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Effect of pooled sampling in the MILFERSK program

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Abstract
The MILFERSK monitoring program is studying the occurrence and biomagnification of contaminants in freshwater ecosystems, mainly Lake Mjøsa. For the last 10 years, individual samples of muscle and liver from brown trout have been analyzed for a wide range of environmental contaminants. In this report we are statistically evaluating the effect of converting to pooled samples, i.e., 5 individuals constituting one composite sample. Main results indicate that analyzing pooled samples could mask or hide extreme concentrations, limiting the possibility to detect early occurrence in the environment. The statistical power will be weaker when studying biomagnification potential and time series, as fewer data points are included in the statistical models.

Keywords: Contaminants, environmental monitoring, statistics
Emneord: Miljøgifter, miljøovervåking, statistikk

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Preface

In accordance with the mandate from the Norwegian Environment Agency (Miljødirektoratet) to investigate the long-term effect of a transition to pooled sample analyzes in the monitoring program MILFERSK, we have carried out an investigation of statistical models based on pooled samples of trout from Mjøsa collected in 2022, as well as a modeling of randomized mathematical pooled samples.

Asle Økelsrud and Morten Jartun have been responsible for coordinating sampling and analysis, as well as writing the first part of the report. Dag Øystein Hjermann has carried out the analysis of randomized, mathematically pooled data, as well as written the section that includes this.

Hamar, 05.01.2024

Summary

We have assessed the effect of converting from measuring environmental contaminants in individual samples to pooled samples in the MILFERSK monitoring program (Monitoring of environmental contaminants in freshwater food webs). This assessment is based on the following:

1. Comparison of statistics for selected contaminants in individual samples (N=15) of brown trout from Lake Mjøsa in 2022 to pooled samples (5 individuals per sample according to length, N=3).

2. The effect of composite samples, hence reduced number of data points, on the reliability of statistical models of trophic transfer, e.g., trophic magnification factors (TMFs).

3. Impact on time trends for selected contaminants, based on a statistical evaluation of randomized, mathematically pooled contaminant levels from historical data in brown trout from Lake Mjøsa

The results show that there is a tendency for outliers to be lost when converting from measuring contaminants in individuals compared to in composite samples (pooling) in the 2022 data (1). This loss of variation, and outliers, may in turn conceal information about emerging compounds with a medium to low detection frequency.

When modeling trophic transfer of contaminants, such as the trophic magnification factor (TMF), pooling data will in turn reduce the number of datapoints (n) in the model (2). This may lead to more uncertain estimates (increased confidence intervals, CIs). In addition, pooling data may produce different estimates of the TMF, while the “extreme” values are left out (outliers).

It appears that for most contaminants in this study, pooled sampling results in a smaller power, which means that the trend needs to be stronger to be «discovered» by statistical analysis. Alternatively, if one uses pooled samples, a time trend of a given magnitude may need some extra years until it is discovered (3).

Sammendrag

Vi har vurdert effekten av å konvertere fra måling av miljøgifter i enkeltprøver til samleprøver i overvåkingsprogrammet MILFERSK (Monitoring of environment contaminants in freshwater food webs). Denne vurderingen er basert på følgende:

1. Sammenligning av statistikk for utvalgte miljøgifter i enkeltprøver (N=15) av ørret fra Mjøsa i 2022 til blandprøver (5 individer per prøve etter lengde, N=3).

2. Effekten av blandprøver, dvs. redusert antall datapunkter, på påliteligheten til statistiske modeller for biomagnifisering, f.eks. trofisk oppkonsentreringsfaktor (TMF).

3. Påvirkning på tidstrender for utvalgte miljøgifter, basert på en statistisk evaluering av randomiserte, matematisk sammenslåtte nivåer av miljøgifter fra historiske data i ørret fra Mjøsa

Resultatene viser at det er en tendens til at statistiske uteliggere (ekstremverdier) går tapt ved konvertering fra måling av miljøgifter i individer sammenlignet med i blandprøver (pooling) i 2022-dataene (1). Dette tapet av variasjon, og statistiske uteliggere, kan i sin tur skjule informasjon om nye forbindelser med middels til lav deteksjonsfrekvens.

Ved modellering av trofisk oppkonsentrering av miljøgifter, slik som trofisk oppkonsentreringsfaktor (TMF), vil sammenslåing av data i sin tur redusere antall datapunkter (n) i modellen (2). Dette kan føre til mer usikre estimater (økte konfidensintervaller, KI). I tillegg kan sammenslåing av data gi forskjellige estimater av TMF, mens de "ekstreme" verdiene utelates (statistiske uteliggere).

Det ser ut til at for de fleste miljøgifter i denne studien resulterer bruken av blandprøver i en mindre statistisk styrke, noe som betyr at trenden må være sterkere for å bli «oppdaget» ved statistisk analyse. Alternativt kan bruk av blandprøver føre til at man trenger noen ekstra år før en oppdager en tidstrend av en gitt styrke (3).

1 Effect of pooled sampling in the Milfersk program

Content

1. Individual vs. pooled samples of brown trout 2022

1.1 Background and method

A wide range of contaminants have been determined in samples of brown trout in Lake Mjøsa annually the last 10 years (see Jartun et al., 2023). Within this period, samples of muscle and liver have been analyzed in individual specimen (N=15).

In this chapter we have compared the concentrations of selected contaminants in individual samples (N=15) of brown trout from Lake Mjøsa in 2022 to pooled samples (5 individuals per sample according to length, N=3). Contaminants in both groups were Hg, siloxanes (cVMS: D4, D5, D6), PBDEs, S/MCCP, PFAS and UV compounds. In the evaluation we have excluded contaminants with no data >LOQ for either group, such as S/MCCP, most UV compounds (except EHMC and octocrylene), and all PFASs except PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOS, brPFOS and PFBSA. See **Figure 1 to Figure 20** for the contaminant groups and selected individual contaminants in the subchapters below.

Furthermore, applying pooled samples may lower the calculated **mean** value for some of the contaminant groups compared to the calculated means for individual samples, see e.g., sum PBDEs and individual BDEs. This is not observed to the same degree for PFAS or siloxanes, however, the variation and outliers become concealed. In this single experiment from brown trout sampled in 2022, we have analyzed samples of individual brown trout (N=15) and sorted these individuals in three groups of five individuals according to length. Equal amounts of matrix (muscle or liver, according to contaminant group) were dissected from individual 1-5, 6-10 and 11-15 to produce pooled sample no. 1 to 3, respectively. As of now, we do not have a justified explanation to the observed findings where pooled sample contain lower concentrations than the calculated mean for individual analyses, but the analytical uncertainty for the methods *may* account for some of these observations. We do however observe that the outliers and extreme concentrations found in individual samples are concealed when calculating the mean, as shown for the total content of PBDEs in Figure 1.

Pooling samples of the same tissue from several individuals will consequently decrease the variation in the dataset, and important information about outliers is lost. This means that detections of potential outliers with higher concentrations, which may indicate an early introduction of given contaminant to the environment, may go undetected. Valuable information may be hidden as the variation are smoothed across several individuals. One example is the UV compound octocrylene, as shown in Figure 14. Of five individual analyses, two were above LOQ. In the pooled samples, when identical amounts of liver from the five same individuals were pooled into one analysis, this compound was below LOQ. There is no doubt that analytical precision may account for some of these differences, as uncertainty for UV compounds (as an example) is approx. 40 %. The empirical information drawn from the contaminants with detections above LOQ all indicate that actual variation of contaminant concentrations in brown trout *may* be lost when using pooled samples. In Figure 15 we have included a graph of how octocrylene would normally be reported, with mean and range for individual samples (N=15) compared to the pooled samples (N=3) in 2022. It is clearly stated that a lot of information on

the variation, potentially concealing extreme values, could be lost for emerging compounds with a medium to low detection frequency.

Another pattern is shown for PFOS in Figure 9, where the mean concentration for individual and pooled samples is similar, but again, the higher concentrations may be lost. This example shows values for 2022 only. Consequently, we may lose valuable information when comparing actual concentration levels to the environmental quality standards (EQS, i.e., 9.1 ng/g for PFOS in this example) when applying pooled sampling.

1.2 Results

1.2.1. PBDEs

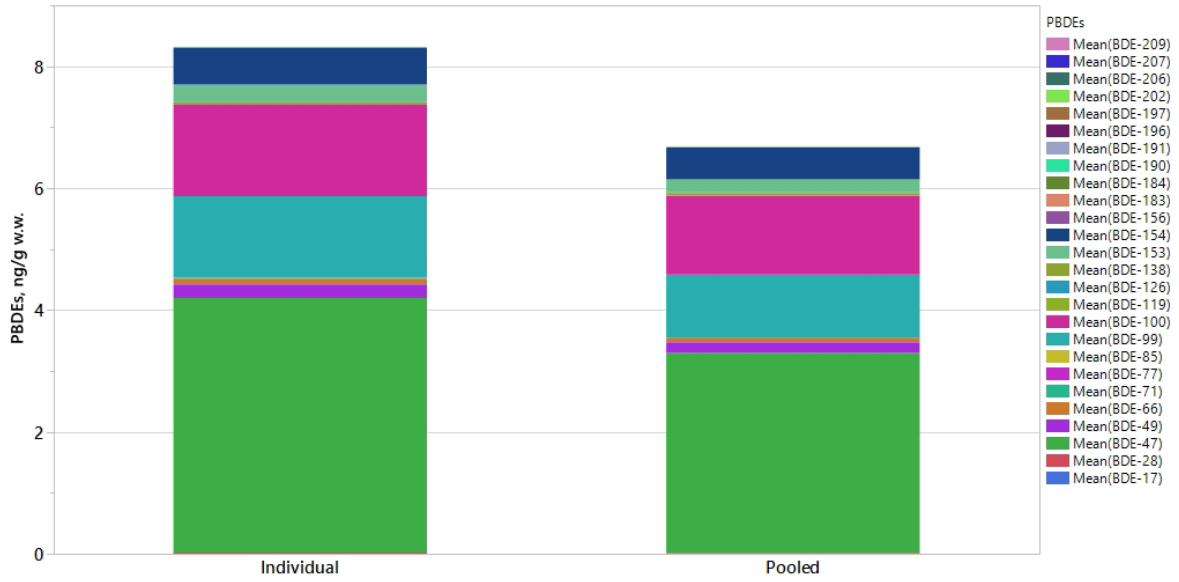


Figure 1 Concentration of PBDEs (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=15), pooled samples (N=3).

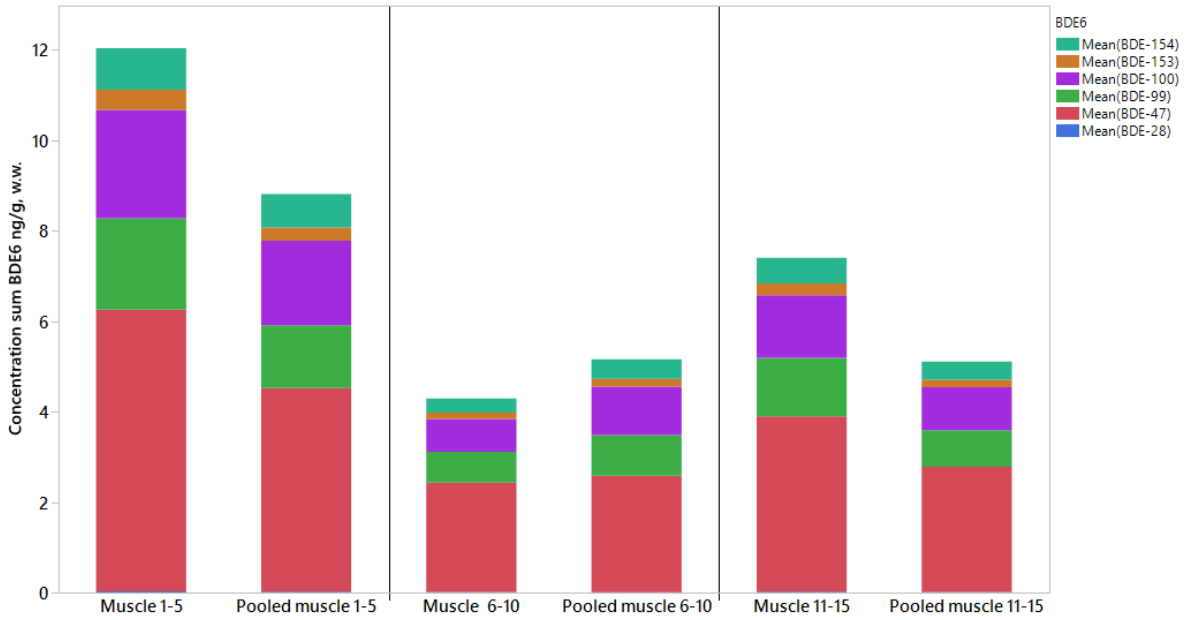


Figure 2 Concentration of sumBDE_6 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

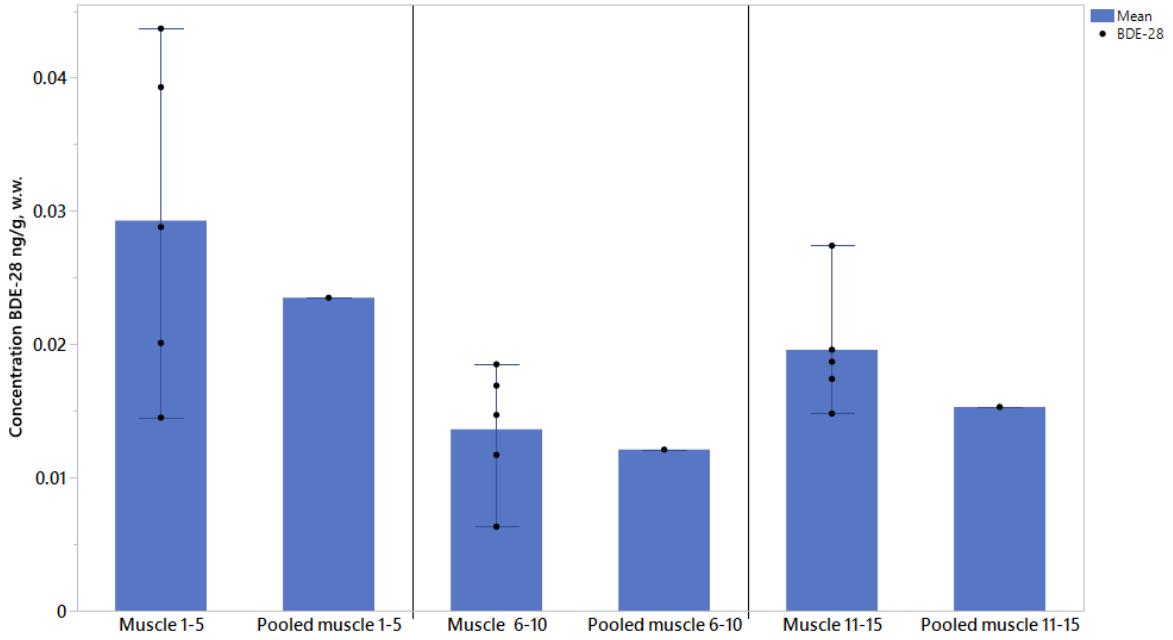


Figure 3 Concentration of BDE-28 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

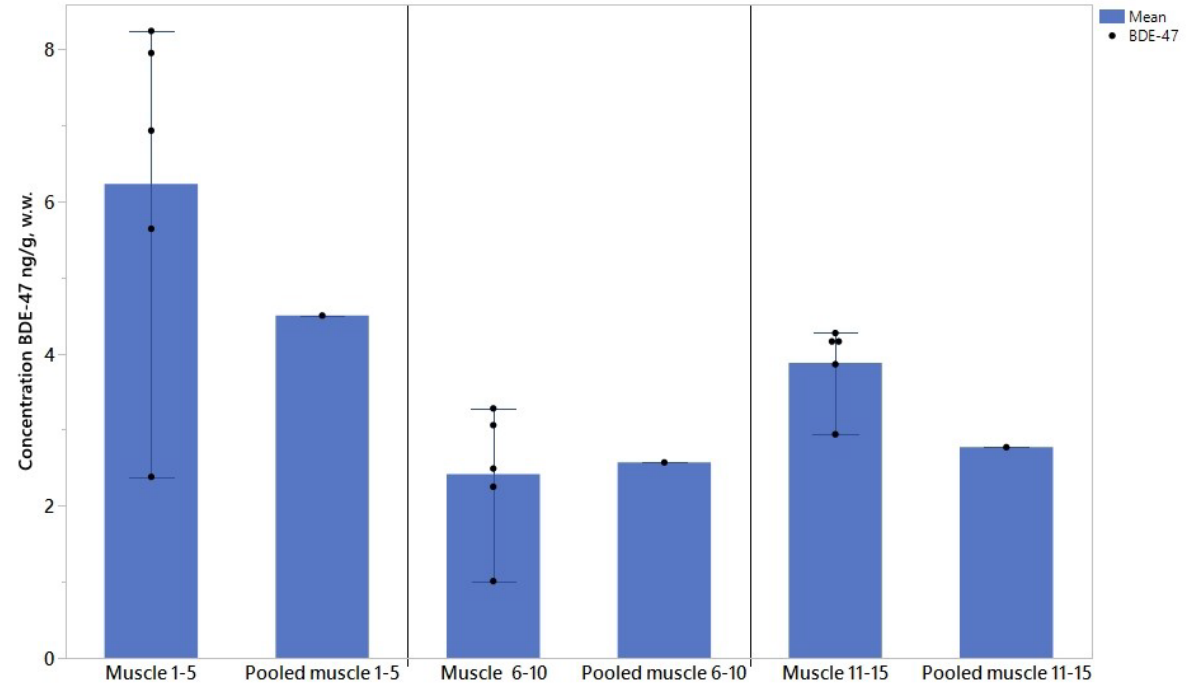


Figure 4 Concentration of BDE-47 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

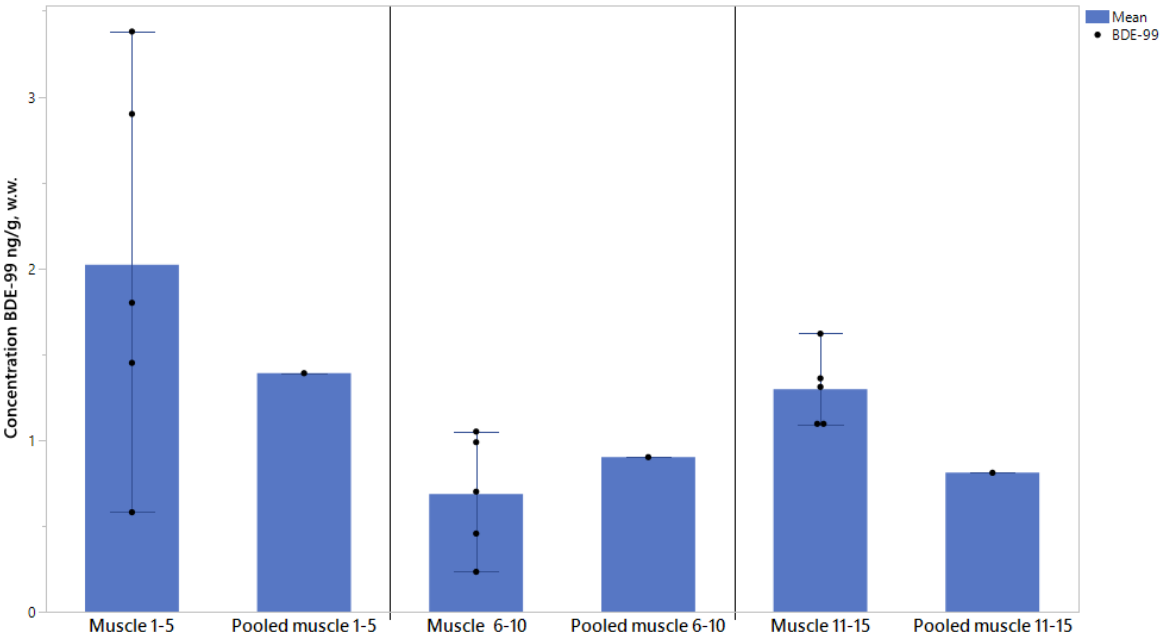


Figure 5 Concentration of BDE-99 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

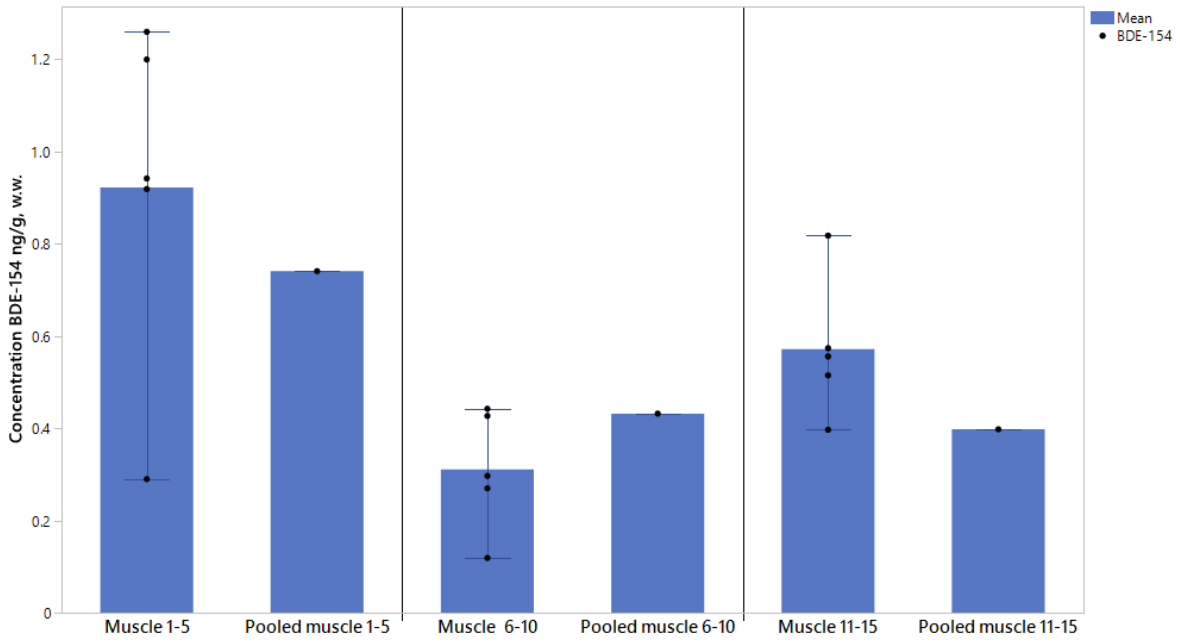


Figure 6 Concentration of BDE-154 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

1.2.2. PFAS

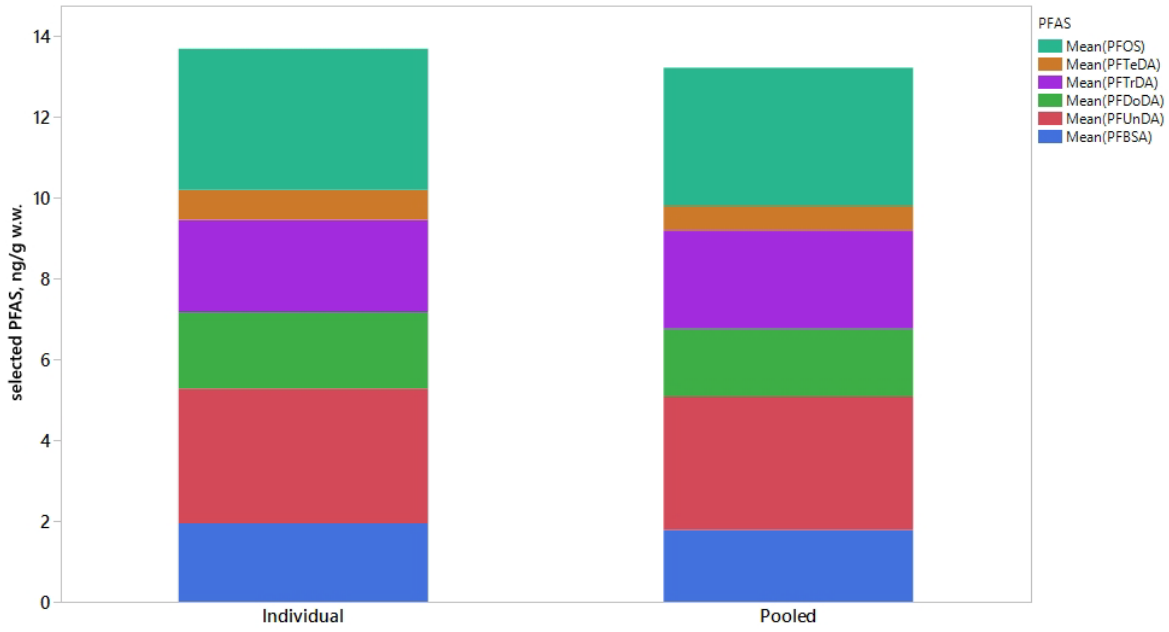


Figure 7 Concentration of detected PFAS (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=15), pooled samples (N=3).

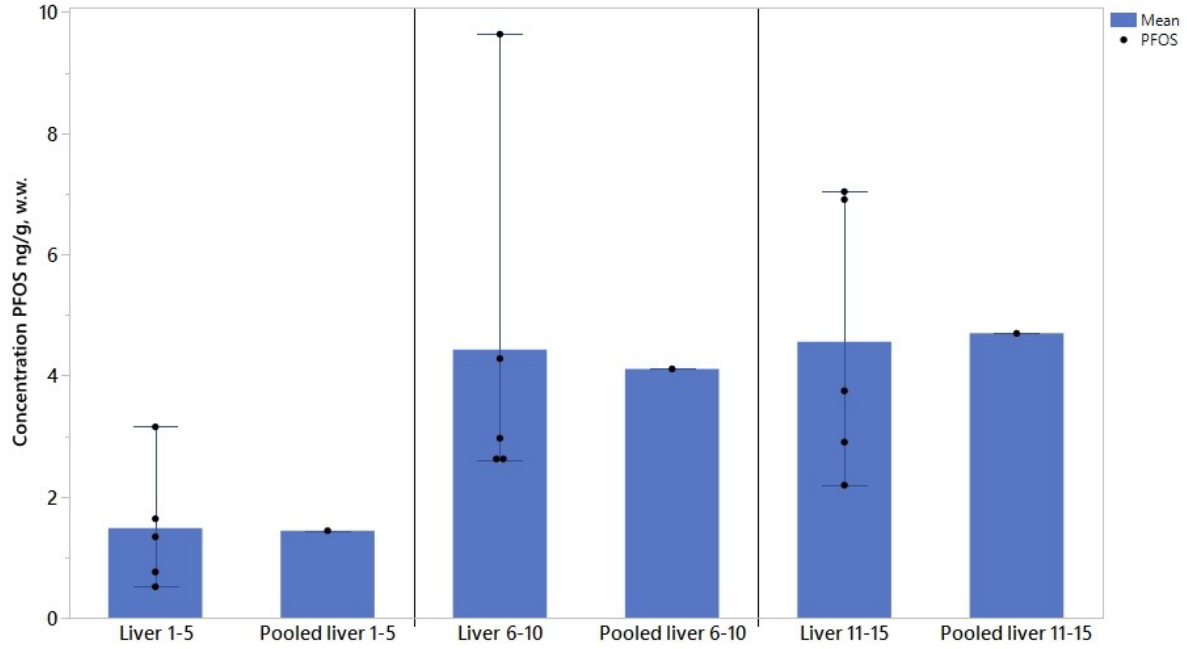


Figure 8 Concentration of PFOS (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

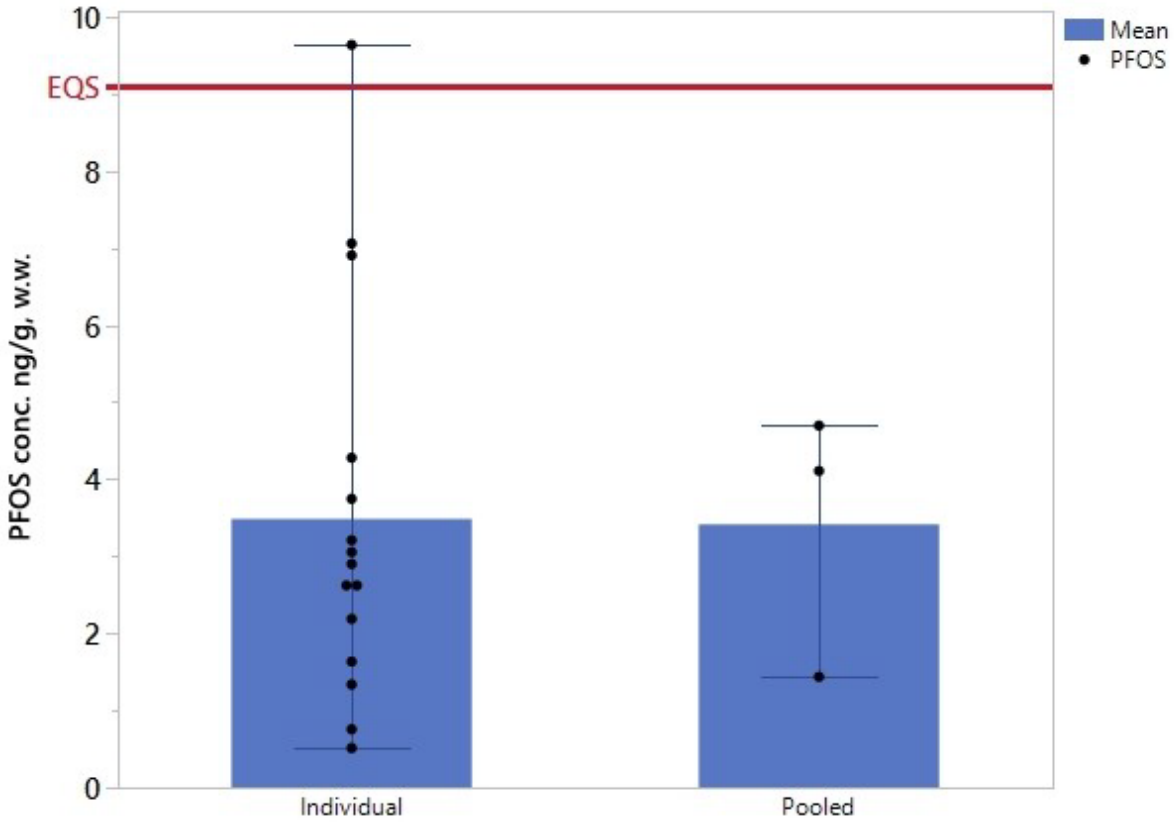


Figure 9 Mean concentration and range of PFOS (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=15), pooled samples (N=3). EQS for PFOS in biota (9.1 ng/g w.w.) indicated with red line.

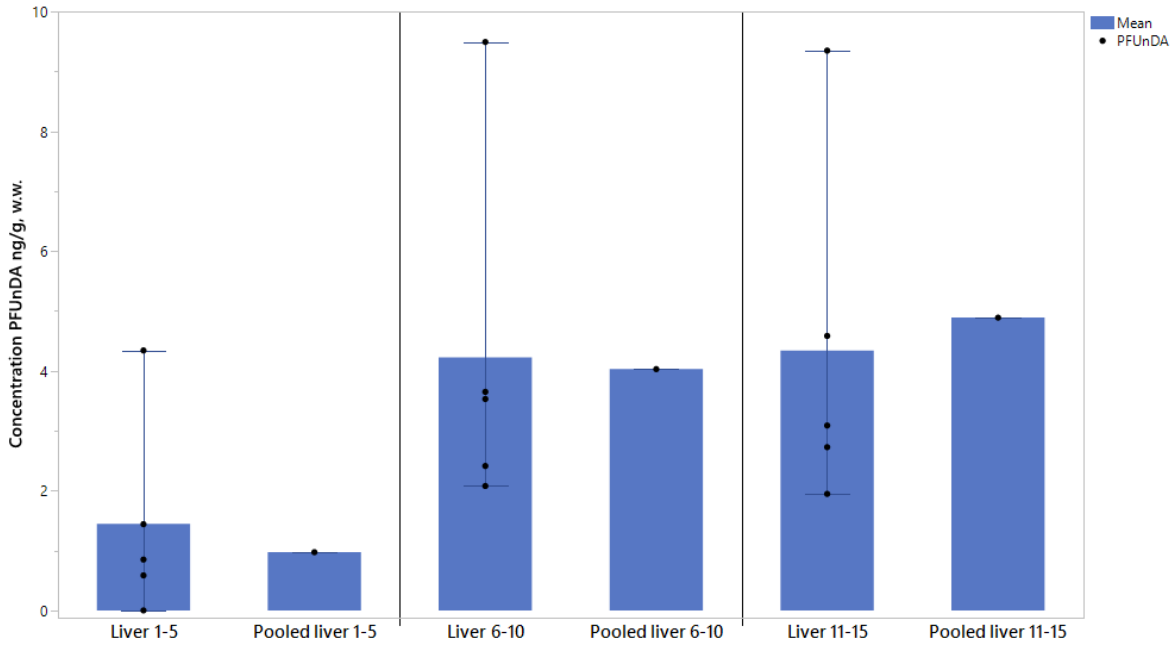


Figure 10 Concentration of PFUnDA (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

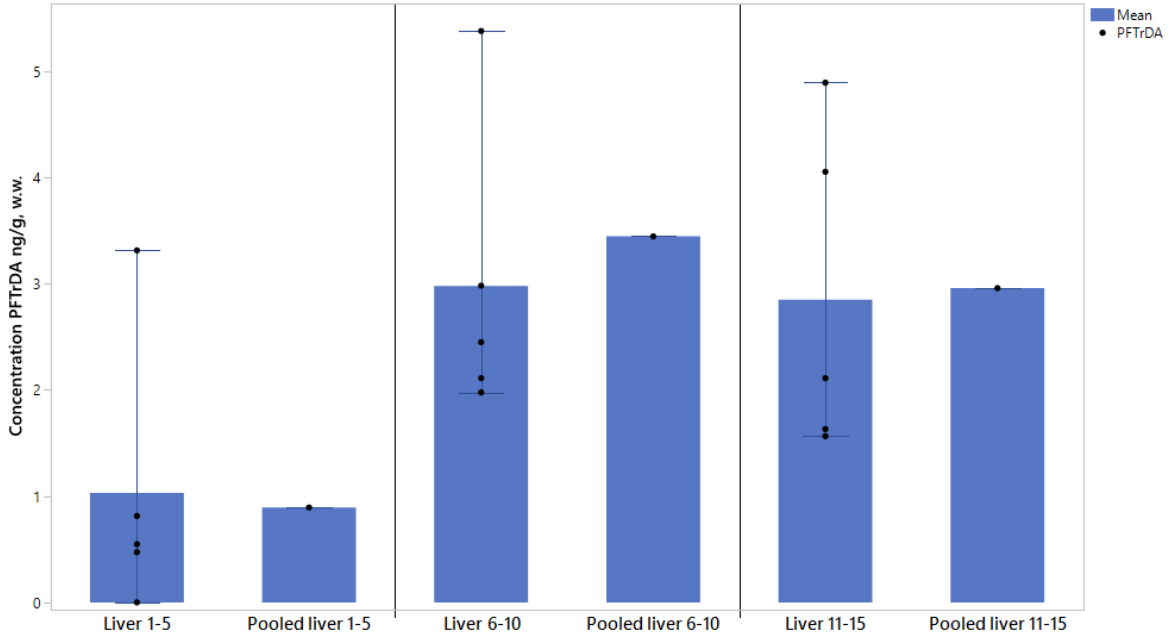


Figure 11 Concentration of PFTrDA (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

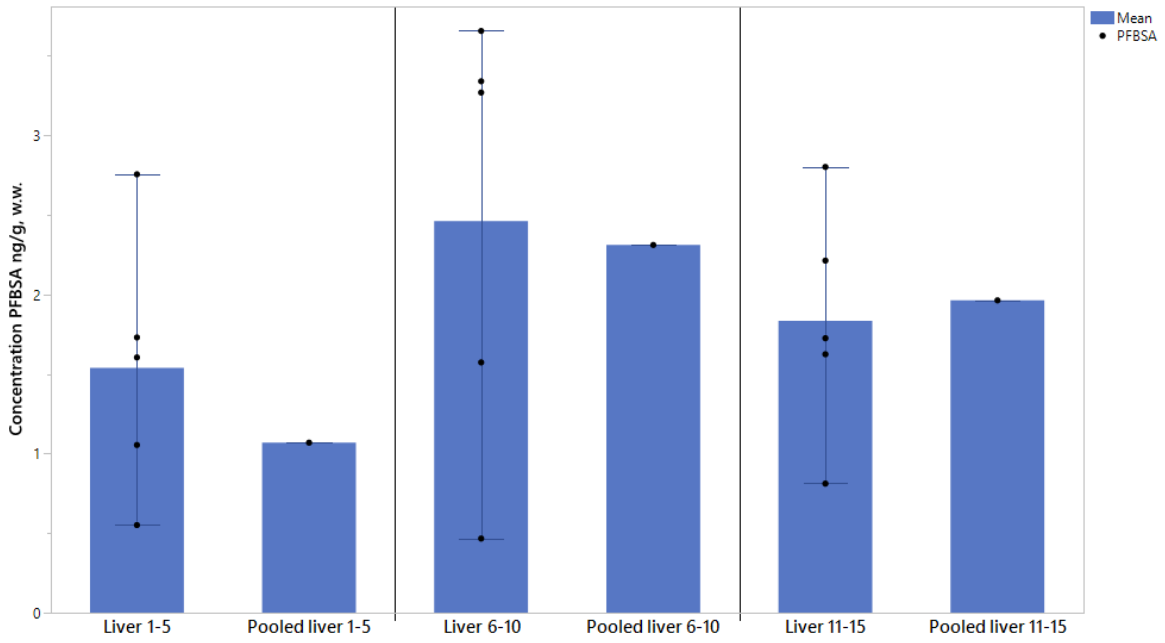


Figure 12 Concentration of PFBSA (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

1.2.3. UV compounds

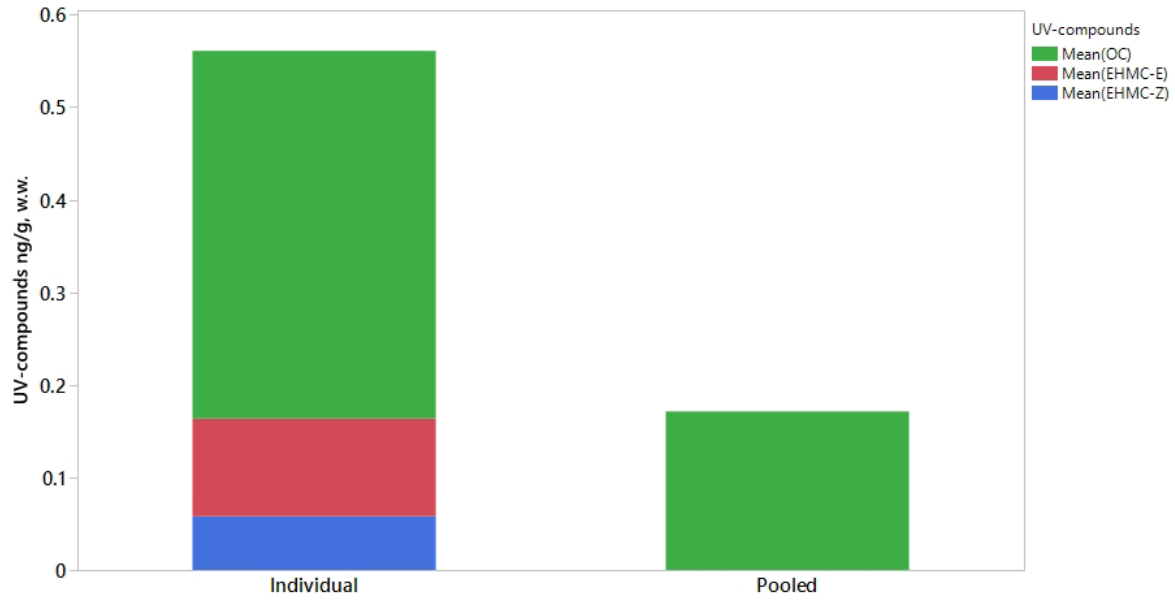


Figure 13 Concentration of detected UV compounds (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=15), pooled samples (N=3).

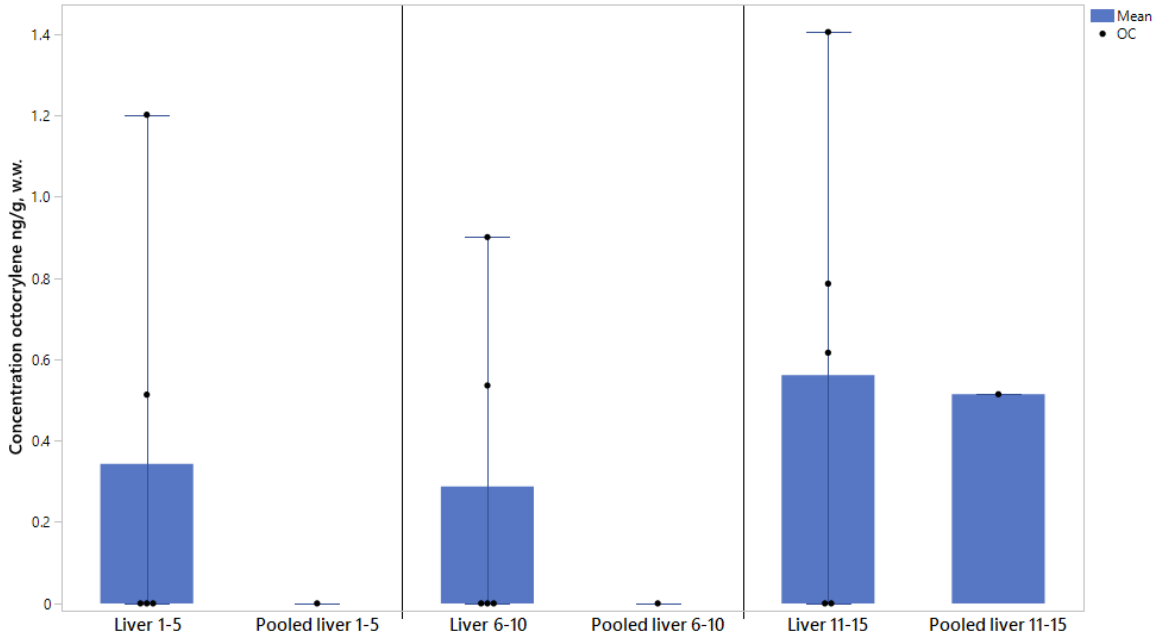


Figure 14 Concentration of octocrylene (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

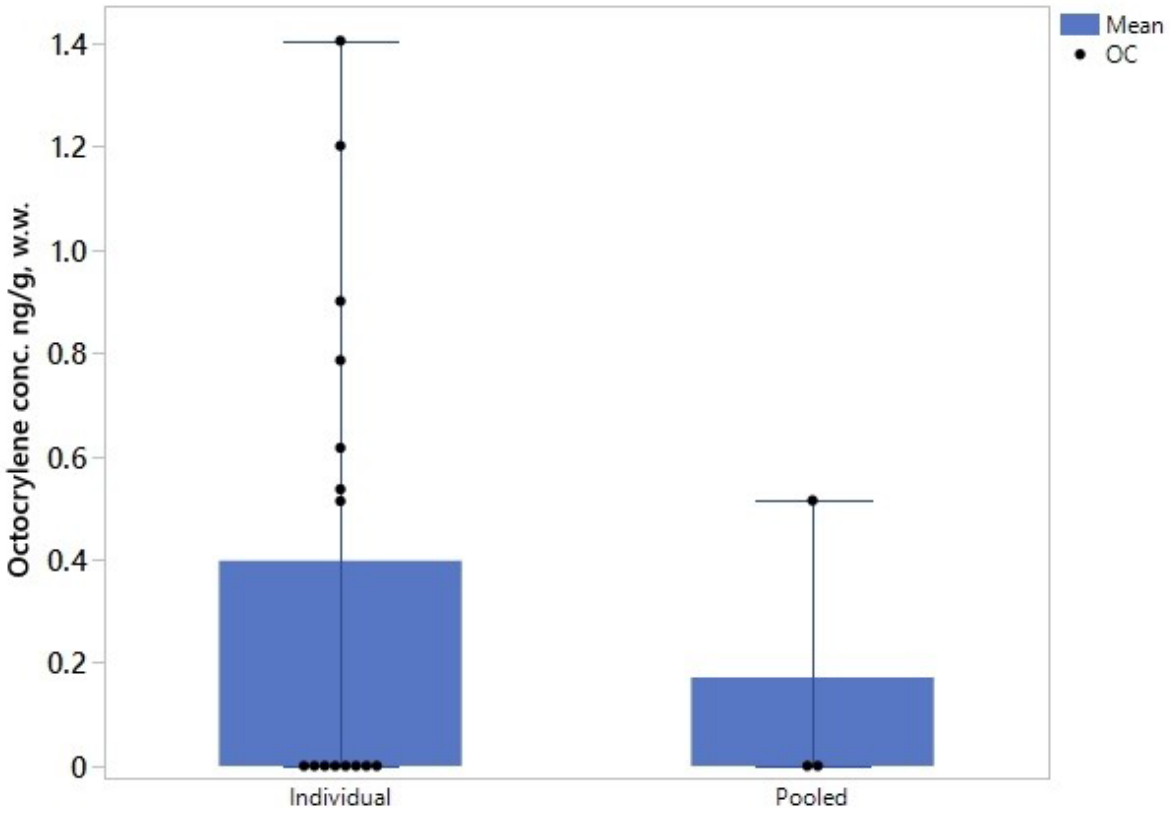


Figure 15 Mean concentration and range of octocrylene (ng/g w.w.) in brown trout liver from Lake Mjøsa 2022. Individual samples (N=15), pooled samples (N=3).

1.2.4. Siloxanes

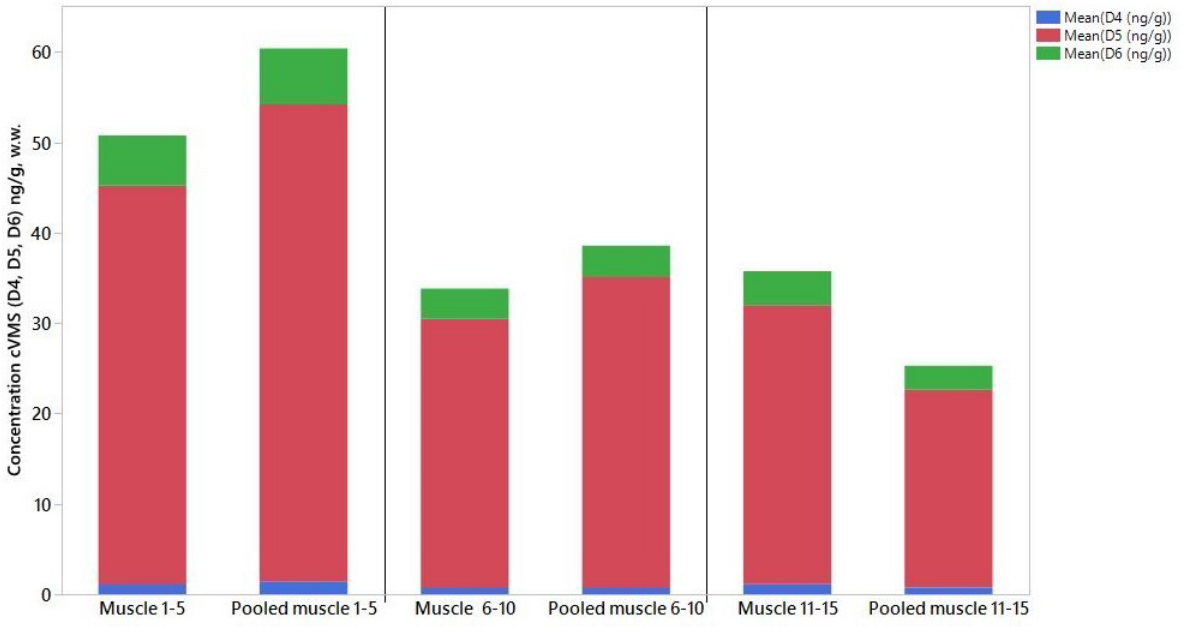


Figure 16 Mean concentration of siloxanes (cVMS: D4, D5 and D6) (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=15 total, N=5 per bar), pooled samples (N=3).

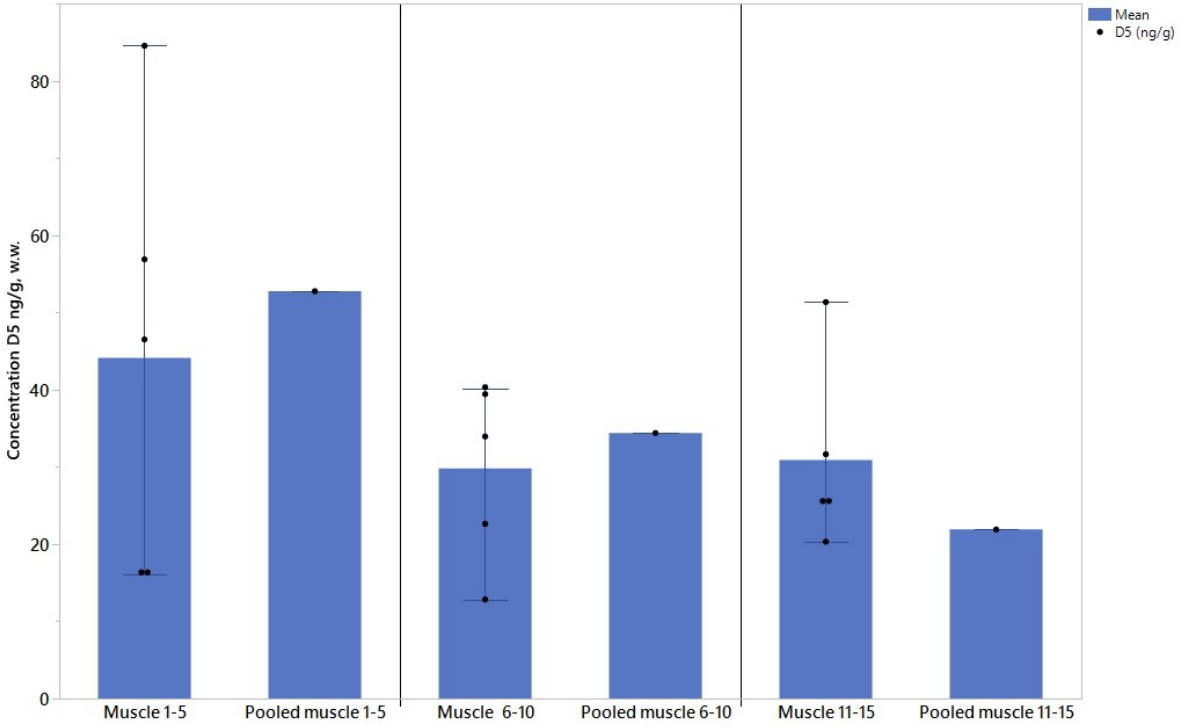


Figure 17 Concentration of siloxane D5 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

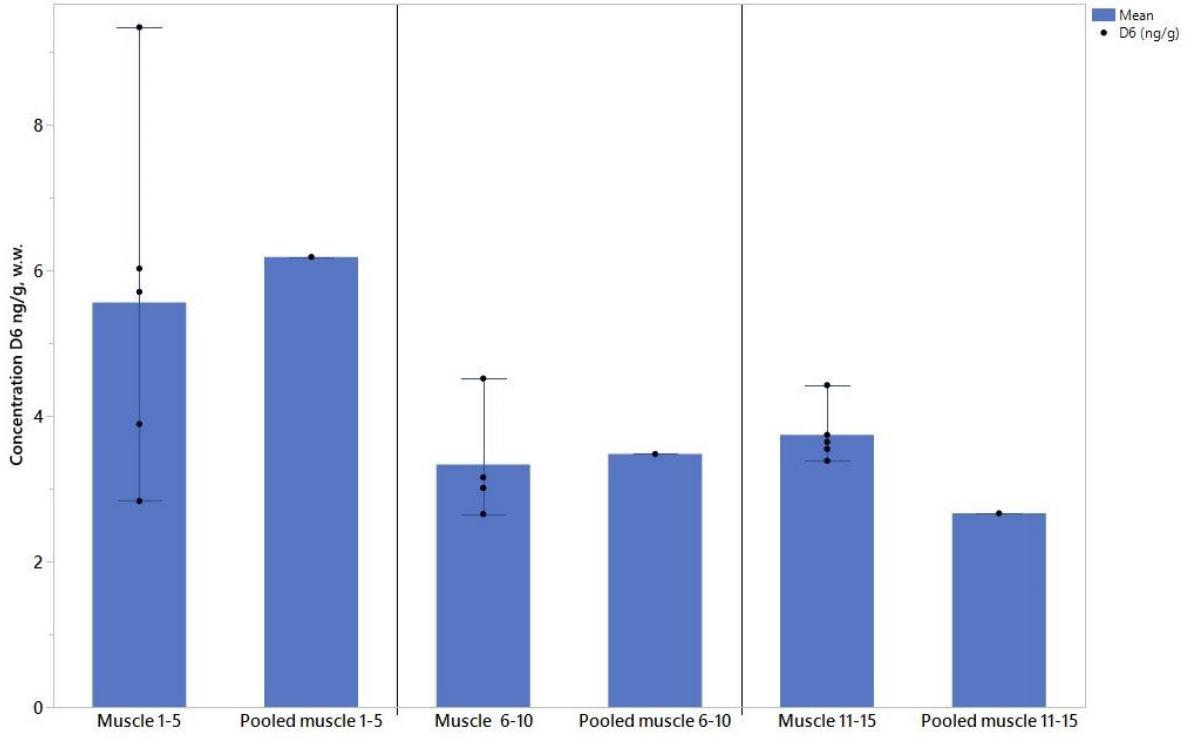


Figure 18 Concentration of siloxane D6 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

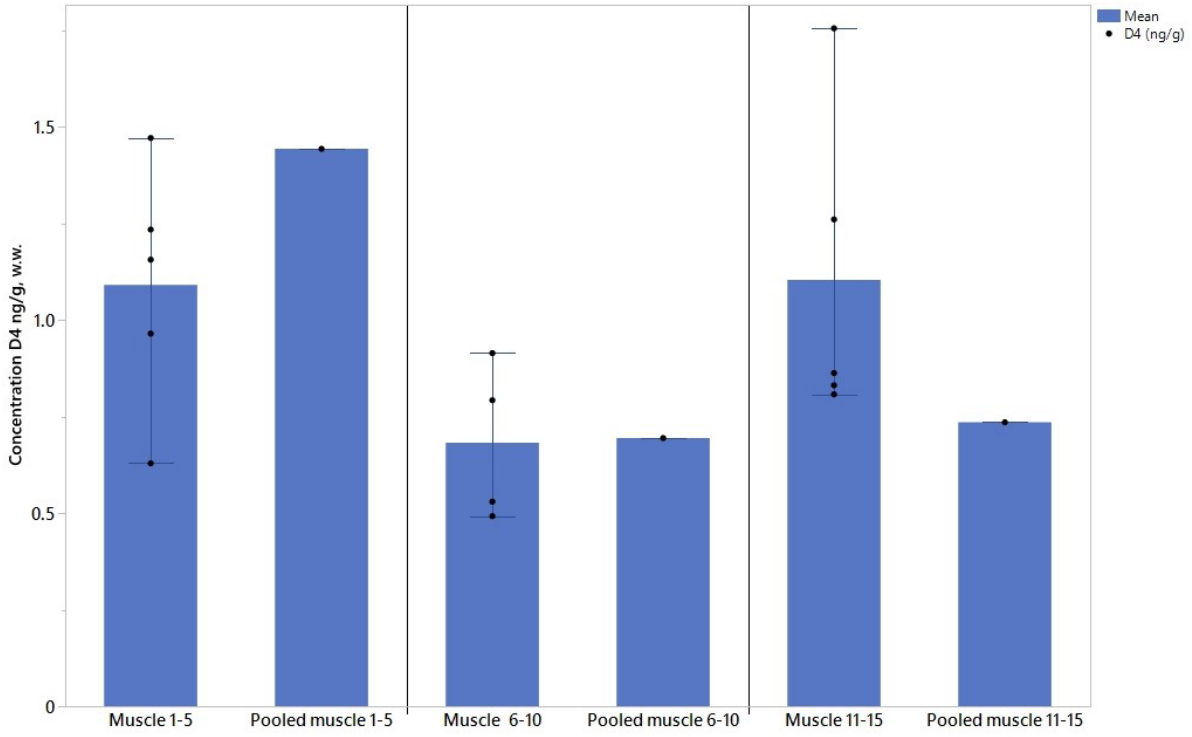


Figure 19 Concentration of siloxane D4 (ng/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

1.2.5. Mercury (Hg)

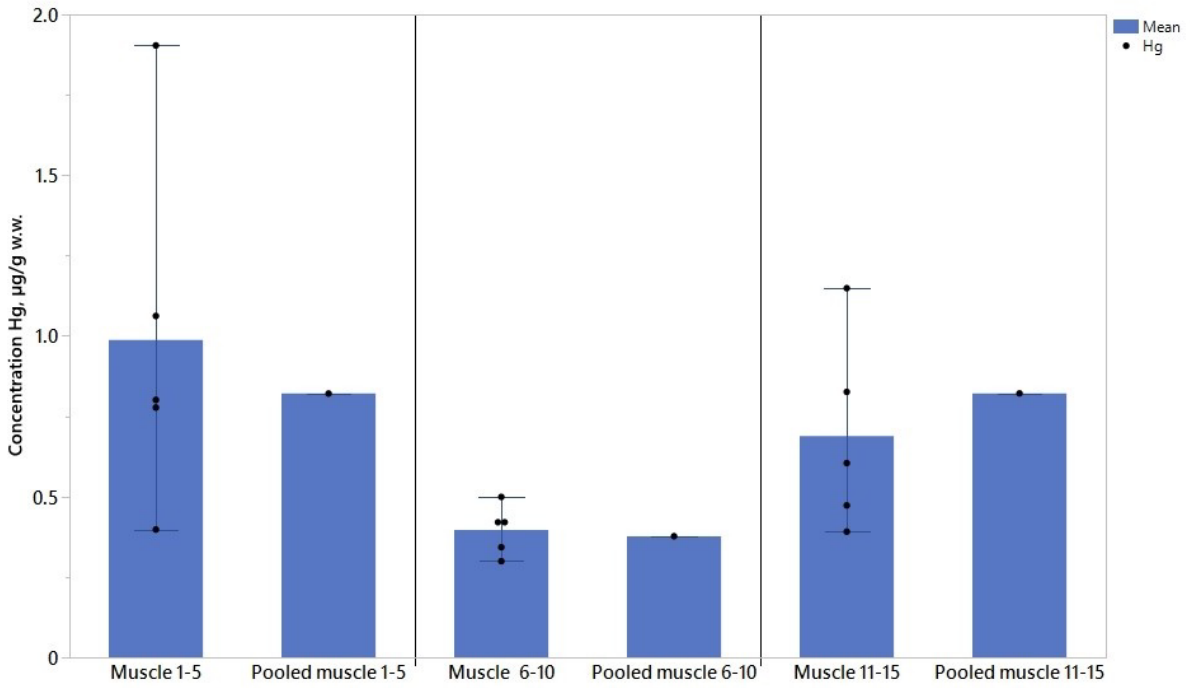


Figure 20 Concentration of mercury (Hg) (µg/g w.w.) in brown trout muscle from Lake Mjøsa 2022. Individual samples (N=total 15; N=5 per bar), pooled samples (N=3).

1.3 Estimates of biomagnification

When modeling trophic transfer of contaminants, such as the trophic magnification factor (TMF), pooling data will in turn reduce the number of datapoints (n) in the model. This may lead to more uncertain estimates (increased confidence intervals, CIs). This can be illustrated (purely as examples) when comparing calculated TMFs for Hg in a dataset with low n (Figure 21), compared to a dataset with high n (Figure 22).

In addition, pooling data may produce different estimates of the TMF, while the "extreme" values are left out (outliers). To better exemplify this, we have created a dataset simulating pooling of historical data and compared the calculated TMF with the TMF calculated from the original dataset (Figure 18). As can be seen in Figure 23 below, only pooling the data for brown trout for the years included lowers the estimate for the TMF.

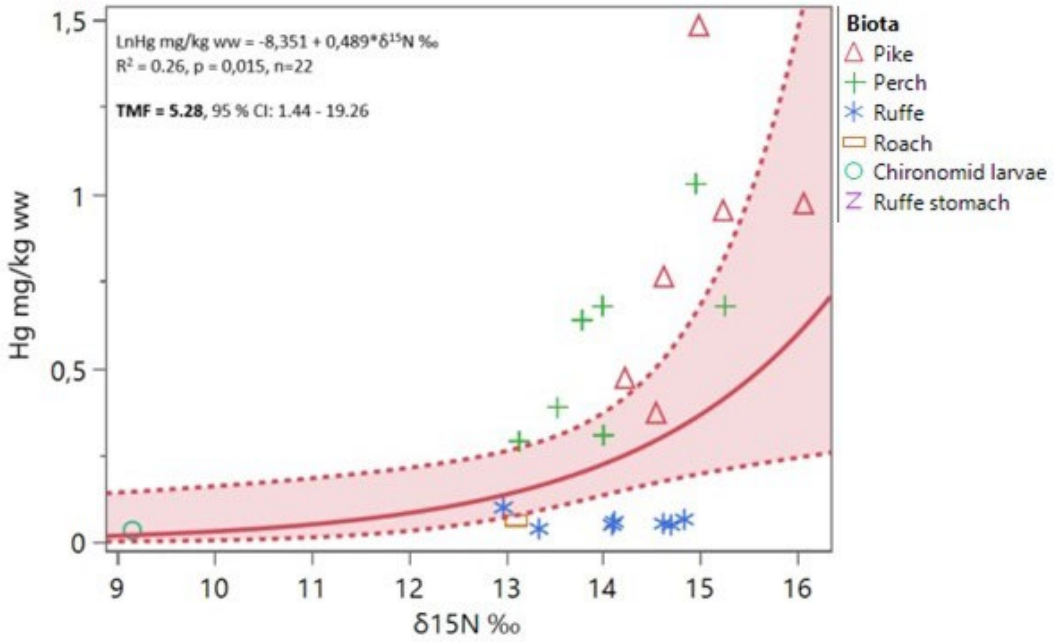


Figure 21 ***Example*** *of biomagnification of Hg in a (benthic) food chain with* ***low N****. An exponential regression, with a* ***wide*** *95 % confidence interval, of Hg concentrations in Lake Mjøsa biota from 2014 to 2020 as a function of measured $\delta^{15}N$. Prediction formula and estimated TMF with 95 % confidence level are shown above the regression curve.*

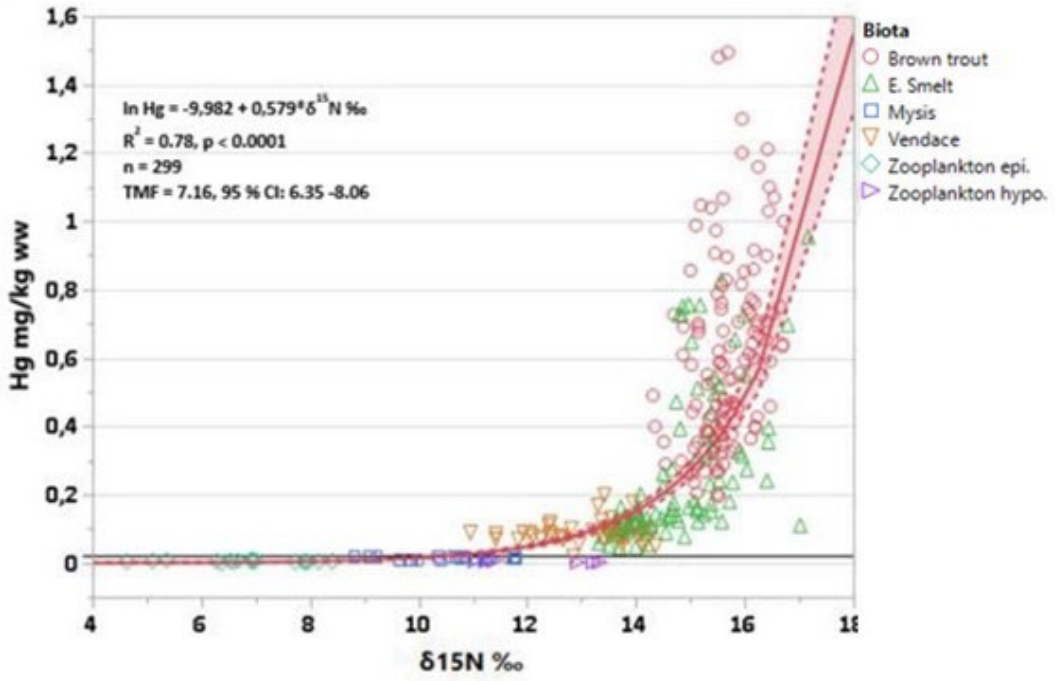


Figure 22 ***Example*** *of biomagnification of Hg in a (pelagic) food chain with* ***high N****. An exponential regression, with a* ***narrower*** *95 % confidence interval, of Hg concentrations in Lake Mjøsa biota from 2014 to 2020 as a function of measured $\delta^{15}N$. Prediction formula and estimated TMF with 95 % confidence level are shown above the regression curve.*

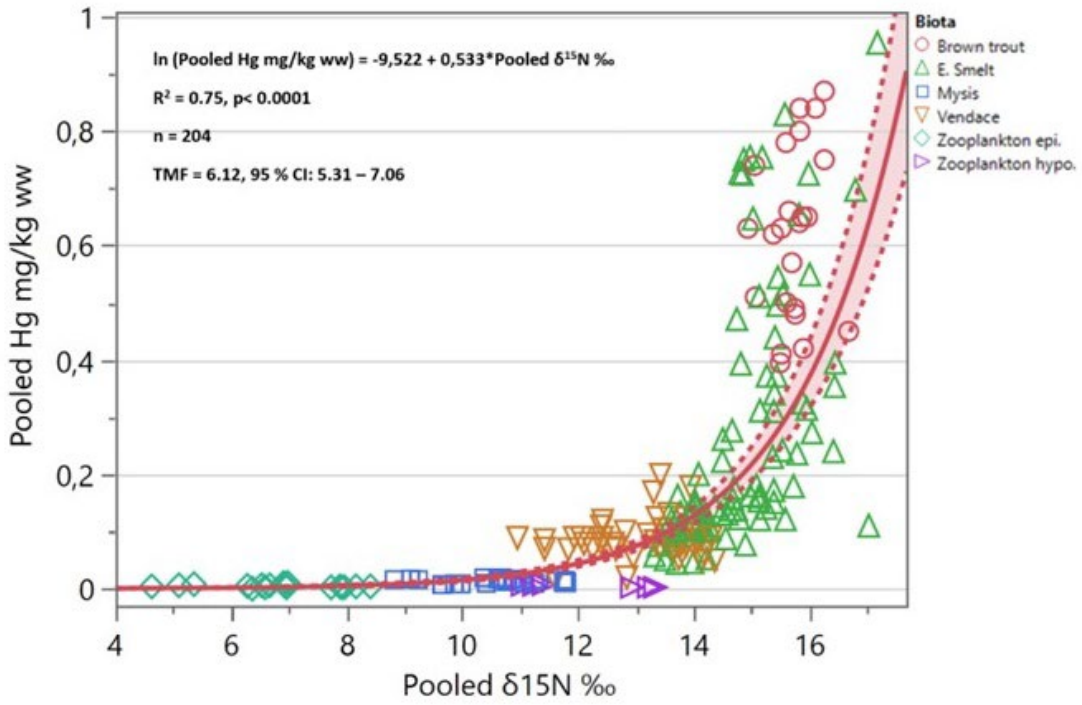


Figure 23 Exponential regression, with 95 % confidence interval, of Hg concentrations in Lake Mjøsa biota from 2014 to 2020 as a function of measured $\delta^{15}N$. All data for brown trout were pooled in groups of five individuals.

1.4 Conclusions – pooled vs. individual for substance detection and biomagnification

Deciding on pooled (composite) vs. individual sampling in environmental monitoring of contaminants will include evaluation of sampling strategies, population dynamics and state, statistical models used, equations (e.g. for TMF), and analytical chemical procedures (uncertainty). It is important also to consider how the user will use the data, such as evaluating time series, calculating TMFs/BAFs, or early warnings and detections for emerging contaminants in the environment.

Composite sampling procedures may reduce sampling variance and reduce analytical costs as number of pretreatments and instrumental analyses are lowered. The process of combining aliquots from separate samples, and analyzing this pooled sample may be beneficial, but researchers must in this process consider detection frequencies, LOQs, sample size (number of individual samples; potential population decrease), pooled sample size, analysis cost, sampling cost, and other factors in order to make a wise decision of whether to composite or not, and how many individual samples to make the pooled sample.

In this part, we have considered the simple statistics for individual contaminants and contaminant groups that occur in a medium to high detection frequency in brown trout in Lake Mjøsa from 2022 where both individual and pooled samples were analyzed.

Table 1. Short summary of evaluations on individual vs. pooled samples in the MILFERSK program. Based primarily on data from 2022 where both individual (N=15) and pooled (N=3 á 5 individuals).

Pros	Cons
Lower analytical cost	May lose information on outliers, e.g., to evaluate an early warning system for occurrence in the environment
For selected contaminant groups no difference is observed when studying ***mean*** concentrations for entire groups. Outliers are still concealed.	For some contaminants a lower mean is observed for pooled samples. Could be partly explained with no correlation between selected metadata to decide which individuals that are included in the pooled samples. E.g.: for PFAS there are no correlation between fish length and matrix concentration. The opposite is observed for e.g., Hg.
	Pooled samples (i.e., lower N in the model) will increase confidence interval (CI) and the statistical power when calculating TMFs.
	Extreme (or just high) concentrations may be hidden, e.g., when comparing with EQSs on individual level.

2 Investigating time trends for contaminants

- A statistical evaluation of randomized, mathematically pooled contaminant levels from historical data in brown trout from Lake Mjøsa

2.1 Background

Chemical analysis of emerging contaminants is costly, and the use of pooled samples (pooling together several samples of the same tissue from individual organism) instead of individual samples (one sample per tissue, e.g., per fish) is therefore an option to reduce costs. Subsequently, it could allow for expanding the sampling program, such as more locations or other matrices for the same cost (Bignert et al. 2014).

If the contribution from inherent specimen variance is considerably larger than the analytical error to the total random or unexplained variation, variation may be reduced by pooling samples. However, also sample size per site and year will decrease. This affects the statistical power, i.e., the probability that a given change in concentrations is detected statistically. In this note, we have pooled individual samples mathematically to explore how the statistical power is affected by pooled sampling.

2.2 Methods

The data basis for the study is the data of chemical concentrations of selected contaminants in brown trout from Lake Mjøsa (the MILFERSK program). For the purpose of this analysis, we found the data for individual fish specimens from the years 2015-2022 to be most fitting for our simulations.

We have focused on contaminants that are frequently determined in concentrations above the LOQ. Following Bignert et al. (2014), we picked time series using the following criteria:

1) at least 20 measurements >LOQ,
2) at least 6 years with some measurements >LOQ,
3) no clearly non-monotonic trends.

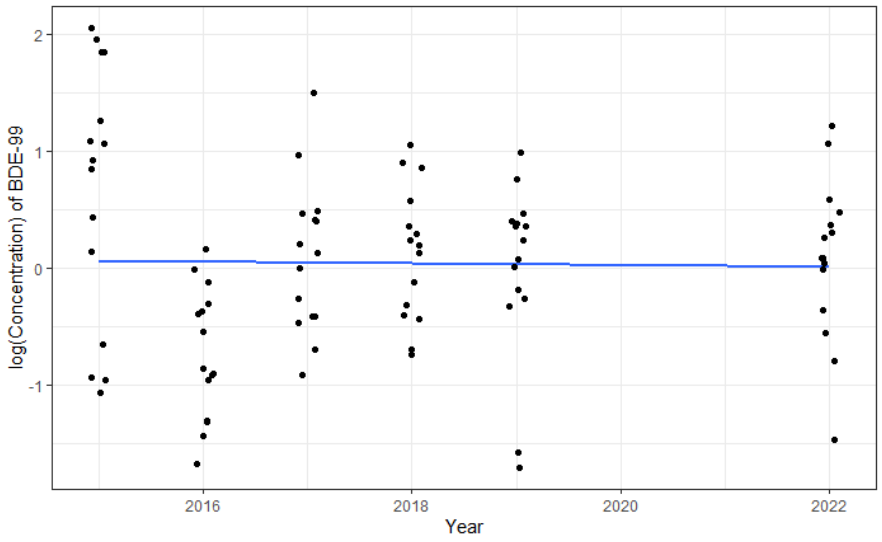
We estimated total variance, expressed as coefficients of variation (CVt), from de-trended time series. Detrending was done using linear regression (Bignert et al. 2014). The approximate chemical analysis variance (CVa) was based on expert judgement and on uncertainties given by chemical labs. Finally, we calculated the specimen variation CVs (the estimated true variance in concentration among specimens) based on the following formula (Bignert et al. 2014):

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

To evaluate how pooling samples would have affected observed time trends, we simulated how the results would have been if the individual samples (15 fish per year) had not been analyzed separately, but combined into 3 pooled samples, basing each sample on 5 fish. The simulation was done by randomly picking the 3 x 5 fish and using the arithmetic mean concentration of the 5 samples as the simulated pooled sample concentration. Thus, we assumed that each pooled sample has equal amount of tissue from the 5 fish. Then, we used linear regression to analyze time trends, using log(concentration) and excluding data below LOQ (*Figure 24*). We used linear instead of non-linear regression as we considered the time series to be too short for non-linear analysis. The simulations were performed 100 times for each

substance, each with random draws. Thus, the simulations are likely to include examples of extremely skewed pooling (e.g., that the 5 highest individual concentrations are chosen for the same pooled sample). Since we used log-transformed concentrations in the time trend analyses, the regression estimates can be transformed to percentage change of concentrations, which in our view is easier to interpret.

(a)



(b)

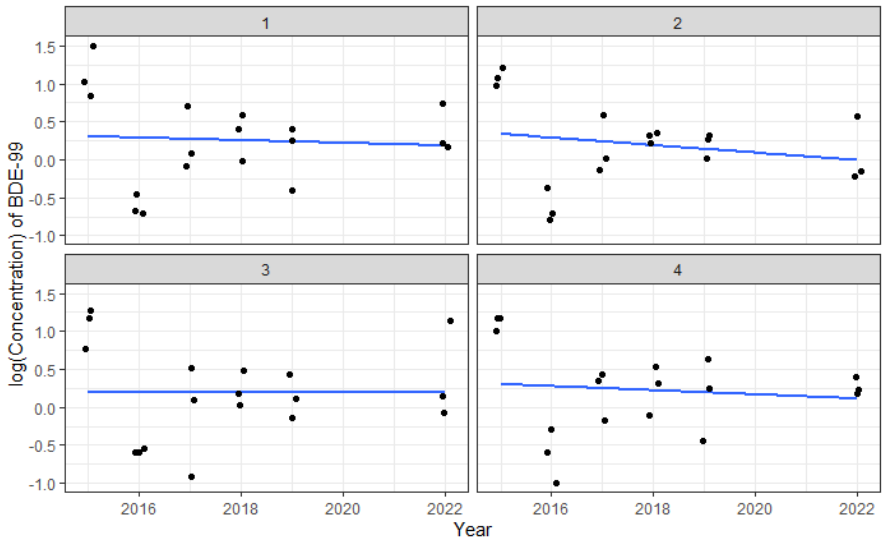


Figure 24. Example of simulating pooled samples (BDE-99 in trout muscle). (a) Original data series, with 15 samples per year. (b) Four examples of simulated time series with 3 "pooled" samples per year. Each pooled sample is the mean of 5 random individual samples. Note that the y axis shows log(concentration), the scale on which we performed time series regression. The points have been given a bit extra variation in the x direction to be able to tell them apart.

The simulations above can compare individual and pooled sampling strategies for each substance for a given time trend, explicitly the time trend observed for each substance. Since there is just one time trend for each substance, we cannot really separate the effect of time trend magnitude from the effect of substance (see explanation in *Figure 25*). In order to explore this, we therefore manipulated the time series strength for each substance to create a range of time trends based on the original data. We did this in the following way:

1) log-transform the data; let Y_i denote the values of log(concentration),
2) perform a linear regression of Y_i as a function of year, and find the expected mean, $Yexpect_{year}$ for each year,
3) for a given trend (given as % increase per year), find the target mean $Ytarget_{year}$ for each year,
4) calculate the manipulated log(concentration) Y'_i by adding/subtracting the difference between Yexpect and Ytarget:

$$Y'_i = Y_i + Ytarget_{year} - Yexpect_{year}$$

In this way we kept the characteristics of the time series, such as the variation among samples, while varying the time trend of the series. These manipulated data were used both to estimate time trends for individual samples, and for simulating pooled samples and estimating the time trends for those.

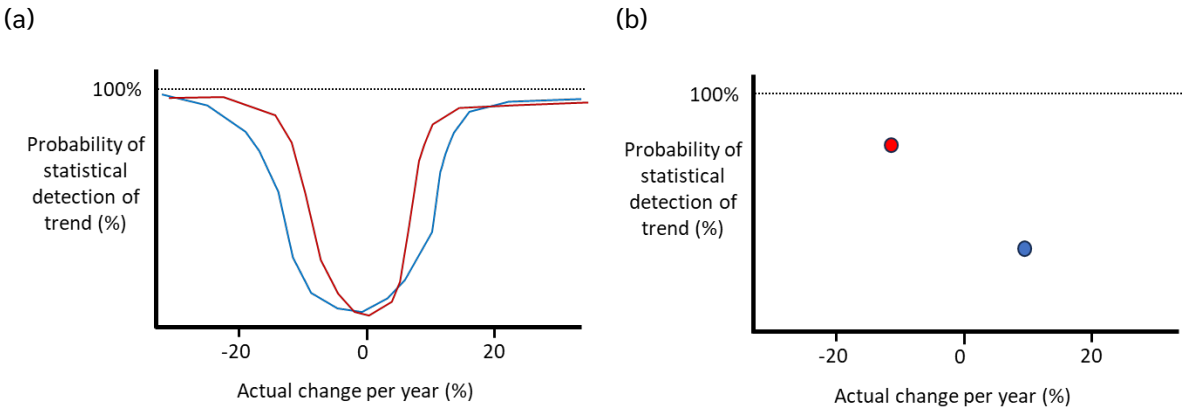


Figure 25. Estimation of power curves. (a) Two theoretical power curves for two different substances, given a certain sample size per year. The x axis is the actual rate of change of concentration per year (downward or upward change), while the y axis shows the power, i.e. the probability that we detect a trend by statistical time trend analysis (achieving P < 0.05 in the statistical test). If there is no actual change (zero on the x axis), the probability of detecting a trend is very small (it is by definition 5%, the Type 1 error rate). If there is a small increasing trend of concentrations (i.e., slightly to the right of zero), we are not likely to achieve P < 0.05 in the statistical trend analysis. For a strong increasing trend (to the extreme right of the graph), the power is close to 100% – i.e., we are almost certain to detect the trend statistically

(achieving $P < 0.05$). How fast power approaches 100% depends on the substance (different substances have different variation among samples for biological reasons, as well as different analytical uncertainty). (b) In this study, we have only one time trend per substance. Thus, for each substance we have only one value for actual change per year as well as one estimate of power. Thus, we cannot estimate the power curve using the raw data alone. However, by manipulating the trend, we can get an estimate of how the curve would have looked like if we had several time series with different actual rates of change.

2.3 Results and discussion

Whereas Bignert et al. (2014) found that their total variance (expressed as coefficient of variation CVt) varied from 12% to 97%, our overall time series indicate a higher variance, with the lowest being 45% for mercury (Hg). Most time series had a CVt of 80-120% (3 individual PBDEs had a lot higher variance). The total variation in measurements (CVt) is generally dominated by variation among specimens (CVs). While there is some uncertainty in the level of chemical analysis variation (CVa), this would still be true even if the actual CVa was considerably larger. Thus, the variation among specimens (CVs) appears substantially larger than in the case of Bignert et al. (2014), who studied PCBs and insecticides in fish and guillemot eggs in the Baltic.

When we analyze time trends for both the original data and for the simulated pooled samples, we find that the pooled sample estimates for the trend (i.e., ignoring the estimates' uncertainty) often tend to be of slightly higher absolute magnitude than the individual estimates. That is, trend estimates from pooled data were slightly more negative for PBDEs and some PFAS (PFOS and PFTrDA), and slightly more positive for PFUnDA (*Figure 26*).

Figure 27 shows the statistical significance of pooled and individual samples. We have used the t value to visualize the degree of statistical significance on the x axis. A rule of thumb is that the trend is statistically significant ($P < 0.05$) when abs(t value) is higher than 2, so these approximate limits are indicated on the figure. The exact limit is slightly below 2 for individual samples and slightly above 2 for pooled samples (because the sample size is lower). For time series with relatively low proportion of measurements over LOQ (BDE 17 and BDE202), the plot indicates that their trends are detected in the individual samples, but not in the pooled ones. (In the case of BDE202, some of the simulations of pooled samples show a trend in the "wrong" direction.) In the case of D4, both individual and pooled samples show trends, but individual samples are further away from zero, indicating a higher degree of statistical significance was obtained. Thus, in some cases, the effect of decreasing the variance among samples does not compensate for the lower sample size. However, the majority of time series have weak, non-significant trends, and in these cases, we cannot see a clear pattern between individual and pooled samples. For some cases (e.g. PFTrDA), the pooled samples show higher power.

Table 2. Overview of statistics (Mean, standard deviation (SD) and total variance as coefficient of variation, CVt) for different compounds in time series of brown trout in Lake Mjøsa 2014-2020. The values are calculated from detrended time series. All units are in ng/g, except for Hg (µg/g).

Compound	Mean	SD	CVt (%)	Cva (%)	CVs (%)
Hg	300	610	49	6	49
BDE17	0.0520	0.0095	546	20	546
BDE28	0.0490	0.0340	145	18	144
BDE47	5.5000	6.6000	84	15	83
BDE49	0.2600	0.2900	88	15	86
BDE66	0.1200	0.1300	91	18	90
BDE77	0.0054	0.0053	103	18	101
BDE99	1.6000	1.7000	93	12	92
BDE100	2.0000	2.1000	95	15	94
BDE119	0.0470	0.0440	107	18	105
BDE126	0.0072	0.0064	113	18	112
BDE153	0.2700	0.3200	84	15	83
BDE154	0.5800	0.7100	81	15	79
BDE183	0.0043	0.0064	67	18	65
BDE184	0.0071	0.0089	80	18	78
BDE202	0.0070	0.0071	100	20	98
BDE209	0.2600	0.0710	365	20	365
D4	1.3000	1.7000	77	8	77
D5	55.0000	47.0000	117	8	116
PFDoDA	2.8000	3.9000	73	10	72
PFOS	5.1000	6.8000	75	10	74
PFTeDA	1.5000	1.8000	82	10	82
PFTrDA	8.7000	7.9000	110	10	110
PFUnDA	4.8000	6.3000	76	10	75

The results from manipulating the time trends of the original data (*Figure 28*) gives us a clearer picture of the statistical power in pooled vs. Individual sampling *Figure 28*. The figures in general shows that around the midpoint of the x-axis, the true (known) time trend is zero or small, and neither individual nor pooled samples obtain a p-value of <0.05 when we perform regression on log(concentration) vs. year (both lines are at 0% on the y axis). When we go to the left (decreasing trend) or right (increasing trend), the time trend analysis more often indicates a significant time trend with p<0.05, resulting in U-shaped curves. When the true trend is strong enough, both individual or pooled samples obtain p<0.05 (in almost all cases, both curves are at 100% both at the left and right side of the graph). However, pooled samples mostly need stronger «true» trends (to move further from zero on the x-axis) to obtain significant results in the regression. Thus, this analysis indicates that **pooled sampling in general has lower power than individual samples**. In some cases, though, the effect is asymmetric: for PFOS, pooled samples have higher power than individual samples for negative (downward) trends, while pooled samples has lower power for positive (upward) trends. It must be noted that the result could have been different if the curves were estimated from actual time series differing in trends, rather than time series with manipulated time trends.

2.4 Conclusion

It appears that for most contaminants in this study, pooled sampling results in a smaller power, which means that the trend needs to be stronger in order to be «discovered» by statistical analysis. Alternatively, if one uses pooled samples, a time trend of a given magnitude may need some extra years until it is discovered statistically (not explicitly demonstrated in this note).

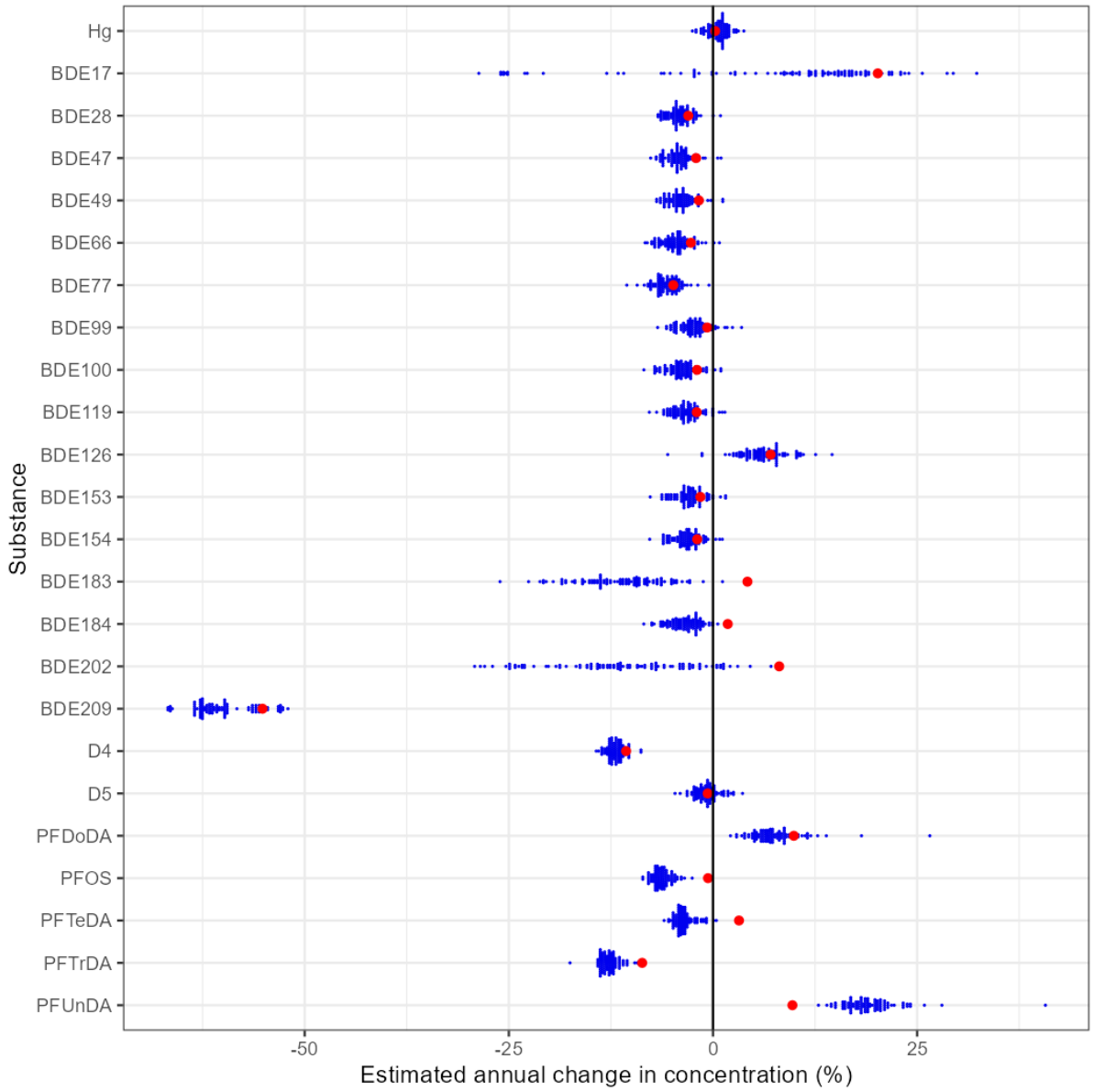


Figure 26. Estimated annual change of concentrations (as percentage of concentration). The small blue dots are the estimates from pooled samples of 5 fish in each (one dot for each of the 100 simulations). The red dots are the estimates from the individual samples.

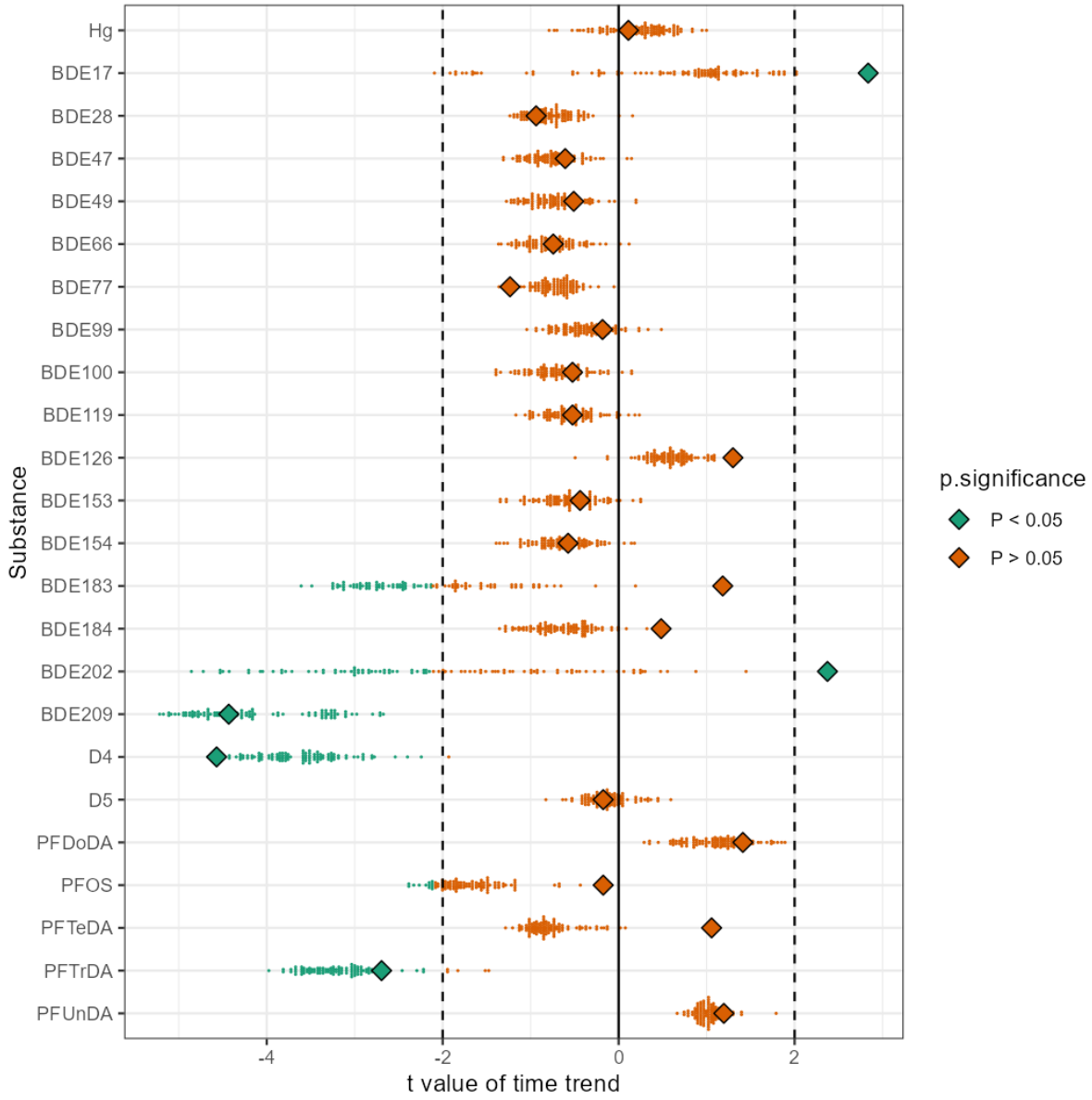


Figure 27. Statistical significance of the annual change. The x axis shows the t value from the regression (the trend estimate divided by its standard error). The lines where abs(t value) = 2 are indicated as dashed lines. Statistically significant trends (p<0.05) are shown in green, while non-significant trends are shown in orange. The small dots represent pooled samples, the larger diamond-shaped dots are the individual samples.

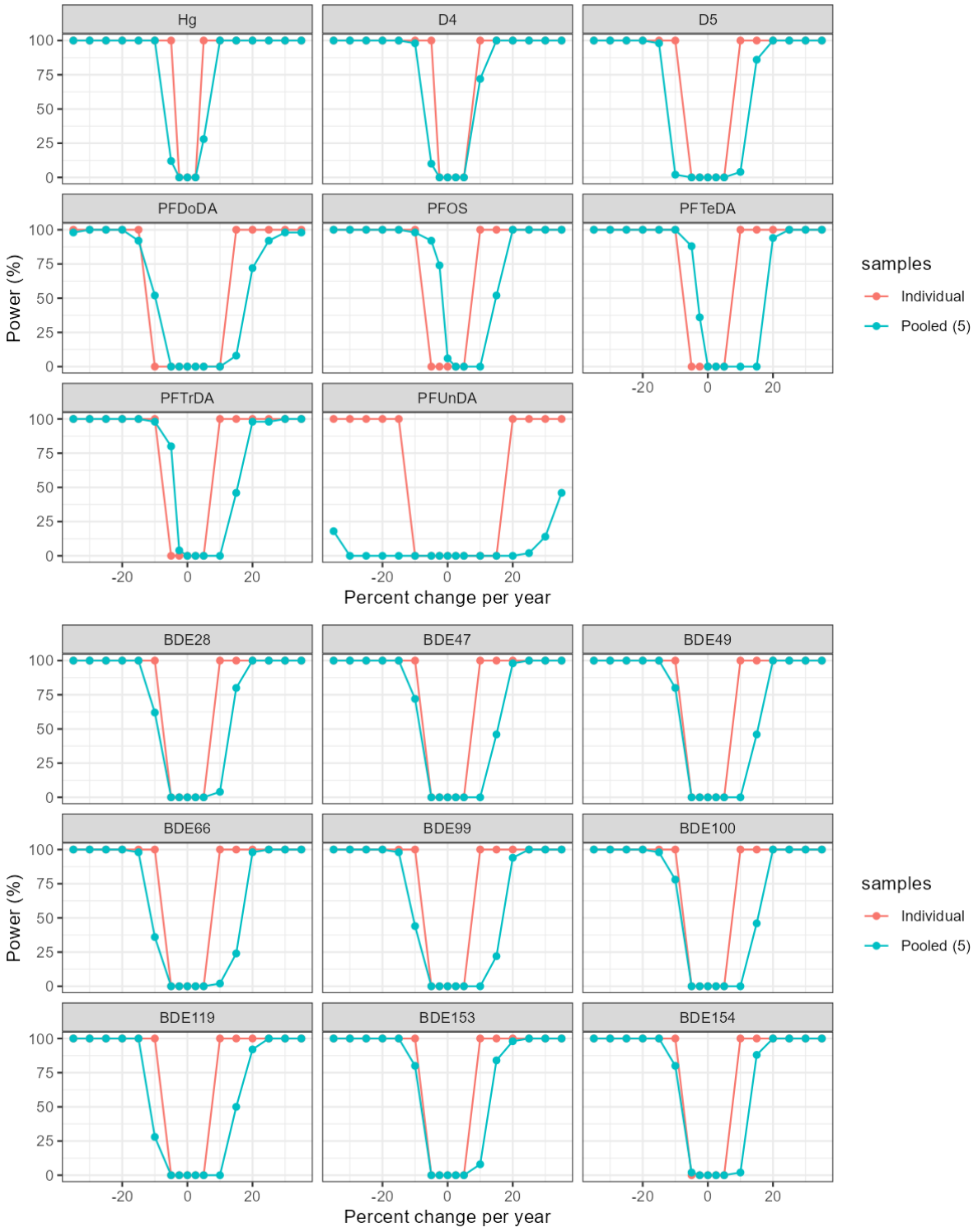


Figure 28. Power of simulated time series with varying degree of change per year. The x axis shows the true (manipulated) trend of the simulated series, while the y axis shows the power, here defined as the percentage of time series that obtain p<0.05 when analyzing the time trends using linear regression. For individual samples, there is only one time series for each value of true trend, so the power is either 0% (the time series did not obtain p<0.05) or 100% (the time series did obtain p<0.05).

2.5 References

Bignert, A., Eriksson, U., Nyberg, E., Miller, A., & Danielsson, S. (2014). Consequences of using pooled versus individual samples for designing environmental monitoring sampling strategies. Chemosphere, 94, 177–182. https://doi.org/10.1016/j.chemosphere.2014.09.096

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