Optimal Monitoring Frequency Estimation Using Confidence Intervals for the Temporal Model of a Zooplankton Species Number Based on Operational Taxonomic Units at the Tongyoung Marine Science Station

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Abstract: Temporal changes in the number of zooplankton species are important information for understanding basic characteristics and species diversity in marine ecosystems. The aim of the present study was to estimate the optimal monitoring frequency (OMF) to guarantee and predict the minimum number of species occurrences for studies concerning marine ecosystems. The OMF is estimated using the temporal number of zooplankton species through bi-weekly monitoring of zooplankton species data according to operational taxonomic units in the Tongyoung coastal sea. The optimal model comprises two terms, a constant (optimal mean) and a cosine function with a one-year period. The confidence interval (CI) range of the model with monitoring frequency was estimated using a bootstrap method. The CI range was used as a reference to estimate the optimal monitoring frequency. In general, the minimum monitoring frequency (numbers per year) directly depends on the target (acceptable) estimation error. When the acceptable error (range of the CI) increases, the monitoring frequency decreases because the large acceptable error signals a rough estimation. If the acceptable error (unit: number value) of the number of the zooplankton species is set to 3, the minimum monitoring frequency (times per year) is 24. The residual distribution of the model followed a normal distribution. This model can be applied for the estimation of the minimal monitoring frequency that satisfies the target error bounds, as this model provides an estimation of the error of the zooplankton species numbers with monitoring frequencies.

Key words: zooplankton, temporal number of species model, confidence interval, Bootstrap method, optimal monitoring frequency

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1. Introduction

Zooplankton is important as an energy messenger in the food chain of the ocean ecosystem. These zooplanktons live in diverse areas, such as the coastal seas, ranging from the surface to deep bottom layers of the ocean (Nishida and Nishikawa 2011). Despite differences in the diverse environments, the homeostasis in species diversity of zooplankton provides important information for the characteristic analysis of the marine ecosystem, the evolution of the marine organisms and the conservation of the ecosystem (Irigoin et al. 2004; Tittensor et al. 2010). The zooplankton species diversity considerably varies with changes in the coastal environment and species-specific life cycles (Nogueira 2001; Lo et al. 2004; Hwang et al. 2011; Lee et al. 2006). The range and frequency are different for the analysis of spatio-temporal changes in species composition. The typical sampling periods range from 4 times (seasonal survey) to 50 times per year within surveyed areas (Moon et al. 2010; Lee et al. 2006; Lee et al. 2012; Hwang et al. 2011).

The ecosystem survey requires an optimal or appropriate monitoring plan for the biological and physiological change analysis because there is a limitation on available cost and labor (Millard and Lettenmaier 1986; Dixon and Chiswell 1996). The increased monitoring numbers for zooplankton reduces the confidence intervals in the estimation error of zooplankton species models based on the monitoring data. The increase in survey numbers, however, means an increase in time and labor necessary for species identification. Thus, studies on the estimation of the minimum optimal monitoring frequency (OMF) are necessary to target acceptable error boundaries. The aim of the present study was to estimate the OMF to guarantee and predict the minimum number of species needed for studies concerning marine ecosystems. The OMF is estimated using the temporal number of zooplankton species models using the bi-weekly monitored zooplankton species data according to operational taxonomic units in the Tongyoung coastal sea. The confidence interval (CI) of the model with monitoring frequency is estimated using a bootstrap method. The CI is used as the reference to estimate the optimal monitoring frequency.

2. Materials and Methods

Materials

A total of 43 samples were collected nearly bi-weekly using a conical net (front half – cylindrical, connected rear half – conical; mouth diameter, 60 cm; mesh size, 200 µm; length, 300 cm; vertical towing) attached flowmeter from March, 2013 to September, 2014 in the Tongyoung Marine Science Station of the Korea Institute of Ocean Science and Technology (34°45.80’N, 128°21.45’E) off the Tongyoung coast. The difference in species number may occur depending on the inflow-quantity. However, in the

| Date (YYYYMMDD) | No. of Species | Date (YYYYMMDD) | No. of Species | Date (YYYYMMDD) | No. of Species |
|-----------------|---------------|-----------------|---------------|-----------------|---------------|
| 20130313        | 14            | 20130327        | 19            | 20130411        | 14            |
| 20130425        | 15            | 20130511        | 20            | 20130525        | 19            |
| 20130610        | 30            | 20130624        | 22            | 20130706        | 24            |
| 20130723        | 24            | 20130808        | 25            | 20130822        | 25            |
| 20130906        | 27            | 20130923        | 25            |                |               |
| 20131007        | 19            | 20140102        | 12            | 20140116        | 6             |
| 20140203        | 10            | 20140217        | 13            | 20140303        | 16            |
| 20140317        | 14            | 20140317        | 14            | 20140401        | 13            |
| 20140401        | 19            | 20140429        | 18            |                |               |
| 20140415        | 19            | 201404514       | 21            |                |               |

Ref. * = Sampled at different time, but regarded as the same day monitoring data set
present study, the difference was found to be insignificant because the species number of zooplankton reached the saturation level of the rare-faction curve.

The independent zooplankton species were identified by using DNA meta-bar-coding process to be explained in detail below and the number of zooplankton species was obtained after counting the available species numbers obtained (selected data based on the threshold reading numbers) at different sampling times (Table 1; KIOST 2016). The genomic DNA (gDNA) of zooplankton was extracted using the QIAGEN DNeasy Blood & Tissue KIT (Qiagen, Inc., Valencia, CA). The gDNA was subsequently amplified using a previously described primer set (miCOLDintF and jgHCO2198, Leray et al. 2013). After generating a library using refined PCR products, we sequenced the PCR products according to the protocol of MiSeq (Illumina Inc., San Diego, CA). The nucleotide sequences for low-quality reads and chimeras were removed using the CD-HIT-OTU program. Subsequently, clustering was performed based on the Molecular Operational Taxonomic Units (MOTUs) at the 98% similarity level (Li et al. 2012; Blaxter et al. 2005; Machida et al. 2009). After searching/comparing the NCBI non-redundant database and MegaBLAST MOTUs with these produced MOTUs, the zooplankton species were confirmed (Zhang et al. 2000), and the MOTUs had a pair-wise identity over 97% and query coverage over 90% (Carew et al. 2013).

**Methods**

**Temporal number of zooplankton species model**

The simplest model of the number of zooplankton species model is the mean-constant model. This model is has only one parameter, but is optimal because it cannot explain temporal changes, such as seasonal and inter-annual changes. The optimal model was selected based on the AIC (akaikes information criteria) values computed using Eq. (1). When the AIC value is minimal, the model can be regarded as optimal (Akaike 1974; Johnson and Omland 2004),

$$AIC = 2k + n \cdot \log(RSS/n)$$  \hspace{1cm} (1)

in which, $k$ is the number of the model parameters, $n$ is the size of the data sample (in this study, $n = 43$), and $RSS$ is the residual sum of squares.

The candidate models comprise the summations of functions with many period components, from the 1-year period to semi-annual, seasonal, and monthly cycle terms. The optimal model is selected after comparing the AIC values computed from the possible models. The temporal number of the zooplankton species model can be expressed as Eq. (2), in general form. The parameters are optimally estimated using the least squares method, minimizing the cost function as the residual (error) sum of squares in the given number of the harmonic functions, $M$, as shown in Eq. (1):

$$N_Z(t) = \bar{N}_Z + \sum_{j=1}^{M} A_j \cos(\omega_j \cdot t_i - \phi_j) + \varepsilon_i$$  \hspace{1cm} (2)

in which $N_Z(t_i)$ is the number of zooplankton species at time $t_i$ ($i = 1, 2, \ldots, n$), $n$ is the number of samples, $\bar{N}_Z$ is the optimal mean of the number of zooplankton species, $M$ is the number of harmonic functions, and $\omega_j$ ($j = 1, 2, \ldots, M$) is the frequency components of the model, defined as $2\pi j/365.25$ (1/days). $A_j$, $\phi_j$ are the amplitude and phase of the harmonic function with the frequency $\omega_j$, respectively; $\varepsilon_i$ is the residual error, defined as the difference between the observed and model-estimated number of zooplankton species at time $t_i$.

The data used in this study were collected for approximately one and a half years. The monitoring date of the data was converted to Julian days to generate intra-annual data. Thus, the model can be considered as a temporal model for understanding intra-annual, not inter-annual, changes. The model can be set up using the number of zooplankton species data accumulated/colllected during many years, assuming no trend. Using this model, it is possible to estimate the expected number (reference number) of times and/or samples to satisfy the target error bounds. The normality on the residuals is assessed using KS and Anderson-darling tests with 95% confidence levels in the ‘nortest’ package in R (Juergen and Uwe 2015). This value can be used to estimate the confidence intervals of the model when the error distribution follows a normal distribution.

There are parametric and non-parametric methods for when the temporal number of a species model is assumed to be a harmonic function and not assumed to be another specific function, respectively. The latter is more flexible than the former because the model is not subject to a specific function. However, the non-parametric methods have more complex expression functions, such as the summation form of functions, making statistical inferences difficult because of the lack of supported closed-form of functions (Wand and Jones 1995; Martinez and Martinez 2005). In the present study, the model is estimated using a non-parametric method. These data are subsequently used as the reference model to determine whether estimating...
the other model using a parametric method is suitable. This non-parametric model, expressed as a locally smoothed regression curve, can be estimated using the ‘locreg’ function in R ‘KernSmooth’ package, and ‘smooth’ function (option='loess') supported through MATLAB. In the present study, we estimated the model using the ‘smooth’ function in MATLAB. To generate the smooth curves in data boundaries (times are indicated near the left (1) and right (365) boundaries), the virtual data sets were added to the original data after adjusting the time index. When the original data set comprises the sampling time in Julian days and number of zooplankton species, the virtual data sets are the same, except for the time. In virtual data, the time was changed to time+365 and time-365 days, and the data size increased 3-fold.

Confidence interval estimation using the bootstrap method

The confidence intervals (CIs, error bounds, also considered as the CI range = the range of the upper and lower limits of the CI) of the temporal number of the zooplankton species (TNZS) model were estimated using the bootstrap method, a powerful method using random re-sampling simulations with substitution when analytical computational methods are not available and the assumption on error distribution is not required. This method is a Monte-Carlo simulation method, estimating the CIs at a given significance level $\alpha$ after computing the specific percentile values of many (about above 1,000 to 1,000,000) simulated results (Efron 1979; Rubinstein and Kroese 2008). This method has been widely used to examine the statistical methods and estimate the optimal monitoring intervals and CIs with given monitoring intervals for the variation pattern analysis of the environmental and ecological parameters (Anttila et al. 2012; Dowd et al. 2004; U.S. Environmental Protection Agency 2010; Cho et al. 2013).

In the present study, the 90%-confidence intervals (significance level 10%, double-sided) of the mean error bound of the model were estimated. The mean error bound is defined as the error bound using the optimal parameter estimation for this model. The simulation number used was 100 for the sake of computational efficiency. The larger the number is, the lower the computational efficiency becomes. The simulation number determines the mean error bound of the estimated error bounds and is suggested analytically as $\pm 1.96 \sigma / \sqrt{n}$ with the assumption of the normality of the error bound distribution. In this method, however, the 95%-CI of the mean error bound was also estimated using the bootstrap method for consistency. In this case, the simulation number was also 100. The CI obtained using the bootstrap method is the error range associated with the model selection (optimal estimation), and this value is different from the error obtained upon estimation of the number of zooplankton species using the model.

3. Results

Estimation error resulting from the level (order) of model complexity

The estimated number of zooplankton species using candidate models was compared with the observed

| $M$ (number of harmonic function sets) | Additional period components (cumulative from the first row) | Number of parameters, $k$ | Residual sum of squares | AIC value |
|--------------------------------------|-----------------------------------------------------------|--------------------------|-------------------------|-----------|
| 0                                    | Mean                                                     | 1                        | 38.38                   | 158.40    |
| 1                                    | + year (annually)                                        | 3                        | 10.45                   | 105.88    |
| 2                                    | + 1/2 year (semi-annually)                               | 5                        | 10.20                   | 108.84    |
| 3                                    | + 1/3 year                                               | 7                        | 9.80                    | 111.12    |
| 4                                    | + 1/4 year (seasonally)                                 | 9                        | 9.27                    | 112.76    |
| 5                                    | + 1/5 year                                               | 11                       | 8.89                    | 114.93    |
| 6                                    | + 1/6 year                                               | 13                       | 8.34                    | 116.17    |
| 7                                    | + 1/7 year                                               | 15                       | 7.91                    | 117.89    |
| 8                                    | + 1/8 year                                               | 17                       | 7.67                    | 120.62    |
| 9                                    | + 1/9 year                                               | 19                       | 6.99                    | 120.57    |
| 10                                   | + 1/10 year                                              | 21                       | 6.71                    | 122.87    |
| 11                                   | + 1/11 year                                              | 23                       | 6.56                    | 125.88    |
| 12                                   | + 1/12 year (monthly)                                    | 25                       | 6.37                    | 128.59    |
numbers after optimal parameter estimation. The estimation error (residual) sum of squares and AIC values are shown in Table 2. The optimal model was selected as a function only with including the 1-year period component because the AIC value was minimal at that condition. The function is shown in Eq. (3), and the simplest mean model is also expressed in Eq. (4) for a basic comparison.

\[ N_z(t) = 17.95 + 7.86 \cdot \cos \left( \frac{2\pi t}{365.25} \right) + N(0, 3.19^2) \]  
\[ N_z(t) = 19.63 + N(0, 6.20^2) \]  

Based on the optimal model, the timings (dates) of the maximum and minimum number of zooplankton species were calculated using the conditions \((2\pi t/365.25) + 2.81 = 0\) and \((2\pi t/365.25) + 2.81 = \pi\), respectively. The computed dates in Julian days were 202 (21st July) and 19 (19th January), respectively, and the numbers of species observed on those dates were 26 (maximum) and 10 (minimum), respectively.

The mean model showed a large AIC value, reflecting large residuals (variance of the data) defined as differences in the observed and estimated number of the zooplankton species. Although the residuals of the more complex models with more parameters decreased, the quantitative effects were not large compared with the increase in the number of parameters used to calculate the AIC values. However, the model estimated using the non-parametric method showed different shapes for the slope of the increasing and decreasing stages and the peak pattern (more flat than the parametric model). However, the non-parametric model was within the 95% error bounds of the

![Fig. 1. CI curves (90%) of the error bounds of the model with the number of survey times. The red line represents the optimal model function. The black and blue lines show the upper and lower limits of the model at 10% and 25% significance levels, respectively. The cyan lines represent the estimated models using the generated samples and the bootstrap method. (a) Seasonal survey (n = 4), (b) Bi-monthly survey (n = 6), (c) Monthly survey (n = 12), and (d) Bi-weekly survey (n = 24)](image_url)
parametric model. The residual components of the optimal model followed the normal distribution based on the KS-test and Anderson-Darling test in terms of 95% confidence intervals (5% significance level). The CIs (estimation error bounds) were approximately ±2 number of species. Confidence interval estimation of the model based on survey times

The survey times are the sample numbers used to determine the confidence intervals. We assumed that the total data on the number of zooplankton species collected at Tongyoung Marine Science Station constituted the reference population. The 90% CI estimation of the model was determined using a bootstrap method. The CI decreased with increasing survey times from 4, 6, and 12 to 24 times (Fig. 1). The analysis of the variation pattern was performed using both 75 and 90% confidence intervals. Both CIs decreased, showing power function patterns. For fewer survey times, the CI rapidly increased. Subsequently, for survey times longer than 24 times, the CI slowly decreased to a negligible extent. The CI with survey times provided in Table 3 can be fitted with the power function shown in Eq. (5). The exponents of the functions were -0.76 and -0.67 at the 90% and 75% confidence intervals, respectively.

\[
Y_{90} = 34.01 \cdot X^{-0.76} \quad (R^2 = 0.960) \\
Y_{75} = 13.41 \cdot X^{-0.67} \quad (R^2 = 0.985) \quad (5)
\]

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Table 3. Mean, lower and upper limits of the model estimation error with the number of survey times

| Survey times | Mean and 95% CI on the 90% CIs of the model estimation error | Mean and 95% CI on the 75% CIs of the model estimation error |
|-------------|-------------------------------------------------------------|-------------------------------------------------------------|
| 4           | 15.57 [12.13, 19.20]                                       | 6.08 [5.29, 6.95]                                           |
| 6           | 8.00 [7.13, 9.10]                                           | 3.89 [3.56, 4.28]                                           |
| 8           | 6.09 [5.58, 6.75]                                           | 3.08 [2.81, 3.41]                                           |
| 12          | 4.57 [4.20, 4.89]                                           | 2.33 [2.10, 2.53]                                           |
| 16          | 3.82 [3.53, 4.07]                                           | 1.97 [1.82, 2.15]                                           |
| 20          | 3.32 [3.04, 3.57]                                           | 1.72 [1.57, 1.90]                                           |
| 24          | 2.97 [2.83, 3.22]                                           | 1.56 [1.43, 1.71]                                           |
| 28          | 2.76 [2.56, 2.92]                                           | 1.43 [1.32, 1.56]                                           |
| 32          | 2.55 [2.32, 2.74]                                           | 1.32 [1.19, 1.47]                                           |
| 36          | 2.41 [2.23, 2.61]                                           | 1.27 [1.13, 1.39]                                           |
| 40          | 2.25 [2.08, 2.43]                                           | 1.18 [1.07, 1.29]                                           |

Ref. The value shown in the cell is the mean, and the values shown in the bracket [ ] are the lower and upper limits, respectively. These values correspond to the number of species.

in which, \( Y \) denotes the CIs, the subscripts of \( Y \) (90 and 75) are the percentage values of the confidence levels, \( X \) is the number of surveys (survey times), and \( R^2 \) is the coefficient of determination. If the acceptable error (unit: number value) of the number of the zooplankton species is set to 3, the minimum monitoring frequency (times per year) is 24.

4. Discussions

Basic information on the number of zooplankton species is essential for understanding the characteristic marine ecosystems. For diverse analyses, such as that of the life cycle, influence on the species due to seasonal environmental parameters (e.g., water temperature) and habitat changes are likely derived using these data (Allan 1976; Conover and Huntley 1991; Machida et al. 2009).

In addition, information on the characteristic parameters of zooplankton can be used as indicators for the assessment and management of the environmental and ecological impacts of global warming and ocean acidification (particularly, decreasing pH, not yet reaching a neutral pH of 7.0) (Link et al. 2002; Bucklin et al. 2010).

However, the accurate identification of zooplankton species diversity is difficult because the number of species increases following the rarefaction curve. The number of species is not linearly proportional to the survey times and the amount (weight) of the samples (Machida et al. 2009).

In the present study, we estimated the optimal survey frequency necessary for the analysis of the species composition and number of species using data for the number of zooplankton species monitored bi-weekly in the Marine Research Platform in the Tongyoung coastal sea. The size of the data set was 43. The optimal model for the temporal number of zooplankton species was selected based on these data. The CI due to survey times was estimated using a bootstrap method (Efron 1979). Based on the optimal model, the dates for the maximum and minimum number of zooplankton species can be calculated. The computed dates, as Julian days, were 202 (21st July) and 19 (19th January), respectively, and the numbers of species observed on those dates were 26 and 10, respectively. These results were strongly expected, as these numbers are approximately consistent with typical water temperature variation patterns. The model estimated using a non-parametric method showed different shapes in the slope of the increasing and decreasing stages and the peak patterns (more flat than the parametric model), as shown in Fig. 2. However, these differences were not
statistically significant. The CI of the model decreased with increasing survey time. The residual distribution of the model followed a normal distribution. In general, the minimum monitoring frequency (numbers per year) directly depends on the target (acceptable) estimation error. When the acceptable error (range of the CI) increases, the monitoring frequency decreases. Based on the results of this study, the minimum frequency is 24. The uncertainty of the annual fluctuation numbers increased as the frequency of zooplankton surveys decreased (Fig. 1). The uncertainty level should be low for the accurate analysis of an ecosystem using the number of species and species composition data for zooplankton.

The species numbers and diversity indices have been suggested as the basic statistics for the analysis of the diverse ecological research fields on zooplankton (Lee et al. 2006; Moon et al. 2010; Lee et al. 2012). However, it is difficult to directly compare these values. The numbers of species are expected to show a large fluctuation with the number of surveys (frequency) based on the model results (see Fig. 1) for the zooplankton occurrence suggested in the present study. Therefore, this model might reflect the determination of the optimal survey times necessary for understanding the ecological zooplankton structure.

5. Conclusions and recommendations

The optimal temporal model for the number of zooplankton species in Tongyoung coastal sea can be expressed as the following model-function:

\[ N_Z(t_i) = 17.95 + 7.86 \cdot \cos \left( \frac{2\pi}{365.25} (t_i - 201.65) \right) \]

The minimum monitoring number is 24 when the acceptable model estimation error is set to 3. It means that at least 24 monitoring times per year (approximately 2 times per month) are required to keep the errors (fluctuation size) below 3. Based on the results of this study, bi-weekly monitoring data are considered sufficient to quantify the annual variation of the number of zooplankton species without any significant error. However, since this study was carried out using only one coastal monitoring station data, it is necessary to be careful to use it as a monitoring standard for the whole coastal waters in Korea. In order to set more general criteria, a comparative study with the results of analysis using data of many different coastal waters is highly required. In addition, an error analysis using data with more short-term time scales (smaller than two weeks interval) is highly recommended. In practice, a continuous monitoring system is the best, when possible.

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