# Analysis of geographical variation of polychlorinated biphenyl congener patterns in harbour seal (*Phoca vitulina*) by principal component analysis.

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## Introduction.

Polychlorinated biphenyl congeners (CBs) are well known environmental contaminants[1-3]. The use of chemical contaminants to discriminate between separate living populations of marine mammals has been discussed in a review by Aguilar[4]. Principal component analysis (PCA) has been demonstrated to classify samples into groups of similar CB congener patterns as reported by Zitko[5], Schwartz and Stalling[6] and Onuska and Davies[7].

## Aim of the study.

The study was initiated by the outbreak of distemper like epizootic among harbour seals (*Phoca vitulina*) in north-west European waters in 1988. The aim of the study was to compare the patterns of the coplanar CB congeners in harbour seals living at three geographical separate locations in Denmark. PCA was used to compare CB congener patterns for different animals and different geographical locations.

## Materials and methods.

**Samples:** Blubber samples from 21 harbour seals were analyzed for toxic coplanar CBs. The 21 samples consists of 7 samples from 1-2 year old animals (both males and females) from three different locations in Denmark (Kattegat, Limfjord and Wadden Sea). Due to the short period of disease, and due to the observed normal blubber thickness of the animals, these animals were considered to be in a *normal* fattening condition. Age determination was based on teeth-cuts[8].

**Chemical analysis:** The analytical procedure, including quality assurance, was described earlier[9-11]. Duplicate analyses of an internal reference sample (unspiked seal oil) was performed for each location (7 samples).

**Statistical methods:** The data have been analyzed in a two-step procedure. The possible characteristic patterns of the CB congeners in the blubber samples were extracted by

results are in agreement with the fact, that migrations of harbour seals are generally not observed[14].

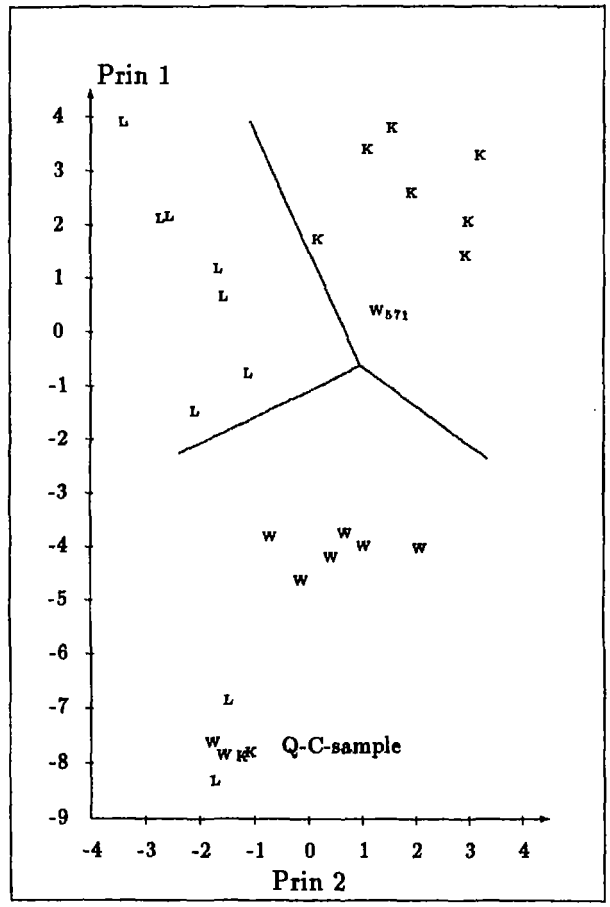


*Figure 1*: The two first principal components based on 17 CB congeners are depicted for 21 seals and for the 6 internal quality control measurements. The principal components are based on 20 seals. L=Limfjord, W=Wadden Sea and K=Kattegat corresponding to the living places of the seals. The labelling of the quality control measurements indicates the group of seals to which any single measurement is connected. The 3 lines are the borders separating the 3 groups of seals as characterized by their living locations. Except for one seal (#571) no misclassifications are seen. The variation between the measurements in the Q-C sample represents measurement errors, and it is seen to be considerably smaller than the variation between seals within the 3 locations.

**Conclusions.**

CB congener patterns have been analyzed by comparing principal components. The two first principal components could separate the samples in groups corresponding to each of three geographical locations. The geographical and biological variations were estimated from the data, and the geographical variation was far the largest.

Three geographically separate populations a single species (harbour seal) were identified. This is in agreement with the fact, that harbour seal is a non-migrating seal species. PCA of CB congener patterns appears to be a very suitable and powerful tool for discriminating between separate living populations of a species. Furthermore, this analysis seems to enable assessment of migratory habits of a wildlife species.

calculating the principal components. With the principal components as explanatory variables and the three geographical locations as classification variable a discriminant analysis has been performed. The observations were normalized to the sum 1 and standardized. Thus, the analyzed variables representing the CB congeners all had the average value zero and the standard deviation one. This procedure is in accordance with the experiences and recommendations reported by others[6]. The statistical methods are given in detail in Storr-Hansen and Spliid[12]. In the discriminant analysis an observation is classified as belonging to that population which has largest density when the observation is inserted. This criterion results in dividing the plane, defined by the first two principal components, in three disjoint parts separated by three lines as shown in figure 1.

The results from the congener specific CB analyses are given by Storr-Hansen and Spliid[12].

**Results and discussion.**

**Quality control:** The internal quality control data consist of three pairs of CB congener patterns measured on the same internal reference seal oil in parallel with the measurements made on the 3x7 seals from the three locations. The measuring procedure with respect to CB congener pattern is seen to be in statistical control, as the principal components for these 6 samples are almost identical (figure 1).

One animal was excluded from the estimation of the principal components, as it was an obvious outlier. The first 2 components explain about 80% of the total variance. The first 2 principal components are depicted in figure 1 where the three locations also are indicated. A clear geographical grouping is seen. Contributions from the higher order components are much smaller. Thus, it was decided to concentrate on the analysis of the first 2 principal components, Prin 1 and Prin 2.

The non-ortho substituted CB congeners are main contributors to Prin 2, in contrast to Prin 1. This could be an effect of these compounds being analyzed in another fraction than the rest of the CBs. However, analysis of the principal components with these four congeners (CB-81/77/126/169) omitted, revealed the same clear geographical grouping of the samples. No grouping was observed, when principal component analysis was performed with only the non-ortho substituted CBs: CB-81/77/126/169.

**Measures of variability:** 80% of the total variance of the standardized measurements can be described by the two first principal components. We estimate, that 82% of the total variance is due to differences between locations. Furthermore, around 14% of the total variability is biological variation between seals within locations, while the remaining 4% is ascribable to measurement uncertainty.

**Comparison with harbour porpoise:** Principal components were also calculated for six CB congeners measured in a number of 1 year old harbour porpoise (*Phocoena phocoena*) from danish waters[13]. The principal components were similar to those of the harbour seals. However, in contrast to the seals no distinct geographical grouping was observed. This indicates that harbour porpoise is a migrating species and/or that harbour porpoise has a broader selection of food compared to the migration and/or food selection of the harbour seals. In our opinion, the former cause is more probable than the latter cause. The present

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