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Diel and Artificial Light Effects on Nearshore Communities Revealed by eDNA Metabarcoding

Kingsly Chuo Beng^{1,2,3}  | Gledis Guri⁴  | Yin Cheong Aden Ip⁴ | Pedro F. P. Brandão-Dias⁴ | Elizabeth Andruszkiewicz Allan⁴ | Diana A. Pazmiño^{5,6} | Adam Brink⁷ | Mei-Hong Zhao⁸ | Muhammad Danie Al Malik⁹  | Fraulein Jan O. Calumpiano¹⁰ | Natalie A. Sawaya¹¹ | Ouattara Koffi Nouho¹² | Abahi Koudjodé Simon¹³ | Florencia Bertoglio¹⁴ | Suzana de Moura Pereira¹⁵ | Yingbei Peng⁸ | Melvie Aulya⁹ | Marianela Veyñ^{16,17}  | Ryan P. Kelly⁴ 

¹Thünen Institute of Sea Fisheries, Bremerhaven, Germany | ²Helmholtz Institute for Functional Marine Biodiversity at the University of Oldenburg (HIFMB), Oldenburg, Germany | ³Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research (AWI), Bremerhaven, Germany | ⁴School of Marine and Environmental Affairs, University of Washington, Seattle, USA | ⁵Galápagos Science Center (GSC), Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Galápagos, Ecuador | ⁶Migramar, Olema, California, USA | ⁷Harbor Branch Oceanographic Institute, Florida Atlantic University, Ft. Pierce, Florida, USA | ⁸Department of Biology, Hong Kong Baptist University, Hong Kong, SAR, China | ⁹Diponegoro Biodiversity Project (DBP), Universitas Diponegoro, Semarang, Indonesia | ¹⁰Marine Science Institute, University of the Philippines, Quezon City, Philippines | ¹¹College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, USA | ¹²Laboratory of Environmental and Aquatic Biology, Nangui Abrogoua University, Abidjan, Côte d'Ivoire | ¹³Laboratoire d'Ecologie, de Santé et de Productions Animales, Faculté d'Agronomie, Université De Parakou, Benin | ¹⁴Departamento de Microbiología, Centro de Investigación en Ciencias Ambientales, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay | ¹⁵School of Health and Life Sciences, Pontificia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil | ¹⁶Centro Austral de Investigaciones Científicas (CADI-CONICET), Laboratorio de Ecología, Fisiología y Evolución de Organismos Acuáticos (LEFyE), Ushuaia, Argentina | ¹⁷Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina

Correspondence: Kingsly Chuo Beng (kingsly-chuo.beng@thuenen.de)

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ABSTRACT

Changes in natural and artificial light presumably have dramatic influences on ecological communities, yet measuring these effects can be challenging due to the difficulty in distinguishing ecological signals from background variability. Here, we used environmental DNA (eDNA) to test for light-associated changes in a nearshore marine community. Over 8 days at Friday Harbor Laboratories, Washington, USA, we sampled seawater at midday and midnight with and without an artificial light treatment, analyzing a total of 84 samples (3 L triplicate samples) using COI and 12S markers on an Oxford Nanopore MinION platform. We detected 229 eukaryotic (COI) and 77 vertebrate (12S) taxa across 11 trophic groups, dominated by primary producers (89 species), benthic invertebrates, and fishes. Metabarcoding captured community restructuring within 30 min, such that we could observe meaningful effects of artificial light treatments at night (particularly among spawning polychaetes), as well as more fundamental community shifts in day vs. night (e.g., with primary producers, gelatinous zooplankton, and filter feeders more common during the day). These techniques offer a means of near-real-time assessment of wholesale changes in ecological communities, and our results here illustrate species- and group-specific effects of light levels that are likely important in structuring nearshore marine communities worldwide.

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1 | Introduction

Light is one of the most fundamental drivers of ecological dynamics in marine ecosystems, influencing energy flow, species interactions, and ecosystem structure (Stibor and Stockenreiter 2023). Natural diel cycles of light and darkness influence predatory-prey interactions, feeding behavior, and movement patterns across a broad range of taxa (Häfker et al. 2022; Hobbs et al. 2021; Storrie et al. 2022). In nearshore environments, where illumination shifts rapidly over the course of a day, species respond with clear and pronounced diel activity rhythms (McFarland 1986; Yeoh et al. 2017). For example, zooplankton often become more active or surface-oriented at night, small fishes may reduce predation risk by seeking shelter during the day, and benthic invertebrates frequently emerge under low-light conditions (Hinojosa et al. 2021; Hobbs et al. 2021).

Artificial light at night (ALAN), such as pier/dockside lighting, can modify these patterns by attracting plankton and forage fishes, while also altering predator distributions and overall community structure (Becker et al. 2013; Bolton et al. 2017; Stanton and Cowart 2024). Understanding how light regimes shape marine communities is crucial for interpreting fine-scale biodiversity patterns in coastal systems and assessing the ecological consequences of light pollution in increasingly illuminated coastal environments. This is particularly important in areas such as coastal ecosystems, known to provide a wide range of ecosystem services (Christoforou et al. 2023).

Studying changes in ecological communities between day and night is particularly challenging because most observational methods require interventions that can themselves alter the patterns being measured. For example, counting individuals in a darkened environment typically requires artificial illumination, and traps or nets can disturb behavior or attract organisms artificially (Brown et al. 2023). Environmental DNA (eDNA) metabarcoding provides a unique opportunity to detect fine-scale community patterns (Turon et al. 2022) and furthermore to explore these diel dynamics by detecting a wide array of species simultaneously, spanning vertebrates, invertebrates, and plankton (Beng and Corlett 2020). This approach offers a broad, non-invasive snapshot of biodiversity that captures molecular traces of organisms without directly disturbing them, providing a complementary perspective on community composition across both day and night conditions. This makes eDNA particularly valuable for assessing diel and light-driven shifts in shallow nearshore ecosystems, circumventing many of the taxonomic or methodological constraints of traditional surveys. However, few studies have found that artificial light disrupts natural diel cycles, resulting in shifts in marine community structure at fine temporal scales (Becker et al. 2013; Bolton et al. 2017; Marangoni et al. 2022; Stanton and Cowart 2024).

Despite the opportunities it provides, eDNA metabarcoding also comes with potential limitations for resolving short-scale ecological changes, given the inherent separation between organism presence and DNA detection. Organisms must first shed DNA before it can be captured in a water sample, creating a small lag between organism presence and detectability; similarly, molecular traces can persist after organisms have moved, meaning eDNA

in effect “trails behind” biological activity (Harrison et al. 2019). Additionally, eDNA can be transported away from its source in nearshore environments, ranging from tens of meters (Ely et al. 2021; Murakami et al. 2019) to hundreds of meters or even several kilometers (Ellis et al. 2022; Shea et al. 2022; Xiong et al. 2025), potentially carrying signals from outside the sampled area. Nonetheless, decay in dynamic nearshore systems is rapid, with half-lives of approximately 4–6 h (Brandão-Dias et al. 2025). Such rapid turnover means that local DNA production generally outweighs lagged or advected DNA, and recovered eDNA is largely endogenous to the sampled area (Kelly et al. 2018; Lamy et al. 2021; O'Donnell et al. 2017). Indeed, recent work has demonstrated that eDNA can reflect diel vertical migration and associated redistribution of fish biomass, e.g., using species specific assays (Trenkel et al. 2025) and multi-species metabarcoding (Canals et al. 2021; Easson et al. 2020; Feng et al. 2022; Govindarajan et al. 2021; Govindarajan et al. 2023). However, the extent to which eDNA resolves such fine-scale temporal dynamics depends on environmental context. In some systems, particularly during winter in open ocean environments, increased eDNA persistence and reduced degradation can smooth over diel signals (Deeg et al. 2023). Consequently, seasonal conditions, microbial activity, and other factors influencing eDNA degradation rates can modulate the temporal resolution of eDNA signals, and thus affect the detectability of diel dynamics.

Shallow nearshore systems, like the waters surrounding Friday Harbor Laboratories (FHL) on San Juan Island, Washington, provide a natural setting for studying these questions. At depths of approximately 4 m, diel changes in detected communities are unlikely to result from classical deep-water vertical migration. Instead, differences are expected to arise primarily from light-mediated behavior: benthic and demersal organisms may alter their microhabitat use, zooplankton may redistribute within just a few meters of the water column, and small fishes may cluster near artificial light sources (Berge et al. 2020). These fine-scale behavioral responses can be captured through eDNA, providing insight into community structure in ways that traditional sampling might miss.

We addressed three central ecological questions: (i) can eDNA be used to detect short-term (30 min) changes in marine community composition in response to natural diel cycles and artificial illumination? (ii) what are the effects of elapsed time between samples, diel phase (day vs. night), artificial light treatment, and biological replication on community dissimilarity? and (iii) within community, how do taxonomic groups differ in their response to changes in ambient light conditions? We predicted that: (1) eDNA would capture compositional shifts within short time scales (30 min); (2) diel phase (day vs. night) and nighttime artificial light would be the dominant drivers of community dissimilarity; (3) predatory fish would increase in abundance under artificial light at night due to attraction to prey aggregations, while prey fish species would decline as a result of predator avoidance; (4) zooplankton and mobile invertebrates would exhibit elevated abundances driven by positive phototaxis, while benthic invertebrates would decrease in response to predator avoidance under artificial light; and (5) phytoplankton would remain primarily day-associated due to photosynthetic requirements regardless of light treatment.

2 | Methods

2.1 | Study Area and Sample Collection

The study was conducted at Friday Harbor Laboratories (FHL), located on San Juan Island, Washington, USA. All samples were collected in a single location, within the waters adjacent to the FHL dock (48.54525, -123.01212). Environmental DNA (eDNA) sampling was carried out from July 27 to August 3, 2025, twice daily—midday (11:00–13:00) and at night (22:00–00:00). Each sampling event included two treatments: light and no light, conducted sequentially to avoid spatial overlap of light conditions. For each sampling period, we first collected the 'no light' treatment samples under ambient conditions (natural daylight at midday, or natural darkness at night). After completing the no-light sampling, an underwater light source (bright white 12V

LED at ca. 15,000lm) was deployed at the surface, positioned ~1m from the sampling point, and switched on 30 min prior to the 'light' treatment sample collection to allow community responses to stabilize (Figure 1). For each treatment, triplicate 3.0 L of surface seawater were collected in sterile 3 L Nalgene Cantene carboys and filtered immediately on site through 5.0 μ m Sterlitech MCE filters (Sterlitech, Kent, WA, USA) using a Smith-Root Citizen Science vacuum pump (Smith-Root, Vancouver, WA, USA). Filter membranes were placed in 1.0 mL DNA/RNA Shield (Zymo Research, USA), transported at room temperature, and stored in the fridge within one hour of collection. Negative controls (3 L of tap water) were processed during each sampling event to monitor potential contamination. A total of 84 seawater samples and 14 negative controls were taken throughout the entire study. All sampling equipment was cleaned with 10% bleach and rinsed with tap water between uses.

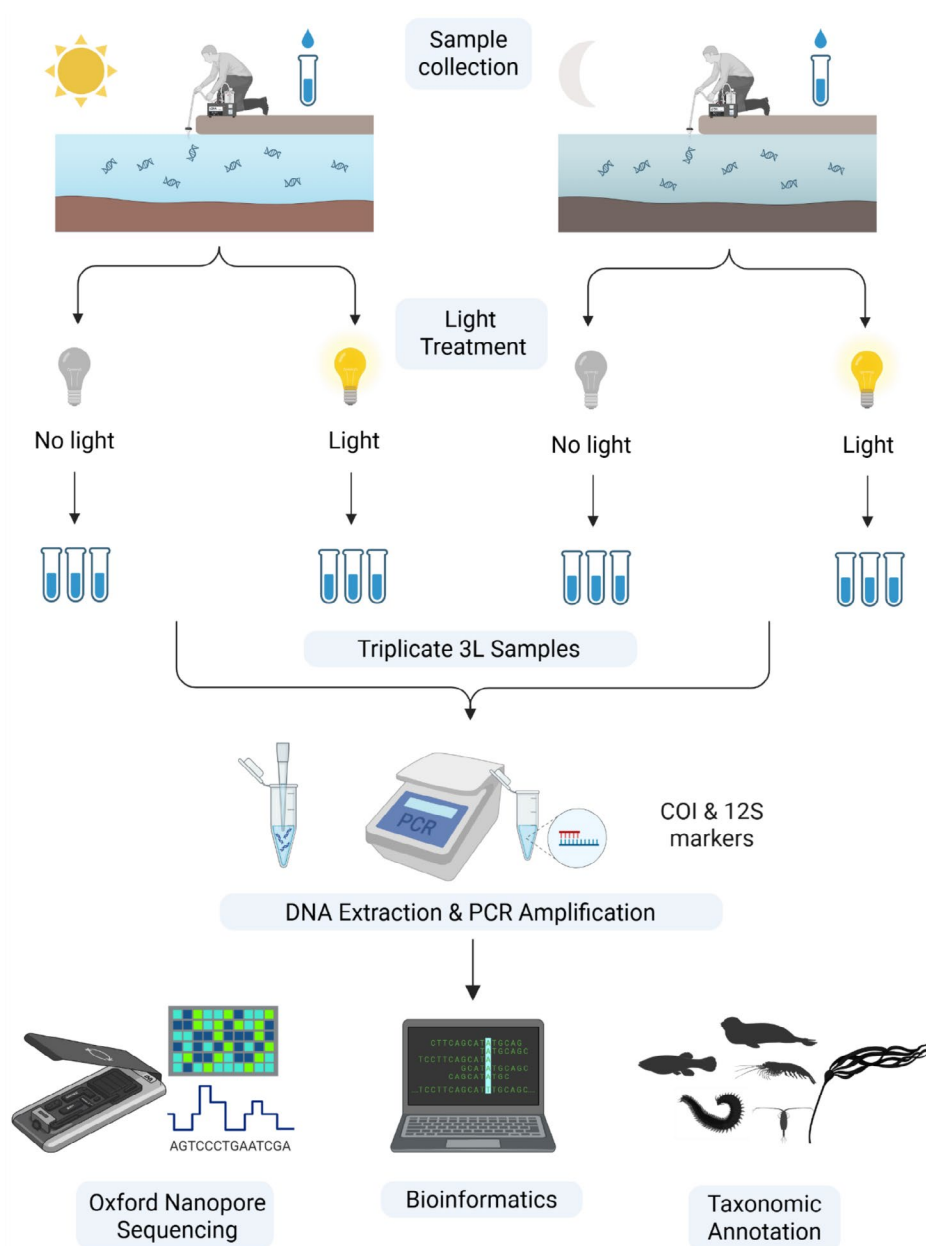


FIGURE 1 | Schematic representation of the study/experimental design.

2.2 | Laboratory Processing and Sequencing

DNA extraction, library preparation, and sequencing were performed according to the protocol outlined in Ip et al. (2026). Briefly, filters were thawed at room temperature, vortexed, and 300 µL of the DNA/RNA Shield preservative was used directly for DNA extraction with the QIAgen DNeasy Blood & Tissue Kit, following the manufacturer's protocol, except for elution in 60 µL of Molecular Grade water. Extracted DNA was quantified using Qubit High-Sensitivity assays and stored at 4°C until amplification. Two primer sets were used to target distinct genetic regions: MarVer1 (MV1; Valsecchi et al. 2020) targeting the 12S region of vertebrates and mCOIintF/LoboR1 targeting the COI region of eukaryotes (Chang et al. 2020; Leray et al. 2013; Lobo et al. 2013) (Table S1). PCR reactions were carried out on three biological replicates, and purified with AMPure XP beads. PCR primers incorporated sample-specific custom barcode tags, enabling single-step barcoding during amplification and eliminating the need for a separate native barcoding ligation step (Ip et al. 2026). Barcoded amplicons from all samples were pooled by marker, and sequencing libraries were prepared using the Oxford Nanopore SQK-LSK114 ligation kit. Each marker was sequenced on a separate FLO-MIN114 (R10.4.1) flow cell on a MinION Mk1D device using MinKNOW v25.05.12 with integrated Dorado v7.9.8 for real-time Super-accurate (SUP) base-calling at a minimum Q-score of 10 (<https://github.com/nanoporetech/dorado>). Each flow cell carried 95 barcoded amplicons (84 field samples, 6 field negatives, 4 extraction negatives, and 2 PCR negatives). The 12S run (~41 h) generated 20.1 million reads (9.5 Gb; 18.5 million pass), and the COI run (~46 h) generated 19.4 million reads (10.3 Gb; 20.2 million pass).

2.3 | Bioinformatics Analysis

Nanopore sequence data were processed following the pipeline of Ip et al. (2026). Raw POD5 files were basecalled real-time during sequencing using Dorado v7.9.8 in Super-Accurate (SUP) mode at 400 bps. Only samples that successfully amplified via PCR and generated usable sequencing reads were retained for downstream analyses. Samples that failed at either the PCR or sequencing stage were excluded, resulting in 63 COI and 74 12S samples for subsequent analyses. FASTQ reads were demultiplexed, and primers and barcodes were trimmed using the OBIMultiplex module of OBITools4 (<https://github.com/metabarcoding/obitools4>), implementing in-del aware settings, allowing up to two mismatches per primer tag and splitting self-ligated concatemers. Within-sample dereplication and clustering were performed with VSEARCH (Rognes et al. 2016) at 98% identity to generate representative OTU centroids. An initial OTU table of read counts was curated using LULU (Frøslev et al. 2017) to remove artifactual OTUs based on sequence similarity and co-occurrence. Filtered OTU centroids were assigned taxonomy via BLASTn (Camacho et al. 2009) against the NCBI eukaryote database ($\geq 96\%$ identity, $e\text{-value} \leq 1e-40$), and the lowest common ancestor (LCA) was determined with TaxonKit (Shen and Ren 2021). Manual curation ensured ecological plausibility, collapsing ambiguous OTUs to genus level when necessary and removing singletons or residual sequencing errors. Background contamination was quantified using the *microDecon* package in R (McKnight et al. 2018) by estimating the mean contaminant

signal from the negative controls and proportionally subtracting it from each true sample based on relative read abundance. The final dataset comprised 80 vertebrate taxa for 12S across 74 eDNA samples, and 265 OTUs for COI across 63 eDNA samples.

2.4 | Taxonomic Annotation

To enable ecological interpretation of eDNA-derived community patterns, all detected taxa were annotated with standardized taxonomy and ecological attributes. Taxon names were cross-checked against the World Register of Marine Species (WoRMS; Ah Yong et al. 2025) to obtain validated classifications and AphiaIDs for marine-only species. Species that did not match marine-only lookup were removed from downstream analyses. For unresolved taxa (e.g., “sp.” or “cf.” designations), names were simplified, and recursive lookups were performed to maximize successful matches. Species were classified into functional ecological groups using a custom hierarchical classification algorithm implemented in R (functionalgroups, R), with taxonomic validation supported by the World Register of Marine Species (WoRMS; <https://marinespecies.org/traits/aphia.php?p=attrdefinitions>) accessed through the WoRMS R package (<https://cran.r-project.org/web/packages/worms/vignettes/worms.html>). The classification function assigned each species to one of 17 trophic levels (including primary producers, parasites, pathogens, symbionts, apex predators, mesopredators, forage species, carnivorous invertebrates, gelatinous zooplankton, planktivores, filter feeders, herbivores, detritivores, scavengers, benthic invertebrates, benthic crustaceans, and decomposers) based on taxonomic information at species or higher taxonomic levels, using a priority-based decision tree with pattern matching on taxonomic names (species → genus → family → order → class → phylum). For example, organisms in phylum Rhodophyta or class containing “phyceae” were assigned to “Primary producers”, while families Caligidae or Pennellidae were classified as “Parasites” and genera like *Salmo* or *Oncorhynchus* were designated “Apex predators”.

2.5 | Statistical Analyses

All analyses and visualizations were performed in R (R Core Team 2022). For all downstream community analyses, read counts were transformed to eDNA index (Kelly et al. 2019), which controls for species-specific amplification bias of the primers used (for community ecology application advantages see Guri et al. 2024). After transformation, we used the Bray-Curtis distance matrix to measure the dissimilarity between pairwise samples, which we will denote here with D . To quantify the magnitude of treatment effects on community dissimilarity, we constructed a beta regression analysis with the form:

$$D \sim \text{Beta}(\mu, \varphi) \quad (1)$$

$$\text{logit}(\mu) = \eta + \gamma d + (1 + \alpha t + \beta_1 I_1 + \beta_2 I_2) + \theta r \quad (2)$$

where D is the pairwise community dissimilarity as a response variable, measured predictor variables are given in Latin characters, and estimated parameters are given in Greek characters. Here, μ is the mean of the beta-distributed dissimilarity (D)

TABLE 1 | Design matrix included in the statistical model (Equation 2). Binary indicator variables (t , l_1 , l_2 , r) determine which coefficients are included in the linear predictor for each set of pairwise comparisons. A value of 1 indicates inclusion of the corresponding parameter, while 0 indicates exclusion. Rows with $t = 1$ and l_1 or $l_2 = 1$ are omitted to avoid interaction between daytime and light treatment, allowing estimation of their independent (marginal) effects. The variable d represents elapsed time (days), and n is the number of pairwise comparisons.

Elapsed time	Daytime	Light (day)	Light (night)	Bio. Rep.	Number of comparisons	Parameter estimated	Remark
d	t	l_1	l_2	R	n		
0	0	0	0	1	56	θ	Included
1–6	0	0	0	0	429	γ	
0–7	1	0	0	0	496	γ, α	
0–6	0	1	0	0	320	γ, β_1	
0–6	0	0	1	0	176	γ, β_2	
0–6	1	0	1	0	271	NA	Excluded
0–6	1	1	0	0	205	NA	

conditional on the covariates (constrained to (0,1) through the logit link), and ϕ is a precision parameter that governs the variance of D (with larger ϕ indicating lower variance). Importantly, the covariates t , l_1 , l_2 , and r are binary indicator (0 and 1) variables within the design matrix (Table 1 and Table S2), such that a value of 1 activates (includes) the corresponding coefficient in the linear model, whereas the value of 0 excludes it. In this way, each row of the design matrix specifies which parameters contribute to a given pairwise dissimilarity comparison (Table 1 and Table S2).

Specifically, d represents elapsed time between pairs of samples (in days), while t encodes differences in day phase (1 = day vs. night; 0 = same time phase), and r indicates biological replicate (where all the other parameters d , t , l_1 , $l_2 = 0$). Light treatment is represented in two indicator variables l_1 and l_2 , for comparing light effect when the pairwise comparison is within same time phase ($t = 0$) corresponding to treatment effect during day and night respectively (1 = light vs. no light, 0 = same treatment). Coefficients (η , γ , α , β_1 , β_2 , θ) then reflect the estimated effect of each of these covariates on the observed dissimilarity matrix. Note that β_1 and β_2 —the effect of light treatment—are nested within each sampling phase linking the treatment effect specifically to day (l_1) or night (l_2). The statistical model was fitted using the *betareg* function in the *betareg* package in R (Cribari-Neto and Zeileis 2010), which implements a beta regression consistent with the response variable here (ecological distance, which is constrained between zero and one).

3 | Results

3.1 | Sampling Effort and Sequencing Depth

After sequencing, 63 samples (36 for day vs. 27 for night) were retained for COI analysis and 74 samples (37 for day vs. 37 for night) for 12S analysis (Table S1). Sequencing yielded over 20 million raw reads for COI and over 18 million for 12S. Following bioinformatic filtering, 36.7% and 25.5% of raw reads were retained for COI and 12S, respectively, with target organism assignment rates of 32.9% for COI and 70.6% for 12S.

Rarefaction and species accumulation curves were generated for each dataset. For the COI dataset, rarefaction curves reached near-asymptotic levels across all samples, demonstrating that sequencing depth was adequate and did not constrain the ability to detect taxonomic diversity in the study area (Figure S1). Species accumulation curves also approached saturation, indicating that sampling effort successfully captured the vast majority of taxa present, with further sampling likely to contribute only marginal additions to observed diversity. For the 12S dataset, rarefaction curves similarly approached asymptotes across all samples, confirming sufficient sequencing depth for detecting taxonomic diversity (Fig. S2). Species accumulation curves likewise tended toward saturation, suggesting that the sampling effort adequately captured most taxa present, with additional sampling expected to yield only minor increases in detected diversity.

3.2 | Community Dissimilarity Across Time and Light Conditions

Overall, both markers (COI and 12S) indicated consistent patterns in the factors driving community dissimilarity (Figure 2). The sampling period (day vs. night) and the light effect during night sampling emerged as the strongest drivers of community dissimilarity in both datasets ($p < 0.001$). The estimated logit-scale effects in increased dissimilarity for the sampling period were 0.169 and 0.152 for COI and 12S respectively, while the nighttime light treatment increased community dissimilarity by 0.111 and 0.278 in COI and 12S respectively (Table 2, Figure 2). The global intercepts (η) differed slightly between markers with -0.692 and -0.346 logit-scale effect sizes (equivalent of 33.3% and 41.5% dissimilarity) respectively for COI and 12S. For both markers, the pairwise dissimilarity decreased among biological replicates (-0.205 and -0.361 logit-scale effect sizes for COI and 12S respectively), indicating higher replicability in community composition within the same group of samples. During daytime sampling, artificial light treatment was associated with a decrease in community dissimilarity relative to the intercept (COI: -0.083 ; 12S: -0.160 ; $p < 0.01$), indicating that artificial light treatment did have an effect on community composition (Figure 2A,B). Lastly, the temporal autocorrelation effects were

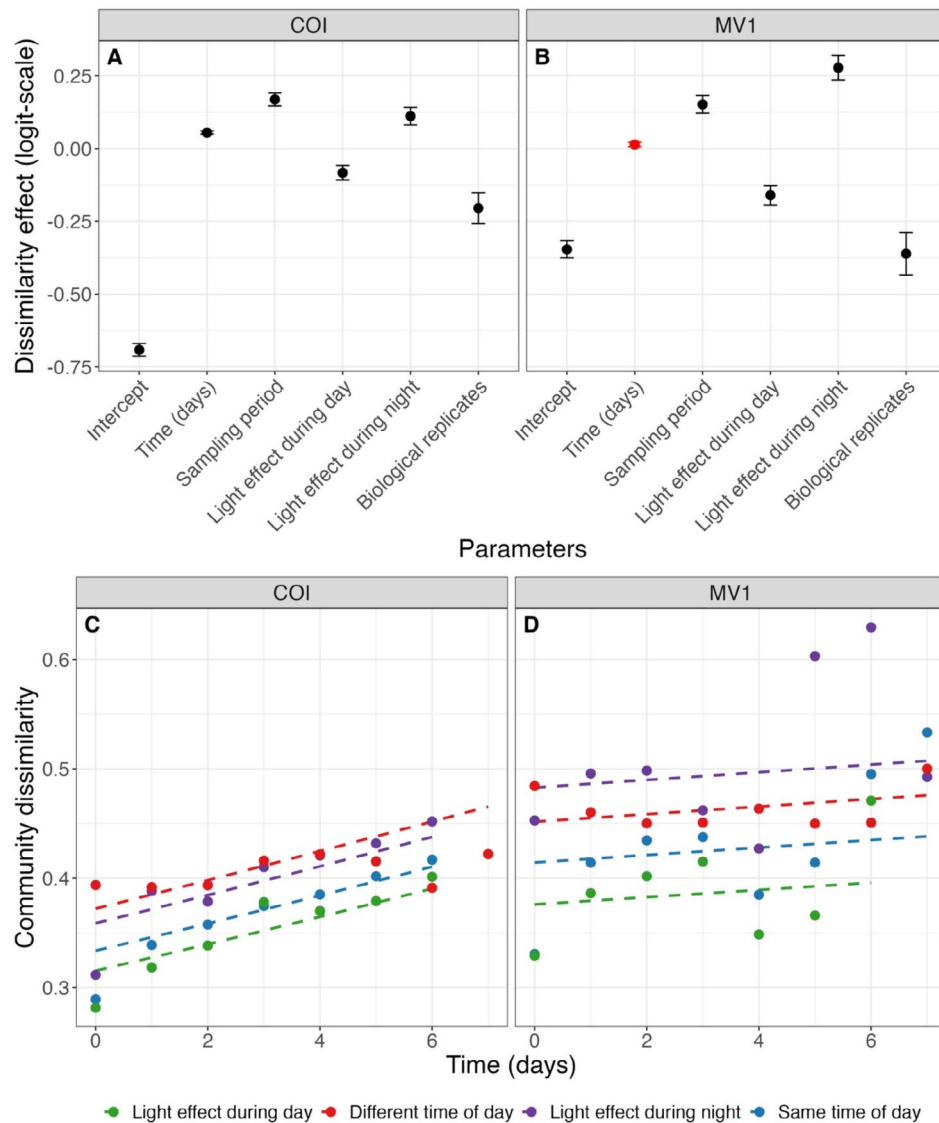


FIGURE 2 | Estimated coefficients (logit scale) of time, sampling period, light treatment, and biological replicates contributing to pairwise community dissimilarity with significant values ($p < 0.05$) in black and non-significant values in red (panels A and B). Predicted (dashed line) and observed (dots) relationship between the pairwise community dissimilarity and time (in days), including the factors such as sampling period (time of day) and the artificial light separately during day and during night; panels (C and D). Panels (A) and (C) show results for the COI marker; panels (B) and (D) show results for the 12S marker.

different between markers. Although both markers indicated an increase in dissimilarity across time, the effect was greater for COI (0.055 logit-scale effect sizes) than for 12S (0.014 logit-scale effect sizes), indicating that as days progressed, the 12S community became more dissimilar but the rate of dissimilarity was smaller compared to that of the COI community (Table 2).

3.3 | Taxonomic, Functional, and Species-Specific Diel-Light Responses

A total of 190 taxa were successfully annotated and used for the analysis of diel and light-associated responses, comprising 156 COI taxa and 34 12S taxa. The COI marker detected nine trophic groups, of which only six had more than one species, while the 12S marker captured three vertebrate-dominated trophic groups. Across both markers, the combined dataset encompassed eight major functional and trophic groups, representing

TABLE 2 | Beta regression model estimates for factors influencing community dissimilarity (Bray–Curtis distance) based on COI and 12S eDNA markers with their respective p -values.

Parameter	COI		12S	
	Estimate	p	Estimate	p
Intercept (η)	−0.692		−0.346	
Time (γ)	0.055	<0.001	0.014	0.053
Sampling period (α)	0.169	<0.001	0.152	<0.001
Light effect during day (β_1)	−0.083	<0.001	−0.160	<0.001
Light effect during night (β_2)	0.111	<0.001	0.278	<0.001
Biological replicate (θ)	−0.205	<0.001	−0.361	<0.001

a broad spectrum of ecological roles from primary producers to apex predators. Primary producers (89 species) were the most species-rich group, showing a strong association with daytime and no-light conditions, with an average eDNA index difference of 0.15 in favor of daytime samples (Figure 3). The dominant taxa—*Micromonas pusilla* (green alga), *Minutocellus* sp. (diatom), and Noelaerhabdaceae (coccolithophore)—all exhibited diurnal activity but variable light sensitivities, perhaps indicating intra-guild niche partitioning. Similarly, diatoms (*Thalassiosira nordenskiöldii*, *Chaetoceros socialis*) maintained day-associated patterns and light avoidance, whereas *Heterocapsa rotundata* (a dinoflagellate) was one of few phytoplankton showing nocturnal preference. Most taxa in this group displayed some degree of light avoidance at night, while daytime responses varied slightly among species (Figure 3). Several other groups—including gelatinous zooplankton, filter feeders, forage vertebrates, and a subset of planktivores—showed diurnal patterns consistent with those of primary producers (Figure 3). Across these groups, only a small number of species exhibited significant positive responses to illumination (Figure 3). Two jellies, *Phacellophora camtschatica* (Scyphozoa) and *Stomatoca atra* (Hydrozoa), were the only taxa that showed consistently positive responses to light, with eDNA index increases of 0.190 and 0.100 under illuminated daytime conditions and 0.110 and 0.0852 at night, respectively. *Aurelia* (Scyphozoa) also responded positively to light at day, showing a mean increase of 0.140, but displayed a negative response during the night (−0.111). In contrast, *Lafoea* (Hydrozoa, with demersal adults) showed opposite patterns, with a positive nighttime response (0.106) and a slight negative effect under daytime illumination (−0.077). Among filter feeders, *Nuttallia* (Bivalvia) exhibited a positive response to light during the day (0.161), whereas *Macoma nasuta* (Bivalvia) responded positively to nighttime illumination (0.0903; likely larval detections from the water column). Among the remaining trophic groups, *Acartia longiremis* (planktivorous copepod) and *Clupea pallasii* (forage fish) were the only species that showed positive responses to nighttime light effects, with mean eDNA index increases of 0.154 and 0.202, respectively.

Benthic invertebrates (27 species) exhibited highly heterogeneous behaviors (Figure 3). Polychaetes such as *Glycinde*, *Glycera americana*, *Glycera robusta*, and *Laonice* were more abundant at night. Polychaetes *Diopatra* and *Alitta* also peaked at night, although the former decreased and the latter increased under illuminated nighttime conditions. In contrast, *Leodice valens* (Polychaeta), *Micropodarke dubia* (Polychaeta), and *Strongylocentrotus* (urchin) were more common during the day, with the latter two showing even stronger daytime abundance when lights were on. *Sabellariidae*, *Nudibranchia*, and *Platynereis bicanaliculata* (Polychaeta) avoided light at night, whereas *Paleanotus* (Polychaeta) showed the opposite pattern. The remaining benthic invertebrate species exhibited no significant diel or light-related effects.

In the vertebrate dataset (12S marker), apex predators were detected predominantly during the day, showed a slight preference for no-light conditions during daytime sampling, and exhibited a modest increase in abundance under illuminated conditions at night (Figure 3). Mesopredators displayed a wide range of behaviors (Figure 3). *Pholis ornata* was more abundant during the day (+0.108), whereas *Aulorhynchus flavidus*, *Gadus*, and *Artedius harringtoni* were more prevalent at night (−0.118, −0.141,

and −0.123 mean eDNA index change respectively), with the latter two showing higher eDNA indices under nighttime illumination (0.0958 and 0.117 mean eDNA index change). Additional mesopredators, including *Nautichthys oculofasciatus*, *Pholis ornata*, and *Phytichthys chirus*, exhibited increased eDNA indices in response to nighttime light alone (0.102, 0.0932, and 0.0903). No mesopredator species showed significant increases in eDNA index under illuminated daytime conditions.

4 | Discussion

4.1 | Assessment of Predictions and Community Patterns

Our study demonstrates that both natural diel cycles and artificial illumination significantly influence nearshore marine communities, with changes detectable within just 30 min of light exposure. All five predictions were supported to varying degrees, revealing substantial behavioral and ecological complexity across taxonomic groups:

Our first prediction—that eDNA would capture compositional shifts within short time scales (30 min)—was supported and aligns with previous studies demonstrating that eDNA can resolve fine-scale temporal variation, including diel vertical migration and biomass redistribution (Canals et al. 2021; Easson et al. 2020; Feng et al. 2022; Govindarajan et al. 2021). However, as shown in other systems, the detectability of such dynamics can be modulated by environmental conditions influencing eDNA persistence (Deeg et al. 2023), suggesting that these patterns may vary across contexts. The ability to detect compositional changes within 30 min highlights the capacity of eDNA to resolve fine-scale temporal dynamics in marine communities. Such rapid shifts are more consistent with behavioral redistribution than with slower processes such as immigration, emigration, or demographic turnover (Ely et al. 2021; Jensen et al. 2022). This suggests that nearshore communities may respond quickly to changes in environmental conditions, including light. From a methodological perspective, these results demonstrate the potential of eDNA to capture transient ecological responses to short-term disturbances that are difficult to detect using conventional sampling approaches.

Our second prediction—that diel phase (day vs. night) and nighttime artificial light would be the primary drivers of community dissimilarity—was also supported. Across both markers, sampling period and nighttime light treatment explained more variation than temporal structure alone, indicating that short-term changes in community composition are more closely associated with immediate responses to environmental conditions than with gradual ecological succession or stochastic processes (Gil et al. 2020; O'Connor et al. 2019). This is consistent with the capacity of eDNA to detect rapid shifts in community composition over short timescales, likely reflecting behavioral redistribution rather than demographic change (Ely et al. 2021; Jensen et al. 2022). The relatively consistent patterns observed across biological replicates suggest that these responses are detectable despite within-treatment variability. The stronger effect of artificial light at night compared to daytime illumination further supports the view that ALAN has its greatest influence when it represents a departure from natural light regimes (Tidau et al. 2022).

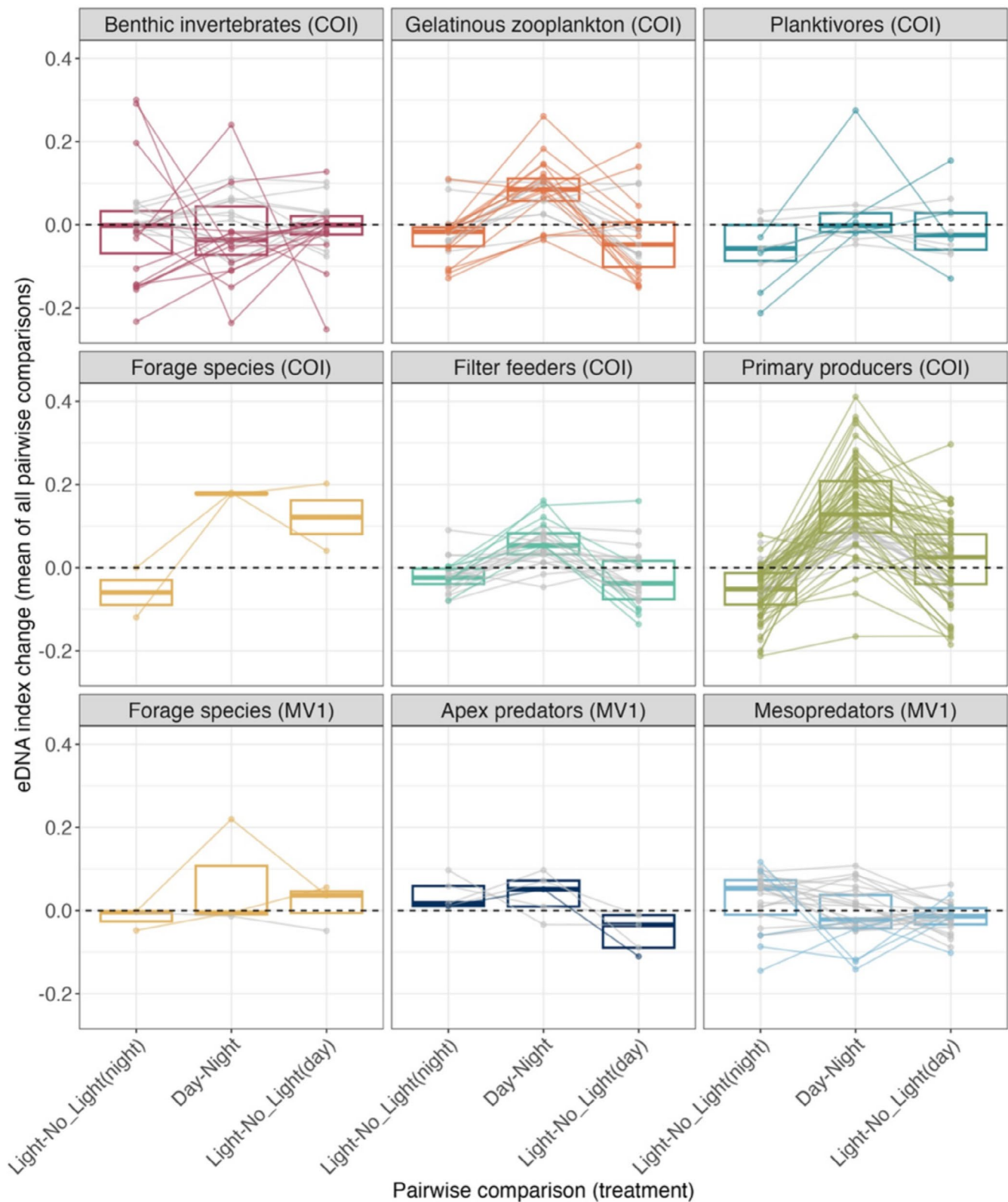


FIGURE 3 | Taxon-specific diel and light-associated responses across trophic groups. Mean eDNA index change (average of all pairwise comparisons) for each species is shown across three treatment contrasts: Day vs. Night, Light vs. No Light at night, and Light vs. No Light during the day. Panels represent major trophic and functional groups detected by COI (top two rows) and 12S (bottom row) markers. Lines indicate species-level responses, while boxplots summarize group-level distributions. Positive values reflect higher eDNA signal under the first condition of each comparison (e.g., Day, Light), whereas negative values indicate higher signal under the second condition (e.g., Night, No Light). Patterns highlight strong daytime associations across primary producers and several consumer groups, limited positive responses to artificial illumination among gelatinous zooplankton and filter feeders, diverse behaviors in benthic invertebrates, and variable diel and light-driven responses among 12S-detected vertebrates.

Our third prediction—that predatory fish would increase in abundance under artificial light due to attraction to prey aggregations, while prey fish would decline through predator avoidance—was largely supported, albeit not as strongly (Figure 3). Apex predators were somewhat more frequently associated with illuminated conditions, whereas planktivores showed patterns consistent with light avoidance, suggesting a spatial decoupling of predator–prey interactions. Similar patterns have been reported in other systems, where artificial light enhances foraging opportunities for visually oriented predators while increasing predation risk for prey (Becker et al. 2013; Bolton et al. 2017). The magnitude of these responses makes it plausible that even localized artificial light sources may contribute to ecological trap-like conditions, in which prey are exposed to elevated predation pressure in illuminated areas, with potential implications for trophic interactions and energy transfer.

Our fourth prediction—that zooplankton and mobile invertebrates would exhibit elevated abundances driven by positive phototaxis, while benthic invertebrates would decrease in response to predator avoidance under artificial light—received partial support with notable taxonomic contrasts. Some mobile invertebrates, particularly polychaetes such as *Alitta* sp. and *Nereis vexillosa*, exhibited increased relative abundances under illuminated conditions, consistent with positive phototactic or altered foraging behavior. We directly observed *Alitta* sp. and other polychaete epitokes swimming around the light source and spawning under the artificial illumination. In contrast, many zooplankton taxa showed patterns consistent with light avoidance, and benthic invertebrates did not exhibit a consistent decline. These divergent responses suggest that invertebrates do not respond uniformly to artificial light, but instead reflect a range of ecological strategies. Such decoupling of functionally related groups indicates that ALAN may disrupt key ecosystem processes, including nutrient cycling and detritus processing, by altering the co-occurrence of functionally complementary taxa (Charpentier et al. 2025). This functional fragmentation implies that the ecological effects of coastal lighting extend beyond shifts in relative abundance to include changes in biogeochemical pathways and food web structure (Bolton et al. 2017).

Our fifth prediction—that phytoplankton would be primarily day-associated due to photosynthetic requirements regardless of light treatment—was supported. Primary producers were strongly associated with daytime conditions, particularly under no-light treatments at night, and exhibited pronounced diurnal patterns. Dominant taxa such as *Micromonas pusilla*, *Minutocellus* sp., and Noelaerhabdaceae all showed consistent day-associated activity, but differed in their responses to artificial light, indicating substantial within-group variability. This heterogeneity suggests that ALAN may alter the taxonomic composition and functional diversity of phytoplankton communities, even where overall abundance remains relatively stable. Such shifts in species composition could influence primary production, nutrient cycling, and the quality of resources available to higher trophic levels (Grubisic 2018). Consequently, restructuring at the base of the food web may propagate through trophic interactions, with potential implications for energy transfer and ecosystem functioning (Maggi and Benedetti-Cecchi 2018).

4.2 | Mechanisms Driving Taxon-Specific Responses

The concordance of patterns across genetic markers supports the role of light as a primary structuring factor, while differences in response magnitude between vertebrates and invertebrates suggest distinct underlying mechanisms. Stronger responses in vertebrates likely reflect both their reliance on visual cues and their higher mobility, which together facilitate rapid aggregation around illuminated areas (Bolton et al. 2017; Tidau et al. 2022).

The aggregation of apex predators under illuminated conditions is consistent with prey-mediated attraction, a mechanism widely documented in marine systems (Becker et al. 2013). In contrast, planktivorous taxa exhibited light avoidance, suggesting behavioral trade-offs between foraging opportunities and predation risk. This spatial decoupling of predators and prey highlights how artificial light can restructure trophic interactions by redistributing organisms along light gradients rather than uniformly altering abundance, with the potential to create localized ecological traps (Bolton et al. 2017; Manríquez et al. 2019).

Invertebrate responses were more heterogeneous and reflect multiple interacting mechanisms. Increased detection of polychaetes (e.g., *Alitta* sp., *Nereis vexillosa*) under artificial light was likely driven by positive phototaxis, altered foraging behavior, or reproductive activity, all of which are known to be light-sensitive (Davies et al. 2014; Last et al. 1999). Elevated eDNA signals may also reflect increased shedding associated with physiological processes such as spawning or molting (Collins et al. 2022). Conversely, the avoidance observed in many zooplankton taxa aligns with established anti-predator strategies under illuminated conditions (Hobbs et al. 2021). Responses in filter feeders may additionally reflect contributions from motile larval stages (e.g., trochophores, veligers), which are often more responsive to environmental cues than their sessile adult counterparts (Crane et al. 2021). Together, these contrasting responses suggest that artificial light may fragment functional roles within invertebrate communities, with implications for processes such as nutrient cycling and detrital processing.

Primary producers showed consistent diel structuring but variable responses to artificial light, indicating that photophysiological differences drive niche partitioning along light gradients. Such variability has been observed in other phytoplankton systems exposed to changing light regimes. Consequently, artificial light may alter species composition without substantially changing total abundance, with potential downstream effects on primary production, nutrient cycling, and trophic transfer (Grubisic 2018; Maggi and Benedetti-Cecchi 2018).

Overall, these findings support the view that responses to artificial light are highly context-dependent and mediated by species-specific traits, including sensory ecology, behavior, physiology, and life-history strategy (Tidau et al. 2022).

4.3 | Temporal and eDNA Dynamics

A key finding of this study is that eDNA metabarcoding reliably detected community compositional shifts within just 30 min, the

interval between our night-light and night-no-light sampling periods. This temporal resolution is unprecedented for multi-species community assessments and demonstrates that eDNA can capture rapid, transient ecological dynamics in nearshore environments. Previous studies have documented diel patterns in fish communities using eDNA (Canals et al. 2021; Easson et al. 2020; Feng et al. 2022; Govindarajan et al. 2021, 2023; Trenkel et al. 2025), but most sampled at coarser temporal intervals (hours to days) and focused primarily on vertical migration in deeper waters. Our results extend these findings to shallow nearshore systems where behavioral responses occur over meters rather than tens or hundreds of meters.

The ability to resolve 30-min changes indicates that eDNA turnover in our study site is rapid enough to track behavioral shifts in near real-time. Given that eDNA half-lives in dynamic nearshore systems are approximately 4–6 h (Brandão-Dias et al. 2025), organisms that alter their vertical positioning or surface activity in response to light will quickly produce detectable changes in the eDNA pool. This rapid turnover means that in situ DNA production generally outweighs lagged or advected DNA, and recovered eDNA is largely endogenous to the sampled area (Kelly et al. 2018; Lamy et al. 2021; O'Donnell et al. 2017).

However, an important open question is whether these community shifts persist after illumination ceases. Our experimental design, with a 30-min pre-illumination period followed by eDNA sampling, primarily captured acute behavioral responses. Organisms aggregated by light might linger after illumination ends, and avoidance behaviors may not immediately reverse, implying that eDNA signals could outlast the lighting stimulus itself. Conversely, if organisms rapidly disperse or resume normal activity patterns once light is removed, the community composition should return to baseline within a few hours as residual DNA degrades. Determining the duration of light-induced community restructuring, and whether repeated or chronic exposure produces longer-lasting changes, represents an important direction for future diel and ALAN studies.

4.4 | Methodological Considerations and Limitations

eDNA metabarcoding provided a non-invasive window into diel and light-driven dynamics, circumventing many of the biases inherent in direct observational methods (Beng and Corlett 2020). Unlike diver surveys, underwater video, or light traps, which introduce disturbance, require illumination for observation, or may artificially attract organisms, eDNA sampling detects molecular traces shed into the environment without directly altering the processes under investigation. This makes eDNA particularly well suited to capture subtle, fine-scale community shifts in response to light regimes (Trenkel et al. 2025), as organisms are not disturbed or influenced by the sampling process itself.

Nevertheless, several methodological caveats remain. First, taxon-specific variation in DNA shedding and degradation rates means that eDNA concentration may not scale linearly with organism biomass, particularly given that factors such as body size, physiology, metabolic rates, stress, temperature, and life stage influence shedding intensity (Jo et al. 2020; Sassoubre et al. 2016).

For example, stressed or actively feeding organisms may shed more DNA than quiescent individuals of the same species, potentially amplifying their signal beyond their numerical abundance. Similarly, organisms with high metabolic rates or those undergoing molting, spawning, or other physiological events may contribute disproportionately to the eDNA pool.

Second, hydrodynamic transport can displace eDNA away from its source, potentially blurring fine-scale temporal signals. Advection and diffusion can redistribute DNA over tens to hundreds of meters or even several kilometers in some systems (Ellis et al. 2022; Ely et al. 2021; Murakami et al. 2019; Shea et al. 2022; Xiong et al. 2025). However, rapid degradation in dynamic nearshore environments (Brandão-Dias et al. 2025) and evidence of diel fish redistribution captured by eDNA (Trenkel et al. 2025) suggest that most signals in this study likely originated locally. At the shallow depth of our study site (~4 m), classical deep-water vertical migrations are improbable. Instead, the observed diel differences likely stem from light-mediated behavioral changes among benthic, demersal, and shallow pelagic taxa that alter their positioning over just a few meters of the water column (Berge et al. 2020).

Third, PCR amplification bias can distort community composition estimates. Variations in primer-template complementarity, GC content, amplicon length, and polymerase efficiency can cause taxa to amplify at different rates (Kelly et al. 2019; Nichols et al. 2018), producing skewed relative abundances in the final dataset. Consequently, apparent shifts in taxon representation may partly reflect differential amplification rather than true ecological differences. While the use of multiple primer sets and rigorous bioinformatic filtering (including quality control, chimera removal, and taxonomic validation) mitigate some of these biases, they cannot entirely eliminate them. We therefore transformed read counts into the eDNA index (Kelly et al. 2019), which controls for species-specific amplification bias of the primers used (Guri et al. 2024), and focused on broad compositional shifts rather than precise relative abundances.

Finally, interpreting eDNA signals in behavioral contexts requires caution. DNA may persist beyond immediate organism presence, capturing both recent and transient activity. This temporal ‘smearing’ complicates precise attribution of signals to specific moments, although the rapid 30-min detection of community changes suggests this effect is relatively minor in our study system. Additionally, eDNA cannot distinguish whether changes in detection reflect altered organism abundance, activity level, vertical positioning, or DNA shedding rates, all of which may vary with light exposure. Despite these limitations, the convergence of results across markers and the alignment with known behavioral ecology of target taxa support the ecological validity of our findings.

4.5 | Ecological Implications and Management Applications

Artificial light at night (ALAN) is increasingly recognized as an ecological stressor in marine ecosystems (Marangoni et al. 2022). By altering natural light regimes, it has the potential to modify predator–prey interactions, feeding behavior, reproductive timing, and habitat use across taxa (Häfker et al. 2022; Hobbs et al. 2021; Storrie et al. 2022). Our findings that both diel phase

and artificial light jointly shape detectable communities underscore that ALAN effects cannot be understood in isolation, but must be contextualized within natural lightscapes. The interaction between time of day and light treatment—with significant effects during night and day times—demonstrates that organisms respond to artificial light primarily when it represents a departure from natural conditions. In coastal systems, organisms have evolved under variable natural illumination ranging from starlight and moonlight (~ 0.001 – 0.3 lx) to bioluminescent flashes, which have long structured ecological rhythms (Häfker et al. 2022; Stibor and Stockenreiter 2023). Artificial light sources, however, typically exceed tens to hundreds of lux, far surpassing natural nocturnal illumination (Becker et al. 2013; Bolton et al. 2017). As observed here, such intense illumination can override natural nocturnal cues within minutes, altering both the composition and variability of shallow-water assemblages. These changes are ecologically significant because nearshore ecosystems function as critical habitats for foraging, shelter, reproduction, and nursery grounds for numerous species (McFarland 1986; Yeoh et al. 2017). Disruption of natural activity patterns could reduce foraging efficiency, increase predation risk, interfere with reproductive behavior, and ultimately alter community structure and ecosystem function.

Seasonal context also likely matters. Our sampling took place during summer at northern latitudes (Friday Harbor, Washington, 48° N), when nights are relatively short (approximately 8 h of darkness in July/August). During winter, longer dark periods may amplify ALAN effects by extending exposure durations and affecting a larger proportion of the diel cycle, potentially producing more persistent community restructuring or cumulative stress effects. Conversely, in summer when natural light periods dominate, organisms may be more resilient to brief nighttime disturbances. Considering seasonal variation in photoperiod will therefore be essential when designing lighting guidelines and assessing ecological impacts across different latitudes and times of year.

Finally, this study demonstrates the power of eDNA metabarcoding for rapid ecological assessment. All field sampling, laboratory processing, sequencing, and analysis were completed within a three-week field course, highlighting the feasibility of this approach for time-sensitive monitoring, educational programs, and resource-limited settings. The portability of nanopore sequencing platforms enables in-field data generation, eliminates delays associated with sample shipping, and provides near real-time ecological information. Such rapid-assessment tools will become increasingly valuable both in the context of basic ecology and for informing evidence-based coastal management decisions.

5 | Conclusion

This study demonstrates that eDNA metabarcoding can capture community shifts at remarkably fine temporal scales, detecting compositional changes within 30 min of artificial light exposure. Both natural diel cycles and artificial illumination shape nearshore marine communities in shallow coastal waters, with different taxonomic groups exhibiting distinct, light-mediated behavioral responses. Moreover, the rapid turnaround enabled by portable sequencing technology allowed complete data collection and analysis within weeks, making this approach particularly valuable for

time-sensitive assessments, educational programs, and resource-limited settings. Being able to produce results rapidly enables near real-time tracking of community shifts and behavioral responses to environmental change. By establishing the capability to capture transient ecological dynamics at scales previously difficult to measure, this work opens new research avenues for investigating behavioral ecology, diel community assembly, and ecosystem responses to environmental change in marine systems.

Author Contributions

Y.C.A.I., P.F.P.B.-D., E.A.A., and R.P.K. conceived and designed the study. K.C.B., G.G., Y.C.A.I., P.F.P.B.-D., D.A.P., A.B., M.-H.Z., M.D.A.M., F.J.O.C., N.A.S., O.K.N., A.K.S., F.B., S.d.M.P., Y.P., M.A., and M.V. conducted field sampling and sample collection. K.C.B., G.G., and Y.C.A.I. performed laboratory work and molecular analyses. K.C.B., G.G., P.F.P.B.-D., and E.A.A. conducted bioinformatics and data analyses. K.C.B. and G.G. prepared figures and tables. K.C.B. wrote the original draft with input from G.G., Y.C.A.I., P.F.P.B.-D., E.A.A., and R.P.K. All authors reviewed, edited, and approved the final manuscript. R.P.K. supervised the project and secured funding.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Metadata and code have been archived in GitHub (https://github.com/invertdna/FHL_2025_eDNA).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Primer information and bioinformatics output. **Table S2:** Design matrix for the beta regression model of pairwise community dissimilarity (D). Columns correspond to predictor variables, and entries (0/1) indicate whether the associated parameter (Greek letter coefficient) is excluded (0) or included (1) in the linear predictor for each condition. Additional variables include elapsed time between samples (d), phase difference (t), biological replicate (r), and the total number of samples included in the pairwise comparisons (N). **Figure S1:** Rarefaction and species accumulation curves for the COI dataset. The different curves in the sequencing depth plot (right) represent different samples. **Figure S2:** Rarefaction and species accumulation curves for the MV1 dataset. The different curves in the sequencing depth plot (right) represent different samples.