In situ imaging reveals how morphology influences Copepod DVM.

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1 Introduction

- Diel Vertical Migration Importance and Background
- Challenges of
- Application of in-situ imaging
- Hypotheses:

In this study, we investigate the applicability of in-situ imaging data to study DVM behavior. We aim to test two hypotheses. First, (H1) Overall copepod morphology, including size and transparency, will impact DVM behavior. It is predicted that copepods which are more visually detectable, e.g. larger and darker, will have to migrate larger distances. Second, (H2) DVM behavior will vary based on environmental conditions related to the risks and rewards of occupying surface waters. Specifically, increased prey availability will increase a copepod's likelihood to travel to surface waters, while increased light availability will force copepods to deeper sections of the water column. To test these hypotheses we build on novel statistical tools to describe morphology of objects sampled by in situ imaging Vilgrain et al. (2021). Additionally we evaluate multiple approaches to describing DVM using in situ imaging data and how to estimate the impact of environmental factors on DVM behavior.

2 2. Methods

2.1 2.1 CTD profiles and UVP imaging of copepods

Data were collected aboard the R/V Atlantic Explorer in collaboration with the Bermuda Atlantic Time-series Study (BATS **cite debbie**). In situ images of plankton were acquired using an Underwater Vision Profiler (UVP5-DEEP, sn: 209, Hydroptic, (Picheral et al. 2010)). The original sampling methodology and insturment specification followed details described in (Barth and Stone 2022). A brief summary of new and relevant sampling methodology are described below. The UVP was attached to the CTD rosette aboard the R/V Atlantic Explorer. The UVP was equipped for monthly cruises into the Sargasso Sea from June 2019 - Aug 2019 and October 2020 - December 2021. A typical monthly cruise included ***XX*** profiles with descents typically to 1200m. Full cast details are available in Supporting Information ***XX***. Only profiles within the BATS region were included (Figure 1). Generally, as described in analyses below, it was assumed that profiles within the BATS region during a cruise sampled a similar water mass. While mesoscale features are known to drive variability in this region, there were no major variations within a cruise (Supplemental Figure 1). The CTD collected continuous environmental parameters on down-casts, including temperature (Dual SBE-03f, Sea Bird Scientific), conductivity (Dual SBE04, Sea Bird Scientific), dissolved oxygen (SBE43, Sea Bird Scientific) and fluorescence (Chelsea Instruments). Because the purpose of this study was to investigate day/night differences in copepod vertical distributions, it was important that there were day and night casts during each cruise. It would be ideal to investigate each profile, without pooling the entire region by cruise, however, the small sampling volume of the UVP and low abundance of plankton in this region create the need to pool or average profiles (Barth and Stone 2022).

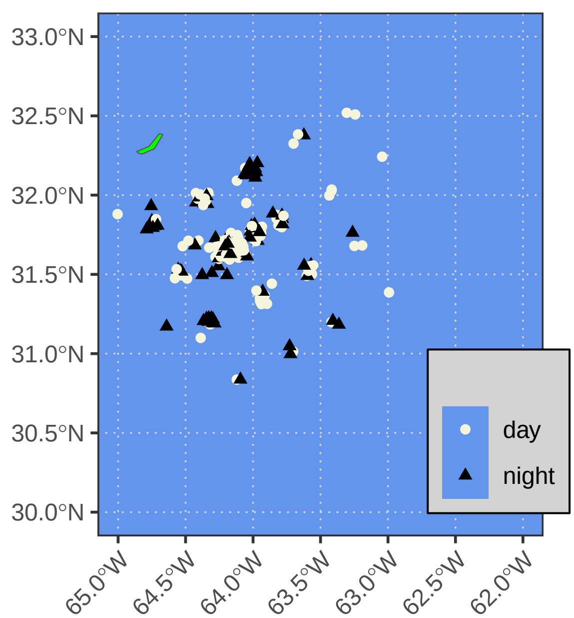

Figure 1: Figure 1. Map of CTD and UVP profiles used in the study. Day and night points from all cruises are shown. Bermuda is shown in green.

The UVP sampled during down-casts of all profiles at a rate of ~15Hz. The UVP records counts of small particles (>184μm Equivalent Spherical Diameter, ESD) and images of large particles (>600μm ESD). However, living particles are not reliably identifiable below 1mm (Barth and

Stone 2022). In the present study, only copepods were investigated. The smallest observed and identified copepod was **xx** mm ESD and the largest was ***xx*** mm ESD. Across all casts, copepods were the most common observed organism at 58.7% of all identified, living particles. Each individual image automatically gets recorded with several morphological parameters related to size, grey value

2.2 2.2 Identification of morphogroups classification

To test the hypothesis of morphological influences on DVM magnitude, we first characterize copepods into distinct morphological-groups (referred to as morphogroups). The UVP automatically measures and collects several morphologically relevant

2.2.1 2.2.1 PCA

2.2.2 2.2.2 K-mean clustering

To select the optimal number of clusters, clusters were added until addition centers did not increase the overall explanatory power $\frac{WithinSS}{TotalSS}$ by more than 10%.

2.3 2.3 Investigating Vertical Structure

Copepods in this system show a clear diel vertical migration. However, it is not clear the maximum depths at which migrations are often done.

2.4 2.4 Quantifying DVM variability across morphogroups

2.5 2.4 Occupancy modelling to investigate drivers of vertical variability.

One challenge from the UVP is the fact that the low sampling volume can lead to inaccurate estimates of true concentration **site bisson**. This problem is compounded when organisms are scarce. In the Sargasso sea, copepod abundances are particularly low **site**. When investigating specific morphotypes, abundances are even more scare as copepod observations are split categorically into new groups. Therefore estimates of copepod abundances, and their response to environmental drivers, are difficult to adequately measure with the UVP. This particular challenge however, can be addressed with an application of site-occupancy models. These models are a hierarchical Bayesian model which allows for the estimation of true occupancy (e.g. the organism of interest actually is occurring in the observed area) and the detection probability. Site-occupancy models have been increasingly popular in terrestrial systems **cite** and offer great potential for analyses of in-situ images with low sampling volumes.

Site occupancy models use a hierarchical model structure to model both the probability a location is occupied by the organism of interest and the probability the organism is detected by researchers. Both the occurrence probability (psi) and detection probability (p) can be separately estimated. To best utilize these models, "sites" must be measured in replicates, or across multiple "surveys." Thus, occupancy probability can be measured in context of covariates across sites while detection probability can be measured in context of covariates across both sites and surveys. This approach is increasingly popular in terrestrial ecology and management work, however has not be implemented in marine ecology. **notes about differences between marines systems that limit this approach**. For this study, we treat each cruise as a "site" and profile during a cruise as a replicate "survey" to that site. By treating each profile as a survey, it effectively pools profiles across one large area, similar to what is done in many UVP studies. However, this approach assumes that observation differences among casts on a single cruise are driven by detection probability rather than occurrence probability.

The model

2.5.0.1 2.4.1 Detetction Probability Estimation

It is clear that volume sampled will have a direct effect on the detection probability. However, it was not clear if detection probability would vary with depth or time of day. Ideally, detection probability should not vary based on time of day. However, **Barth&Stone(2022)** displayed UVP avoidance behavior of large, mobile zooplankton. It was hypothesized this avoidance is driven by avoidance of the CTD rosette. If copepods are visually detecting the CTD rosette,

To test the variability of detection probability with depth and time of day, occupancy models were ran for each.

2.5.0.2 2.4.2 Environmental Data Formatting

Several metrics were used to evaluate environmental drivers of variability. Ohman and Romagnan (2016) showed a clear relationship between the diffuse attenuation coefficient and diel vertical migration magnitude. Photosynthetically available radiation (PAR) and the diffuse attenuation coefficient (1/k490) were calculated using data from the Aqua MODIS **insert more satellite detail**. Data from the Aqua MODIS were obtained in 4km resolution over a grid which encompassed the entire study area (MAP 1) over daily intervals. PAR and diffuse attenuation coefficient values were averaged over the entire study area for each day. Average values were then matched to specific UVP/CTD casts which occurred on the given day. For a few cases (34 total days), AquaMODIS coverage was not available for the exact date of a corresponding CTD cast. In these instances, the nearest available date was used, with a maximum of 3-days difference. All environmental factors were averaged over a cruise, separating day/night. These cruise-average environmental variables were then able to be used as site covariates in an occupancy model. test (Gastauer et al. 2022).

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