mortality risks (say, all copepodites) are probably better determined in aggregate (Constant or Step models) than for each stage separately or for adjacent pairs of stages (Ratio model). In Simulation 2, substantial changes in mortality between stages could be reliably detected only in the absence of variation, i.e. known stage durations and immense counts (Fig. 2, Table IV). Thus, while mortality may differ from one stage to the next, heroic efforts may be required to reduce variability enough to be able to detect these differences. In addition, calculating mortality across several stages avoids the unintuitive assumption that mortality is independent and potentially very different between adjacent pairs of similar stages. It also provides a reasonable constraint on the rate of change of mortality with stage. Exploratory analysis is always warranted for any mortality estimate to see which method is most appropriate to the species and life stages under study, and which gives the best fit, but there is no substitute for an analytical method that uses all the data.

Every effort should be made to estimate and reduce uncertainty in stage duration and to use an appropriate adjustment for field conditions under food limitation. Proxies for stage duration (e.g. based on biochemical measurements, Ohman, 2012) should be used only if their prediction errors are small, and prediction errors should be carried through to the mortality estimates as for CV_D and Φ discussed above.

Regardless of the estimation model, the analytical method should take variability into account. The Bayesian approach provides full statistical distributions of mortality estimates and other parameters, including means across stages and means among samples, and also probability distributions of expected numbers per stage. These numbers can be compared with observed counts to validate the underlying estimation model. For example, counts of early naupliar stages in the analysis for *L. tetraspina* were consistently lower than expected, indicating that these stages had been undersampled, and a revised analysis of naupliar mortality used only the later stages.

Although the method described here gives confidence limits, these should be interpreted cautiously and with attention to the assumptions of the models. If these assumptions are violated, the confidence intervals around the estimates are too tight, and bias is likely (e.g. Fig. 3). Violations may be detected by examining predicted and observed counts (above), rates of change of abundance over time (Figs 3 and 4) and spatial and temporal gradients in relative abundance of life stages, and considering any biological reasons for sharp changes in mortality from one stage to the next (e.g. diapause).

Egg production rate of egg-carrying copepods is somewhat dependent on mortality of the adult female, and strictly speaking estimates should be corrected to account for that dependence (Ohman *et al.*, 1996). However, egg production rate by the egg-ratio model (Edmondson *et al.*,

1962) is one of the easiest measurements to make and can result in very precise estimates of reproduction. Correcting this precise estimate with a very imprecise estimate of female mortality would severely degrade the precision of reproductive rate estimates and may not improve their accuracy. Therefore, if this correction is applied, it should be based on the mean or median mortality rate determined from as many samples as possible rather than from individual estimates, and the effect of this correction on precision should be examined.

Regarding the difficulties in mortality estimates, "what is to be done?" (Ohman, 2012). Mortality estimates are not feasible in some, maybe most, situations. In the ideal situation of a closed population of abundant copepods with a stable age distribution and with no ontogenetic change in habitat or vulnerability to samplers, precise estimates of stage duration and a substantial counting effort are still required for mortality estimation to provide usable results. It is up to investigators (and reviewers) to determine whether a given situation allows for these estimates, and to scrutinize their data and models for the various sources of bias and imprecision.

Mortality of *L. tetraspina*

The individual mortality estimates for *L. tetraspina* had large confidence intervals (Fig. 5), despite the high precision of laboratory estimates of stage duration (Gould and Kimmerer, 2010). Nevertheless, the overall pattern is clear: mortality decreased from nauplii to copepodites to adults, and the seasonal increase in mortality was most pronounced in nauplii and absent in adults.

Mortality of adults is probably due mainly to predation by planktivorous fish, because gelatinous plankton are uncommon in this region of the estuary (Orsi and Mecum, 1986, Kimmerer, 2006) and macroinvertebrate predators such as mysids have declined greatly (Orsi and Mecum, 1996). Most of the planktivorous fishes of the brackish region are near historically low abundance. Northern anchovy (*Engraulis mordax*), once abundant in the low salinity waters of the estuary, declined sharply in 1987 (Kimmerer, 2006). Other fishes have also declined in abundance, and most of the remaining planktivores are visual feeders such as larval and juvenile longfin smelt *Spirinchus thaleichthys* and striped bass *Morone saxatilis*. These species consume copepods during early development but *L. tetraspina* is not a major part of their diets (Bryant and Arnold, 2007). The endangered delta smelt *Hypomesus transpacificus* feeds on copepods through its first summer and autumn, but apparently feeds on *L. tetraspina* only when other prey are scarce (Slater and Baxter, 2014). These findings are consistent with the small size (<0.5 mm total length) and cryptic behavior of this copepod.

Nauplii, in contrast, are vulnerable to incidental predation by clams (*Potamocorbula amurensis*, Kimmerer *et al.*, 1994) and the predatory copepod *Acartiella sinensis* (York *et al.*, 2014). Consumption rates of these species are highest in late summer (Kimmerer and Thompson, 2014; A. Slaughter, SFSU, submitted). Copepodites are also somewhat vulnerable to predation by *A. sinensis* (York *et al.*, 2014) and are small enough to enter clam siphons, but adults appear less vulnerable than nauplii to both clams and predatory copepods.

The analysis of mortality in *L. tetraspina* was made feasible by the limited geographic range of the copepod population, the slow rate of change of the population during the sampling period and the very tight confidence limits on laboratory stage durations. The total numbers counted were sufficient to make RSD_m <50% in nearly all cases, and only one estimate of mortality for copepodites was <0 (Fig. 5). Thus, despite the shortcomings of the mortality method presented here, individual, unreplicated mortality estimates are consistent enough to be interpreted.

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