

Compared to DNA-based techniques, Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) used as a mass spectrometric profiling method provides rapid and cost-effective identification results. Creating a MALDI-TOF *Vibrio* reference database leads to a clear discrimination between vibrios. Hence, whole environmental *Vibrio* populations can easily be screened for potentially human pathogenic species.

## MALDI-TOF Principle

Colonies of Overnight-Cultures are getting picked. Transferring to target plates occurs either directly or by an ethanol / formic acid extraction. Covering with Matrix (cinnamic acid) yields to crystal structures. Therein embedded proteins getting desorbed by Laser shots. Simultaneously these molecules become ionized due to proton transfers. After electric field acceleration, protein ions passing a vacuum flight tube. The flight duration differs depending on masses and charges of these molecules ( $m/z$ ). Thus, there is a time-dependent detection of ion impact intensities. The outcome are spectra with the  $m/z$  ratio (molecular masses) on the abscissa and measured intensities on the ordinate (Fig. 1).

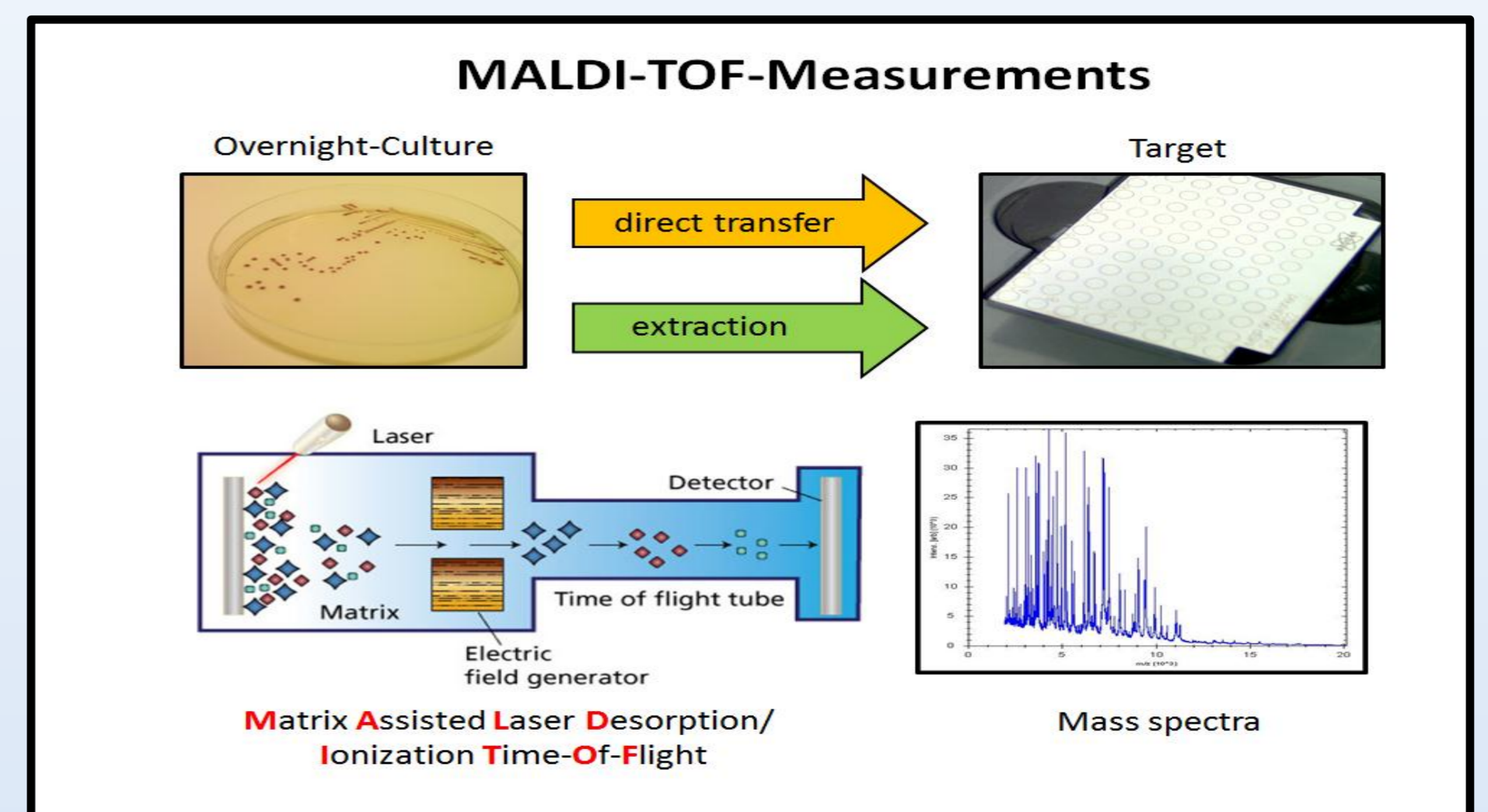


Fig. 1: Sample preparation approaches and measurement principle of MALDI-TOF.

	Number of isolates	<i>Vibrio alginolyticus</i>	<i>Vibrio cholerae</i>	<i>Vibrio parahaemolyticus</i>	<i>Vibrio vulnificus</i>
<i>Vibrio alginolyticus</i>	15	0,69	0,19	0,57	0,37
<i>Vibrio cholerae</i>	11	0,19	0,80	0,21	0,23
<i>Vibrio parahaemolyticus</i>	15	0,57	0,21	0,85	0,45
<i>Vibrio vulnificus</i>	15	0,37	0,23	0,45	0,89
<i>Vibrio diazotrophicus</i>	1	0,33	0,23	0,32	0,29
<i>Vibrio fluvialis</i>	1	0,32	0,20	0,23	0,23
<i>Vibrio harveyi</i>	1	0,55	0,23	0,44	0,34
<i>Listonella anguillarum</i>	1	0,22	0,16	0,21	0,20
<i>Shewanella putrefaciens</i>	1	0,11	0,08	0,12	0,07

Fig. 3: Average Correlation component scores of *Vibrio* isolates.

## Species Identification

In a pilot study, 61 *Vibrio* reference mass spectra were included in one correlation component matrix. In Fig.2 matching scores over 0.66 are coloured red, partly matches (0,33 to 0,66) are yellow and the coloured background of matching scores lower than 0,33 is green. The average correlation score between vibrios of the same species varies from 0,69 to 0,89 (CV: 0,14 to 0,21). Clustering results in a clear separation of single species (Fig.3). Mass spectra generated from one isolate correlate with a score of 0,97 and a standard deviation of 2 %.

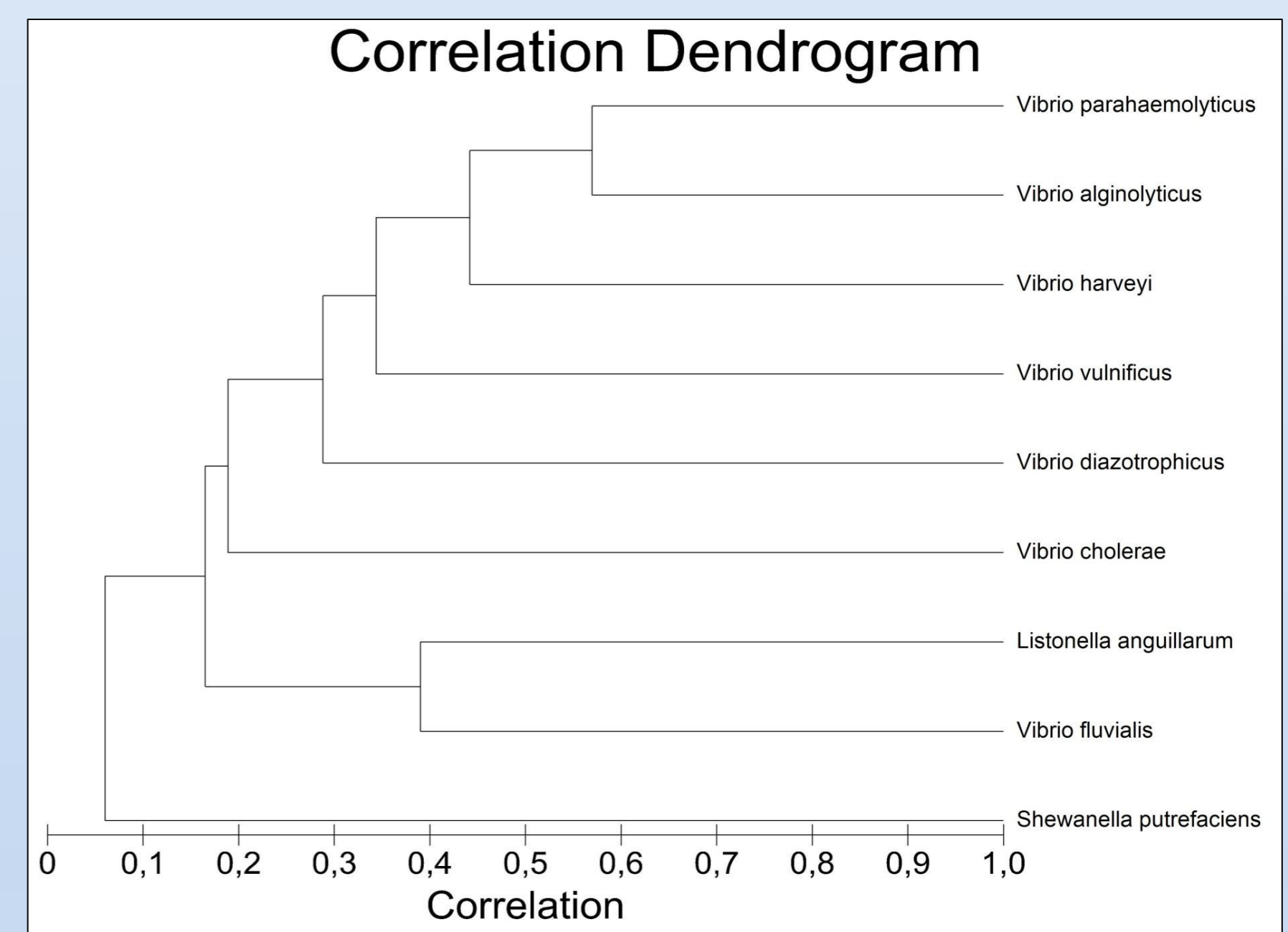


Fig. 3: Cluster Analysis Dendrogram according to data from Fig.2.

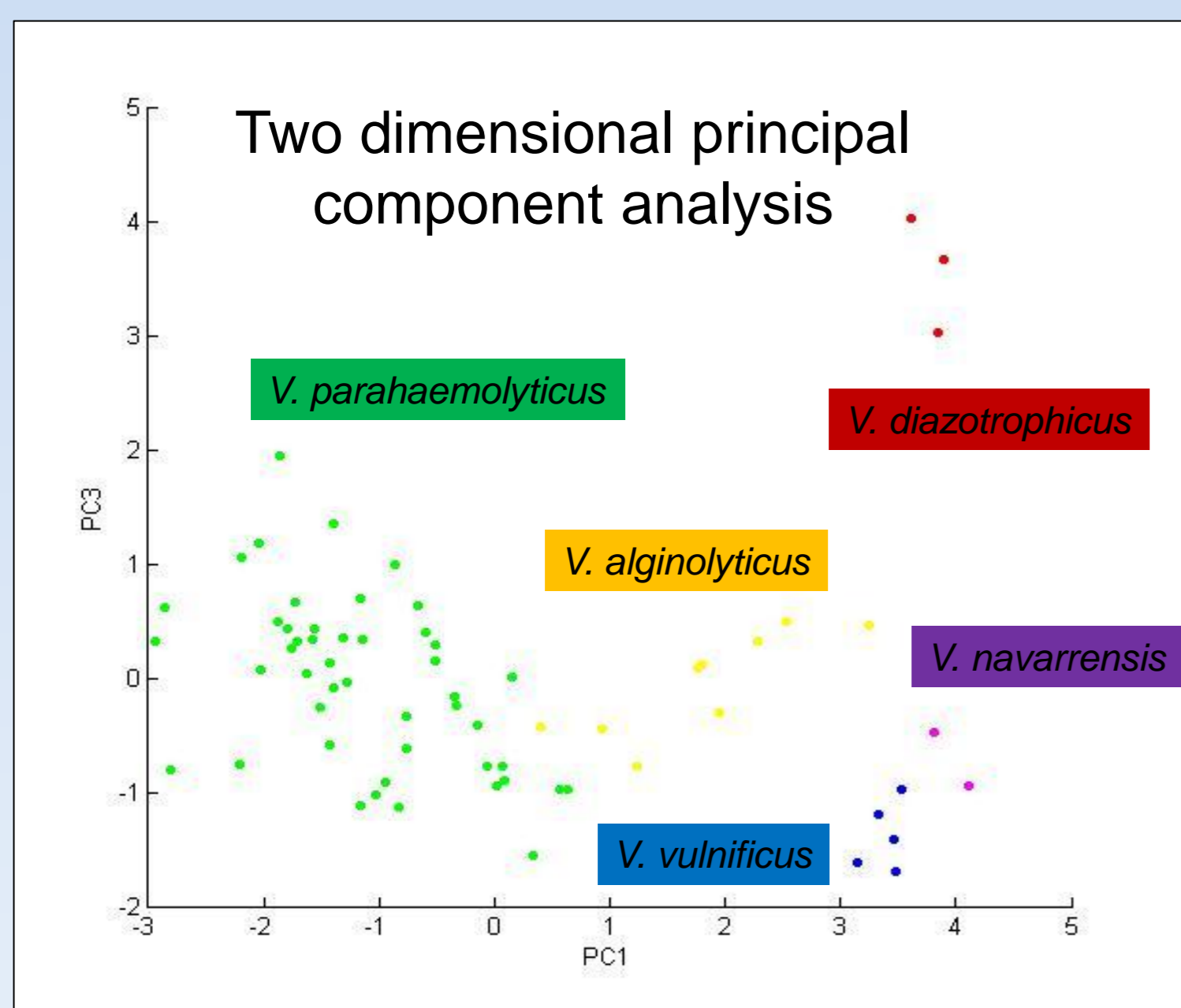


Fig. 4: Two dimensional component analysis of single spectra, acquired from environmental isolates.

## *Vibrio* Population Analyses

In August 2011, vibrios of the North and Baltic Sea were isolated during a cruise with the research vessel Heincke. Comparison of single spectra shows distinct species-specific Cluster, even in the case of closely related species like *V. parahaemolyticus* / *V. alginolyticus* and *V. vulnificus* / *V. navarrensis* (Fig.4). An even clearer differentiation of species cluster can be reached by multi-dimensional scaling (Fig.5).

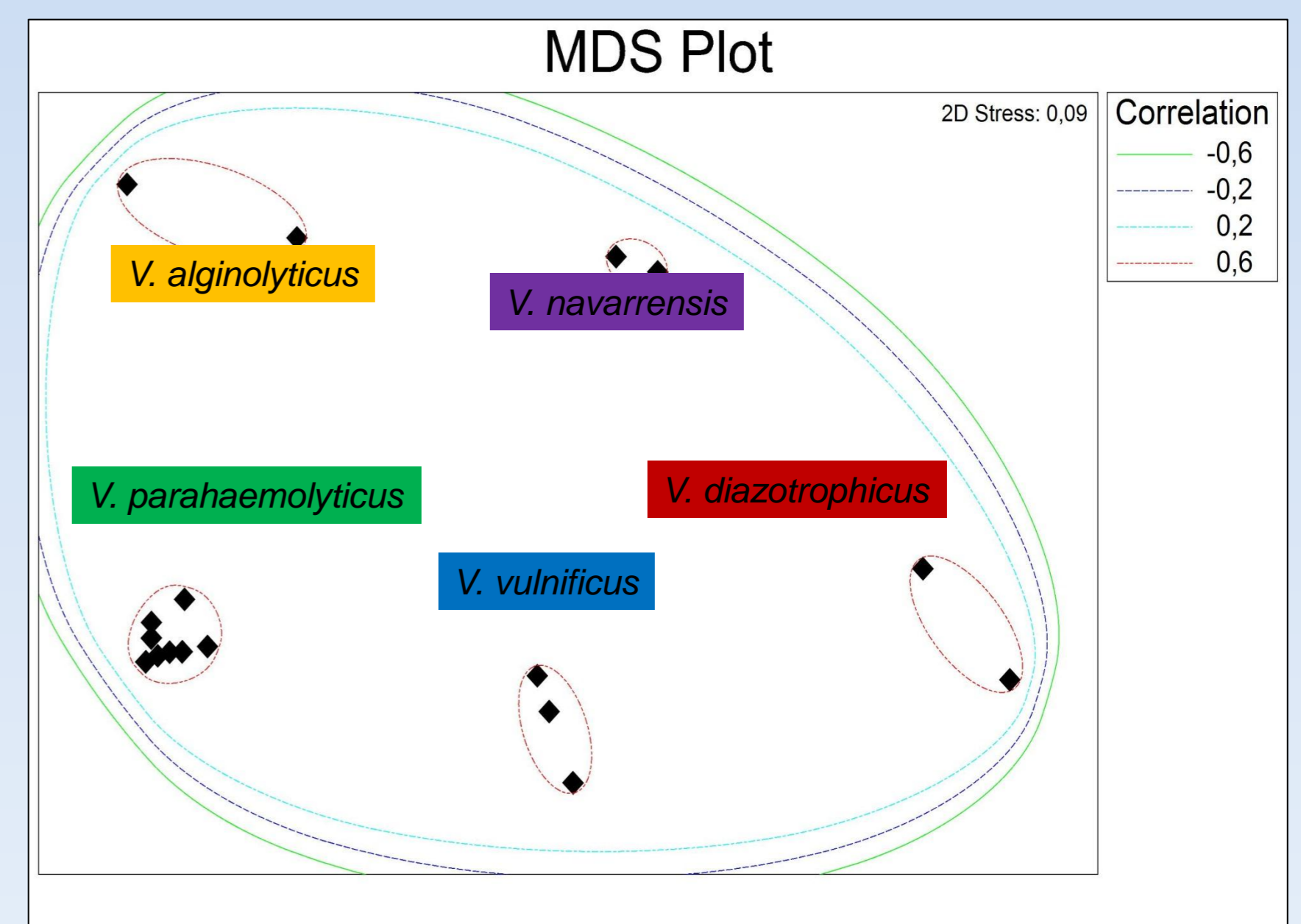


Fig. 5: MDS Plot of correlation data from Heincke isolates.

## Summary & Outlook

MALDI-TOF can be a powerful tool in *Vibrio* population analyses. The approach is currently tested on monitoring cruises to observe the relative proportion of *Vibrio* species in the German Bight. In addition to this quantitative application of MALDI-TOF, Cluster Analyses can be performed to detect interspecific, maybe pathogenic-associated groups. Our goal is to establish this method in *Vibrio* research, which would offer the opportunity for *Vibrio* population research on a larger scale.

