

Review and testing of universal and specific primers for the amplification of the barcode in marine epipelagic copepods.

Authors: Beltrán-Castro Juan Ramón, Hernández-Trujillo Sergio, Gómez-Guitérrez Jaime, Blanco-Jarvio Anidia.

Abstract

The amplification of the CO1 partial region (Genetic Barcode, 650pb) uses as standard protocol a pair of "universal" (HCO2198 and LCO1490) primers for metazoan. However, the use of such universal primers has not been implemented, so far, with success. The development of degenerate primers and several primer blends are two methodological alternatives to increase the probability of amplification success. Here we do the first literature review of the efficiency of primers for the amplification of the Genetic Barcode copepods to identify the main primers used to amplify CO1 region and to standardize the PCR protocol for the copepods collected in the Gulf of California. Seven publications of Calanoid copepods (belonging to 13 families and 21 genera) have used 17 primers with different efficiencies of CO1 amplification. Seven of those primers (41%) were aligned with the CO1 region and Barcodes of the Bold database while the rest 10 (59%) cannot be aligned with success. From the sequence of the copepod *Tigriopus japonicus*, we estimated the total number of base pairs of the CO1 gene is 1528 base pair. Comparing the complete *T. japonicus* gene with the partial fraction of interest to amplify (barcode) with other species of pelagic copepods the CO1 is located between 40 and 693 base pair. Therefore, we estimate the length of CO1 in pelagic copepods is about 653 base pair. Currently we are standardizing the PCR technique and testing different selected primers obtained from literature with copepod specimens collected in the Gulf of California.

Keywords: Standardized protocol, success of amplification, PCR, Calanoids.

Diet of *Sardina pilchardus* larvae in the Bay of Málaga (Alboran Sea, SW Mediterranean) assessed by mitochondrial COI metabarcoding.

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Abstract

The most common small pelagic fish species in Málaga Bay (SW Mediterranean Sea) is the sardine (*Sardina pilchardus*). Despite its commercial importance in the region, little is known about the ecology of their early life stages and their role in the trophic web dynamics. However, the importance of zooplankton in the diet of sardine larvae in productive regions of the Mediterranean Sea and Iberian Atlantic littoral has been recognized. Here we present the results of a 26 hours survey during which we followed a shoal of sardine larvae (ranging 6 - 21 mm standard length) and the associated zooplankton community in the northern Alboran Sea. The diet of the sardine larvae was analyzed by mitochondrial COI metabarcoding, and compared with the zooplankton community composition, analyzed both with morphological and molecular tools. Diel variability was observed in plankton abundance, both in the field and in larval gut contents. Sardine larvae preys included several copepod species, but also cladocera, euphausiid, gastropod and hydrozoa were detected, suggesting an opportunistic foraging behavior, rather than a selective one.

Keywords: Diel cycle, integrative taxonomy, larval diet, Málaga Bay, mitochondrial COI metabarcoding, *Sardina pilchardus*, trophic ecology

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DNA elucidates identification, distribution and cohabitation of *Calanus* spp. in the North Atlantic and Arctic Oceans

Authors: Marvin Choquet, Janne Søreide, Galice Hoarau

Abstract

Calanus species dominate the zooplankton biomass in the North Atlantic and Arctic Oceans where they play a key role as grazers and as preys for many commercially important fishes. Due to their distinct environmental preferences, *Calanus* species are among the fastest organisms to respond to climate variability by shifting their distributions, and are thus often used as climate change indicators. Therefore, accurate identification of the different species and documentation of their distribution range is essential to detect warnings of climate change. This knowledge has been, so far, almost entirely based on the use of morphological characters for species identification, despite the inaccuracy of this approach has been documented. We used a combination of 6 molecular markers type InDels as a tool for species identification in order to redraw the distribution range of each *Calanus* species in the North Atlantic and Arctic Oceans. This large-scale investigation revealed much wider and overlapping distributions than previously described. To explain this discrepancy, we used our set of molecular markers to assess the validity of each of the morphological features proposed as species-specific and commonly used for species identification and showed that none of them is reliable at 100%. Furthermore, as it has been suggested that *C. finmarchicus* and *C. glacialis* may hybridize, we used our markers designed in the purpose of detecting hybrids to explore this theory in two areas where both species co-occur and reproduce locally, and we showed that hybridization is unlikely to happen at all.

Keywords:

Calanus; species identification; morphology; molecular markers; hybridization.

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Comparative molecular (metabarcoding) and morphological analysis of zooplankton diversity on the NW Atlantic continental shelf during 2002-2012.

Authors: Bucklin, A., H.D. Yeh, J.M. Questel, B. Reese, N.J. Copley, and P.H. Wiebe

Abstract

Morphological taxonomic and molecular (metabarcoding) analysis of zooplankton biodiversity was compared for 27 samples from three regions (Gulf of Maine, Georges Bank, Mid-Atlantic Bight) of the NW Atlantic continental shelf over 10 years (2002-2012) during surveys of the US NOAA-NEFSC Ecosystem Monitoring (EcoMon) Program. Metabarcoding analysis used the V9 hypervariable region of nuclear small-subunit (18S) rRNA; clustered sequences and operational taxonomic units (OTUs) were identified, classified, and counted based on alignment to the SILVA database (Release 128). Definitive analysis included a total of 1,600,269 clustered sequence reads spanning 29 taxonomic groups of invertebrate zooplankton. Numbers of clustered sequences by group showed significant differentiation among regions over the 10 years; sequence numbers were significantly correlated ($p < 0.05$) with latitude for three groups (Calanoid Copepoda, Gastropoda, Peracarida). Comparative analysis focused on seven groups for which morphological taxonomic species counts were available from NOAA-NEFSC. All seven groups showed positive correlations between sequence numbers and total species abundances, with significant correlations ($p < 0.05$) for three groups (Calanoid Copepoda, Gastropoda, Chaetognatha). Shannon Diversity Index (H) values were calculated using both sequence numbers and species counts for the seven groups; H values based on molecular and morphological data were highly significantly correlated for each of the three regions over 10 years. This study provides evidence of statistically similar time/space patterns of zooplankton biodiversity based on morphological taxonomic and molecular (metabarcoding) analysis. Additional research is needed to allow direct application and integration of metabarcoding analysis of zooplankton biodiversity for ocean ecosystem assessment and management.

Keywords: zooplankton, metabarcoding, integrative taxonomy, biodiversity

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Reference COI barcode database for Arctic zooplankton: a critical resource for integrative taxonomy

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Abstract

The high biodiversity of marine metazoan holozooplankton assemblages in the Arctic Ocean presents challenges for routine assessment of these pelagic ecosystems and detection of the impacts of climate change. Analysis of patterns of species distribution and abundance, and their relationship to environmental conditions, require discrimination and accurate identification of species, including rare and morphologically-cryptic species. We report progress toward a comprehensive, region-specific reference DNA database for Arctic Ocean metazoan holozooplankton species for the cytochrome oxidase I (COI) barcode region. Reference sequence databases are critically important, since species can only be identified based on COI barcodes by matching to available sequences for specimens identified by morphological taxonomic experts. DNA reference barcode databases are also key to accurate identification and improved taxonomic resolution of molecular operational taxonomic units (MOTUs) resulting from metabarcoding analysis. Our goal is to encourage collaborative work toward region-specific reference DNA databases for marine zooplankton that will ensure accurate species identification for characterization of pelagic biodiversity and allow continued progress toward use of barcoding and metabarcoding for assessment and management of pelagic ecosystems.

Keywords: zooplankton, Arctic Ocean, DNA barcoding, biodiversity, integrative taxonomy

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Species-specific patterns of distribution and abundance of the cryptic copepods *Pseudocalanus moultoni* and *P. newmani* on Georges Bank (NW Atlantic Ocean) 1995-2012

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Abstract

Species-specific patterns of zooplankton diversity, distribution, and abundance are essential for accurate assessment and monitoring of pelagic ecosystems, since even closely-related, morphologically-cryptic species may exhibit significantly different patterns of time/space variation. Real-time quantitative PCR (qPCR) was used to discriminate two morphologically-cryptic species of the calanoid copepod genus *Pseudocalanus*, *P. moultoni* and *P. newmani*, which occupy key positions in the pelagic food web of the NW Atlantic continental shelf. Time-series analysis was conducted on distribution and abundance data for the pooled species (based on morphological taxonomic analysis) and environmental data collected on Georges Bank (NW Atlantic Ocean) by the NOAA Northeast Fisheries Science Center (NEFSC) during 1977-2012. Comparative analysis was done for the individual species (discriminated by qPCR) using samples from US GLOBal Ocean ECosystem Dynamics (US GLOBEC) cruises (1995-1999) and NEFSC Ecosystem Monitoring (EcoMon) surveys during 2002-2012. The abundance of pooled *Pseudocalanus* spp. based on morphological analysis was not strongly correlated with depth-averaged temperature and salinity in the Georges Bank region, although correlations between annually-averaged abundances and environmental data were significant. Molecular discrimination of the species provided evidence of species-specific differences in abundances and abundance anomalies, including different responses to environmental variables, particularly depth-averaged temperature, over the time frame studied. This study demonstrates that accurate resolution of species diversity and detailed analysis of species-specific patterns of time/space variation are critically needed for analysis of pelagic ecosystems and prediction of the responses to environmental variability and climate change.

Keywords: zooplankton, cryptic species, integrative taxonomy, time-series analysis

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Integrative metabarcoding analysis of mesopelagic biodiversity based on new sampling and sensing technologies

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Abstract

We are evaluating and integrating metabarcoding biodiversity assessments with new sampling and sensing technologies to explore mesopelagic biodiversity. Metabarcoding, or the sequencing of diagnostic DNA markers from bulk environmental samples, is quickly gaining traction as a method for marine biodiversity assessments. Challenges include the lack of reference sequences associated with accurately identified specimens, understanding the relationship between genetically defined “operational taxonomic units” and species, and taxon bias associated with different genetic protocols. Here, we present and evaluate results from metabarcoding samples collected from traditional plankton net tows and organism and environmental DNA (eDNA) samplers adapted from the Suspended Particulate Rosette sampler (SUPR) system. Organism samplers filter relatively large water volumes (e.g., >30 liters) and target small zooplankton and larvae that are trapped on a mesh, while eDNA samplers filter relatively small water volumes (e.g., < 5 liters) on submicron filter paper and are preserved *in situ*. The samplers will be mounted on and deployed on DEEP-SEE, a new towed broadband acoustic and imaging system. The samplers are also being adapted for integration into *Mesobot*, an autonomous underwater vehicle that will have imaging and tracking capabilities. By combining sampling with AUVs and sensors, sampling efforts can target discrete, ecologically relevant patches of water. Recently, the mesopelagic zone has attracted significant attention as new studies have suggested that its biomass and biodiversity have been vastly underestimated. Metabarcoding, especially in conjunction with autonomous sampling, imaging, and acoustics, is an important tool for understanding this vast and biologically rich region.

Keywords: metabarcoding, mesopelagic zone, AUV sampling

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Challenges in appendicularian integrative taxonomy and barcoding

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Abstract

Appendicularians are a group of planktonic tunicates which usually comprise about 10% of total mesozooplankton. Despite their importance in marine plankton communities little has been done in their integrative taxonomy or barcoding. Appendicularians possess very high evolutionary rates which makes designing of universal barcoding primers a challenge. This problem is further aggravated with the discovery of poly-T insertions in mitochondrial genes of *Oikopleura dioica* which renders mitochondrial genes practically unsequenceable by common sequencing methods. Special challenges in sequencing appendicularian genes in combination with very few available taxonomic experts has lead to a situation where there are practically no appendicularian population genetic studies and very few available *cox1* and 18S sequences.

In order to gain insight in appendicularian genetic diversity and phylogenetic relationships, we produced a comprehensive set of appendicularian 18S sequences as well as ITS1-5.8S-ITS2-28S fragment. Based on obtained data we re-assess the status of currently established genera and validity of some appendicularian subspecies. We also discuss problems in appendicularian sampling and some potential candidates for barcoding genes.

Keywords: Appendicularia, integrative taxonomy, barcoding

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First environmental DNA assessment of planktonic metazoan assemblages at a Mediterranean Long Term Ecological Research site

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Abstract

In the Omic-Era an increasing number of studies of nucleic acids from natural assemblages have provided new insights into the diversity of marine prokaryotes and protists. We used this approach to assess the diversity of metazoan assemblages in surface waters of the Long Term Ecological Research site MareChiara (LTER-MC) in the Gulf of Naples (Mediterranean Sea) over a three-year period (2011-2013). The Illumina sequencing of the V4-18S rDNA fragments from 48 samples revealed a total of 13,040,961 clean reads, of which 40% (5,243,851 reads) were assigned to metazoans. The Crustacea Maxillopoda represented 46% of these reads and were dominated by Copepoda (94%). Anellida accounted for 46% of the reads, with over two millions reads (98%) assigned to the polychaet *Hydroides elegans*, an invasive benthic species successfully established in the Mediterranean Sea. With the exclusion of this species, the reads assigned to meroplankton dropped to about 8% but showed a high diversity, covering 11 of the 14 different phyla, from Porifera to Craniata, found in the metazoan dataset. The results of this environmental DNA analysis expand our knowledge of zooplankton diversity in the coastal Gulf of Naples, highlighting the remarkable contribution of developmental stages of benthic organisms in the surface layer. This component of the metazoan assemblages is hardly identifiable with traditional morphological methods, which confirms metabarcoding as a powerful approach to be integrated in the monitoring at LTER sites.

Keywords: zooplakton, high-throughput DNA sequencing, biodiversity, LTER-MC site

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Can proteomic fingerprinting help identify zooplankton diversity?

Authors: Janna Peters, Silke Laakmann, Sven Rossel, Pedro Martínez Arbizu, Jasmin Renz

Abstract

Taxonomic knowledge is a key premise for reliable zooplankton monitoring. However, in many cases identification to species level remains difficult and too time-consuming for routine analysis. This is an issue even for many calanoid copepod species, despite their overwhelming abundance, wide distribution and pivotal position in marine food webs. In many regions, species of the genus *Paracalanus/Pseudocalanus*, *Acartia*, *Calanus*, *Metridia* or *Centropages* need to be grouped, specifically when it comes to juvenile life stages. The failure to adequately account for species identification may cause serious errors in estimations of zooplankton diversity, species-specific abundance and production, in zooplankton-mediated fluxes and trophic interactions as well as in shifts in species ranges and ecosystem status. Species discrimination and identification using specific mass profiles of peptides and small proteins (proteomic fingerprinting) are well established and applied in microbiology and bacterial diagnostics. A common method for this analysis is the matrix-assisted laser/desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS), a time- and cost-efficient alternative to existing molecular species discrimination techniques. Pilot studies on proteomic fingerprinting of metazoan taxa strongly suggest the possibilities to identify individual organisms on species level. Our study aimed to test the applicability of proteomic fingerprints to identify the most abundant copepod species in the ICES monitoring area.

Keywords: taxonomy, proteomic, diversity, copepods

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Diversity of echinoderm larvae in Svalbard waters based on DNA barcoding

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Abstract

Global change, especially pronounced in the Arctic, influences many marine ecosystems, and causes shifts in species distribution. It concerns especially planktonic organisms, which are under impact of water currents. Predicting results of these processes is difficult, as in order to understand it, the first step should be to describe biodiversity. This research focuses on pelagic larvae of Echinodermata, one of the meroplankton most abundant component. Similarity and small sizes of the larvae does not allow for the accurate morphological species identification. In this study, we use DNA barcoding (based on 16S rDNA) to assign larvae to the lowest possible taxonomic resolution. To obtain this goal, reference library of DNA sequences from adult organisms was created, thanks to which, sequences of the species that earlier were not deposited in GenBank were obtained. Phylogenetic tree of adult and larval sequences was created and as a result, we were able to identify species of Echinodermata larvae from several fjords in Svalbard Archipelago. Reference library contained following classes of Echinodermata: Echinoidea, Asteroidea, Holothuroidea and Ophiuroidea, however within larvae only Echinoidea and Ophiuroidea were found. Seven larval taxonomic units were found with strong domination of Ophiuroidea. The highest diversity of larvae was noticed in the cold-water northern fjords. In further research, we recommend monitoring of the Arctic harbor waters as with warming there is a possibility for species of more boreal origin to appear in this area.

Keywords: molecular identification, meroplankton, marine benthic invertebrates, 16S rDNA, larvae

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DNA metabarcoding as tool for zooplankton biodiversity monitoring: the case of the Venice lagoon

Authors:

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Abstract

The Lagoon of Venice is a mediterranean microtidal lagoon in the North Adriatic Sea, affected by an array of anthropogenic factors. It is part of the Long-Term Ecological Research network (LTER), a global monitoring network of research sites located in a wide array of ecosystems. Zooplankton plays a key role in marine trophic networks and its study has become a part of important monitoring projects such as European Marine Strategy Framework Directive (MSFD). Here we discuss the zooplankton biodiversity in the Venice lagoon and the nearby coastal area.

With the aim of verifying the reliability and suitability of DNA metabarcoding (a DNA based approach of taxonomic identification in multiple samples) for zooplankton monitoring programs in transitional waters, we compared the taxonomic profiles obtained by such genetic approach targeting the mitochondrial COI gene with those estimated by morphological screening.

Metabarcoding is less time consuming and more cost-effective than morphological screening, and it may become suitable for zooplankton biodiversity investigations, whenever high effort in terms of sampling frequency and number of sites is required.

Furthermore, our study indicates that the overall estimation of zooplanktonic biodiversity is much higher in metabarcoding data compared to morphological taxonomy data, as we are able to infer more species with this approach especially among cryptic species and meroplanktonic stages. The biodiversity estimation of copepods instead resulted much lower. As metabarcoding does also lack in providing information about population structure, we would suggest to combine both methods to get a more complete estimation of zooplanktonic biodiversity.

Keywords:

DNA metabarcoding, COI, zooplankton, lagoon, biodiversity, integrative taxonomy

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Assessment of cephalopod biodiversity and distribution around the Cape Verde Archipelago using environmental DNA

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Abstract

Traditional monitoring techniques (e.g. nets, in situ observations) are often expensive, invasive and ineffective at detecting rare or elusive species. The analysis of DNA directly from environmental samples (eDNA) is an increasingly used technique for biodiversity assessment and unravelling marine ecological processes. The technique is non-invasive, very sensitive, and only small amounts of DNA from water or sediment samples are necessary. eDNA could be a promising tool for the detection of cephalopod species that can escape approaching nets and cameras or that are not very abundant, but so far it has not been used in any cephalopod studies.

The aim of this study is to test and optimize eDNA analysis for biodiversity and distribution analysis of cephalopods and other pelagic invertebrates in oceanic ecosystems. During a recent R/V *Poseidon* cruise (journey POS520) in the eastern tropical Atlantic around the Cape Verde Islands, we isolated eDNA from seawater samples, collected at a series of three stations in depths between 50 and 1000 m, at three different stations (off Fogo, Santa Antão and north of São Vicente). Water was filtered and the filter with eDNA was then frozen at -80° C until PCR amplification of marker genes. We used next-generation DNA sequencing to sequence the PCR amplicons that were present in the samples. As a reference DNA barcode library we used tissues obtained from specimens captured in nets during the same cruise and from previous cruises, as well as sequences already available in GenBank. We investigated local cephalopod community compositions, and also looked at the distribution patterns of elusive species such as *Vampyroteuthis infernalis*, *Architeuthis* spp. and *Taningia danae*.

Tropical biodiversity assessment of shelf eukaryotic communities via DNA metabarcoding

Authors: Judith Bakker, Owen S. Wangensteen, Dayne Buddo, Demian D. Chapman, Austin Gallagher, Tristan L. Guttridge, Heidi Hertler & Stefano Mariani

Abstract

The understanding of marine communities and their functions in an ecosystem relies heavily on our ability to detect and monitor species distributions and abundances. However, one of the most critical issues in marine conservation is the lack of efficient and reliable tools to comprehensively assess and quantify biodiversity. Currently, the use of environmental DNA (eDNA) metabarcoding is increasingly being applied for the rapid assessment and monitoring of aquatic species. But this has thus far mostly pertained to the identification of a few species or groups simultaneously. However, the application of eDNA at a much broader taxonomic scale, such as the description of full marine eukaryotic communities, may be greatly beneficial for a more holistic perception of biodiversity and ecosystem functioning. Here we investigate the potential of eDNA COI metabarcoding, for the characterisation of the biodiversity of complex natural marine communities in tropical coastal shelf habitats. We screened 67 samples from five Caribbean locations and detected a high level of species richness. However, a disproportionally large number of eukaryote taxa remained unassigned, suggesting that the sampled communities host an astonishing amount of yet undescribed micro-eukaryotic diversity. Nonetheless, we were able to characterize marine eukaryotic diversity and spatial patterns across the five locations, and identify some key environmental drivers of the observed patterns.

Keywords: marine communities, pelagic ecosystems, environmental DNA, metabarcoding

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Molecules and morphology: two new and three well-known species of pelagic polychaetes from the Arctic Ocean

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Abstract

Pelagic polychaetes collected in the deep Eurasian Basin (Arctic Ocean) during the RV “Polarstern” cruises PS95 (September-October 2015) and PS101 (September-October 2016) were investigated morphologically and using the molecular analysis of mitochondrial 16S and nuclear 18 and 28S gene sequences. Two holoplanktonic species (*Pelagobia longicirrata*, Fam. Lopadorrynchidae and *Typhloscolex muelleri*, Fam. Typhloscolecidae), common for the Arctic Ocean, were identified in the studied collections. Both of them were collected in the upper mesopelagic, from the depths 0—800 m. *Phalacrophorus pictus borealis*, Fam. Iospilidae, was collected from the depths 0—4000 m. Basing both on morphological and molecular data, we suppose the validity of *P. borealis*, previously synonymized with *P. pictus*. Along with them we found two new species. The first of them belongs to the genus *Pelagobia*; it was collected from the bathypelagic layer 3000—3860 m. The second one was also collected from the bathypelagic depths (2000—4830 m) and belongs to genus *Bathypolaria*, Fam. Polynoidae. The morphological description of the new Polychaeta species found during our studies supplied with molecular data will help to produce more comprehensive species inventory and reference DNA database for Arctic Ocean biodiversity.

Keywords: Annelida, Polynoidae, *Austropolaria* sp. nov., Lopadorrynchidae, *Pelagobia* sp. nov., taxonomy, DNA 16S, 18S, bathypelagic, Arctic Basin.

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Sequencing, cytometry, and imaging provide complementary assessment of plankton communities in the MVCO time series

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Abstract

Sustained time series observations of plankton provide essential insight on responses of marine ecosystems to environmental change. If the appropriate taxonomic resolution is accessible, time series records can be used to quantify shifts in ecological phenomena such as population dynamics, phenology, and community structure. At the Martha's Vineyard Coastal Observatory (MVCO), we have collected multi-year records to compare information available from high throughput sequencing, flow cytometry (FlowCytobot, FCB), and imaging-in-flow cytometry (Imaging FlowCytobot, IFCB). Both FCB and IFCB are automated submersible instruments that have been used to provide long-duration (>10 years) high resolution (~hourly) records of taxonomically resolved plankton assessment at MVCO. In recent years, we have complemented the in situ observations with discrete samples (~monthly) collected for high throughput DNA sequencing (V6-V8 region of 16S rRNA gene for prokaryotes, V4 region of 18S rRNA gene for eukaryotes, and targeted portion of the SSU rDNA gene for ciliates). This presentation will highlight results for example taxa where combining two observational approaches provides deeper insight into the interactions between seasonality and population dynamics for the picocyanobacterium *Synechococcus*, seasonal aspects of diversity and likely feeding mode in Spirotrich ciliates, and ecological interactions between parasites and the chain-forming diatom *Guinardia delicatula*.

Keywords: plankton, time series, seasonality, diversity, cytometry, imaging, nucleic acid sequencing

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