

STATISTICAL EVALUATION OF LIFE HISTORY VARIATION USING AN INVERSE MATRIX MODEL

BU-1484-M

MAY, 2000

Nancy Tisch
Department of Biometrics
Cornell University
Ithaca, NY 14853

and

Saran Twombly
Department of Biological Sciences
University of Rhode Island
Kingston, RI, 02881

Keywords: demographic estimation, life history, transition matrix, copepods, amphipods.

Abstract

For many organisms, variation in life history traits is poorly understood because we are unable to measure demographic rates such as growth and survival directly in field populations. While many mathematical and statistical methods have been developed to estimate these rates from abundance data, most of them require a priori information about development rates or survival rates, neither of which is known. One method, an inverse matrix technique (Caswell and Twombly, 1989) allows simultaneous estimates of growth and survival probabilities.

Here we document a number of improvements to the inverse matrix method including the application of a non-parametric bootstrap to characterize bias and generate confidence intervals and the incorporation of more than one reproductive stage making the model more flexible. We then apply the modified method to three data series that vary with respect to the number of reproductive stages included and in the quality of the abundance estimates based on sample variability among replicate samples and variability between successive samples.

Our results address a number of problems associated with demographic estimation techniques. We found that the modified method produced bias corrected confidence intervals that allowed statistical inference regarding temporal variation of demographic rates. Increased sampling noise had two effects on the results: 1) increased width of the confidence intervals and 2) introduction of bias to the original estimates. Modification of the model to include more than one reproductive stage allows for application to a broader number of species. Most importantly, the improved inverse matrix model provides a robust method for simultaneous estimation of stage-specific growth and survival probabilities.

Running head: Inverse Matrix Estimation

Statistical evaluation of life history variation using an inverse matrix model

Nancy Tisch¹ and Saran Twombly²

¹ Biometrics Department
Cornell University
Ithaca, New York 14853-7801 USA
(607) 255-7826
e-mail nt34@cornell.edu

² Department of Biological Sciences
University of Rhode Island
Kingston, RI 02881-0816 USA
(401) 874-2609
e-mail stw4877u@uri.edu

ABSTRACT

For many organisms, variation in life history traits is poorly understood because we are unable to measure demographic rates such as growth and survival directly in field populations. While many mathematical and statistical methods have been developed to estimate these rates from abundance data, most of them require *a priori* information about development rates or survival rates, neither of which is known. One method, an inverse matrix technique (Caswell and Twombly, 1989) allows simultaneous estimates of growth and survival probabilities.

Here we document a number of improvements to the inverse matrix method including the application of a non-parametric bootstrap to characterize bias and generate confidence intervals and the incorporation of more than one reproductive stage making the model more flexible. We then apply the modified method to three data series that vary with respect to the number of reproductive stages included and in the quality of the abundance estimates based on sample variability among replicate samples and variability between successive samples.

Our results address a number of problems associated with demographic estimation techniques. We found that the modified method produced bias corrected confidence intervals that allowed statistical inference regarding temporal variation of demographic rates. Increased sampling noise had two effects on the results: 1) increased width of the confidence intervals and 2) introduction of bias to the original estimates. Modification of the model to include more than one reproductive stage allows for application to a broader number of species. Most importantly, the improved inverse matrix model provides a robust method for simultaneous estimation of stage-specific growth and survival probabilities.

keywords: demographic estimation, life history, transition matrix, copepods, amphipods

INTRODUCTION

Organisms respond to environmental variation by altering their vital demographic rates of birth, growth and survivorship. Ecologists observe these changes as fluctuations in abundance of individuals over time; the underlying vital demographic rates are often difficult or impossible to measure directly. This is especially true for organisms that are too small, too mobile or too endangered to be successfully marked and recaptured. Knowledge of the way in which organisms respond to environmental change is useful for a number of reasons: it can predict changes in the population at some time in the future, it can reveal sublethal responses to environmental perturbation, or it can provide insight into the ecological and evolutionary significance of intraspecific life-history variation.

When vital rates cannot be measured directly in the field, efforts have focused on methods which allow their mathematical estimation from stage-structured abundance data (reviewed in Manly, 1989; Manly, 1990; Aksnes et al., 1997). Recent methods rely on fairly complex mathematical models; all require *a priori* estimates of developmental rates that are usually not available. For example, Wood (1994) used a population surface method to estimate stage-specific mortality over time. His method requires both stage abundance data and some *a priori* knowledge of stage duration but there are no constraints on sampling frequency and the method is robust to noisy data. The method relies on fitting bicubic spline functions to the observed data. Aksnes and Ohman (1997) suggested using a static life table approach for estimating mortality. Their method has the advantage of requiring only a limited number of samples taken at one time rather than a time series of abundance data, which is often difficult to collect. The resulting demographic rates are time specific. This approach does not assume a closed population, but instead it accounts for the demographic effects of advection (i.e. immigration and emmigration due to water movement). Knowledge of stage duration is again required to solve for estimates of mortality. An inverse matrix method (Caswell and Twombly, 1989) provides some advantage over these models because it does not require prior

knowledge of stage duration. It does, however, assume that the population is closed and that sampling intervals are equal and shorter than the duration of the shortest stage in the model. The choice of which model is most appropriate depends largely on the available data and the ability to sample frequently at regular time intervals.

Because we study field populations for which developmental rates are not known and not easily estimated, we have focused our research on the inverse matrix method (hereafter IMM, Twombly, 1994, Tisch, 1997). In addition to its rigorous sampling requirements, the IMM has other drawbacks. The original model provided only single point estimates of stage specific rates, it introduced bias to the estimates and it was developed for organisms (copepod crustaceans) with a single reproductive stage. In addition, the model generated survival probabilities that were greater than one (Caswell and Twombly 1989; Twombly 1994), which may have been caused by the introduction of bias. Here we improve the model with the development of a nonparametric bootstrap to characterize bias and to provide confidence intervals for demographic rate estimates. We also generalize the model by including more than one reproductive stage.

Using the improved model to analyze three data series, we address problems with demographic estimation in general and particular problems associated with the inverse matrix method. The first of these problems is that most other demographic estimation methods require independent estimates of development rates. Researchers generally rely on temperature to estimate these rates (e.g. Ohman and Wood, 1996) despite mounting evidence that developmental rates are influenced by factors other than temperature (e.g. food quality and food quantity, Twombly et al., 1998, Xu and Burns, 1991). Small errors in these estimates result in large deviations of rate estimates from known values, especially using the population surface method (Wood, 1994; personal observation). One major goal of our study has been to improve the inverse matrix method because it can provide simultaneous estimates of development and survival probabilities.

Secondly, when applied to field data, the inverse matrix method frequently yields probability estimates that are greater than 1 (Twombly, 1994). Without associated confidence intervals, it is impossible to interpret these estimates. Values greater than 1 suggest that some important demographic transition may be missing from the model, or that bias inherent in the technique may produce biologically unrealistic results. Lack of confidence intervals also provides no way to detect statistically significant trends over time, or to determine whether spurious estimates result from a faulty model or noisy data. A second goal of the study, then, was to re-evaluate Twombly's *Diaptomus negrensis* data (1994) with confidence intervals to address these problems.

Field data are often very noisy, and this noise may destabilize matrix estimates. A third goal of the study was to apply the inverse matrix method to data from a closed mesocosm population that are less noisy than field data, allowing us to assess the problems associated with noisy data.

Lastly, the inverse matrix method was developed for, and has been applied to, copepod crustaceans that are relatively easy to sample and have only one reproductive stage. A great many other problematic (from a demographic perspective) organisms don't fit this model well, and a fourth goal of the study was to make the model more flexible by including a number of reproductive stages. We applied this modified version of the model to an organism that is also more difficult to sample and patchier in distribution allowing us to revisit the issue of sample noise with increased levels of noise.

MATERIALS AND METHODS

The Data

Diaptomus negrensis: The first data series used was for a freshwater calanoid copepod, *Diaptomus negrensis*, which was sampled every second day from a tropical floodplain lake along the Orinoco River, Venezuela (Twombly, 1994). The life cycle is characterized by 12 developmental stages—6 naupliar stages and 6 copepodite stages--

the last of which is the adult stage (Figure 1A). Abundance of each stage and single point estimates of vital demographic rates have been reported previously (Twombly, 1994).

Here, we report point estimates with bias corrected confidence intervals with the expectation that bias correction will provide more biologically meaningful results.

Acartia hudsonica: In contrast to field data, mesocosm data represent closed populations in which all stages of the life cycle are present in the water column. Experiments designed to evaluate the effects of eutrophication on zooplankton population dynamics were conducted at the Marine Ecosystem Research Laboratory (MERL) at the University of Rhode Island's Bay Campus located on Narragansett Bay, Rhode Island (E.G. Durbin, personal communication). Plankton samples were collected twice weekly from April 9 to May 22, 1985, from two 13.1 m³ mesocosm tanks using pumps. Abundance of each stage of *Acartia hudsonica* was enumerated. The life cycle of *A. hudsonica* is similar to that of *D. negrensis* (Figure 1A). Here, we report abundance data and demographic model estimates for the control tank only.

Jassa marmorata: Lastly, abundance data of the marine amphipod *Jassa marmorata* were collected at Fort Wetherill on Narragansett Bay, Rhode Island. *J. marmorata* is a tubicolous amphipod. Sampling of the substratum (algae or fouling crust) provides estimates of abundance for all stages in the life cycle. Amphipods were sampled at weekly intervals from May 1993 - October 1993 (Tisch, 1997). The stages enumerated included eggs, recruits (0.8-1.5 mm body length), juveniles (>1.5-3.5 mm), small adults (>3.5-5.5mm), medium adults (>5.5-7.5mm) and large adults (>7.5 mm) (Figure 1B). All of the adult stages are reproductive.

The Model

Caswell and Twombly (1989) proposed that stage specific survivorship and growth rates could be estimated from population stage vector data using matrix projection models. Their model makes only minimal assumptions: they do not assume that the

population is characterized by a stable age distribution, they do not assume a constant environment and therefore the transition matrix can vary with time and environmental conditions. Lastly, cohorts do not need to be identified. The basis for the model is a typical life cycle graph in which individuals within a stage can survive and remain in stage i (P_i) or can survive and develop into stage $i+1$ (G_i) during a given time (sampling) interval (Figure 1). The probabilities G_i and P_i can be expressed in terms of basic survival and growth probabilities as follows:

$$G_i = \sigma_i \gamma_i$$

$$P_i = \sigma_i (1 - \gamma_i)$$

where σ_i is the survival probability of stage i and γ_i is the probability of growth from stage i to stage $i+1$. Reproductive individuals can produce new recruits; F_i equals the fertility or average number of recruits produced per individual in stage i per unit time. (For one organism, we will include an egg stage and F_i will represent fecundity, see the section *Increasing the number of reproductive stages*). The projection matrix (a Lefkovitch matrix) contains P_i 's on the diagonal, G_i 's on the subdiagonal and F_i 's in the first row. The matrix projection equation can be written as

$$n_{(t+1)} = A_t n_{(t)}$$

where $n_{(t+1)}$ is a vector of population stages at time $t+1$, $n_{(t)}$ is the corresponding vector at time (t) and A_t is an $s \times s$ matrix (s = number of stages) whose entries incorporate the demographic transitions.

The method works by solving a series of simultaneous equations. For stages 2 through the final adult stage these equations are written as follows

$$n_{i(t+1)} = n_{i(t)} * P_i + n_{i-1(t)} * G_{i-1}$$

$$n_{i(t+2)} = n_{i(t+1)} * P_i + n_{i-1(t+1)} * G_{i-1}$$

$$n_{i(t+3)} = n_{i(t+2)} * P_i + n_{i-1(t+2)} * G_{i-1}$$

$$n_{i(t+4)} = n_{i(t+3)} * P_i + n_{i-1(t+3)} * G_{i-1}$$

where i = stage. Rearrangement of these terms yields:

$$\begin{bmatrix} n_{i-1(t)} & n_{i(t)} \\ n_{i-1(t+1)} & n_{i(t+1)} \\ n_{i-1(t+2)} & n_{i(t+2)} \\ n_{i-1(t+3)} & n_{i(t+3)} \end{bmatrix} \begin{bmatrix} G_{i-1} \\ P_i \end{bmatrix} = \begin{bmatrix} n_{i(t+1)} \\ n_{i(t+2)} \\ n_{i(t+3)} \\ n_{i(t+4)} \end{bmatrix}$$

which can be written in the form:

$$N\beta = d$$

where N is a matrix and d is a vector of census data; β is a vector of unknown transition probabilities. To solve for β the equation is rewritten as:

$$\beta = N^-d$$

where N^- equals the generalized inverse of the matrix N .

Caswell and Twombly (1989) used simulated data to determine the number of simultaneous equations that were required to yield stable estimates of P_i and G_i ; they found that at least 5 data points were necessary but more than 5 did not substantially improve the estimates. The five data points are referred to as the sample window. Thus, A_t does not vary between each successive sample but varies smoothly between successive sample windows instead. They evaluated a number of regularization techniques to deal with sampling noise and found that truncated singular value decomposition overcame problems resulting from noise that is typical of field data. They also recognized that singular value decomposition introduces bias to the estimates.

Modifications to the model

Bias and confidence intervals

To characterize the bias introduced by singular value decomposition, we generated bootstrapped estimates for the unknown parameters in each sample window. We used one-step forecasting to generate stage vectors of error terms within each sample window as follows:

$$\begin{aligned}\hat{n}_{t+1} &= \hat{A}_t n_t \\ \hat{n}_{t+1} - n_{t+1} &= \varepsilon_t\end{aligned}$$

All possible combinations of the error vectors with the original data vectors produced 256 matrices that mimicked the original sample window. Thus 256 bootstrapped estimates of each parameter in the transition matrix were made.

Preliminary evaluation of the distributions of each of the parameter estimates revealed the presence of statistical outliers in some of the distributions. If outliers were identified (using the criterion of 3 times the interquartile range (Kuehl, 1994)) they were removed. Following the removal of outliers, confidence intervals were generated using BCa, a non-parametric bias-corrected and accelerated method (page 184, Efron and Tibshirani, 1993). The interval of coverage $1-2\alpha$ for any of our demographic parameter estimates is given by

$$\text{BCa} : (\theta_{lo}, \theta_{up}) = (\theta^{*(\alpha_1)}, \theta^{*(\alpha_2)})$$

where $\theta^{*(\alpha_1)}$ and $\theta^{*(\alpha_2)}$ indicate the $100*\alpha$ th percentile of the B bootstrap replicates $\theta^*(1), \theta^*(2), \dots, \theta^*(B)$. In contrast to the percentile method, the interval endpoints depend on two numbers – a and z_0 – which are the acceleration and bias correction respectively. The endpoints are calculated as follows:

$$\alpha_1 = \Phi \left(\hat{z}_0 + \frac{\hat{z}_0 + z^{(\alpha)}}{1 - \hat{a}(\hat{z}_0 + z^{(\alpha)})} \right)$$

$$\alpha_2 = \Phi \left(\hat{z}_0 + \frac{\hat{z}_0 + z^{(1-\alpha)}}{1 - \hat{a}(\hat{z}_0 + z^{(1-\alpha)})} \right)$$

The value of the bias-correction z_0 is obtained directly from the proportion of bootstrap replications less than the original estimate θ :

$$\hat{z}_0 = \Phi^{-1} \left(\frac{\#\{\hat{\theta}^*(b) < \hat{\theta}\}}{B} \right)$$

where $\Phi^{-1}(\cdot)$ indicates the inverse function of a standard normal cumulative distribution function. The value of a , or the acceleration, is calculated using a jackknife and represents the rate of change of the standard error of θ with respect to the true parameter value θ . Efron and Tibshirani (1993) provide the following simple expression for the acceleration:

$$\hat{a} = \frac{\sum_{i=1}^n (\hat{\theta}_{(\cdot)} - \hat{\theta}_{(i)})^3}{6 \{ \sum_{i=1}^n (\hat{\theta}_{(\cdot)} - \hat{\theta}_{(i)})^2 \}^{3/2}}$$

Efron and Tibshirani (1993) also describe two important theoretical advantages of the BCa method. First, it is transformation respecting and therefore automatically chooses its own best scale. Secondly, it is second-order accurate while the standard and percentile methods are only first-order accurate.

Increasing the number of reproductive stages:

The presence of more than one reproductive size class (Figure 1B) would necessitate a larger sample window for simultaneous estimation of F_i 's and P_1 as described above (Twombly, 1994). This need would, in turn, require us to assume that environmental conditions remain constant over a longer sampling period. To avoid this requirement, we estimated F_i 's and P_1 using a more empirical approach. For the amphipod *Jassa marmorata*, we include the egg as the first stage in the life cycle and

assume that egg development time is solely a function of water temperature. This did not seem unreasonable given that eggs are a non-feeding stage. Nair and Anger (1979) reported egg development times for *Jassa marmorata* at three different temperatures (10, 16 and 20 °C). We averaged water temperature data for each sample window (5 sampling dates) and used linear interpolation of Nair and Anger's data (assuming a smooth relationship between temperature and development time) to estimate development times at intermediate temperatures. We used the inverse of average egg development time as an estimate of γ_1 . The probability of an egg hatching during a time interval was estimated as G_1 using the inverse technique. The values for G_1 and γ_1 were used to calculate σ_1 and P_1 was using the relationships of the first two equations above (under the section heading *The Model*).

Because the egg stage is included as a discrete stage, the F_i 's estimated by the modified IMM (Figure 1B) represent fecundity or the average number of eggs produced per individual per unit time. (This is in contrast to the original model in which the F_a term incorporates egg production and subsequent egg survival to the nauplius 1 stage. For copepods then, F_a is a measure of fertility or recruitment.) To estimate fecundity for each of the adult stages in *Jassa marmorata*, we first needed to determine the total number of new eggs produced from time t to time $t+1$. We used the following equation:

$$\text{Eggs}_{(t+1)} = P_1 * \text{eggs}_{(t)} + \text{Births}_{(t,t+1)}$$

where $\text{eggs}_{(t+1)}$ and $\text{eggs}_{(t)}$ are the number of eggs at times $t+1$ and t respectively, P_1 is the probability that an egg survives and does not hatch and $\text{births}_{(t,t+1)}$ represents the production of new eggs in the time interval t to $t+1$.

Once an estimate of $\text{births}_{(t,t+1)}$ was made, we assumed that the new egg production was distributed among the adult size classes in the same relative proportion as the total number of eggs. This assumption is based on our observation of no pattern in the distribution of embryonic development stages among different size classes of females. We used sample data to calculate values for m_x the average number of eggs per adult for

each size class x . Next we summed m_x over all reproductive stages and calculated the proportion that each size class contributed to total egg number in each sample. This proportion was multiplied by the births $(t, t+1)$ to estimate size-specific fecundity for each sample window.

RESULTS

We report water temperature, stage-specific abundance, and corresponding demographic estimates for each of the three species investigated. For both copepod species, the sampling intervals (twice weekly, and every second day) did not meet the requirement of being shorter than the shortest stage duration. Therefore, we combined successive stages as follows: nauplius 1 and 2, nauplius 3 and 4, nauplius 5 and 6, and copepodite 1 and 2. We did not include the egg stage for either of the copepod data series; therefore, F_a represents fertility or the average number of nauplii produced per adult per unit time. Fertility incorporates both egg production rate (fecundity) and survival rate to the nauplius 1 stage and therefore represents recruitment rate.

For each species, the time series of demographic estimates and their associated confidence intervals allow us to infer statistically significant temporal variation in demographic rates. We use a conservative approach of making this inference only in instances for which the 90% confidence intervals do not overlap.

Diaptomus negrensis

Water temperature of Laguna Orsinera fluctuated between 27° and 30 °C over the course of the study. The abundance data presented in Figure 2 are a subset of the data reported in Twombly (1994) representing a period of rapid growth and subsequent decline in the *D. negrensis* population during which the sampling protocol did not change. The numbers of individuals (per stage) for the entire lake are reported. Sample densities were adjusted to total population size by multiplying by lake volume to account for the rapid change in volume that occurs in tropical floodplain lakes. The coefficient of

variation among replicate samples varied between 3% and 113% with an overall average (over sampling dates and stages) of 30%. Peak abundance was reached in August and was followed by a decline in September; there is no obvious cohort structure in the data.

Using the modified model, survival probability varied among stages of *D. negrensis* and values greater than 1 persisted throughout the time series for the N1-N2 combined stage (Figure 3). Demographic estimates or the upper bound for the confidence intervals for the remaining stages often exceeded 1, however, the pattern was not consistent. We inferred statistically significant temporal variation in survival probability whenever we observed non-overlapping confidence intervals. Survival of the combined N3-N4, and the N5-N6 stages varied significantly over time. Other stages exhibited variability but the confidence intervals were too broad to infer significant temporal variation.

All of the growth probabilities for *D. negrensis* were within the bounds of [0,1]. The confidence intervals were generally narrow except for the C4 and C5 stages. We found evidence for statistically significant temporal variation in the developmental rates for the N1-N2 and the N5-N6 combined stages (Figure 4). Fertility estimates revealed several peaks in recruitment to the population in July and August (Figure 5) which accounted for the observed population maximum.

The demographic rates that we report here are similar to those reported by Twombly (1994). The critical difference is that now we are able to infer statistically significant temporal variation. Additionally, we can rule out the introduction of bias (introduced by the estimation method) as a contributing factor to the survival probabilities that are greater than 1.

Acartia hudsonica

During the period April 9 to May 22, 1985 the temperature in the mesocosm tank increased from about 6.8 to 15.8 °C with a mean temperature of 11.0 °C. Stage-specific

abundance data for *Acartia hudsonica* (Figure 6) revealed a significant decline in abundance by the time copepods reached the combined N5-N6 stage. After sample number 8 there was an apparent decrease in mortality of the late naupliar (N5-N6) and juvenile (C1 through C5) stages. A continual increase in the numbers of adults through the end of the sampling period provides evidence of an accumulation of individuals after they have reached the adult stage. No estimates of sample variability were available for these data but careful comparison with the *D. negrensis* data suggests a smoother time series for *A. hudsonica* probably due to less sampling variability.

Survival probability varied among the different stages of *Acartia hudsonica* (Figure 7). As we found for the *D. negrensis* analysis, the survival probability for the combined N1-N2 stage was consistently greater than one. This suggests negative mortality that we assume is not possible. Survival probabilities greater than one were also found consistently for the C5 stage. For the remaining stages, biologically meaningful results revealed survival probabilities that decreased early in the experiment and then leveled off or increased slightly during the remainder of the sampling period.

All values for γ_i , the stage-specific growth probabilities, fell within the range $0 < \gamma_i < 1$ (Figure 8). Here, γ_i approximates the inverse of the stage duration; higher values are therefore correlated with more rapid development. Our results suggest that many of the stages have shorter development times as temperature increases (combined N1-N2, N5-N6, and C1-C2 stages) while the remaining stages were characterized by a general increase in development time (or decline in γ_i). Fertility estimates were all quite low (always < 0.25 offspring per adult). There was a general increase in fertility over the sampling period (Figure 9).

We analyzed mesocosm data to evaluate the effects of reduced sampling noise (a smoother time series) on model results. Our results are clear: less variable data produce tighter confidence intervals and the IMM performs better as the field data used improve

in quality. These results highlight the importance of data quality (in addition to or instead of, complex mathematical models) when attempting to estimate demographic rates.

Jassa marmorata

Water temperature at Fort Wetherill ranged from 11.5 to 21 °C. Egg abundance and egg development time (estimated from temperature data and laboratory estimates) are shown in Figure 10. The decrease in egg development time corresponds with the increasing temperature during the summer season. There were two peaks in egg number on 16 June and on 25 August. Abundance data for each of the remaining five size-classes of amphipods are presented in Figure 11. The maximum number of recruits was found on 11 August and was just over 5000 per quadrat. Peak abundances of juveniles, small adults and medium adults coincided and were correlated with the second peak in egg production on 25 August. The largest adult size class was only present early in the sampling season. Sample variability was high: the coefficient of variation for replicate counts of stage abundance varied from 10 to >100 % in some instances. Additionally, there was considerable week to week variability in the time series for each stage.

Generally, survival probabilities for all stages (eggs and size classes) of *J. marmorata* fell between 0 and 1 although upper bounds of the confidence interval often exceeded 1 (Figure 12). Estimates show that egg survival improved during the first half of the sampling period and then declined. Additionally, the confidence intervals for egg survival were large (nearly 0 to 1) during the latter part of the season. A similar temporal pattern of survival was found for the recruit stage. Juveniles did not exhibit any clear trend in survival, while all three adult stages were characterized by a general decline in survival over the course of the season. For a few of the survival estimates (e.g. juvenile survival during sample window 6) the original estimate is not within the confidence interval. These types of observations indicate a large bias and a bias-correction of the estimate would constrain it to fall within the confidence interval. We did not perform bias correction because there were few cases in which it would improve our results.

In general, the growth probabilities were characterized by small confidence intervals. Recruits and juveniles exhibited statistically significant temporal variation in growth probabilities as evidenced by non-overlapping confidence intervals for some of the sample windows. Recruits experienced a decline in growth around the middle of the sampling season while juveniles had increasing growth during the first 6 sample windows followed by a gradual decline during the remainder of the season. Small adults had little temporal variation of growth rate while the medium sized adults exhibited a rapid decline in growth rate early in the season.

Fecundity varied among the three *J. marmorata* adult size classes (Figure 14). Not surprisingly, average fecundity increased with body size. There was little variation in the fecundity of the adult 1 size class and sometimes the confidence intervals included negative numbers. As fecundity increased among the size classes of adults, so did the variability in the estimates. While none of the original fecundity estimates is unreasonable, the confidence intervals include estimates that are beyond the physiological limit of egg production for this species especially for the largest adult size class.

For these data, our goal was to test the modifications to the model that allow the incorporation of more than one reproductive stage. Because these are field data for which the true fecundity parameters are unknown, we cannot verify the results of this modification to the model. However, the results that we obtained for fecundity estimates are reasonable given our knowledge of the relationship between body size and fecundity in amphipods. Increased sampling noise observed for this particular species often produced large confidence intervals and in a few isolated cases resulted in biased estimates.

DISCUSSION

The results presented here represent a unique opportunity to explore the utility and robustness of a demographic estimation method by comparing results from three different time series of stage structured abundance data. Each time series differs with respect to sampling variance and perhaps to sampling adequacy within and between stages. In addition to inferences that can be made about model performance in relation to the quality of data, we are also able to identify stage-specific temporal variation of demographic rates (for each species) that is worthy of additional investigation.

Model improvements

Reanalysis of the *Diaptomus negrensis* data yielded confidence intervals that were relatively narrow. Narrow confidence intervals allowed us to rule out the possibility that survival probabilities greater than 1 were caused by bias introduced into the method (Caswell and Twombly, 1989; Twombly, 1994; Wood, 1994). If the method itself had generated biased estimates, we would have found confidence intervals that were within the interval [0,1]. We now can focus on two possible alternative explanations for estimates greater than 1. The first of these is that the earliest developmental stages (N1 and N2) are not adequately sampled. All demographic estimation techniques require data in which the sampling bias is equal among all stages (Aksnes et al., 1997) and there are a number of reasons that the earlier stages are likely to be underrepresented (Miller and Tande, 1993). The second possible explanation is that the model does not adequately represent the life cycle. Calanoid copepods are known to produce diapausing eggs (Hairston & Bohonak, 1998) that later hatch from the egg bank. Although resting stages are not commonly reported for tropical copepods, they could produce overestimates of survival in the earlier stages like those we observed for *D. negrensis*.

In addition to improving our understanding of the potential underlying mechanisms for estimates greater than 1, the development of confidence intervals

allowed us to make inference about the time-varying nature of survival and growth probabilities of other stages. For example, the survival probabilities of the combined naupliar stages (N3-N4 and N5-N6) exhibited significant temporal variation. While Twombly (1994) highlighted low overall survival of the combined N5-N6 stage, she was unable to detect statistically significant temporal variation and missed significant changes in survival of the N3-N4 stage. In addition, there is a general decrease in survival of the combined C1-C2 stage that also was not detected in Twombly's (1994) initial analysis. We found a few instances of statistically significant temporal variation in growth probabilities of the N1-N2 and N5-N6 stages. When translated to estimates of development time, these differences resulted in a maximum difference of 0.7 days. While statistically significant, these results provided relative consistency in estimates of development, which was expected because of the temperature dependent nature of copepod development rates and the small fluctuation in water temperature between 27 and 30° C. Adult fertility estimates exhibit marked temporal variation as evidenced by small confidence intervals.

Acartia hudsonica abundance data were collected from a closed population and yielded a smoother time series with less day-to-day variation than our other data series. Our analysis of these data produced very small confidence intervals for all of the demographic estimates. In this case, as was true for *D. negrensis*, sample noise had little effect on bias as illustrated by the fact that all of the original estimates fell between the bias-adjusted confidence intervals. Although we obtained tighter confidence intervals, we found little significant temporal variation in demographic rates. The temporal variability in fertility was statistically significant but inferring biological significance of fertility values that change gradually from 0.03 to 0.15 is questionable.

Some of the stage specific survival probabilities for *A. hudsonica* were also greater than 1 with narrow confidence intervals. The systematic occurrence of high survival probabilities (>1) for the combined N1-N2 stage in *A. hudsonica* is likely due to

a missing transition in the life cycle or to inadequate sampling of this stage. Similarly high estimates characterized the *D. negrensis* analysis. Persistent survival estimates > 1 for the C5 stage are puzzling, however. A possible explanation is that the sampling distribution of this stage differs from that of the other stages, even though these data were collected by pump sampling from a tank. Pump sampling can be an effective alternative to the sampling bias that is often introduced when using net tows (Miller and Judkins, 1981).

The *Jassa marmorata* data series exhibited the greatest amount of sampling error (as represented by the coefficient of variation among replicate samples) and the largest fluctuations between consecutive samples. Not surprisingly this produced bias in the original model estimates (exhibited by the fact that the bias corrected confidence intervals do not always envelope the original estimates) and large confidence intervals. For this species, only the recruit stage had survival probabilities that were significantly greater than 1. Despite large confidence intervals, we were able to detect significant temporal variation in survival probability of the recruits and the two smaller of the adult stages. We were also able to find significant temporal variation in all but the adult 1 growth probabilities.

Stage specific *Jassa marmorata* survival probabilities exhibited different temporal patterns, suggesting that environmental effects on survival probability differ among size-classes. This is not surprising given that the species ranges from a minimum of about 1 mm in body length to a maximum size of 12 mm. The decline in adult 3 survivorship is difficult to interpret because of the complete disappearance of the largest adult size class from the population by late June or early July. It is not clear whether individuals grow to the largest size class and are subsequently preyed upon or if they never grow that large. Body size in ectotherms is often smaller when water temperature increases (Berrigan and Charnov, 1994, Sibly and Atkinson, 1994, Hartnoll, 1982). There is a rapid decline in adult-2 growth rate that is also difficult to interpret for the same reasons.

Modifications to the model allowed the successful estimation of fecundity for more than one reproductive size class. This was possible partly because of the reproductive biology of amphipods: females brood their young and thus estimates of the number of eggs per individual were relatively easy to obtain (Tisch, 1997). Secondly, the assumption of egg development times that were solely dependent on temperature reduced the number of model parameters that required estimation by the inverse matrix method. This assumption seems reasonable given that the eggs are clearly a non-feeding stage. Modifications of this type make the model more generally applicable to organisms that continue to grow after the onset of reproduction.

The IMM requires no *a priori* information on development rates; this is an important consideration when evaluating demographic rates in field populations. The method is now substantially improved in a number of ways: 1) bias corrected confidence intervals allow for statistical evaluation of temporal (and spatial) variation, 2) narrow confidence intervals for estimates that are consistently greater than 1, refute the hypothesis that such estimates are the result of bias introduced by the estimation method itself, and 3) the incorporation of more than one reproductive stage provides more flexibility to the model. Importantly, we show that the quality of the field data collected affects demographic estimates, width of confidence intervals, and the interpretations possible (see Ohman and Wood (1996) for an alternative view of the importance of data quality).

Remaining issues require investigation. The first among these is to address the effect of sampling bias among different stages on the model results. Sampling bias among different stages may not be known (or even knowable) for any given time series of stage specific abundance data in field populations. Wood's model (1994) incorporates a matrix that characterizes this bias and adjusts stage specific mortality estimates accordingly, but it is not clear to us that as field ecologists we can know *a priori* what that bias is. Second, the issue of sampling noise requires resolution. There are two

potential solutions to this problem. The first is to acquire high quality data by increasing sample size and replication, both of which are expensive especially in terms of research hours. An alternative solution is to apply a simple smoothing technique to the abundance data time series. For the inverse matrix method, we already know that this results in the generation of tighter confidence intervals (Tisch, 1997). The problem remaining is to evaluate how much smoothing, if any, is adequate or appropriate. As a last resort mathematical optimization routines are available for constraining model estimates within biologically meaningful bounds. We recommend that they not be used until stage specific sampling bias (using simulations) is shown to be unrelated to unrealistic estimates. In its current state, the model at least identifies estimates that are unrealistic and these may occur because the data are not representative of the true abundance or the model has not adequately described the life cycle.

In summary, our present analyses have shown first, that modifications to the inverse matrix method result in bias corrected confidence intervals that are often narrow and allow statistical comparison on a temporal scale. Second, estimates greater than 1 are likely due to sampling inadequacies or a missing transition in the life cycle such as the emergence of resting stages. Third, relatively simple modification of the model allowed us to apply the method to an organism with a different life cycle. Most importantly, taken together, these results suggest that the inverse matrix method is a robust method that does not rely on any *a priori* information. We plan to continue our evaluation of this method using simulated data to address the remaining questions.

ACKNOWLEDGMENTS

This paper is written as partial fulfillment of the requirements for a Ph.D. degree in the Biological Sciences at the University of Rhode Island (NT). Hal Caswell, Michael Fogarty, Jim Heltsche and Mike Clancy provided useful discussion for improvement and modifications of the model. Rick Grosberg, David Avery and two anonymous reviewers

made suggestions to improve an earlier version of this manuscript. Financial support was provided by a Sigma Xi Grant in Aid of Research.

LITERATURE CITED

- Aksnes, D.L., C.B. Miller, M.D. Ohman, and S.W. Wood, 1997. Estimation techniques used in studies of copepod population dynamics—a review of underlying assumptions. *Sarsia* 82:279-296.
- Aksnes, D.L. and M.D. Ohman, 1996. A vertical life table approach to zooplankton mortality estimation. *Limnology and Oceanography* 41:1461-1469.
- Berrigan, D. and E.L. Charnov, 1994. Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos* 70:474-478.
- Caswell, H. and S. Twombly, 1989. Estimation of stage specific demographic parameters for zooplankton populations: methods based on stage classified matrix projection models. Pages 94-107 *in* L. McDonald, B. Manly, J. Lockwood, and J. Logan, editors. Estimation and analysis of insect populations. Lecture Notes in Statistics 55. Springer-Verlag, Berlin, Germany.
- Efron, B. and R.J. Tibshirani, 1993. An Introduction to the Bootstrap. Chapman and Hall, New York, NY.
- Hairston, Jr., N.G. and A.J. Bohonak, 1998. Copepod reproductive strategies: life-history theory, phylogenetic pattern and invasion of inland waters. *Journal of Marine Systems* 15:23-34.
- Hartnoll, R.G., 1982. Growth, *in* The biology of Crustacea, edited by L.G. Abele, Academic Press, New York, pp.111-195.
- Kuehl, R.O., 1994. Statistical Principles of Research Design and Analysis. Duxbury Press, Belmont, CA.
- Manly, B.F.J., 1989. A review of methods for the analysis of stage frequency data. Pages 1-69 *in* L. McDonald, B. Manly, J. Lockwood, and J. Logan, editors. Estimation and analysis of insect populations. Lecture Notes in Statistics 55. Springer-Verlag, Berlin, Germany.

- Manly, B.J.F., 1990. Stage-structured Populations: Sampling, analysis and simulation. Chapman and Hall, New York, NY.
- Miller, C.B. and D.C. Judkins, 1981. Design of pumping systems for sampling zooplankton, with description of two high-capacity samplers for coastal studies. *Biological Oceanography* 1:29-56.
- Miller, C.B. and K.S. Tande, 1993. Stage duration estimation for *Calanus* populations, a modelling study. *Marine Ecology Progress Series* 102:15-34.
- Nair, K.K.C. and K. Anger, 1979. Experimental studies on the life cycle of *Jassa falcata* (Crustacea, Amphipoda). *Helgolander wiss. Meeresunters.* Vol. 32, pp.444-452.
- Ohman, M.D., and S.N. Wood, 1996. Mortality estimation for planktonic copepods: *Pseudocalanus newmani* in a temperate fjord. *Limnology and Oceanography* 41:126-135.
- Sibly, R.M. and D. Atkinson, 1994. How rearing temperature affects optimal adult size in ectotherms. *Functional Ecology* 8:486-493.
- Tisch, N., 1997. Ecology and evolution of life history variation in the marine amphipod *Jassa marmorata* Holmes. Ph. D. Thesis. University of Rhode Island, USA.
- Twombly, S., 1994. Comparative demography and population dynamics of two coexisting copepods in a Venezuelan flood plain lake. *Limnology and Oceanography* 39:234-247.
- Twombly, S., N. Clancy and C.W. Burns, 1998. Life history consequences of food quality in the freshwater copepod *Boeckella triarticulata*. *Ecology* 79:1711-1724.
- Wood, S.N., 1994. Obtaining birth and mortality patterns from structured population trajectories. *Ecological Monographs* 64:23-44.
- Xu, Z. and C.W. Burns, 1991. Development, growth and survivorship of juvenile calanoid copepods on diets of cyanobacteria and algae. *Internationale Revue der gesamten Hydrobiologie* 76:73-87.

FIGURE CAPTIONS

Figure 1. Life cycle graphs in which P_i represents the probability that an individual survives and remains in stage i , G_i represents the probability that an individual survives and grows from stage i to stage $i+1$. A. Compressed life cycle for the calanoid copepods. F_A represents combined egg production and egg survival to the nauplius stage. The ... represents a number of "missing" stages that undergo the same transition probabilities as those included in the illustration. The naupliar stages and the first two copepodite stages are combined in successive pairs to account for the fact that the sampling interval is not shorter than the shortest stage duration. B. Life cycle graph for *Jassa marmorata*. Each stage is defined as follows: stage 1 = eggs, stage 2 = recruits (0.8 - 1.5 mm), stage 3 = juveniles (>1.5 - 3.5 mm), stage 4 = adult 1 or small adults (>3.5 - 5.5 mm), stage 5 = adult 2 or medium adults (>5.5 - 7.5 mm) and stage 6 = adult 3 or large adults (>7.5mm). F_i represents the average number of eggs produced per individual in stage i .

Figure 2. Abundance data for *Dipatomus negrensis* collected in Laguna Orsinera, Venezuela. Numbers on the y-axis reflect the whole lake abundance for each stage. The x-axis represents time, the interval between samples is 2 days; sampling was begun in late June and continued every second day until mid-September.

Figure 3. Stage specific survival probabilities for *Diaptomus negrensis*. Sample window is on the x-axis; symbols are original model estimates and dotted lines represent 90% confidence limits. Note the change in scale of the y-axis for stages N1&N2, and C5.

Figure 4. Stage specific growth probabilities for *Diaptomus negrensis*. Symbols and x-axis are the same as Figure 8.

Figure 5. Fertility estimates of adult *Diaptomus negrensis*. Fertility is the average number of new recruits (nauplii) produced per adult per time interval. Symbols and x-axis are the same as Figure 8.

Figure 6. Abundance data for the calanoid copepod *Acartia hudsonica* sampled from mesocosm tanks at the MERL facility at the University of Rhode Island. The sample days on the x-axis are 3 to 4 days apart and sampling was begun April 9, 1985 and completed on May 22, 1985. Numbers on the y-axis are the number of individuals m^{-3} . Note the change in scale of the y-axes.

Figure 7. Stage-specific survival probabilities for *Acartia hudsonica*. Sample windows on the x-axis represent estimates made from 5 successive data points. An increment in the sample window is accomplished by "sliding" ahead one time-step and re-estimating the parameters. The symbols represent the original model estimate of survival and the dotted lines represent 90% confidence bands. Note the change in scale of the y-axis for the N1& N2 stage and the C5 stage.

Figure 8. Stage-specific growth probabilities for *Acartia hudsonica*. Symbols and x-axis are the same as Figure 4.

Figure 9. Fertility estimates of adult *Acartia hudsonica*. Fertility is the average number of new recruits (nauplii) produced per adult per time interval. Symbols and x-axis are the same as Figure 4.

Figure 10. Egg abundance estimates for *Jassa marmorata* collected at Fort Wetherill, RI. Also plotted are estimates of egg development time derived from a smoothed function of

development time and water temperature. The decrease in development time corresponds to increasing water temperature.

Figure 11. Abundance of post-embryonic stages of *Jassa marmorata* collected at Fort Wetherill, RI from May 26, 1993 to November 6, 1993. Samples were collected at weekly intervals.

Figure 12. Size-specific survival probabilities for *Jassa marmorata*. Sample window is on the x-axis; symbols represent the original model estimates and dotted lines represent 90% confidence limits.

Figure 13. Size-specific growth probabilities of *Jassa marmorata*. Symbols and x-axis are the same as Figure 13.

Figure 14. Size-specific fecundity for 3 adult size-classes of *Jassa marmorata*.

Fecundity is the average number of eggs produced per adult per time interval.

Symbols and x-axis are the same as Figure 13. Note the change in scale on the y-axes.

FIG. 1

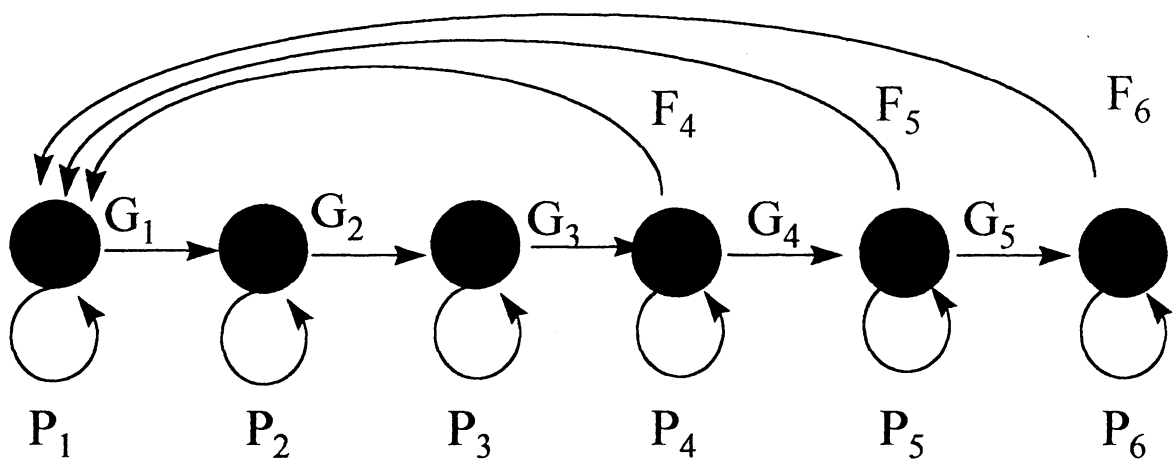
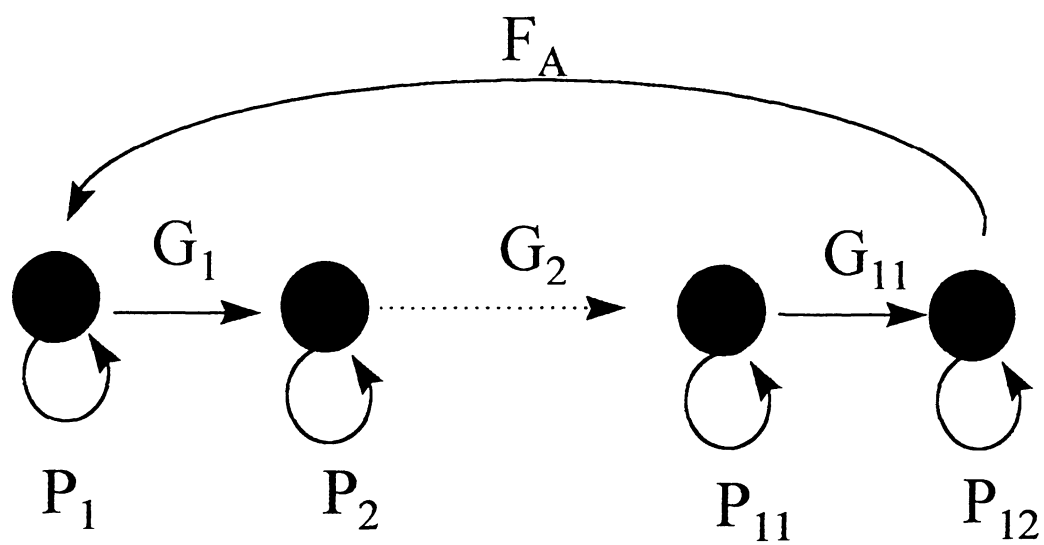


Fig 2

Diaptomus negrensis

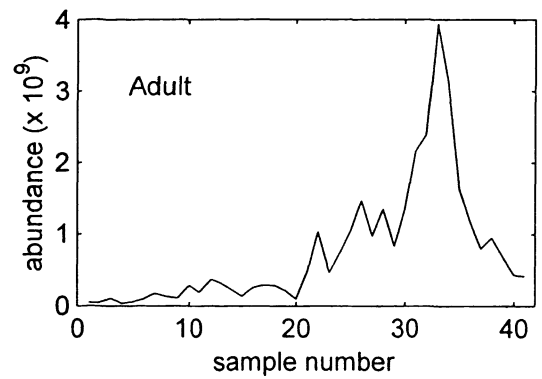
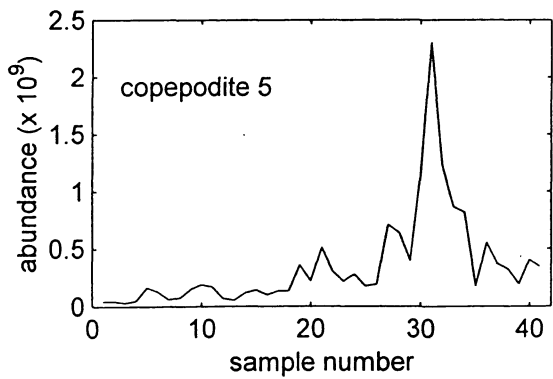
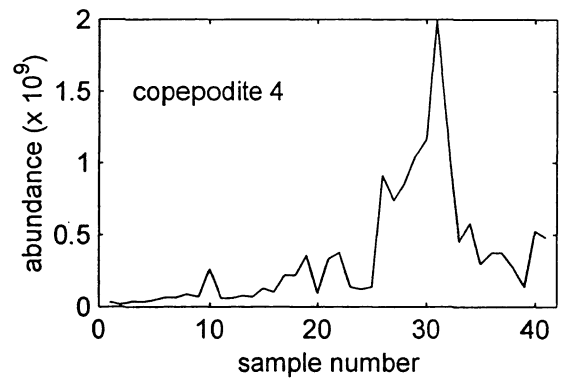
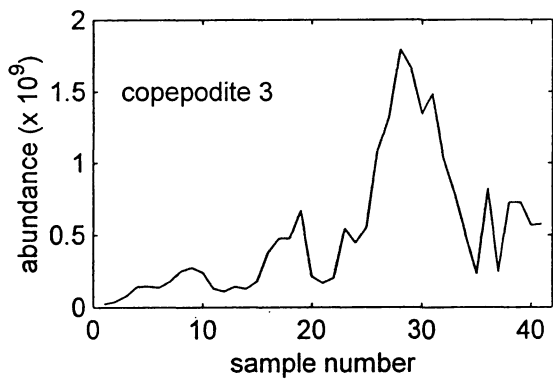
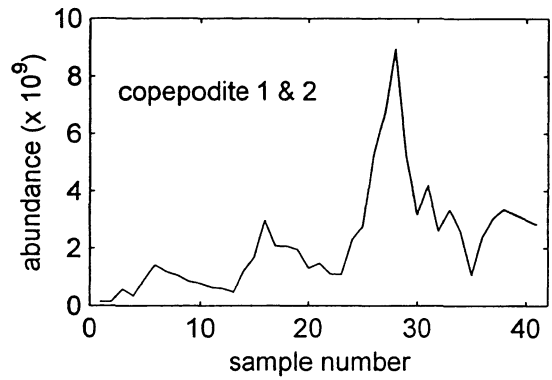
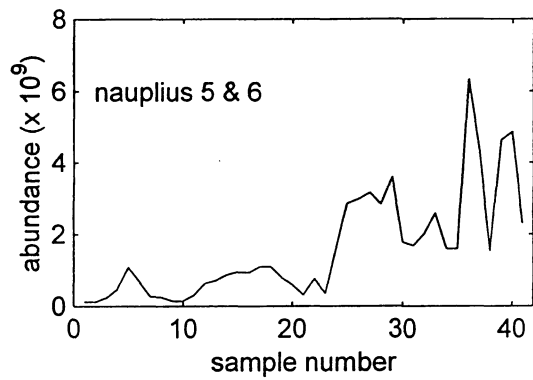
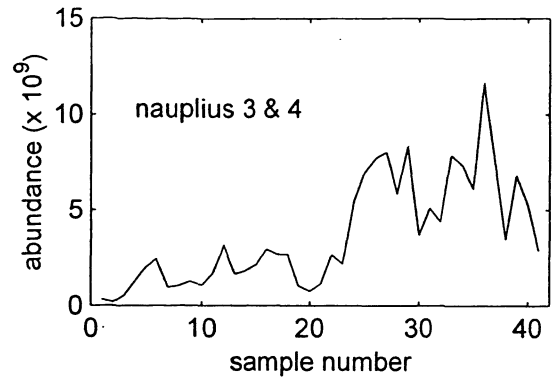
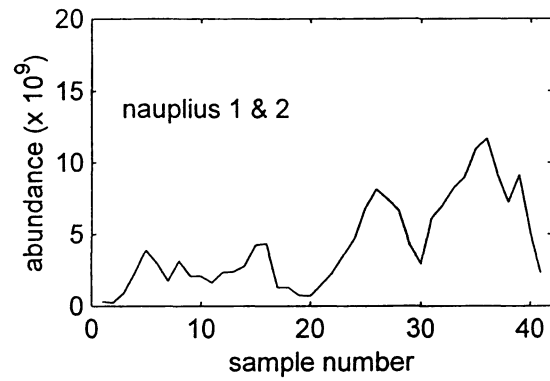


Fig 3

Diaptomus negrensis

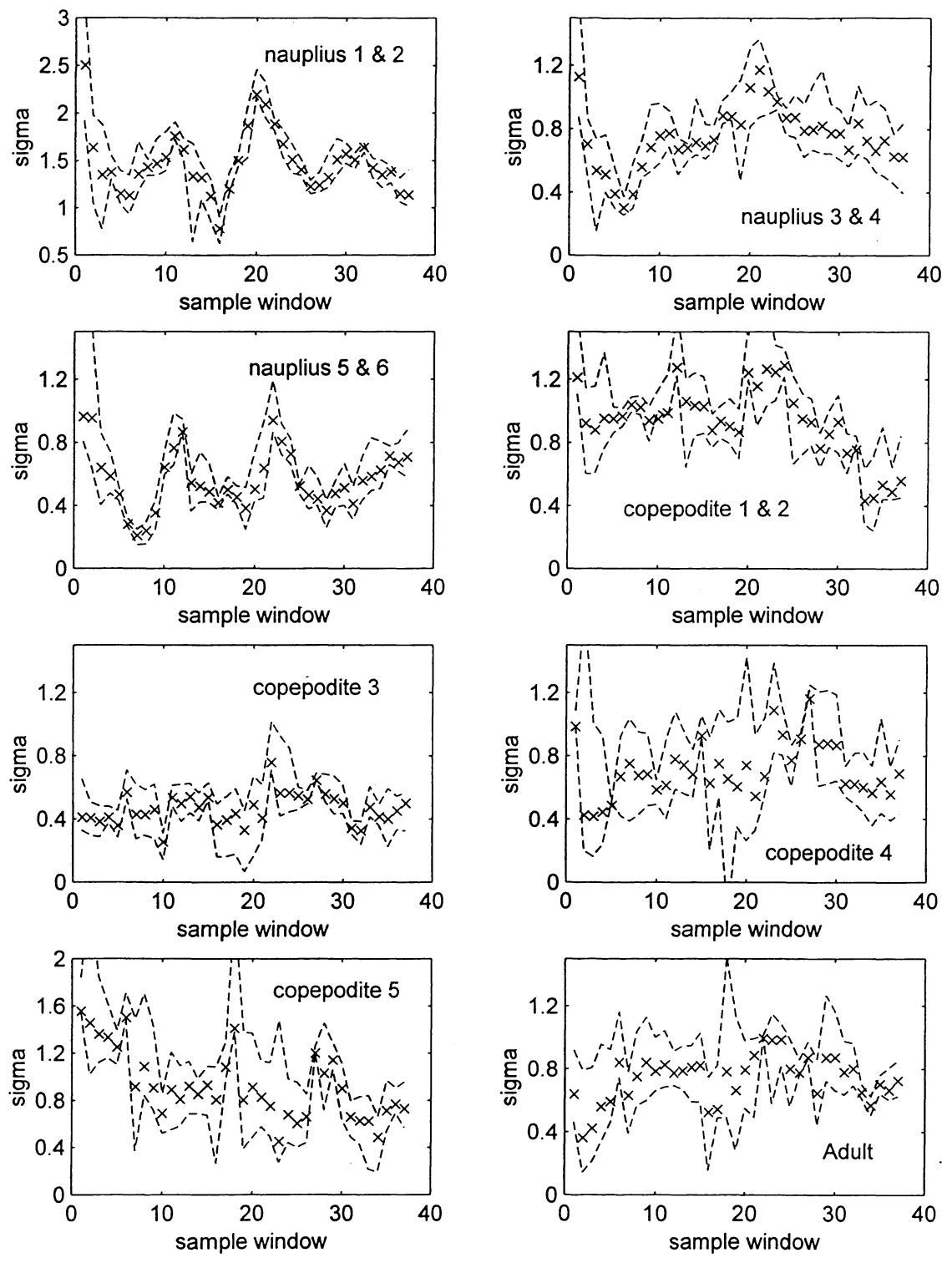


Fig 4

Diaptomus negrensis

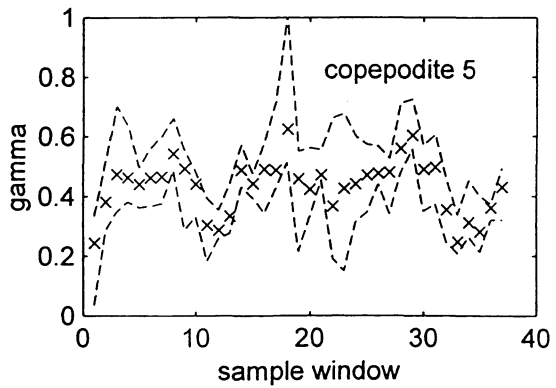
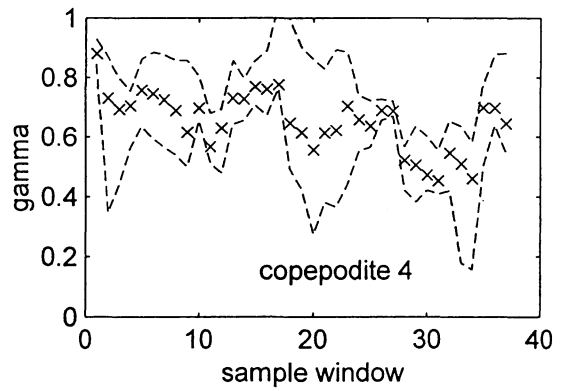
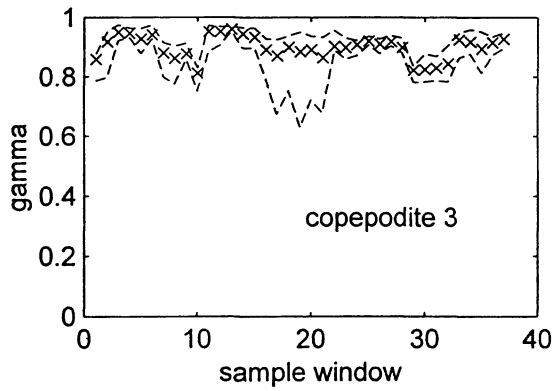
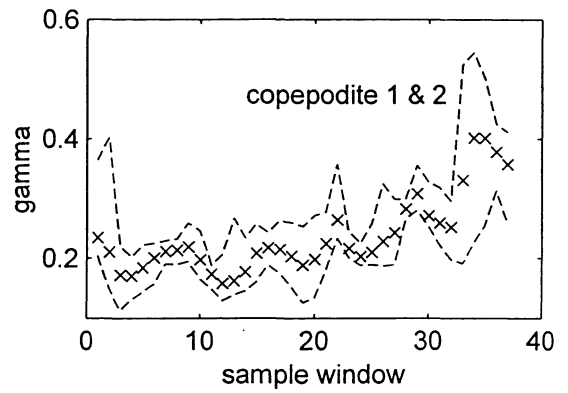
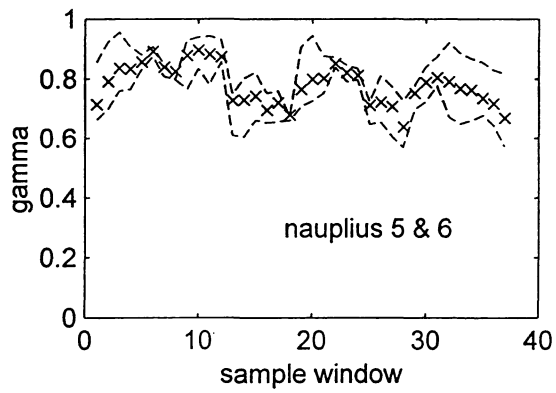
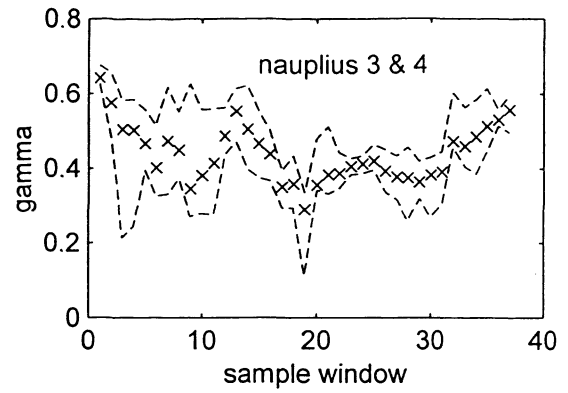
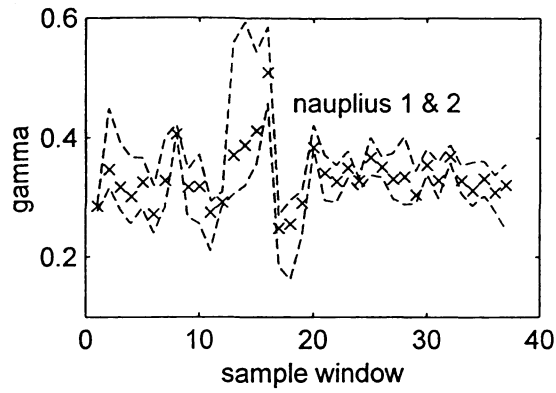


Fig 5

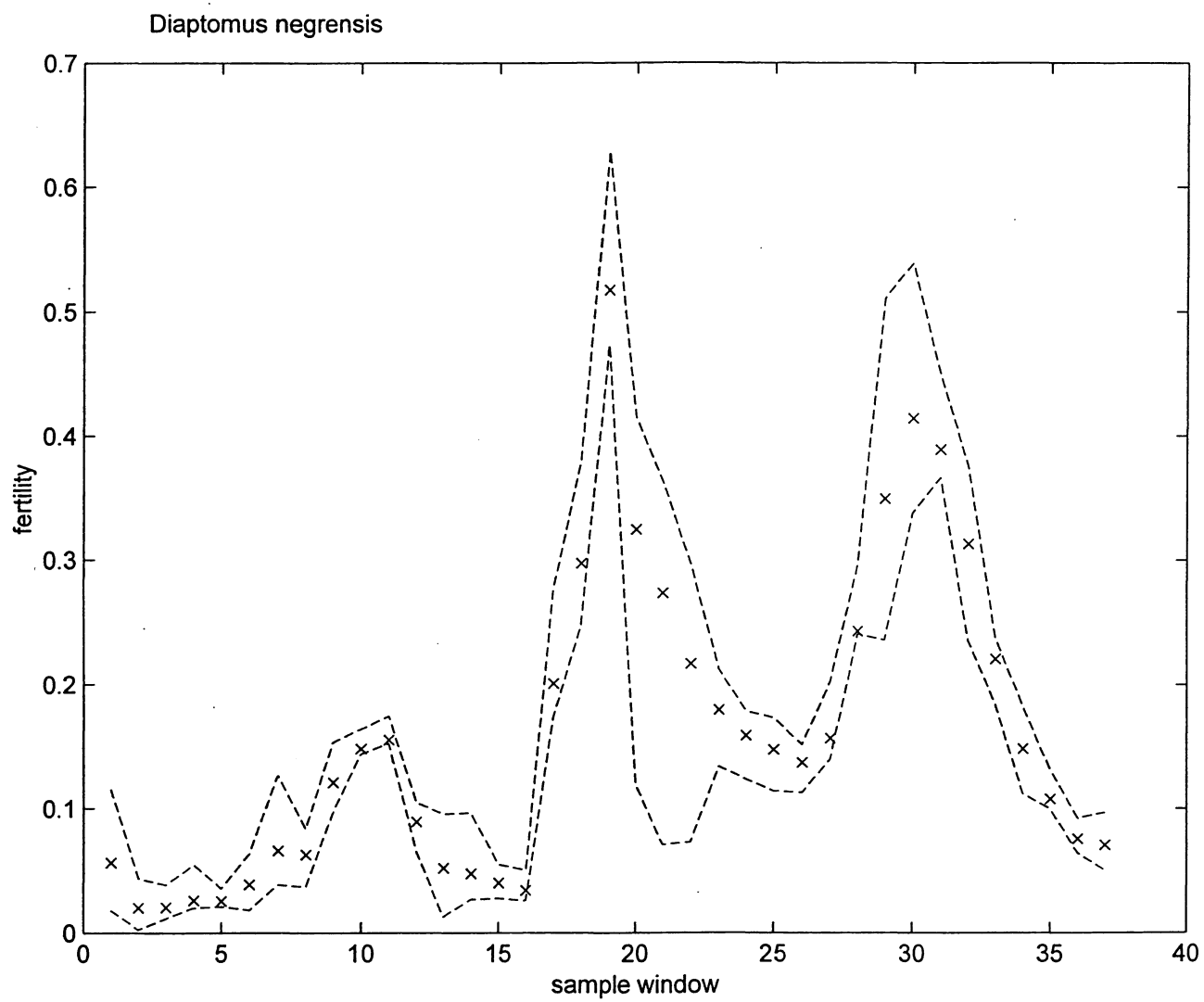


Fig 6

Acartia hudsonica

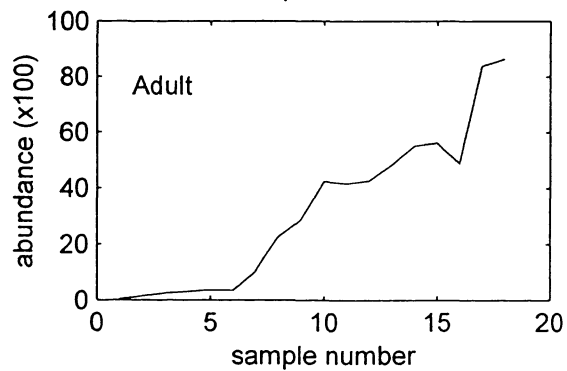
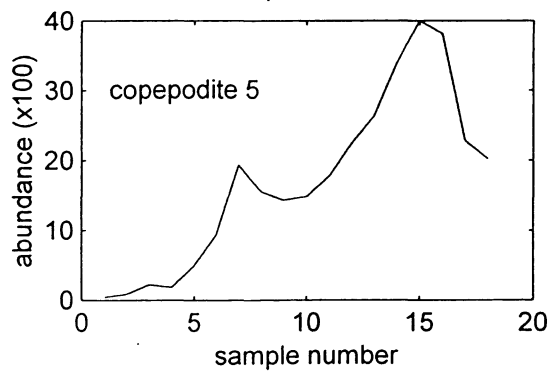
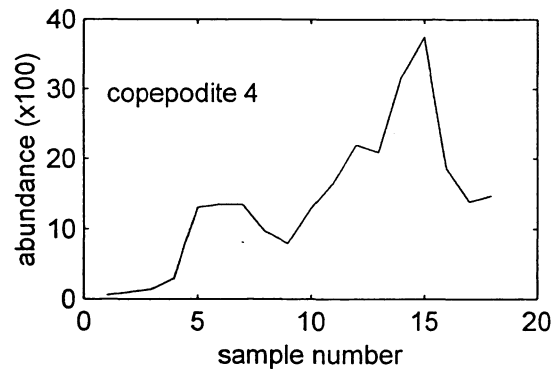
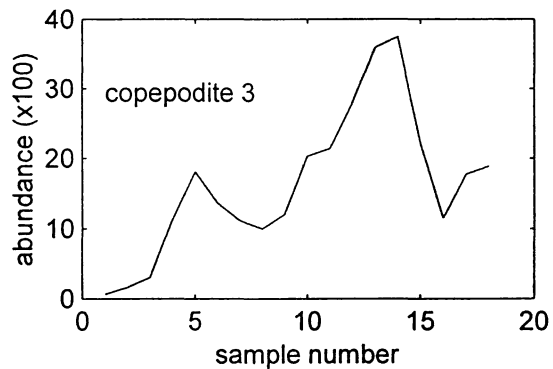
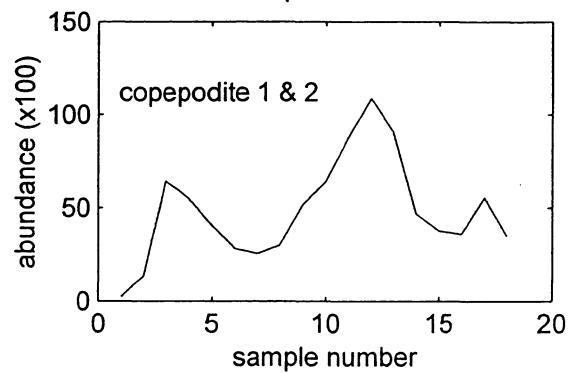
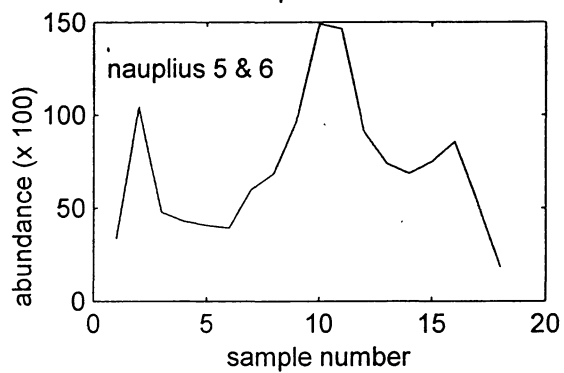
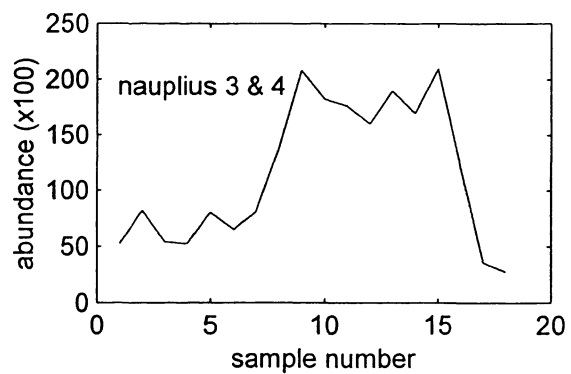
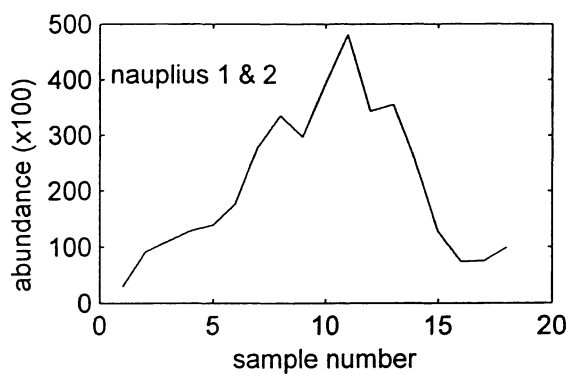


Fig 7

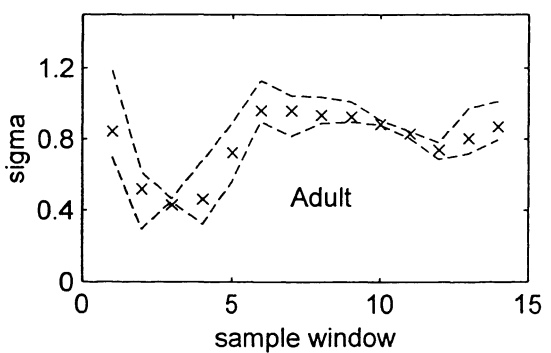
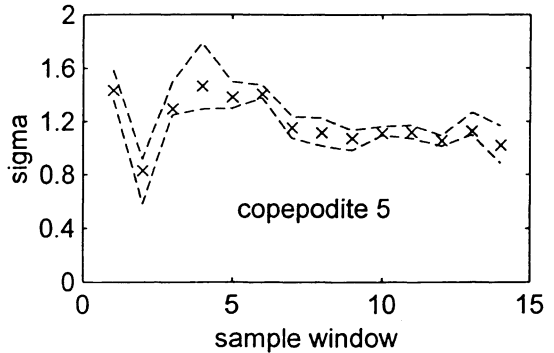
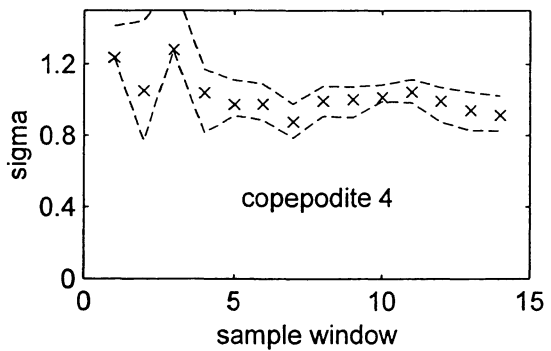
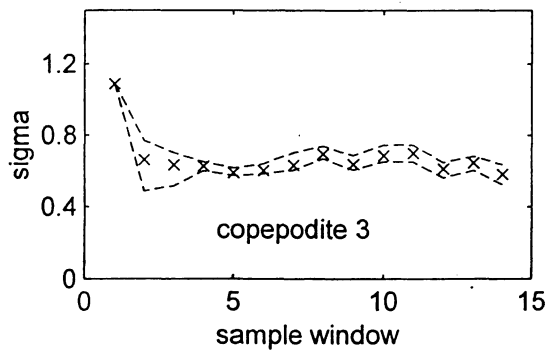
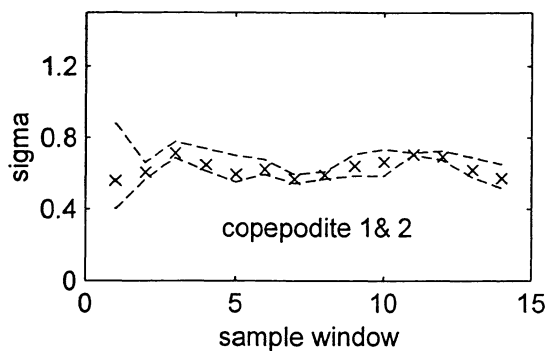
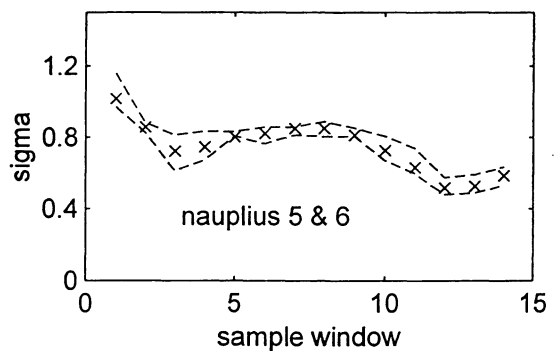
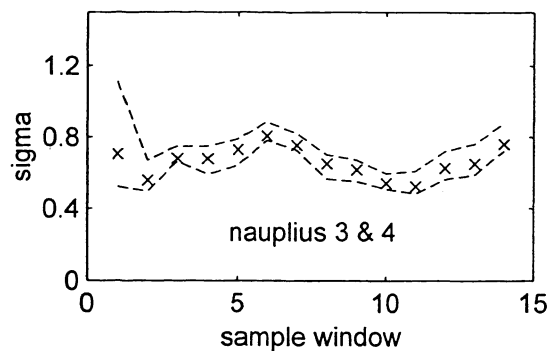
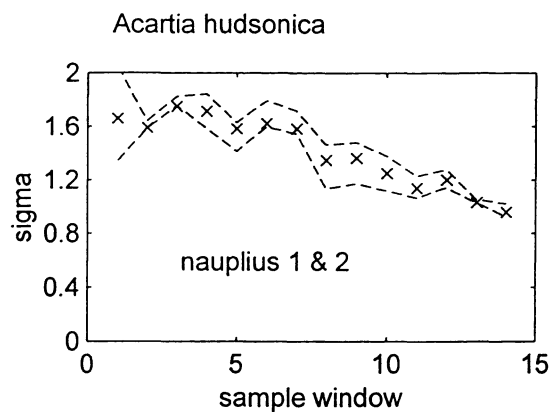


Fig 8

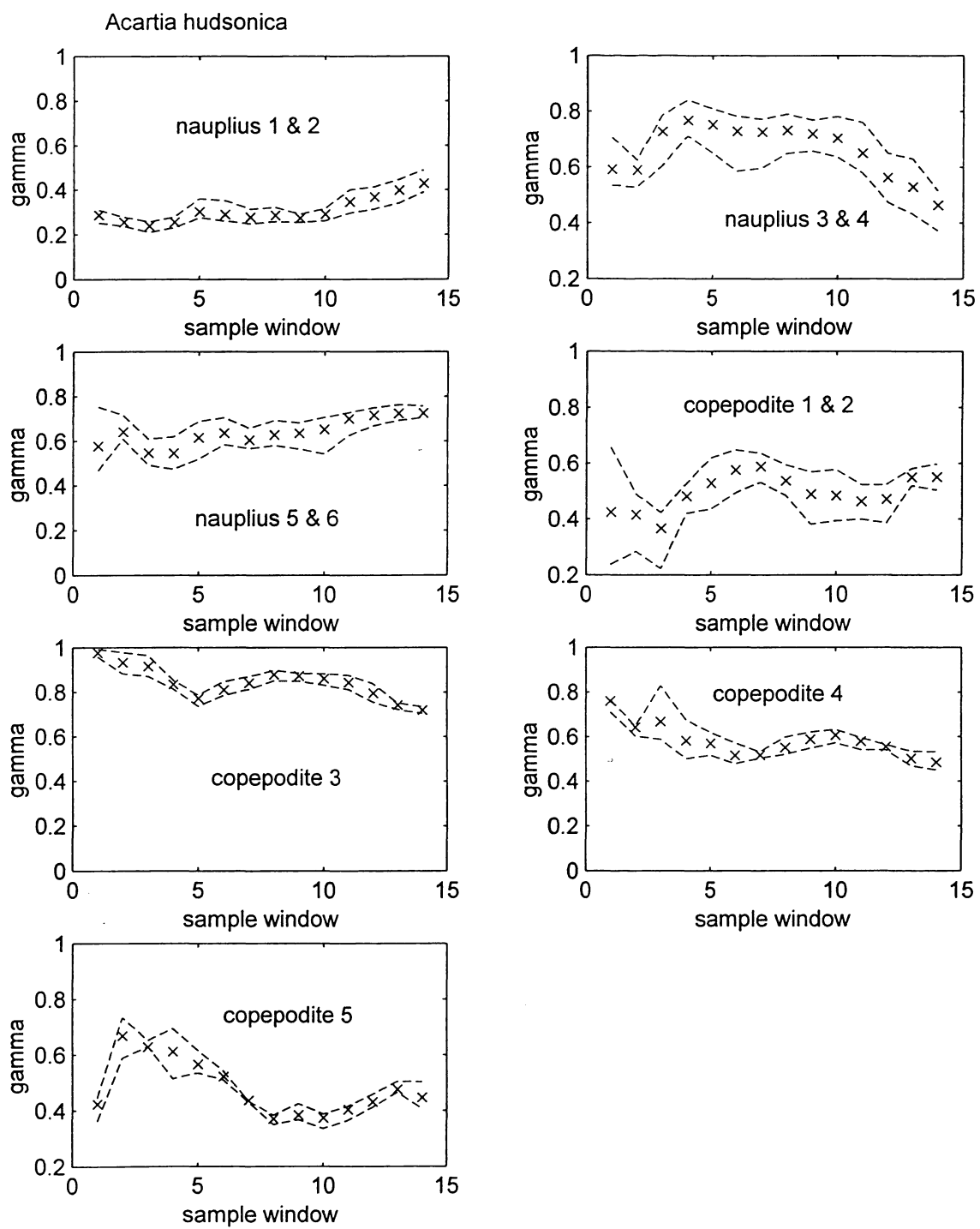


Fig 9

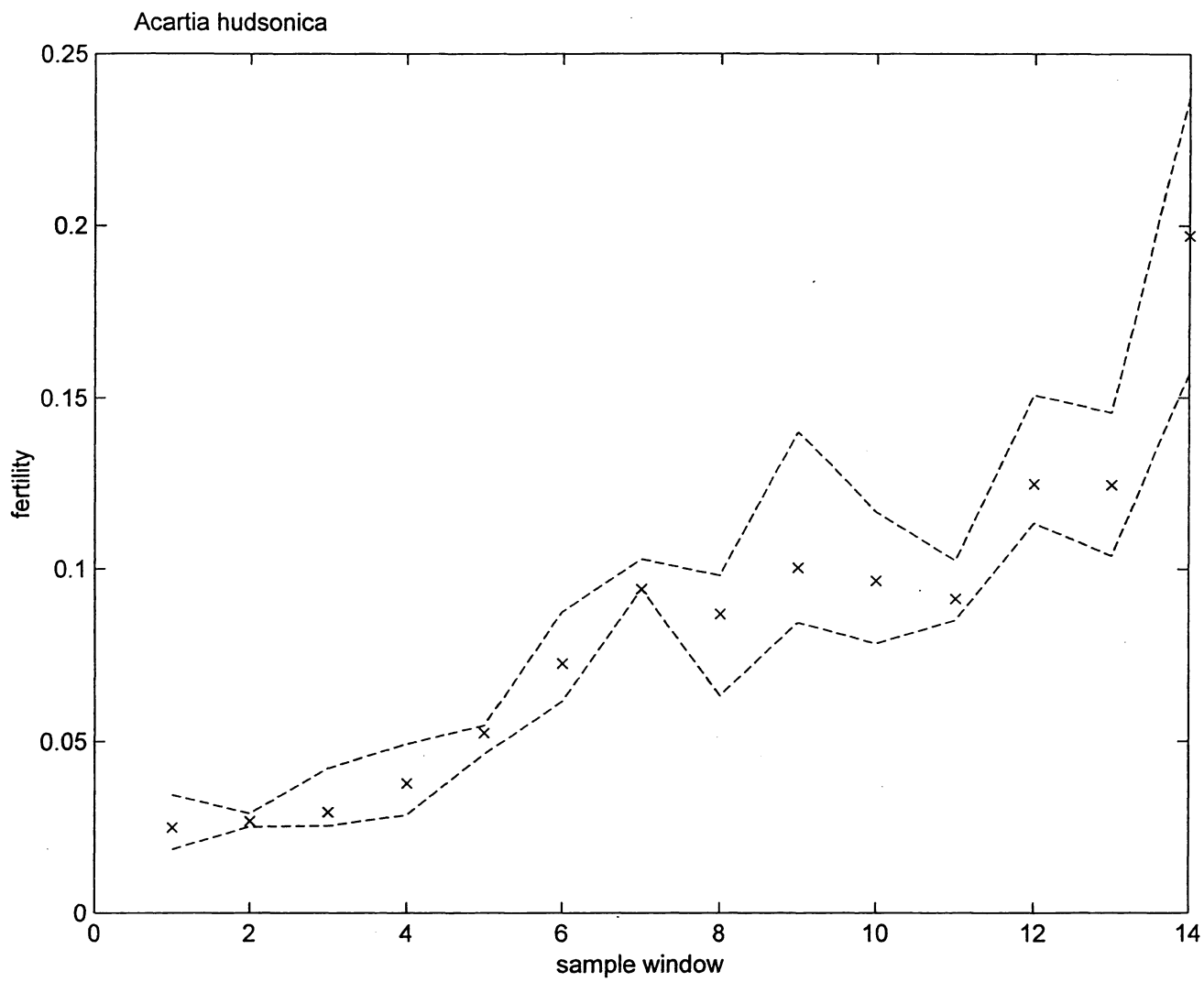
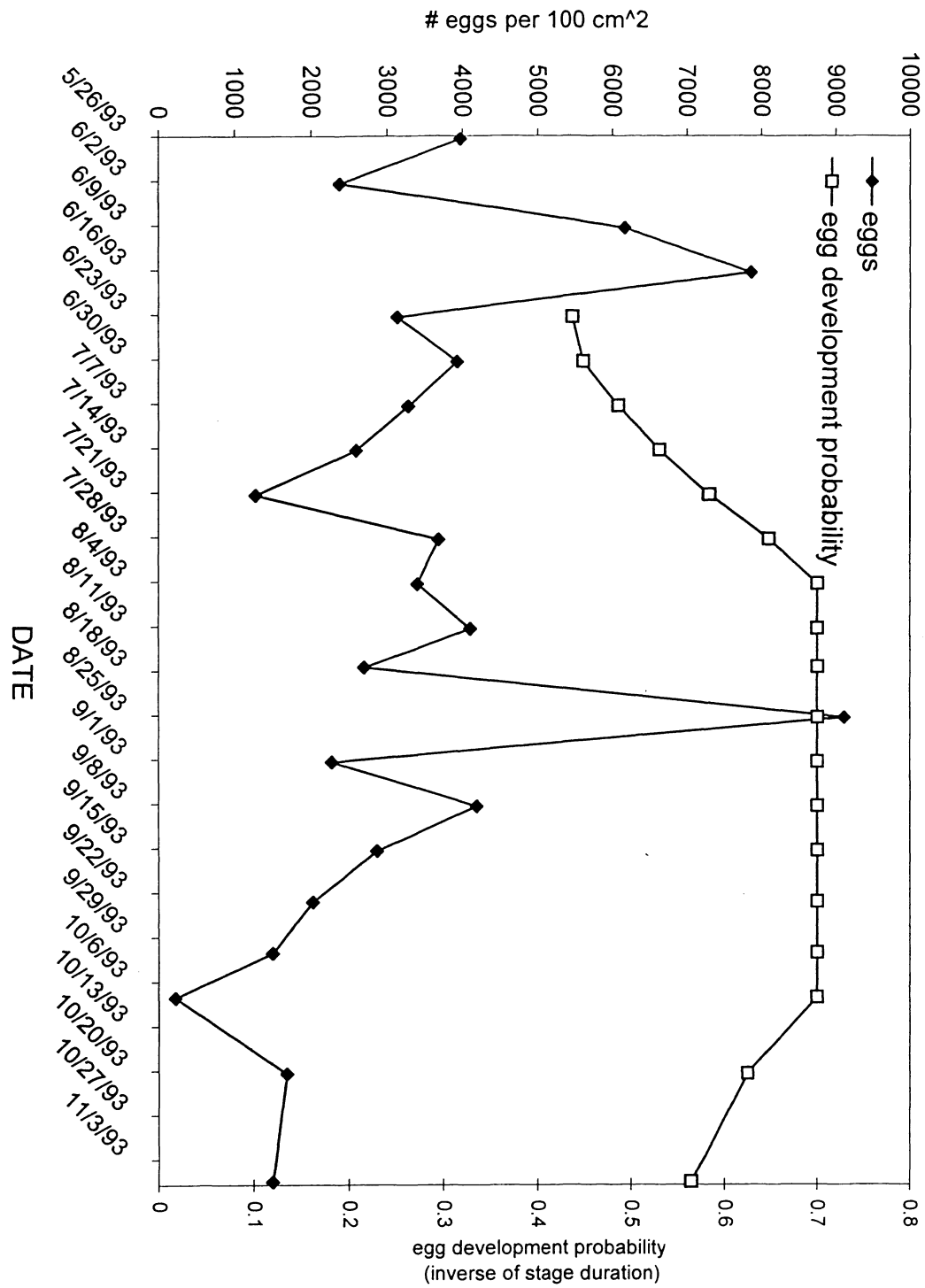


Fig 10



FW 1993 Abundance

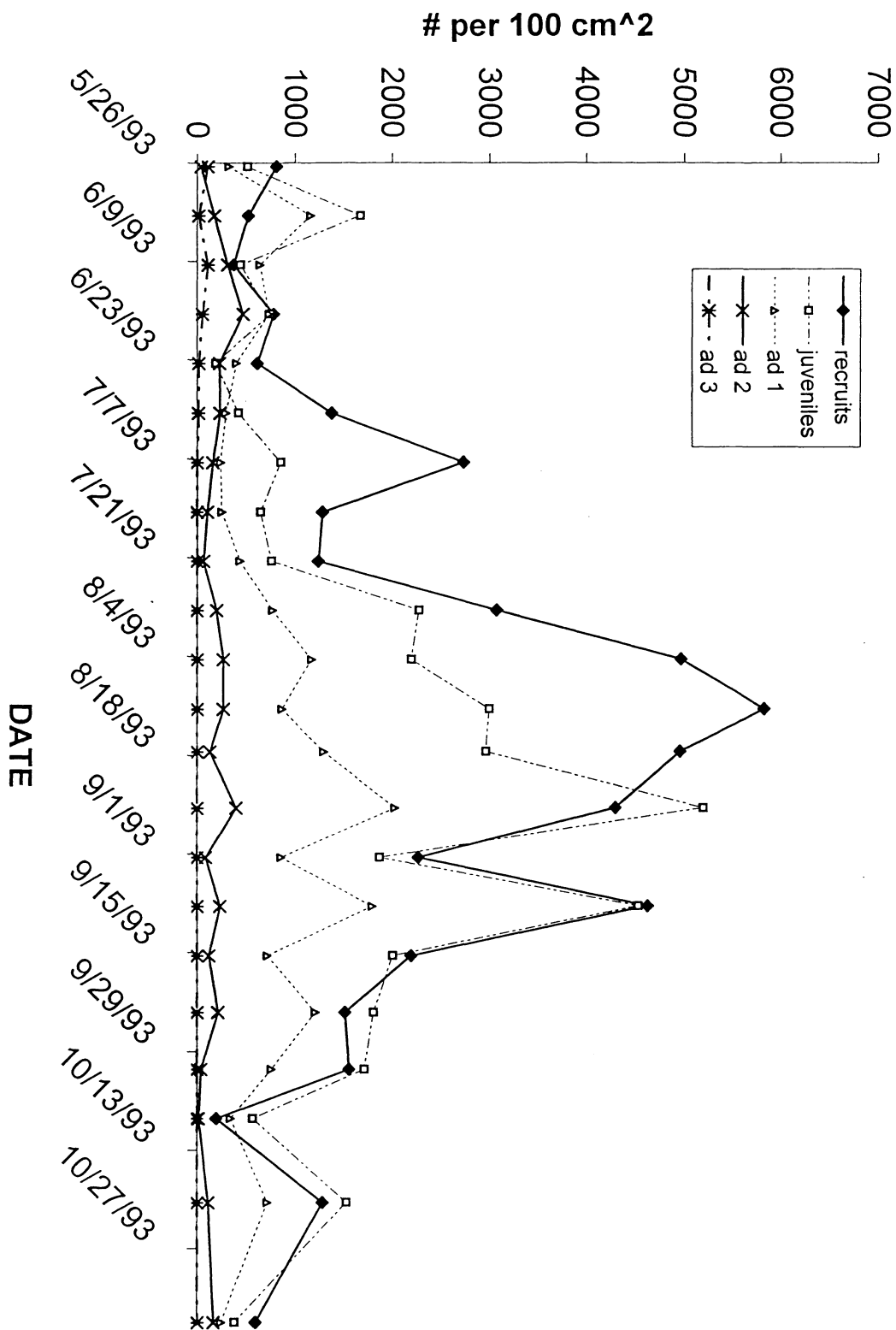


FIG 11

Fig 12

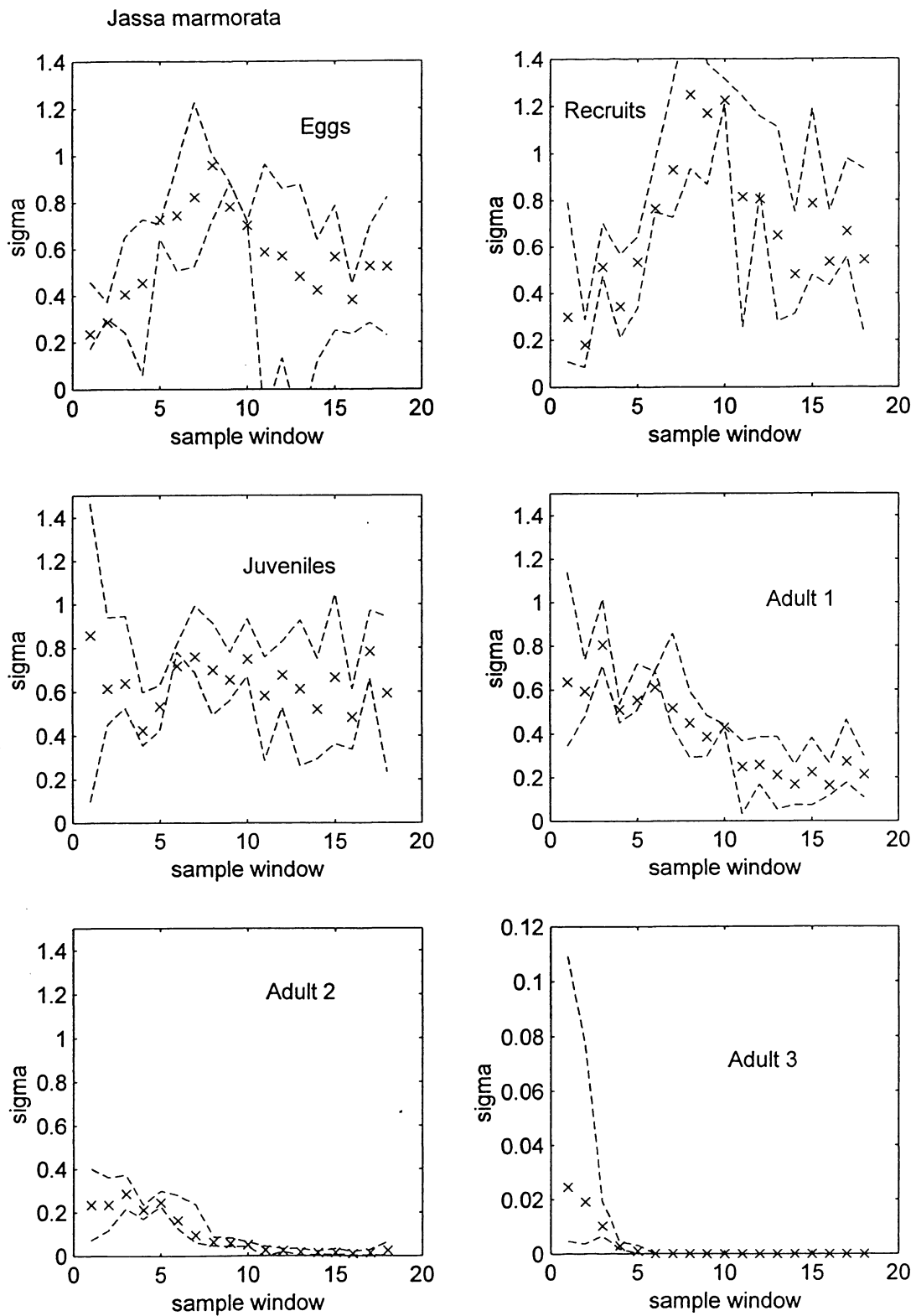


Fig 13

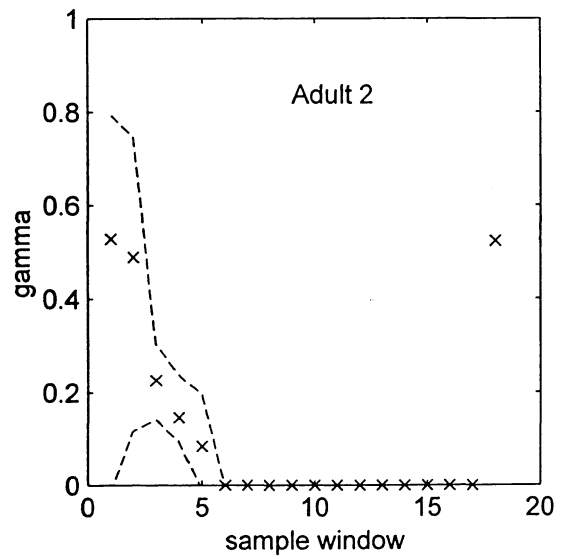
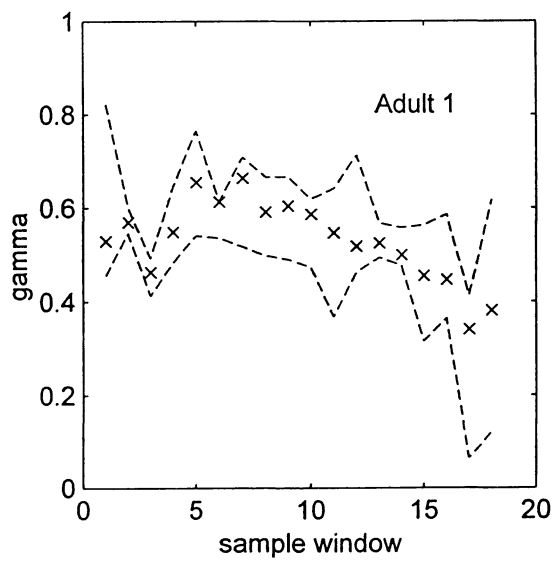
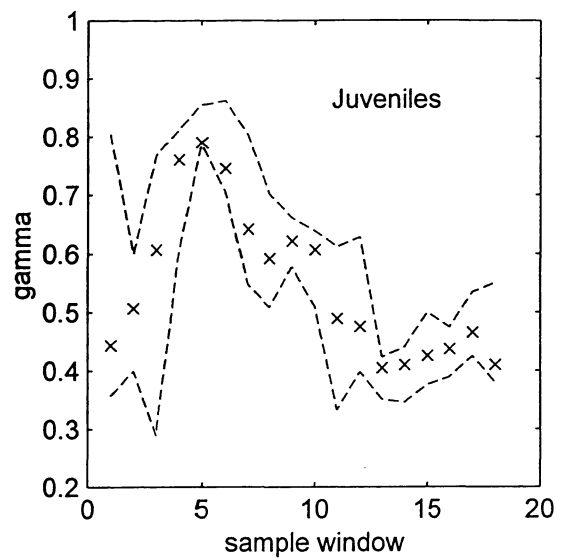


Fig 14

