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Consequences of using pooled versus individual samples for designing environmental monitoring sampling strategies

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HIGHLIGHTS

- Use of individual or pooled samples is important when designing sampling strategies.
- Variation caused by e.g. chemical analysis or sample variation need to be considered.
- Various solutions are offered using different numbers of individual/pooled samples.
- Results allow the design of cost-efficient, statistically sound sampling strategies.

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ABSTRACT

Choosing an appropriate sampling strategy for chemical analysis within environmental monitoring includes the important decision of whether to sample and store individual or pooled samples. This choice impacts on future analyses from Environmental Specimen Bank samples. A number of advantages exist to support using either individual or pooled samples for temporal trend studies. However, it is important to know the total and analytical variance to be able to design the best sampling strategy. Statistical power in temporal or spatial studies is determined by the random/unexplained sample variation. The relationship between chemical analytical error and other sources of variation, as well as the cost for collection, preparation of samples and chemical analysis, will determine the number of individuals in each pool, and the number of pools that should be analysed to achieve high cost efficiency and good statistical power. Various scenarios of different numbers of individual samples, different numbers of pooled samples containing various numbers of individual specimens, the relationships between chemical analytical error and other sources of sample variance, have been compared by simulating random sampling from computer generated populations using realistic measures of variation from ongoing monitoring activities. These results offer guidance in the design of a cost-efficient, statistically sound sampling strategy.

1. Introduction

When choosing a sampling strategy for chemical analysis within environmental monitoring, or when storing samples in Environmental Specimen Banks (ESBs) for future analyses, the important decision of whether to use individual or pooled samples i.e., multiple individual samples homogenized into one sample for chemical analysis, should be emphasised. This decision can impact on many things, from the sensitivity to detect changes in contaminants in the environment, to the potential for future and/or retrospective analyses. Strategies outlines here are applicable to both environmental and human samples.

A number of advantages are gained from using individual samples (Bignert et al., 1993). Information about variance is important in itself to identify the sample distribution e.g., normal or log-normal distribution, and changes in variance are often the first sign of a change in contaminant burden. In this study, the variance estimates are based on biological samples from the Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPCMB). Information about the maximum value can be crucial when the threshold level for a substance is set at the maximum value. It may also be essential for risk analyses. Individual samples allow the freedom to choose an appropriate central measure (Caudill et al., 2007; Caudill, 2012). Environmental contaminant data often display a right skewed distribution, in which case geometric mean values are more appropriate. By contrast, pooled samples reflect approximate arithmetic means, although this can be compensated for to some extent (Caudill et al., 2007). Sampling of individual organisms facilitates direct adjustments for

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confounding factors, for example fat content, age, and size (length and weight), and the detection of extreme values. Further to this, storage of individual samples allows the estimation of within-specimen variation and distribution.

However, in cases where sampling and sample preparation are considerably less expensive than the chemical analytical costs, and where the contribution from inherent specimen variance due to e.g., physiological factors in biological samples, is considerably larger than the analytical error to the total random/unexplained variation, variation may be reduced, while keeping within the same budget, by using pooled samples. Hence, the statistical power to detect changes over time or differences between groups can be increased or the spatial distribution can be better defined for the same cost by using pooled samples. A reduction in the random/unexplained between-year variation implies that a smaller annual change can be detected, or that a specified lowest trend that needs to be detected will be identified in a shorter period of time (Bignert et al., 2004). Pooled samples may also allow for chemical analysis to be conducted when individual samples are too small to provide enough material e.g., small fish or zooplankton (Gewurzt et al., 2011), resulting in fewer non-detects. Further, a better defined spatial distribution enables e.g., comparison of quality status between lakes and other types of surface water within the Water Framework Directive (2000/60/EC) (WFD) and the Marine Strategy Framework Directive (2008/56/EC) (MSFD).

Within the SNMPCMB, temporal trend studies of polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexanes (α, β- and γ-HCH), 1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane (*p,p′*-DDT), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethene (*p,p′*-DDE) and 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane (*p,p′*-DDD) have been conducted. To study these contaminants, a number of individual samples have been collected from the same station within the same sampling season each year (Bignert et al., 2013). From the beginning, individual samples from 12 sampling sites were used, but during the last 10 years, due to the use of pooled samples, the SNMPCMB has been able to expand the number of sites sampled from 12 to 24. One of the objectives of the SNMPCMB is to estimate long-term time trends and the rate of the changes found. The quantified objective is to detect an annual change of approximately 5% within a time period of 10 years with a power of 80% at a significance level of 5%. This objective will be used as the target values in this study.

There are clearly a number of potential advantages and disadvantages in certain situations to using either pooled or individual samples. Here, we outline various sampling strategies for chemical analysis based on using different numbers of individual or pooled samples to provide several possible outcomes, which can then be used to guide those designing sampling strategies for environmental monitoring or working with the storage of specimens in ESBs. Although in this study the variance estimates are based on biological samples from the SNMPCMB, the results presented here should also be applicable for monitoring of human and abiotic samples if similar variance estimates are calculated for such matrices.

2. Materials and methods

This study was based on simulations using computer generated values. However, the estimated variances, the ratios between specimen variation and the chemical analytical measurement errors in the various scenarios were based on real values collected during the last 10 years of environmental contaminant monitoring within the SNMPCMB. This program is ongoing, and consists of locally unpolluted areas. Samples are collected annually. Herring (*Clupea harengus*) is the most frequently used species in the SNMPCMB, although cod (*Gadhus morhua*), eelpout (*Zoarces viviparous*), perch (*Perca fluviatilis*) and guillemot (*Uria aalge*) eggs are also monitored, as well as other species not included here. Since 1996, 10 individual cod, perch, eelpout, guillemot eggs and spring samples of herring, and 12 individual samples of autumn-caught herring from 12 sites along the Swedish coast were collected within the SNMPCMB and analysed and stored in an ESB. Since 2007, the SNMPCMB has expanded to include 24 sampling sites along the Swedish coast due to the resources saved by using pooled samples. However, all samples collected are individually stored in the ESB at the Department of Environmental Research and Monitoring, Swedish Museum of Natural History, Stockholm, Sweden. The chemical measurement errors handled in this study were estimated from the analyses carried out at the Department of Applied Environmental Science (ITM), Stockholm University, Sweden.

Beginning with 229 time series based on the above collected data, a number of filters were applied to eliminate series that were deemed unsuitable for this analysis. Total variances, expressed as coefficients of variation (CVt), were estimated from de-trended time series from the SNMPCMB (Bignert et al., 2013) for the last 10 years from 12 different sites. The removal of possible trends was achieved by subtracting the predicted annual mean value from a linear regression line from each individual observation in the same year. Eight time series with a CVt above 100% were removed because they were not representative of the majority of samples. A further 7 time series were excluded, mainly because of gaps (missing years) and non-monotonic trends. Another filter was applied to achieve robust data sets, which specified that a) the contaminant concentration should be quantified in at least 20 samples per site (23 time series excluded), and b) there should be at least 6 out of 10 years with quantifiable values (15 time series excluded). After filtering, 176 of the original 229 time series remained. The highest CVt observed for one time series was 97%, the lowest 12% and the median value 52% with an interquartile range of 35–62% (Table 1).

The precision of the chemical analysis, also expressed as a coefficient of variation (CVa) (within and between years), was based on approximately 6000 analyses from four different internal reference materials. These had a lipid content ranging from 0.5% to 11.3% analysed for 7–9 years over a period of 20 years i.e., not all reference materials were available for the entire period (two reference materials were used for 7 years; when these ran out, new ones were made and used for approximately 9 years, thus partly overlapping). A sample of 10 g of muscle tissue was extracted with a mixture of polar and non-polar solvents and analysed on a gas chromatograph equipped with a μ-electron capture detector (μECD) (Jensen et al., 1983; Eriksson et al., 1997) at ITM, Stockholm University. The standard deviation, expressed as CV%, was plotted against the arithmetic mean value for the log concentration on a lipid weight basis for each contaminant for each reference material. A regression line was fitted to the values for each contaminant group using a power function, so that the variation decreased when the concentration increased (Horwitz and Albert, 2006). The CV% of the geometric mean values for the contaminant concentrations for each species and sampling site within the SNMPCMB for the last 10 years was then calculated from the corresponding regression lines. The highest CVa was 16% and the lowest 4%. The median value was 10% with an interquartile range of 8–12% (Table 1).

Specimen variance (within and between years) reflecting e.g., physiological differences such as sex, age, condition and reproduction phase, or other factors possibly induced by abiotic factors e.g., temperature, and salinity, as well as long-term storage and sample treatment, expressed as coefficients of variation (CVs), were calculated from the CVt and the CVa using the relationship (Råde and Westergren, 1990):

$$CVt = (CVs^2 + CVa^2)^{0.5}.$$