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A new quantitative model of multiple transitions between discrete stages, applied to the development of crustacean larvae

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Abstract—Many biological processes are described in terms of transitions between discrete stages. For example, crustacean larvae generally pass through a number of stages that are punctuated by transitional molting events. On the other hand, some continuous processes, such as embryo development, are frequently described in terms of discrete stages. Despite the widespread use of such conceptual models, a mathematical model that quantitatively describes the transitions between multiple stages has not been developed for crustacean larvae. I describe a model of multiple transitions between stages that can be fitted to such data and that holistically describes the processes and allows explicit, quantitative comparisons among treatments or studies. The base of the model is the logistic equation that is frequently used to model a transition between 2 stages. By summing together multiple logistic equations, one for each transition between stages, the model can accommodate multiple stages. Variance is modeled by treating each transition as a binomial distribution and summing the variance from each transition. To demonstrate, I fitted the model to data on larval development of red and blue king crabs (*Paralithodes camtschaticus* and *P. platypus*). The model provides an excellent fit for these data and quantitatively describes the process of larval development for these crab species.

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Biologists often describe biological processes as discrete stages, either on the basis of a natural underlying stepwise process or to simplify a complex continuous process. For example, crustacean larval development generally encompasses discrete stages that are punctuated by molting events (e.g., Costlow and Bookhout, 1959; Haynes, 1982). For simplicity's sake, embryo development of various species is frequently divided into stages defined by particular characteristics, although development in some stages is considered continuous rather than discrete (e.g., Kimmel et al., 1995; Bas and Spivak, 2000; Stevens, 2006). More broadly, diseases and communities are also described as transitioning between stages. Although such developmental processes are commonly described in the biological literature, no model has been developed that quantitatively describes processes which involve sequential transitions between multiple discrete stages and allows explicit comparisons among treatments or species.

Frequently, each stage is considered independently (e.g., Paul and Paul, 1999; Andrés et al., 2010; Walther et al., 2010) and each measured or estimate variable, such as stage duration, is analyzed separately by using univariate statistics (e.g., analysis of variance [ANOVA] or *t*-tests). This approach is unsatisfactory because any comparisons among treatments require a large number of statistical tests, increasing the frequency of type-I errors—problems that are similarly caused by the use of a series of univariate statistics to analyze a multivariate data set (Quinn and Keough, 2002). In addition, in studies on larval development, the method used for determining average interstage duration is often not defined (Paul and Paul, 1999; Andrés et al., 2010; Walther et al., 2010). The method is not defined because of the inherent difficulties in determining when a replicate container with many larvae has reached the next stage. Does the next stage occur the first larva transitions or when the last one does? Or does it occur on the first day when at least half have transitioned?

Red king crab and blue king crab (*Paralithodes camtschaticus* and *P. platypus*) are commercially fished species in Alaska and have a wide and overlapping distribution (Somerton, 1985). In both species, mature females molt, mate, and extrude a batch of eggs in the spring and brood the eggs for about a year (Jensen and Armstrong, 1989; Stevens and

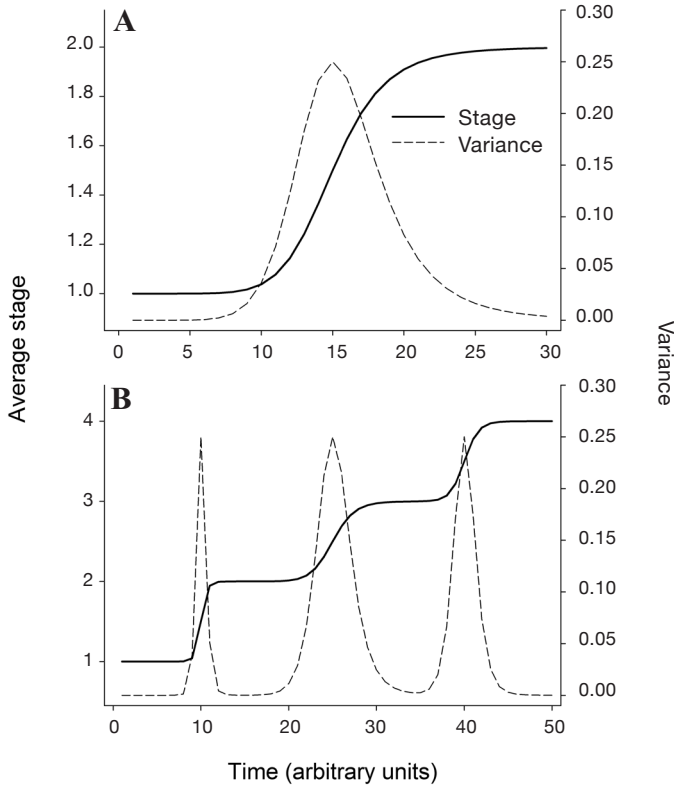

Figure 1

Relationships of stage transitions and predicted variance to time, measured in arbitrary units, from the (**A**) single-stage transition model (time at which 50% of the individuals have made the transition [$t_{50,1}$]=15, slope parameter at the transition that describes the abruptness of the transition [s_1]= −8) and the (**B**) Model of multiple transitions between stages with 4 stages and 3 transitions ($t_{50,1}$=10, $t_{50,2}$=25, $t_{50,3}$=40, s_1=−30, s_2=−20, s_3=−50).

Swiney, 2007). The species have a similar size–fecundity relationship (Herter et al., 2011; Swiney et al., 2012; Swiney and Long, 2015), but blue king crab reproduce only once every 2 years whereas red king crab produce a clutch annually (Jensen and Armstrong, 1989).

The larvae of both species are planktonic for 2–3 months before the glaucothoe settle into benthic habitats (Shirley and Shirley, 1989; Stevens et al., 2008). Because newly settled king crabs are highly vulnerable to predators (Stevens and Swiney, 2005), the glaucothoe typically remain planktonic until they find a complex habitat suitable for settlement (Stevens and Kittaka, 1998; Stevens, 2003; Tapella et al., 2009). Red king (Pirtle and Stoner, 2010) and blue king (Daly and Long, 2014a) crabs are vulnerable to predation from both conspecifics (Stoner et al., 2010; Daly and Long, 2014b) and other predators (Daly et al., 2013), but predation is reduced in complex habitats such as cobble, shell hash, and macroalgae (Stoner, 2009; Long et al., 2012; Long and Whitefleet-Smith, 2013). In red king crab, individuals transition into podding behavior as they grow too large to be cryptic (Powell and Nickerson, 1965; Dew, 1990), but nothing is known about blue king crab at this age. Both species mature at a carapace length of about 90 mm (Somerton and MacIntosh, 1983; Blau, 1989), although size at maturity varies among populations (Pengilly et al., 2002).

In this study, I present a simple model that describes such stepwise processes. It is flexible enough to be expanded to multiple stages and allows for explicit comparisons among species or treatments in a holistic way. Throughout this article I refer to this model of multiple transitions between stages as the MT (multiple transitions) model. To illustrate this model, I fitted larval development data from laboratory-reared red and blue king crabs. Larvae of both species pass through 4 zoeal stages (ZI–ZIV) and 1 glaucothoe stage (G) before they metamorphose to the first benthic crab stage (C1) (Sato and Tanaka, 1949; Hoffman, 1968); therefore, these larval stages provide an opportunity to explore the utility of this model.

Materials and methods

Description of the multiple transitions model

The basis of the MT model is the logistic family of equations, which are frequently used to describe a transition from one stage to another, for example, from life to death as a function of time (e.g., Long et al., 2008) or from immature to mature as a function of size (e.g., Somerton, 1980). For the power-function version used to describe the transition between 2 stages of development, the equation would be parameterized as follows:

$$Stage = 1 + \frac{1}{1 + \left(\frac{t}{t_{50,1}}\right)^{s_1}}, \quad (1)$$

where t = the independent variable (time);
$t_{50,1}$ = the time at which 50% of the individuals have made the transition; and
s_1 = the slope parameter at the transition that describes the abruptness of the transition.

This equation could be simplified as $Stage=1+p_1$, where p_1 is the probability of an individual having undergone the transition. Larger absolute values of s indicate a more rapid transition between states. The lower and upper limits for this function are 1 and 2, respectively.

This equation has the desired properties of the function being 1 at values of t far below $t_{50,1}$, rising sigmoidally to 1.5 at $t_{50,1}$, and rising toward an asymptote of 2 as t increases above $t_{50,1}$, with the amount of time both stages are present being a function of s_1 (Fig. 1A).

This function is easily expanded to n stages with n–1 transitions:

$$Stage = 1 + \sum_{i=1}^{n-1} \frac{1}{1 + \left(\frac{t}{t_{50,i}} \right)^{s_i}}. \quad (2)$$

This function ranges from 1 to n and increases in a stepwise fashion (Fig. 1B). Although I have used the power function in this study because the parameterization is convenient for interpretation, other sigmoidal functions could be substituted in the equation without otherwise altering the model.

Modeling the expected variance employs a similar logic. Because the variance is expected to vary continuously in this model, it is imperative to model the variance as well as the mean in a statistically valid manner (Bolker, 2008). When the logistic function (Eq. 1) is used to describe the transition between 2 states, a binomial distribution is often assumed (e.g., Long et al., 2013a) and variance is given with the following equation:

$$var = p(1 - p), \quad (3)$$

where var = the variance; and
p = the probability of the event occurring.

This variance structure is appropriate because the variance is 0 at a probability of 0 (i.e., none of the population have made the transition), maximum at a probability of 0.5 (i.e., at t=t_{50}, the point at which there is transition between the states), and 0 again at a probability of 1 (i.e., all of the population has made the transition; Fig. 1A).

In the MT model, a pure binomial distribution cannot be assumed because the total number of states is greater than 2; however, a similar variance structure can be achieved by treating each of the transitions as a separate binomial distribution and summing the variances together. Therefore, variance for the expanded MT model (Eq. 3) can be given with the following equation:

$$var = Cov(X_j, X_k) + \sum_{i=1}^{n-1} (1 - p_i), \quad (4)$$

where $Cov(X_j,X_k)$ = the covariance between each combination of stage transitions (where $i \neq k$) and p_i is the probability of an individual undergoing the i^{th} transition.

Because the covariance between any 2 stage transitions will be 0 if the stage transitions happen at different times (i.e., if only one of the transitions is occurring at the same time), this term is 0 under most circumstances. If, however, there is substantial overlap between 2-stage transitions (i.e., if there are times when 3 different stages are present at the same time), the covariance between those 2 stage transitions should be included in the model. This circumstance should be rare for the majority of uses for which this model is intended.

Equation 4 has properties similar to those of Equation 3 in that the variance is highest at values of t that are near one of the transitions but approaches 0 at values between transitions when all the individuals are expected to be in a single stage (Fig. 1B). At a given variance, the binomial distribution can be approximated by the normal distribution (Bolker, 2008). Although such an approximation is not as good at values of p close to 0 or 1, this approximation affects only the estimates of the tails of the error distribution and not the mean and, therefore, should not affect the fit of the model. By assuming normal distributions of error with variances that change according to Equation 4, the model allows more than 2 stages and therefore overcomes the 2-state limit of the binomial distribution. This approach allows the variance to change as if it were a binomial distribution, providing a good mechanistic match to the data structure.

Model applied to larval development

In the winter of 2010, 9 and 11 ovigerous female red king crab and blue king crabs, respectively, were collected in baited commercial pots in the Bering Sea. Crabs were identified according to the methods of Donaldson and Byersdorfer (2005). Red king crab were transported to the Kodiak Fishery Research Center in the "live well" of a commercial fishing vessel, and blue king crab were transported in coolers by air cargo. In the laboratory, the crabs were held in flow-through seawater supplied from Trident Basin, Kodiak, at ambient temperature and salinity and fed to excess on a diet of chopped frozen fish and squid.

Larval rearing procedures were similar to those of Swingle et al. (2013). In brief, larvae were collected at hatching and larvae of red and blue king crabs were pooled and each stocked in a separate 2000-L tank. Larval red king crab were stocked at 50 larvae/L, the amount collected in a single day from 8 females that were hatching at the time. Because only 6 female blue king crab produced larvae simultaneously, larvae of blue king crab were collected over 3 days and were stocked at 30 larvae/L. Because of differences in thermal tolerances (Stoner et al., 2013), the red king crab were reared at 8.8°C (standard deviation [SD] 1.0), and the blue king crab were reared at 6.5°C (SD 0.6). While the larvae were in the zoeal stages, they were fed a diet of DC DHA Selco[1] (INVE Aquaculture, Salt Lake City) enriched *Artemia* nauplii. The glaucothoe stage is a stage when larvae are not feeding (Abrunhosa and Kittaka, 1997a, 1997b); therefore, no food was provided. Each day, from stocking to the point when all of the larvae had molted to the first crab stage, 10 larvae from each species were removed, the developmental stage of each was determined, and the mean developmental stage of the 10 larvae was calculated.

[1] Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Table 1

Ranking of models of larval development in red and blue king crabs (*Paralithodes camtschaticus* and *P. platypus*) with the use of Akaike information criteria, corrected for small sample sizes (AIC_c). Common parameters (species the same) or different parameters (species different) were used in the models. K=number of parameters. Likelihood=likelihood of each model relative to all the models considered.

Model	K	AIC_c	ΔAIC_c	Likelihood	AIC_c weights
Species different	20	−5974	0.00	1.00	1.00
Species the same	10	−2249	3725	0.00	0.00

The mean stage on each day was fitted to the MT model (Eq. 2) in R vers. 2.14.0 (R Development Core Team, 2011), by using maximum likelihood (mle function, stats4 package, vers. 3.1.0) and by assuming a normal distribution of errors with a variance structure defined by Equation 4. Time was expressed in degree-days (a measure that accounts for both time and temperature) to control for the difference in rearing temperatures (e.g., Stevens, 1990; Long et al., 2013b; Swiney et al., 2013) and calculated as

$$DD_m = \sum_{t=0}^{m-1} Temp_t \times 1\, day, \quad (5)$$

where DD_m = the degree-days on day m;
t = the time in days; and
$Temp_t$ = the temperature (in Celsius) on day t.

Two models were fitted, one in which the parameters were common between red and blue king crabs and one in which parameters differed between the species. Models were compared with the Akaike information criteria, corrected for small sample sizes (AIC_c):

$$AIC_C = -\log(L) + 2K\left(\frac{n}{1-K-1}\right), \quad (6)$$

where$L$ = the likelihood of the model;
K = the number of parameters in the model; and
n = the sample size; and

where the AIC_c was used to select the best model (Burnham and Anderson, 2002). Normality of the errors was checked through examination of the standardized residuals of the best model.

Results

The model of larval development with independent parameters for red and blue king crabs was the one in which red and blue king crabs provided the best fit (Table 1) with a coefficient of determination (r^2, calculated with the raw data) for red and blue king crabs of 0.98 and 0.97, respectively (Fig. 2). The model in which they did not differ had a ΔAIC_c of 3700, indicating that there was no support for this model (Burnham and Anderson, 2002).

In terms of degree-days, larvae of red king crab molted to the ZII, ZIII, and ZIV stages earlier than larvae of blue king crab, both species molted to the G stage at about the same time, and blue king crab molted to the C1 stage earlier than red king crab (Fig. 2, Table 2). The stage transitions of red king crab were more rapid than those of blue king crab (Fig. 2, Table 2), although the precision in the estimates for s

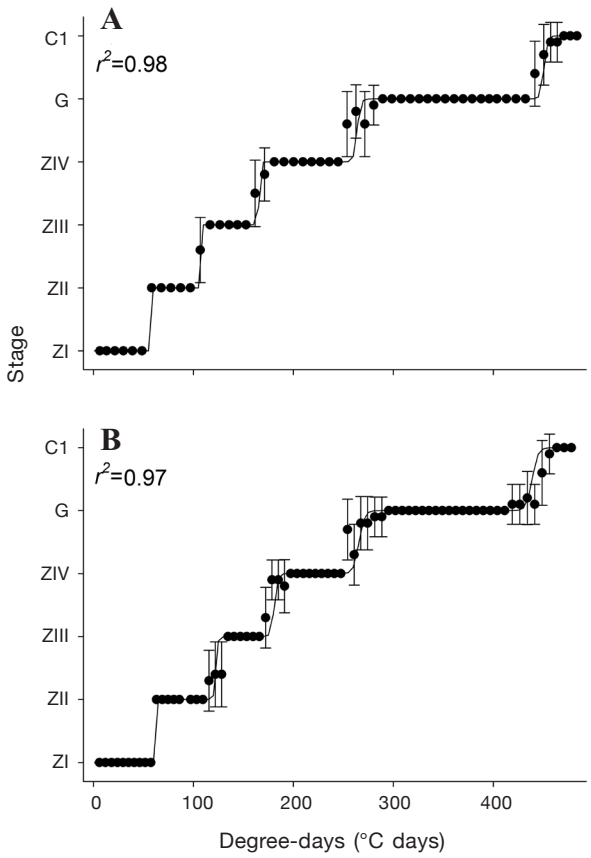

Figure 2

Larval development of (**A**) red king crab (*Paralithodes camtschaticus*) and (**B**) blue king crab (*P. platypus*) from the first zoeal stage through the first crab stage. Points represent the mean stage as determined each day throughout development. Error bars are one standard deviation; note that on days when all the larvae were at one stage the standard deviation was 0. Lines represent the best-fit stage-transition model for each species. The larval stages shown are the 4 zoeal stages (ZI–ZIV), the glaucothoe stage (G), and the first benthic crab stage (C1). r^2=coefficient of determination.

Table 2

Mean estimates, with standard errors in parentheses, of the t_{50} parameter (the time at which 50% of individuals have made the transition) and the s parameter (the slope at the transition) for the transitions between each stage in the larval development of red and blue king crabs (*Paralithodes camtschaticus* and *P. platypus*). The stages are the 4 zoeal stages (Z1–IV) and the glaucothoe stage (G). The stage given in the first column indicates that the transition is from that stage to the next one (e.g., "G" indicates the transition from the glaucothoe stage to the first crab stage). The estimates for t_{50} are given in degree-days.

	Red king crab			Blue king crab		
Stage	t_{50}	s	$-s/t_{50}$	t_{50}	s	$-s/t_{50}$
ZI	57.0 (SE 0.0002)	−1743 (SE 2929)	30.57	61.5 (SE 0.0004)	−1434 (SE 58)	23.31
ZII	107.2 (SE 0.6)	−642 (SE 1208)	5.99	122.5 (SE 0.1)	−129 (SE 1.2)	1.05
ZIII	165.7 (SE 0.1)	−225 (SE 4)	1.36	180.9 (SE 0.3)	−113 (SE 1.3)	0.62
ZIV	263.9 (SE 0.1)	−158 (SE 0.1)	0.60	265.6 (SE 0.1)	−101 (SE 0.1)	0.38
G	450.6 (SE 0.2)	−286 (SE 6)	0.64	438.9 (SE 0.8)	−158 (SE 6.8)	0.36

were low for several stages because of the rapidity of the transition.

Discussion

In this article, I describe a new method for modeling biological processes that involve multiple transitions between discrete stages. The summing of simple logistic equations, which are frequently used for stage-transition models, is analogous to commonly used time-series analyses that model periodic phenomena as a sum of multiple cosine waves (e.g., Linhart and Zucchini, 1986). The method provides a concise, mathematical description of such transitional processes, is mechanistically sound, and provides easily interpreted parameters. The t_{50} for each transition is used easily to determine the length of time between stages, is objective and quantitative, allows for explicit comparisons among studies, and avoids problems with qualitative determinations, such as the time when molts are observed (e.g., Swingle et al., 2013). The s parameter, which is proportional to the rate of change between stages, may also be of interest to investigators.

The MT model provided an excellent fit to the data for larval development of red and blue king crabs and provided estimates of the standard error (SE) in the estimates of parameters that allow comparisons among studies. It is worth noting that I used these data as example data (the original purpose for rearing the larvae was to produce crabs for use in other experiments), and no conclusions can be drawn about reasons for the differences between the red and blue king crabs in this experiment because there was no replication and multiple factors (e.g., species, stocking density, and temperature) differed between the tanks. However, the estimates for development time can be compared with those of other studies, and they agree well with them.

Kurata (1960) compiled results from a number of experiments on larval rearing of red king crab and reported a range of 260.4–397.8 degree-days (mean: 325.0) from hatching to the G stage and a range of 392.4–514.8 degree-days (mean: 462.8) from hatching to the C1 stage. My estimates of 263.9 degree-days (from hatching to G) and 450.6 degree-days (from hatching to C1) fall within both ranges from that earlier study. Similarly, larvae of red king crab from the Barents Sea had stage durations of 66.0, 68.7, 69.3, and 79.1 (284 total) degree-days for the Z1–Z4 stages (Kovatcheva et al., 2006) compared with my estimates of 57.0, 50.2, 58.5, and 98.2 (263.9 total). Blue king crab have been studied less than red king crab, but our estimate of 265.6 degree-days for hatching through the G stage and 438.9 degree-days for hatching through the C1 stage are very similar to the 254.4 degree-days (from hatching to G) and 439.4 degree-days (from hatching through C1) averages found by Stevens et al. (2008).

In general, the estimates for the s parameters were good, but on a couple of the transitions, particularly the first 2 transitions for the red king crab, the estimates had poor precision (Table 2). In these cases, there were 0–1 observations of the actual transition, and, therefore, the MT model could not precisely estimate the rapidity of the transitions. Values of s that approach infinity are possible given the data; therefore, the SE in the parameter estimate is high. If the estimate of s is of particular interest, then the precision of the estimate can be increased by increasing the frequency of observations.

Theoretically, there is no limit to the number of stages that can be modeled with this approach. I originally developed this technique to model embryo development in golden king crab (*Lithodes aequispinus*) and was able to obtain a good fit for a 13-stage model (Long and Van Sant, 2016). However, as the number of stages and the number of parameters increase, it becomes more difficult for the algorithms to find the global minimum in the log-likelihood surface (Bolker, 2008), and the model fitting becomes more sensitive to the starting values for the parameters (Appendix).

The starting values for the t_{50} parameters are fairly easily estimated by simply examining the data for the times when the transitions are occurring; however, the s parameters are more difficult to estimate. Therefore, the use of an iterative process to determine reasonable starting values for these parameters may be helpful. When fitting the MT model or any model with a large number of parameters, it is highly recommended to fit the data under multiple sets of starting parameter values, and it is imperative to graph the model and data together to ensure that the fit is optimal and realistic.

In most cases, t_{50} will be a parameter of interest; however, there are times when the rapidity of the transition between stages may be relevant to the question posed by an investigator. For example, many crab species are cannibalistic, especially immediately after molting, when soft crabs are particularly vulnerable (e.g., Borisov et al., 2007). Therefore, to minimize cannibalism, a hatchery may find it valuable to determine under what conditions molting is highly synchronous among individuals within a tank (because all the individuals transition within a short space of time). The s parameter, as stated previously, indicates how quickly the transition between one stage and another occurs. However, s values cannot be compared directly with each other without first normalizing them to the t_{50} values. The derivative of Equation 1 evaluated at t_{50} is

$$\frac{dp}{dt}(t_{50}) = \frac{-s}{4t_{50}}. \quad (6)$$

This derivative demonstrates that the slope at t_{50} is dependent on both s and t_{50}.

In cases where comparisons in the rate of the stage transitions are important, it is necessary to calculate the ratio of s to t_{50} to make the comparison. For example, in Figure 1B, the first transition, which has an s of –30, occurs more rapidly than the third transition, which has an s of –50. Interpreting the s values alone would indicate that the third transition should be the most rapid, and it is not. However, the ratios of s to t_{50} for the first and third transitions are –3.0 and –0.8, respectively, and comparing the absolute values of these ratios allows an investigator to make a correct interpretation of the relative rapidity of transitions (Fig. 1B).

The data on larval development provide an example of how this ratio can be used to compare the rapidity of stage transitions. For both red and blue king crabs, the rapidity of the transition between stages decreases with each additional stage transition (Fig. 2, Table 2). The individual variance in developmental time leads to this decrease, which has been previously observed in both species (Stevens et al., 2008; Persselin and Daly, 2010), and it is reasonable to conclude that individual differences in feeding and growth rates would result in a larger spread in molting times later in development. In addition, the larvae of red king crab consistently had faster transitions between stages than did the larvae of blue king crab (Fig. 2, Table 2). This difference is most likely a result of the red king crab having been stocked in a single day, compared with the blue king crab, which were stocked over 3 days.

The MT model presented in this article provides a flexible and holistic approach for a quantitative description of complicated biological processes. This model is particularly well suited to crustaceans and indeed to arthropods in general, given that they develop though a series of transitional molts; however, any biological process that is divided into discrete stages (e.g., Kimmel et al., 1995) can be modeled with this technique. Treating the process with a single model affords investigators the ability to compare treatments by using model selection techniques (Burnham and Anderson, 2002), while avoiding the increase in the type-I error rate inherent in analyzing a large number of response variables with univariate statistics (Quinn and Keough, 2002).

Metadata for the data produced in the study described in this article are available at InPort (website).

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Appendix

This appendix provides R code with annotations for fitting data (by using maximum likelihood) to a multiple-stage transition model with 3 stages. The code can be expanded by adding more stages. The procedure requires that data to be in a data frame called "Stg" that consists of at least 2 vectors, one called "stage," which contains the stage for each sample, and one called "time," containing the time at which each sample was measured. Annotations are in italics.

```
library(stats4)   #The stats4 library contains the required mle function

Stage=function(T50_1,T50_2,s1,s2){      #this function calculates the negative log likelihood
                                        #given the data and a set of parameters. Additional stages
                                        #would require further parameters to be included here.
    ave=1+(1/(1+(Stg$Time/T50_1)^s1))+   #"ave" is the average stage at each time
```

```
        (1/(1+(Stg$ Time /T50_2)^s2))              #additional stages would be added by
                                                  # summing additional logistic equations here. A
                                                  #fourth stage would require 2 more parameters:
                                                  #T50_3 and s_3
        var=(1/(1+(Stg$ Time /T50_1)^s1))*(1-(1/(1+(Stg$ Time /T50_1)^s1)))+   #calculates
        (1/(1+(Stg$ Time /T50_2)^s2))*(1-(1/(1+(Stg$ Time /T50_2)^s2)))        #the variance
                                                  #additional stages require the variance for each
                                                  #transition to be added here.
        -sum(dnorm(Stg$Stage,ave,var^.5,log=TRUE))  #calculates the negative log likelihood
        }
param=list(T50_1=10, T50_2=25,s1=-10,s2=-20)   #initial parameter estimates. If the model is
                                                  #expanded to more stages, then the
                                                  #necessary parameters and estimates need
                                                  #to be added to this list.
mStage=mle(Stage,start=param)                     #fits the data to the model through the
                                                  #use of maximum likelihood
summary(mStage)                  #gives a summary of the fit, including estimates of the
                                 #parameter, their standard errors, and the –2 log
                                 #likelihood of the fit.
```