Monterey Bay Aquarium
Research Institute

Temporal and Spatial Biological Variability in Monterey Bay from AUV eDNA Samples

Grant Sanderson, University of Hawai'i at Hilo

Mentors: Monique Messié and Kathleen Pitz

Summer 2022

Keywords: eDNA, Autonomous Underwater Vehicles, Seasonality, Monterey Bay

ABSTRACT

Since 2006, environmental DNA (eDNA) samples have been regularly collected in the Monterey Bay National Marine Sanctuary (NBNMS) for the purpose of monitoring biological communities. While these samples are useful to study how biological communities in Monterey Bay change over time, they cannot adequately describe the spatial variation present in these communities. As an alternative, Autonomous Underwater Vehicles (AUVs) have collected eDNA samples within the Monterey Bay since 2016. These samples are distributed along a wide spatial range within the bay, so these samples are ideal for describing biological variation across both space and time. Using 181 samples collected across a five-year period, this project shows that eDNA samples collected by AUVs can be effectively used to describe both spatial and temporal variation from the same set of samples. Based on the findings of this project, it is recommended that autonomous eDNA sample collection be continued into the future, so that a wider time frame can be studied.

INTRODUCTION

In recent years, ecosystem monitoring has become more important than ever before, due in part to widespread ecological shifts brought about by anthropogenic climate change. As such, there is an increasing need for reliable and scalable ecosystem monitoring solutions to measure the impact of climate change, and to inform future management strategies (Yoccoz, 2012, Pace et al., 2015). Several recent advancements in the marine sciences have allowed marine ecosystems to be studied more effectively, and one of these advancements is the use of environmental DNA for metabarcoding. "Environmental DNA," or eDNA for short, is an umbrella term used to refer to fragments of unincorporated genetic material in an environment. These fragments may result from shed tissue cells, waste products, or from cellular death in the case of free-living single-celled organisms (Chavez et al., 2021). In marine environments, this genetic material can stay intact and suspended in the water column for a week or more, and is subject to the movement of water currents (Saito & Doi, 2021). These genetic fragments can be collected for analysis by pushing the water sample through a micron-scale filter. The genetic material can then be extracted from the filter, amplified using polymerase chain reaction (PCR), and finally sequenced into a digital format for data analysis (Chavez et al., 2021, Coissac et al. 2012). If the resulting sequences are compared to a reference database of known sequences, it can be determined which organisms they originated from. eDNA can be used to detect many different species from every domain of life, which lends itself well to many applications for ecological studies (Wang et al., 2020).

Traditionally, eDNA has only been used with primers designed specifically for a single species, a technique referred to as "active" monitoring. This method targets a specific, known region of the organism's genome for PCR amplification, and is useful for monitoring the population dynamics of specific species, or to detect rare species that may be overlooked in traditional surveys (Yamahara et al., 2019). However, genetic information from thousands of different organisms may be present within a sample of seawater, so eDNA samples can potentially be used to study entire biological communities at once, providing insight into ecosystem-scale community dynamics (Wang et al., 2020, Bohmann et al., 2014). In this "metabarcoding" approach, many different species can be sequenced at once by targeting a barcode sequence; a portion of

the genome that is present in many different organisms, but is variable enough to resolve differences between organisms (Coissac et al., 2012). Some of the most common genetic markers used as barcode sequences include the 12S ribosomal RNA gene, the 18S ribosomal RNA gene, and the Cytochrome Oxidate Subunit I (COI) mitochondrial DNA gene. Each marker provides different information on different groups of organisms, so the choice of a genetic marker will depend on the goals of a study (Drummond et al., 2016).

Since 2006, the Monterey Bay Aquarium Research Institute (MBARI) has collected eDNA samples as part of a decades-long Monterey Bay Time Series describing oceanographic conditions within the Monterey Bay National Marine Sanctuary (MBNMS). These samples are taken regularly, which allows them to be used for monitoring temporal variation within local biological communities. Prior analysis of these samples has shown that biological communities in Monterey Bay tend to form clustered subnetworks that vary independently throughout time (Djurhuus et al., 2020). However, these samples have only been taken from three discrete points in the Monterey Bay region, and thus, cannot adequately describe the spatial variation of these biological communities (Bálint et al., 2018). Ecosystem management strategies rely on accurate measurements of biological community composition across both time and space in order to be effective. Therefore, in order to produce the most complete model of biological variation in Monterey Bay through population genetics, a new method of eDNA sampling is required (Yamahara et al., 2019).

eDNA can provide information about organisms from all trophic levels and all domains of life from just a water sample, so it is not technically necessary to be physically present when collecting the sample. This allows for the possibility of collecting samples remotely, and filtering the samples from a centralized location. Autonomous Underwater Vehicles (AUVs) are unmanned robotic sampling devices that are capable of autonomously performing physical measurements in the water column, imaging the seafloor, and collecting water samples (Caress et al., 2008). Since 2016, MBARI has pioneered the use of upper-water-column AUVs to autonomously collect eDNA samples in the Monterey Bay. Each AUV is capable of collecting ten samples of seawater, which are filtered and processed in the lab following the completion of the

AUVs mission (Yamahara et al., 2019). Samples have been collected across a wide geographic area within Monterey Bay, which allows for the possibility of ecosystem monitoring across both space and time. The goal of this study was to determine whether these autonomously collected samples could be used to measure both spatial and temporal variation among the biological communities in Monterey Bay from the same set of samples. If this is possible, AUV-based eDNA sampling has the potential to be an immensely useful tool for ecological studies, especially in remote regions where in-person sampling efforts are difficult.

MATERIALS AND METHODS

SAMPLE COLLECTION AND SEQUENCING

The sample data for this project were collected by autonomous underwater vehicles in the Monterey Bay National Marine Sanctuary by the Monterey Bay Aquarium Research Institute's Biological Oceanography Group. The water samples were collected along a diamond-shaped quadrilateral transect, starting from the right-most corner of the diamond, at a point labeled C1. After reaching this point, the vehicle moved in a counter-clockwise direction along the diamond, passing through point M1, and arriving back at C1 to complete a survey after a period of about fifteen hours. Samples were collected between the surface and 25m deep in the water column. In total, 377 samples were collected between March 30, 2016, and July 1, 2021. Of these, 181 samples were selected for sequencing across 29 AUV surveys (Fig. 1). After the AUV returned to shore, the water samples were retrieved, and filtered through a 0.22 μm membrane filter (MiliporeSigma, USA). After filtration, the filters were immediately frozen in liquid nitrogen, and stored at -80° C to await amplification and sequencing.

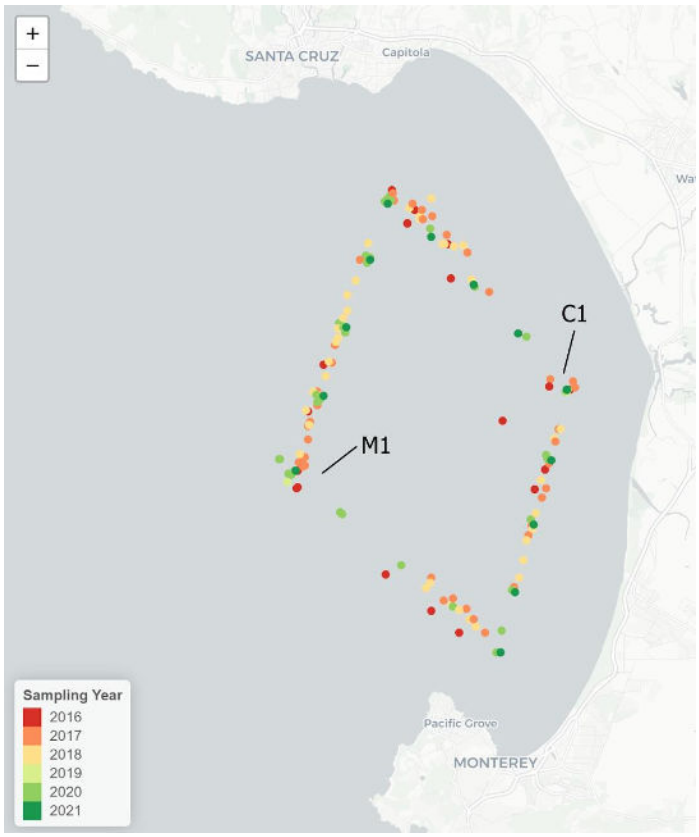

Figure 1: A map showing the sampling locations of the 181 samples used for the study. The samples were collected by an autonomous underwater vehicle (AUV) from March 2016 to July 2021. All samples were taken between the surface and 25m depth, and had a measured fluorescence greater than 0.001. All surveys began at the point labeled C1, and proceeded in a counter-clockwise direction around the diamond-shaped transect.

DNA was extracted from the filters using the DNeasy® Blood and Tissue Test Kit (QIAGEN, USA), following directions provided by the manufacturer. PCR amplification reactions were conducted using 3 µl of DNA extract from each sample in a 96-well plate. Following PCR amplification, the presence of the target sequence in the product was verified using gel electrophoresis, as well as the absence of non-specific amplicon products. The primary products were then selected for size using the AMPure XP bead purification system. Sequencing services were provided by the Research Technology Support Facility Genomics Core at Michigan State University, and the samples were sequenced using the Illumina MiSeq sequencing platform. The raw sequencing files were processed using a modified version of the Banzai pipeline developed by MBARI. Primer

sequences were removed from the files using Atropos, the sequences were merged using DADA2, chimera sequences were removed, and taxonomy was assigned through nucleotide BLAST searches using NCBI GenBank's Non-redundant nucleotide database.

MBARI amplifies three different genetic markers for metagenomic analysis: The 12S ribosomal RNA gene, the 18S ribosomal RNA gene, and the Cytochrome Oxidase Subunit I (COI) mitochondrial DNA gene. Of these three, the 12S rRNA gene and the COI mtDNA gene markers were selected for this project. These samples were originally collected in order to study phytoplankton productivity in Monterey Bay, so as a result, only the samples which were collected from water observed having a measured fluorescence greater than 0.001 were selected, since high fluorescence was expected to correlate with high phytoplankton productivity.

BIOINFORMATICS AND DATA ANALYSIS

The bioinformatics portion of this project was performed within the R Statistics platform version 3.6.3 (R Core Team, 2020). In particular, the Phyloseq package was heavily used in order to process the genomic data (McMurdie 2013). The analyses for both the 12S rRNA and COI mtDNA markers were conducted identically, following the same steps. In order to assess the temporal variation present across the time span covered by the dataset, the samples were divided into groups based on the year they were collected in. Additionally, all years were merged and separated by month, in order to determine variation within each year. To this end, the samples were also split into seasonal groups, which were determined by the summer and winter solstices, and the spring and autumnal equinoxes.

In order to avoid relying on measurements of physical distance between samples to determine spatial variation, each sample was assigned an angle from the center of the quadrilateral transect. The angle from the center of the transect to the MBARI campus at Moss Landing, California was chosen to be 0 degrees, with the angle increasing with counter-clockwise motion around the transect (Fig. 2). It should be noted that the AUVs collected the samples moving in a clockwise direction, opposite of the growth of the angle measurement, but this does not affect the analysis.

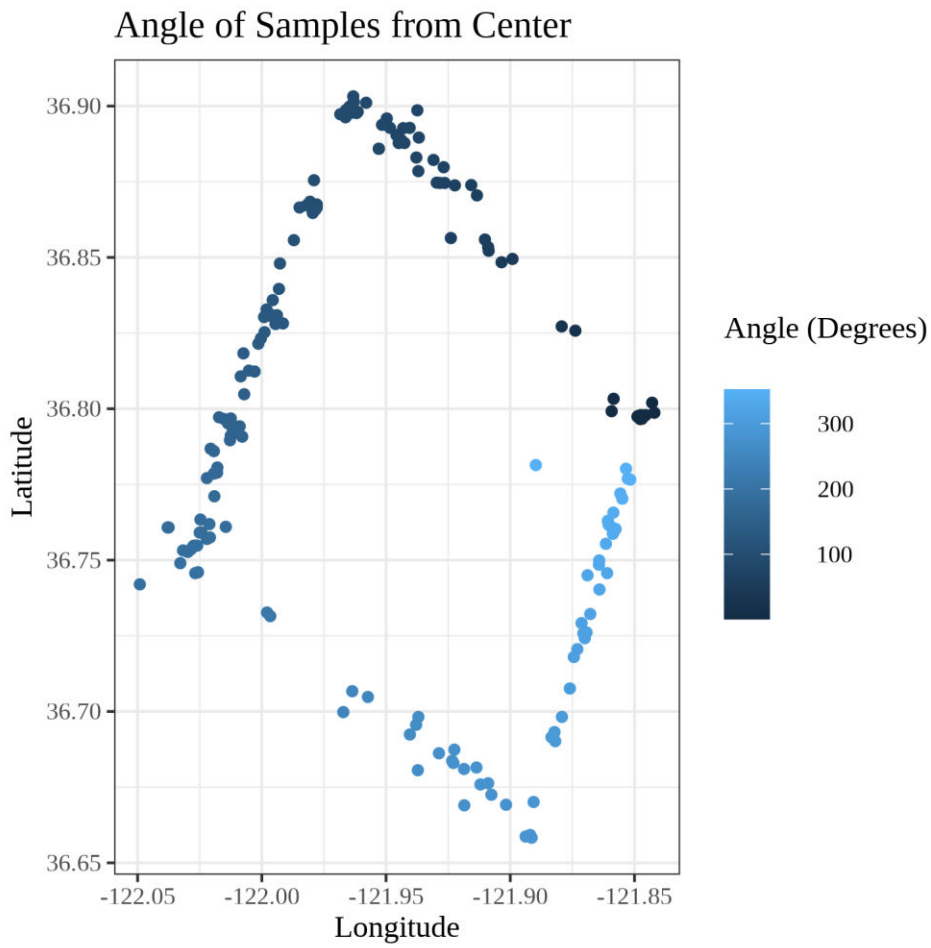

Figure 2: A chart showing the layout of the samples contained in the dataset, colored according to the angle assigned to each. The easternmost point of the quadrilateral transect closest to Moss Landing, CA was designated as 0 degrees, with the angle increasing with counter-clockwise motion around the diamond.

In order to compare spatial variability, the samples were placed into groups based on their angle around the diamond-shaped transect. Firstly, the samples were separated into one of four groups divided into 90 degree segments which covered each of the four corners of the transect (Fig. 3a). This allowed for separate analysis of communities in the northern end of the bay near Santa Cruz, CA, the eastern area of the bay near the Elkhorn Slough, the southern area of the bay near Monterey, CA, and the offshore portion of the transect. Secondly, the samples were divided into 180 degree segments representing nearshore and offshore environments (Fig. 3b).

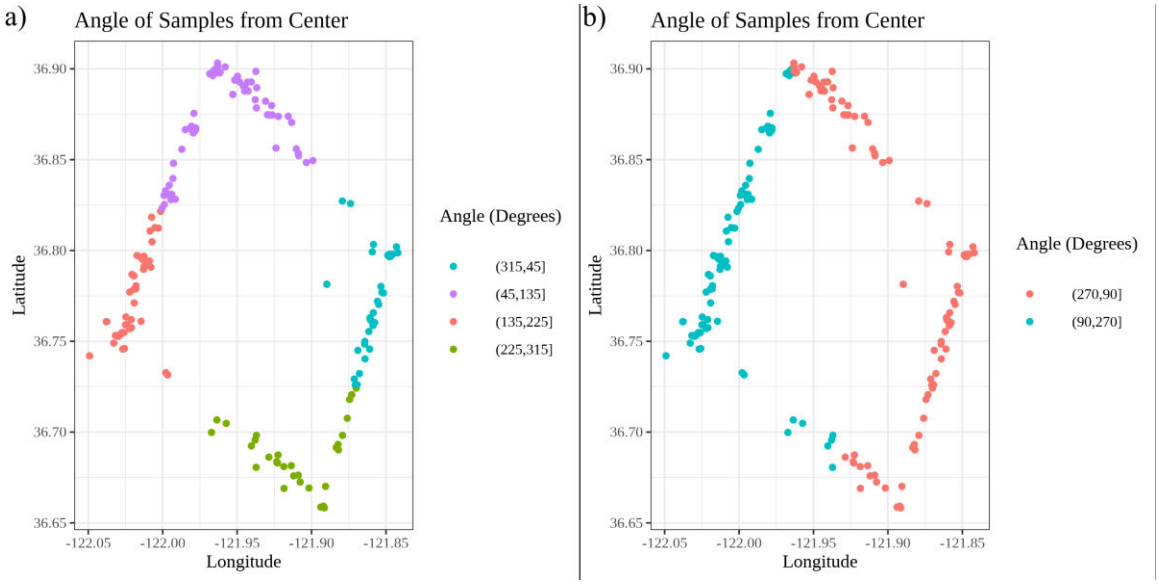

Figure 3: Charts showing the breakdown of groups to measure spatial variation. In chart a), the angle was split into four categories covering the four corners of the quadrilateral transect. In chart b), the angle was split into two categories, in order to measure inshore/offshore variation.

Before any analysis was done, the samples were rarefied to an even depth, so that direct comparisons could be made. For all of the categories, the community composition of each of the groups was compared using a stacked bar plot, which were separated by taxonomic order. The same groups were then tested for clustering and variance using a non-metric multidimensional scaling (NMDS) ordination plot. The samples were also used to generate plots showing Alpha Diversity for all of the groups, in order to compare and corroborate with the bar plots and NMDS plots. Both Chao1 and Shannon-Wiener alpha diversity metrics were used.

RESULTS

TEMPORAL VARIATION

Overall, the observations from the COI mtDNA marker were largely dominated by Zooplankton, Marine Fungi, and Eukaryotic algae. The communities described by the COI data showed a very high degree of temporal variability across all groups tested (Fig. 4). In particular, the temporal variation appears to be driven by the taxonomic orders

Saccharomycetales (An order of marine fungi) and *Ploima* (An order of rotifers). 2016 appears to have been dominated by *Saccharomycetales*, but by 2021, this dominance appears to have shifted to *Ploima.* Interestingly, the samples from 2021 appear to form two distinct clusters in the NMDS plot; a feature which should be investigated for future analyses. The shift between dominant orders is also seen in the plots depicting seasonal variation, with *Ploima* appearing to dominate during the summer, and shifting to *Saccharomycetales* in the winter. Furthermore, the months of August and December appear to show a high amount of sequence reads assigned to order *Phaeocystales*, while the month of July also appears to show an unusually large signal for *Ploima.*

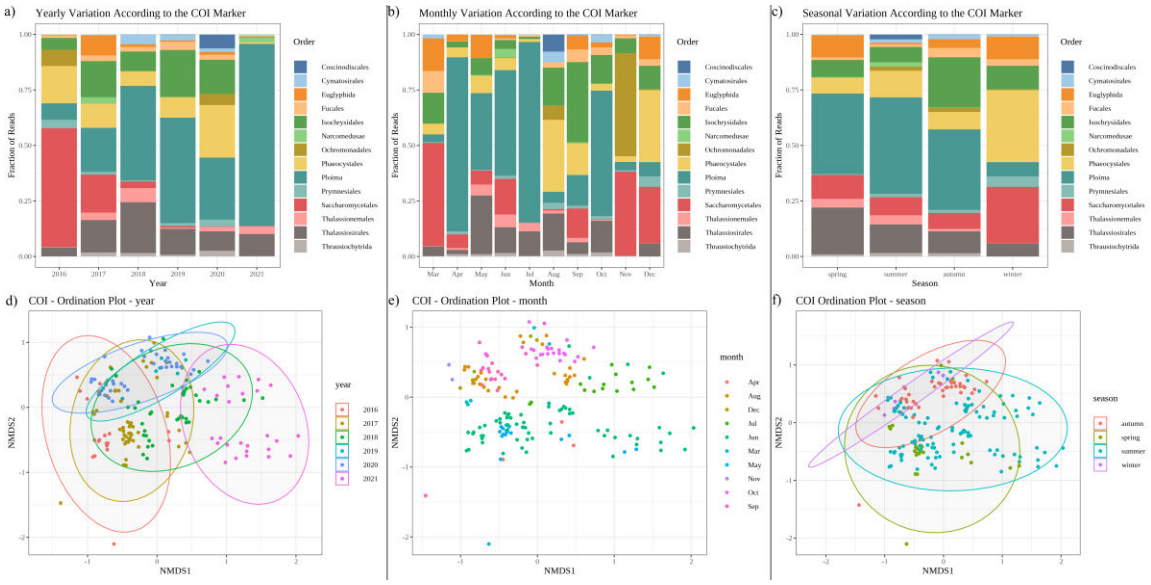

Figure 4: A Comparison of Temporal Variation in Monterey Bay, CA, as shown by the Cytochrome Oxidase Subunit I mitochondrial DNA marker. Figures a) and d) show variation from year to year, b) and e) show monthly variation within the year, and c) and f) show seasonal variation within the year.

The primers used for amplifying the 12S rRNA gene in this project have been designed to target bony fish, and this group makes up nearly the entirety of the observations for this marker, aside from a handful of observations for order *Cetacea* and several land vertebrates. The communities described by the 12S rRNA marker were mostly dominated by order *Clupiformes*, which itself is mostly represented by sardines and anchovies. However, a high degree of temporal variation is seen here as well (Fig. 5). The samples from 2016 show the highest degree of diversity, with a relatively high

number of observations for organisms within orders *Gadiformes* (Cod) and *Pleuronectiformes* (Flatfish). In contrast, 2021, the most recent year represented in the dataset, showed the lowest diversity, with very few sequence reads being identified as non-*Clupiformes* organisms. This contrast can also be seen in the NMDS plot, where the samples from 2021 are clustered tightly together, while the samples from 2016 are broadly spread across the plot. The plots depicting seasonal variation show the highest diversity in the summer, and the lowest in the winter. However, it is worth noting that there have been many more samples taken during summer months than there have been during winter months, so this analysis may be affected by statistical outliers. However, it is worth noting the month of July, where there appears to be very few observations for organisms outside of order *Clupiformes.*

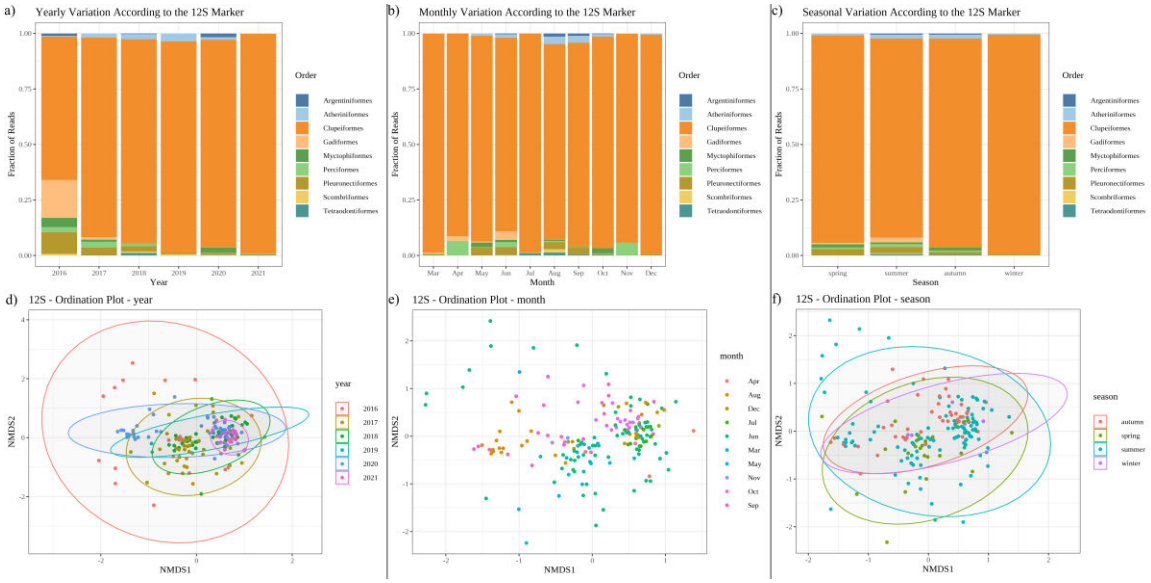

Figure 5: A Comparison of Temporal Variation in Monterey Bay, CA, as shown by the 12S Ribosomal RNA marker. Figures a) and c) show variation from year to year, b) and e) show monthly variation within the year, and c) and f) show seasonal variation within the year.

SPATIAL VARIATION

Unlike the plots depicting temporal variation, the plots measuring spatial variation show little difference between groups. For both the COI mtDNA data and the 12S rRNA data, the group which shows the most difference appears to be the 90-degree increment

covering the offshore portion of the Monterey Bay. For the COI data, this group shows fewer observations for *Ploima*, and more observations for *Thalassosirales* (An order of marine diatoms) (Fig. 6a). Though less apparent, this pattern in also seen in the 180-degree segment representing the outer Monterey Bay (Fig. 6b). There is no significant clustering seen in the NMDS plots (Fig 6c, Fig 6d.)

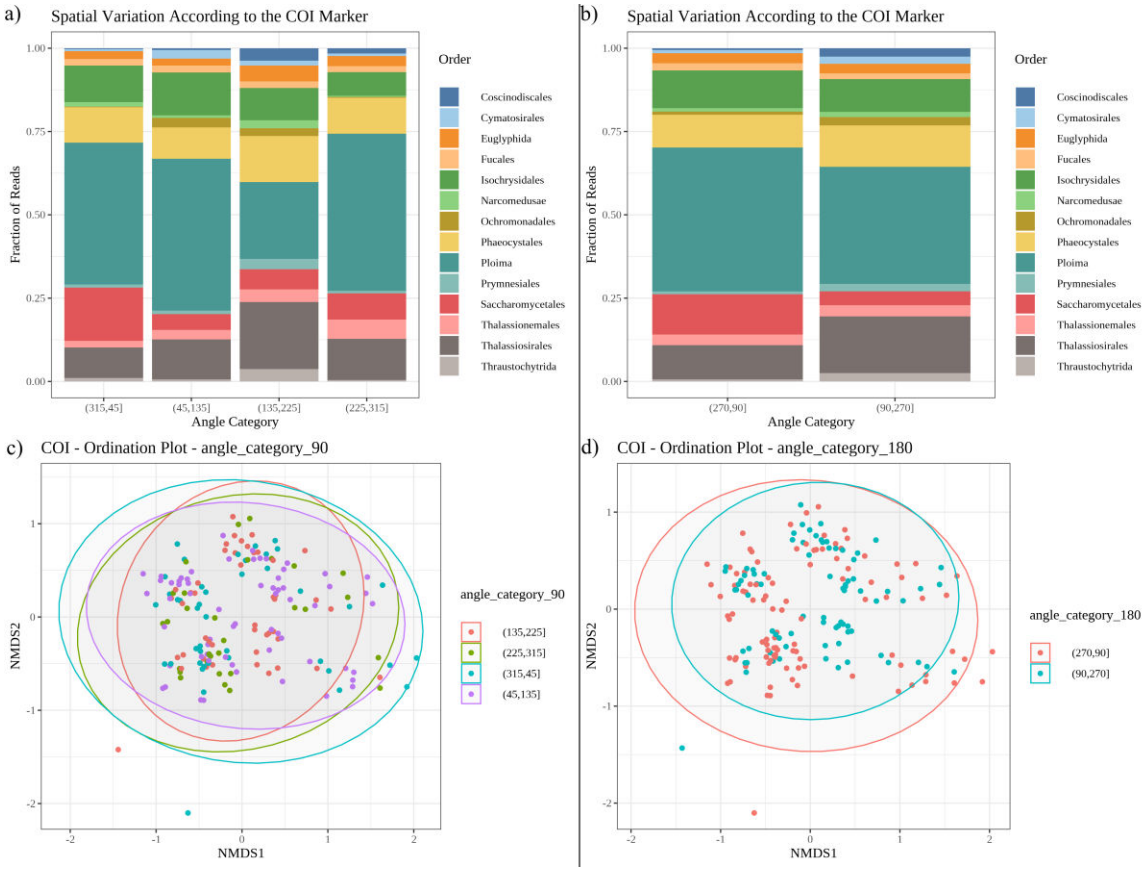

Figure 6: A Comparison of Spatial Variation in Monterey Bay, CA, as shown by the Cytochrome Oxidase Subunit I mitochondrial DNA marker. Figures a) and c) show variation between four corners of a quadrilateral transect throughout Monterey Bay, while b) and d) represent nearshore/offshore variation

Like the COI data, the 12S data show little spatial variation. Like the analysis for Temporal variation, most of the sequence reads have been assigned to order *Clupiformes.* However, mirroring the observations from the COI data, there appears to be slightly more diversity in the groups depicting the offshore environment. In particular, there is a stronger signal for *Pleuronectiformes* in the offshore samples (Fig. 7a, Fig. 7b). Like the

plots for the COI data, there is little significant grouping shown in the NMDS plots (Fig 7c, Fig. 7d).

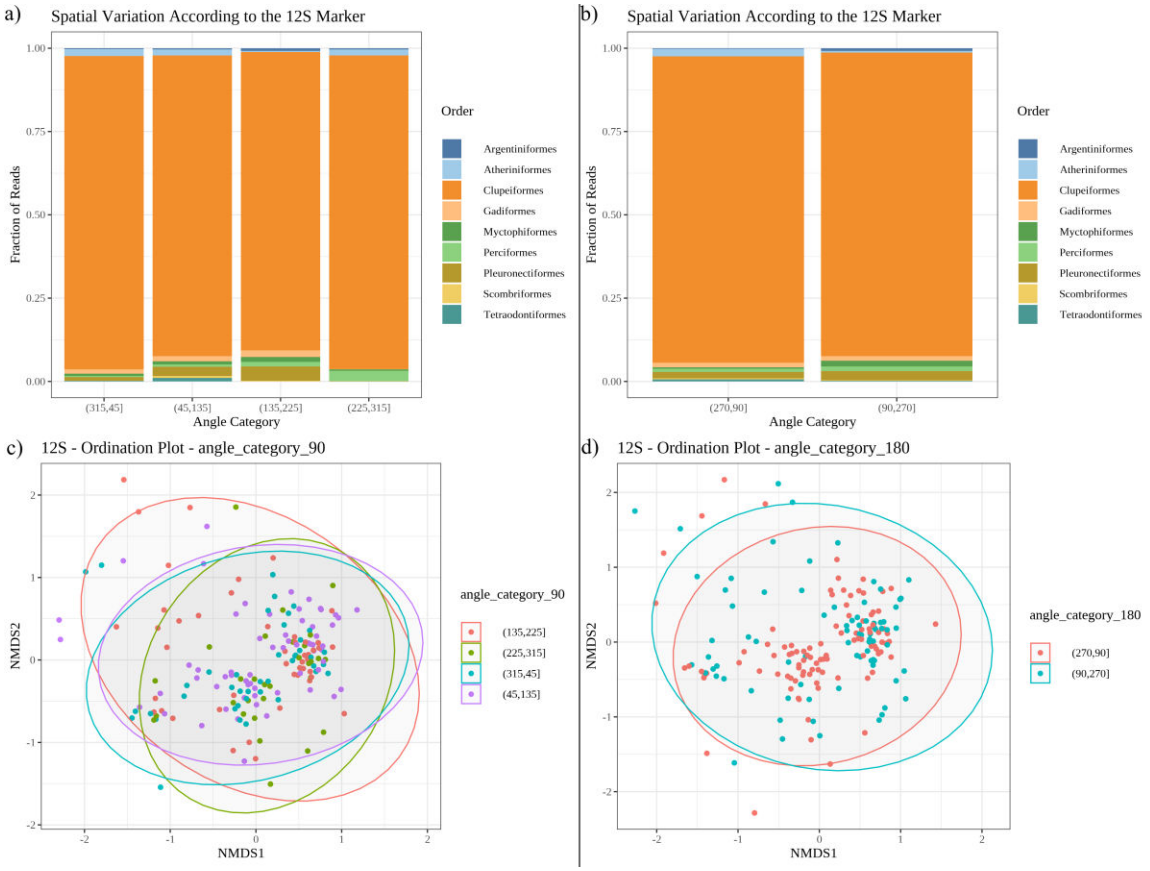

Figure 7: A Comparison of Spatial Variation in Monterey Bay, CA, as shown by the 12S Ribosomal RNA marker. Figures a) and c) show variation between four corners of a quadrilateral transect throughout Monterey Bay, while b) and d) represent nearshore/offshore variation

ALPHA DIVERSITY

The plots depicting temporal variation in alpha diversity mirror many of the observations made with the bar plots and NMDS plots. For both the COI mtDNA and 12S rRNA data, the year 2021 stands out, showing significantly less alpha diversity than in other years (Fig. 8a, Fig. 8d). This corroborates what was seen in the other plots, where *Ploima* and *Clupiformes* dominated the COI and 12S data respectively. The 12S marker saw particularly high alpha diversity in 2020 (Fig. 8a). Interestingly, the month of July

appears to show very little alpha diversity for both genetic markers; a trend that was also seen in the bar plots and NMDS plots (Fig. 8b, Fig. 8e).

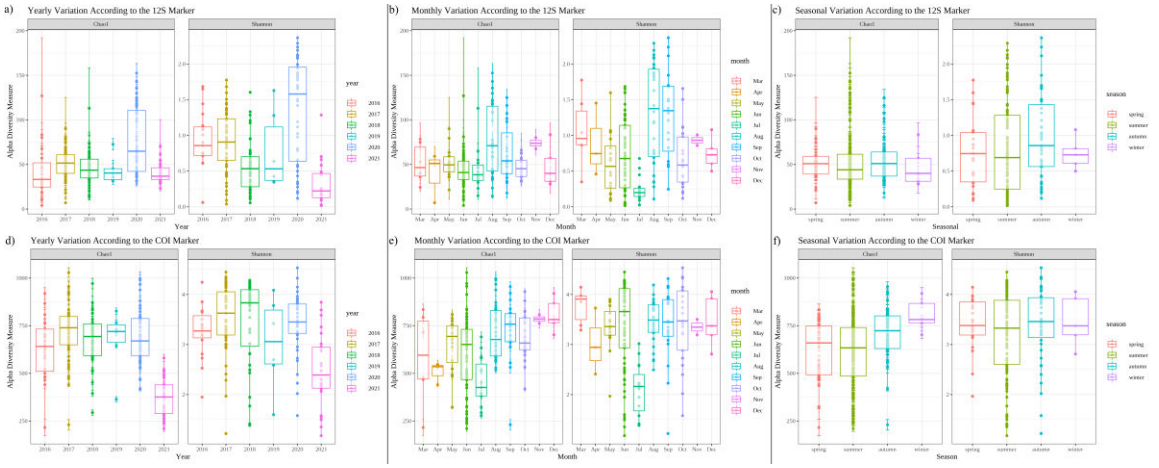

Figure 8: A Comparison of Temporal variation in Chao1 and Shannon Alpha Diversity in Monterey Bay CA. Figures a), b), and c) show variation according to the 12S Ribosomal RNA marker, and figures d), e), and f) show variation according to the Cytochrome Oxidase Subunit I mitochondrial DNA marker. Figures a) and d) show variation between years, figures b) and e) show variation between months within a year, and figures c) and f) show variation between seasons within a year.

As seen with the bar plots and NMDS plots, there appears to be little spatial variation among the groups shown here, with no significant difference between any two groups (Fig. 9). It is worth noting, however, that the highest alpha diversity was seen in the offshore groups, which supports the observations from the 12S marker bar plots.

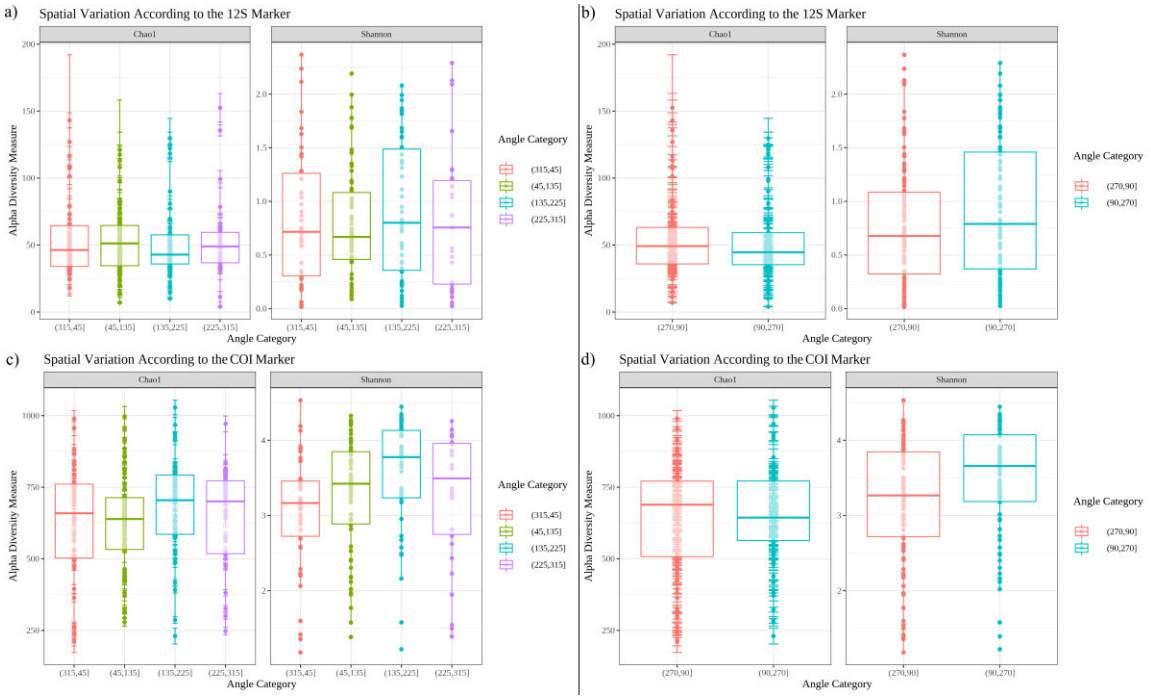

Figure 9: A Comparison of Temporal variation in Chao1 and Shannon Alpha Diversity in Monterey Bay CA. Figures a) and b) show variation according to the 12S Ribosomal RNA marker, and figures c) and d) show variation according to the Cytochrome Oxidase Subunit I mitochondrial DNA marker. Figures a) and c) show variation between the four corners of the diamond-shaped transect, while figures b) and d) show nearshore/offshore variation.

DISCUSSION

This analysis shows that eDNA samples collected by AUVs can be used to study the variation within biological communities in the Monterey Bay National Marine Sanctuary across both space and time. This collection of samples showed very little spatial variation in the communities described by both the 12S rRNA and COI mtDNA markers, suggesting that seasonal and yearly changes in oceanographic conditions play a larger role in determining community composition and trophic interactions than differences in physical location within the bay. These results are consistent with previous studies, which have observed a strong seasonal shift among the biological communities in Monterey Bay (Djurhuus et al., 2020). By limiting this analysis to samples taken within a short period of time, and thus eliminate the influence of temporal variation, it may be possible to accurately resolve spatial differences in biological communities where this

analysis could not. This should be considered for future studies adapting the methods shown here.

The analysis of the 12S samples showed communities which were dominated by sardines and anchovies in the order *Clupiformes.* This observation is consistent with previous studies, as the dominance of *Clupiformes* in the Monterey Bay is well known. In fact, previous studies have used eDNA to document the shift from an Anchovy-dominated community to a sardine-dominated community; a change which occurs on a multidecade time scale (Chavez et al. 2021, Chavez et al. 2003). Given the fact that eDNA is able to determine differences between individual species, a longer time period covered by this eDNA dataset could provide valuable insight into the drivers behind this phenomenon. As a result, it is important that eDNA sample collection continues into the future. The stronger signal seen for *Pleuronectiformes* flatfish seen in the offshore environment with the 12S marker is unusual, since the fish in this order are typically benthic-dwelling. These fish often inhabit deeper habitats, and previous studies have observed some amount of connectivity between the deep and shallow habitats of some flatfish (Barkley et al., 2017). However, it is unknown if this could be responsible for the offshore observations seen here, and his feature of the analysis should be investigated by future studies.

Temporal variation as described by the COI marker appears to be mostly driven by the orders *Saccharomycetales* and *Ploima*, with a clear transition from *Saccharomycetales* to *Ploima* shown in the species composition from year to year. The samples from 2016 saw a large number of reads for *Saccharomycetales*, while the samples from 2021 were mostly dominated by *Ploima*. Order *Phaeocystales* also shows strong seasonal variation, with more observations occurring during the winter. The seasonality of plankton communities in Monterey Bay has been well-documented for many years. In particular, phytoplankton like those in order *Phaeocystales* become much more abundant during periods of upwelling in Monterey Bay. This upwelling tends to occur during the month of august, which could potentially explain the drastic changes in community composition observed during that month in this study (Garrison, 1979).

The analysis of alpha diversity for the communities described by the 12S rRNA and COI mtDNA markers corroborates the observations made from the bar plots and NMDS plots, with low biodiversity shown in 2021 for both markers, as well as low diversity in the month of July overall. The 12S marker in particular showed high diversity during the month of August. As discussed above, previous studies have found that strong upwelling conditions cause nearshore primary producers to become more abundant, which may contribute to the observed rise in biodiversity among bony fishes during this month (Satterthwaite et al., 2020). Though there were no significant differences between the groups used to measure spatial variation, it is worth noting that the offshore groups saw slightly higher biodiversity than the nearshore groups.

These samples currently cover a relatively short time frame compared to the data from the Monterey Bay Time Series (Chavez et al., 2021). As a result, analyses of temporal variation using the AUV data will be less robust than analyses using the time series data, and some of the groups shown here were prone to being affected by outliers due to the low number of samples in the group. As a result, it is important to continue collecting samples into the future, for this reason and for reasons discussed above.

CONCLUSIONS/RECOMMENDATIONS

This analysis has shown that eDNA samples collected by autonomous underwater vehicles can be used to measure biological variation across both temporal and spatial distances from the same set of samples. The results shown here indicate that changes across time play a larger role in determining the community composition in Monterey Bay than differences in physical space do, and the observations of community composition are consistent with literature on the topic. The approach of using AUVs to collect eDNA samples has great potential for future ecological studies within the Monterey Bay and elsewhere. Therefore, it is recommended that autonomous eDNA sample collection in Monterey Bay be continued into the future, so that a wider time frame than the one shown here may be studied. Furthermore, it is important to collect more eDNA samples during the winter months, since these are under-represented in the dataset, which may subject the analysis to outliers. With a more robust collection of AUV

eDNA samples, this dataset can complement the Monterey Bay Time Series data, and provide a fuller understanding of the biological communities in the Monterey Bay National Marine Sanctuary. This information may be used to inform future ecosystem management strategies to combat climate change and other anthropogenic stressors on the marine environment. Future studies may also wish to include other genetic markers during analysis, such as the 18S rRNA gene (Which MBARI already sequences), since this would aid in creating a more complete view of biological variability in the Monterey Bay. As eDNA sampling methods and AUV technology continue to improve, these methods could be deployed outside of the Monterey Bay, to study marine environments which are too remote to be sampled regularly, and provide a clearer understanding of community dynamics across many different environments.

ACKNOWLEDGEMENTS

The MBARI Summer Internship Program is generously supported through a gift from the Dean and Helen Witter Family Fund and the Rentschler Family Fund in memory of former MBARI board member Frank Roberts (1920-2019) and by the David and Lucile Packard Foundation. Additional funding is provided by the Maxwell/Hanrahan Foundation. I would personally like to thank Dr. Monique Messié, and Dr. Kathleen Pitz for their advice and guidance over the course of this project, as well as Dr. Francisco Chavez, Dr. Lis Henderson, and Jacoby Baker for their help. Furthermore, I would like to thank Dr. George Matsumoto, Megan Basset, and Lyndsey Classen for their efforts in running the MBARI internship program.

References:

Bálint, M., Pfenninger, M., Grossart, H., Taberlet, P., Vellend, M., Leibold, M.A., Englund, G., Bowler, D. (2018) Environmental DNA Time Series in Ecology. *Trends in Ecology and Evolution* **33**: 945-957. https://doi.org/10.1016/j.tree.2018.09.003

Barkley, A.N., Fisk, A.T., Hedges, K.J., Treble, M.A., Hussey, N.E. (2017) Transient movements of a deep-water flatfish in coastal waters: Implications of inshore-offshore connectivity for fisheries management. *Journal of Applied Ecology,* **55:** 1071-1081. https://doi.org/10.1111/1365-2664.13079

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M. (2014) Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology and Evolution,* **29**: 358-367. https://doi.org/10.1016/j.tree.2014.04.003

Caress, D.W., Thomas, H., Kirkwood, W.J., McEwen, R., Henthorn, R., Clague, D.A., Paull, C.K., Paduan, J. (2008) High-Resolution Multibean, Sidescan, and Subbottom Surveys Using the MBARI AUV D. *Allan B. Marine habitat mapping technology for Alaska*, 47-69. https://doi.org/10.4027/mhmta.2008

Chavez, F.P., Min, M., Pitz, K., Truelove, N., Baker, J., LaScala-Grunewald, D., Blum, M., Walz, K., Nye, C., Djurhuus, A., Miller, R.J., Goodwin, K.D., Muller-Karger, F.E., Ruhl, H.A., Scholin, C.A. (2021). Observing Life in the Sea Using Environmental DNA. *Oceanography*, **34:** 102-119. https://doi.org/10.5670/oceanog.2021.218

Chavez, F.P., Ryan, J., Lluch-Cota, S.E., Ñiquen Carranza, M. (2003) From Anchovies to Sardines and Back: Multidecadal Change in the Pacific Ocean. *Science,* **299**: 217-221. https://doi.org/10.1126/science.1075880

Coissac, E., Riaz, T., Puillandre, N. (2012) Bioinformatic challenges for DNA metabarcoding of plants and animals. *Molecular Ecology,* **21**: 1834-1847. https://doi.org/10.1111/j.1365-294X.2012.05550.x

Djurhuus, A., Closek, C.J., Kelly, R.P., Pitz, K.J., Michisaki, R.P., Starks, H.A., Walz, K.R., Andruszkiewicz, E.A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F.E., Chavez, F.P., Boehm, A.B., Brietbart, M. (2020) Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications* **11**: 254. https://doi.org/10.1038/s41467-019-14105-1

Drummond, A.J., Newcomb, R.D., Buckley, T.R., Xie, D., Dopheide, A., Potter, B.C.M., Heled, J., Ross, H.A., Tooman, L., Grosser, S., Park, D., Demetras, N.J., Stevens, M.I., Russel, J.C., Anderson, S.H., Carter, A., Nelson, N., (2015) Evaluating a multigene environmental DNA approach for biodiversity assessment. *Gigascience* 4: s13742–015–0086–1. https://doi.org/10.1186/s13742-015-0086-1

Garrison, D.L. (1979) Monterey Bay phytoplankton I. Seasonal cycles of phytoplankton assemblages. *Journal of Plankton Research* **1:** 241-265. https://doi.org/10.1093/plankt/1.3.241

McMurdie, P.J. and Holmes, S. (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE.* **8**(4):e61217. https://doi.org/10.1371/journal.pone.0061217

Pace, M.L., Carpenter, S.R., Cole, J.J. (2015) With and without warning: managing ecosystems in a changing world. *Frontiers in Ecology and the Environment* **13**: 460-467. https://doi.org/10.1890/150003

R Core Team (2020). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Saito, T., Doi, H. (2021) A Model and Simulation of the Influence of Temperature and Amplicon Length on Environmental DNA Degradation Rates: A Meta Analysis Approach. *Frontiers in Ecology and Evolution,* **19**: 623831. https://doi.org/10.3389/fevo.2021.623831

Satterthwaite, E.V., Morgan, S.G., Ryan, J.P., Harvey, J.B.J., Vrijenhoek, R.C. (2020) Seasonal and synoptic oceanographic changes influence the larval biodiversity of a retentive upwelling shadow. *Progress in Oceanography,* **182:** 102261. https://doi.org/10.1016/j.pocean.2019.102261

Wang, S., Yan, Z., Hänfling, B., Zheng, X., Wang, P., Fan, J., Li, J. (2020) Methodology of fish eDNA and its applications in ecology and environment. *Science of The Total Environment,* **755**: Article 142622. https://doi.org/10.1016/j.scitotenv.2020.142622

Yamahara, K.M., Preston, C.M., Birch, J., Walz, K., Marin, R., Jensen, S., Pargett, D., Roman, B., Ussler, W., Zhang, Y., Ryan, J., Hobson, B., Kieft, B., Raanan, B., Goodwin, K.D., Chavez, F.P., Scholin, C. (2019) In situ Autonomous Acquisition and Preservation of Marine Environmental DNA Using an Autonomous Underwater Vehicle. *Frontiers in Marine Science,* **6**: 1-14. https://doi.org/10.3389/fmars.2019.00373

Yoccoz, N.G. (2012) The future of environmental DNA in ecology. *Molecular Ecology,* **21**: 2031-2038. https://doi.org/10.1111/j.1365-294X.2012.05505.x