Multiple environmental stressors drive changes in fish communities: evidence from a tropical natural analogue to future oceans

Michael Izumiyama[1,2], Davide Spatafora[2,3,4], Gabriele Turco[4,5], Nicolas Dierckxsens[1], Roger Huerlimann[1], Billy Moore[1,2], Erina Kawai[1,2], James Davis Reimer[2,6,7], Sylvain Agostini[2,8], Ben P. Harvey[2,8], Shigeki Wada[2,8], Riccardo Rodolfo-Metalpa[2,9,#] and Timothy Ravasi[1,2*,#]

[1]Marine Climate Change Unit, Okinawa Institute of Science and Technology Graduate University, 1919-1 Tancha, Onna-son, Okinawa 904-0495, Japan

[2]International CO_2 Natural Analogues Network (ICONA), Japan

[3]Department of Integrative Marine Ecology, Sicily, Stazione Zoologica Anton Dohrn, Lungomare Cristoforo Colombo, 90149 Palermo, Italy

[4]National Biodiversity Future Centre (NBFC)

[5]Department of Earth and Marine Sciences (DiSTeM), University of Palermo, via Archirafi 20-22, 90123 Palermo, Italy

[6]Molecular Invertebrate Systematics and Ecology (MISE) Lab, Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan

[7]Tropical Biosphere Research Center, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan

[8]Shimoda Marine Research Center, University of Tsukuba, 5-10-1 Shimoda, Shizuoka 415-0025, Japan

[9]ENTROPIE, IRD, Université de la Réunion, CNRS, IFREMER, Nouméa 98848, New Caledonia

[#] Shared lead position

[*] Corresponding author Timothy Ravasi (timothy.ravasi@oist.jp)

Abstract

Anthropogenically-driven climate change will very likely alter marine ecosystems in the future. Unique environments (i.e., volcanic CO_2 seeps and semi-enclosed bays) that act as natural analogues of future oceans are valuable resources for studying the effects of climate change on marine ecosystems. Our study examined fish assemblages at the semi-enclosed bay of Bouraké, New Caledonia, where coral reefs are subjected to multiple environmental stressors such as temperatures, pH, and dissolved oxygen close to, or even worse than projected conditions under future climate change. By utilizing environmental DNA (eDNA) metabarcoding and underwater visual surveys (UVC), we detected a shift in fish assemblages between the natural analogue of Bouraké and a nearby control reef.

We detected eight species from the Acanthuridae, Chaetodontidae, and Pomacentridae families, which seem to be utilizing the Bouraké, suggesting that some species can take advantage of the environmentally driven shifts in habitat due to extreme environmental conditions in Bouraké. Additionally, some species of Labridae and Scaridae were absent from Bouraké and may be less tolerant to extreme conditions. The combination of eDNA and UVC surveys highlights the strength of combining both methods to characterize the fish assemblage and the importance of natural analogues in expanding our understanding of the ecosystem-level responses of fishes to future ocean conditions.

Keywords

Environmental DNA, Underwater Visual Census, Climate Change, Bouraké natural analogue

Introduction

Anthropogenetic activities have led to an increase in atmospheric CO_2, contributing to climate change, with a wealth of evidence supporting the detrimental effects of increasingly higher CO_2 levels on the environment [1]. As atmospheric CO_2 rises, temperature increases, and this heat is partially absorbed by the ocean, leading to increased sea surface temperatures and increased occurrences of prolonged heatwaves [1, 2]. The ocean's rising carbon content lowers pH levels, causing ocean acidification (OA) [3]. The consequences of increased temperature and carbon dioxide in the water alter factors such as thermal stratification, gas solubility, and biological metabolic activities, resulting in reduced oxygen levels in the water [4]. The combination of the deadly trio of hot, acidic, and deoxygenated water is expected to have detrimental consequences for marine ecosystems [5, 6].

Research on the impact of climate change on marine organisms has shown varying responses among different taxa and populations [5, 7]. However, these studies have primarily been conducted under laboratory conditions and have often been limited to short-term stressor exposure experiments [8], which may potentially underestimate the broader ecological effects of climate change [9-12]. Natural analogues (i.e., volcanic vents and semi-enclosed bays) are unique environments that mimic future climate conditions, which may provide insights for more realistic predictions of climate change effects on marine communities and ecosystems [13]. Research in natural analogues has found shifts in species compositions, creating losers and winners [14], a reduction of reef-building corals [15], and shifts from hard scleractinian to soft corals [16] and other anthozoans [17]. Other research has shown shifts from hard corals to macroalgae [18] or turf algae [19] or a general reduction of calcareous species due to competition from fleshy algae [20]. Ocean acidification conditions have also been implicated in shifts in the distribution of sea urchins

[21, 22], acclimation in polychaetes [23], and dwarfing in gastropods [24]. Shifts in community composition, such as increased algae, can lead to more frequent coral disease outbreaks [25]. These results demonstrate the crucial role natural analogues play in advancing our knowledge of the effects of climate change on reef ecosystems.

Work on fishes at natural analogues has mainly examined the behavior [26-29] or molecular responses to OA [30, 31] of wild fish chronically exposed to elevated CO_2 conditions. However, only three studies at natural analogues have investigated the effect of future ocean conditions on fish community dynamics. Munday *et al.* [27] reported a reduction of coral reef complexity at CO_2 seep compared to nearby control sites but little to no differences in fish communities between seep and control. Nagelkerken and Connell [32] documented a shift from kelp and seagrass to turf algae at CO_2 seeps, which was hypothesized to have caused a loss of fish predators. Lastly, Cattano *et al.* [33] reported a loss of benthic complexity, leading to decreased fish diversity and selection for species adapted to the simplified ecosystem due to a shift from calcified to non-calcified habitat. In all of these studies, the impact of OA on these sites altered the habitat; however, the resulting changes in fish communities ranged from minor to moderate.

Most of the previous work has examined the effects of OA at CO_2 seep natural analogues. Our study focused on the semi-enclosed bay of Bouraké in New Caledonia, a unique ecosystem where scleractinian coral populations thrive despite the adverse effects of the trio of high temperatures, low pH levels, and low dissolved oxygen (DO) levels [34], as well as high nutrients [35]. Fish assemblage in Bouraké has also been well characterized. [36, 37]. Bouraké is an ideal site to study the multifaced effect of climate change in a natural setting. Long-term monitoring of the location has found that during a semi-diurnal tidal cycle, temperature, pH, and dissolved oxygen periodically fluctuate, from extreme values at low tide to close-to-normal values at high tide, with

swings in temperature of up to 6.50°C, pH of 0.69 pH_T, and dissolved oxygen up to 4.91 $mgO_2\ L^{-1}$ between tides [35]. Despite the extreme and regularly fluctuating environmental parameters, Bouraké's ecosystem is comprised of a diverse and high-cover community of macroalgae, sponges, and scleractinian corals [34, 35].

Identifying marine fish communities has traditionally been conducted through underwater visual census (UVC) [33, 38]. However, more recent studies have increasingly made use of environmental DNA (eDNA) metabarcoding [39-41]. There is no perfect method to detect fish assemblages, as all surveying methods are subject to limitations [42, 43], such as biases against mobile and small cryptic species with UVC, the inability to determine fish sizes or abundances, and false-positive and false-negative detections with eDNA. To overcome this, an increasing number of studies have shown that combining UVC and eDNA methods may provide more accurate insights into fish communities [44, 45].

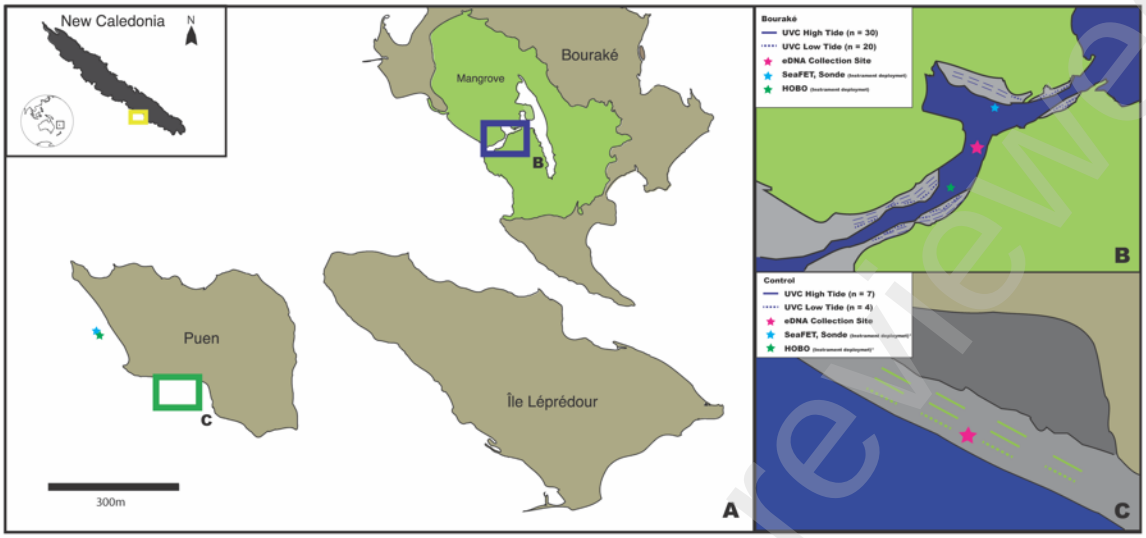
Here, we examined the fish community in the semi-enclosed bay of Bouraké and a nearby control site via UVC and eDNA methods. We expected that unique environmental and biological conditions would drive changes in the species richness of the fish communities. Additionally, given that the strong tidal fluctuation regulates most of the physical and chemical parameters of Bouraké [35], we assessed potential fish community changes between low and high tides in Bouraké. We then discuss the significance of our findings for the field of climate change and natural analogue research and suggest potential future avenues of research.

Materials and Methods

Study sites and water parameter measurements

89 Our study utilized the semi-enclosed coral reef bay of Bouraké (South Province, Grande Terre,
90 New Caledonia, from here on referred to as Bouraké), which has a channel 80 m wide and 0.5 to
91 6 m deep that penetrates a dense mangrove forest. Extensive surveys have shown the physical and
92 chemical parameters inside Bouraké consistently fluctuate according to a semi-diurnal tide [35].
93 For example, tidal activities subject these reefs to swings in temperature of up to 6.50 °C, pH of
94 0.69 pH_T, and dissolved oxygen of up to 4.91 mgO_2 L^{-1} [35]. We utilized a nearby reef as a control
95 to compare to the bay in Bouraké (Figure 1).

96 In addition to the long-term monitoring previously assessed at study sites, water parameters were
97 measured across control and Bouraké sites from June 15 to June 22, 2022. Four HOBO® pH and
98 Temperature Data Logger MX2501 (HOBO, with 5-minute logging intervals) and two Seabird
99 SeaFET™ pH loggers (SeaFET, with 10-minute intervals) were placed in Bouraké as well as the
100 control site to measure pH_T (total scale) and temperature (°C, Figure 1). In addition, we used two
101 Sonde YSI 600 OMS-M (Sonde, with 10-minute intervals) to measure depth (as a proxy for the
102 tide at the control site) and dissolved oxygen concentrations (DO, in mg L^{-1}) at the control and
103 Bouraké sites (Figure 1).



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105 Figure 1. A. Map of the study site in Bouraké, New Caledonia. B, semi-enclosed coral reef bay of
106 Bouraké; C, control site. Lines and dotted lines represent 25m long and 2 m wide belt transects
107 where underwater visual surveys were conducted. Pink stars represent eDNA collection sites, blue
108 stars Seabird, SeaFET™ pH loggers and Sonde YSI 600 OMS-M placement, and green stars
109 HOBO® pH and Temperature Data Logger MX2501 (n=2 per site) placement. *Control SeaFET,
110 Sonde, and HOBO were deployed at the site depicted in map A.

111 **Underwater visual surveys**

112 Underwater visual surveys (UVCs) were conducted between 10:00 and 15:00 from June 15 to 22,
113 2022, by scuba diving with replicated belt transects (25m long and 2 m wide) using single GoPro
114 cameras (GoPro Hero 8 Black, 1080p, 60fps, wide FOV). Transects were conducted parallel to
115 shore at both Bouraké (total n=50; Low Tide n=20, High Tide n=30) and control reef (n=11) during
116 high (n=7) and low tide (n=4) at 1–2 m depth on mixed sandy/rocky substrates (Figure 1).

Videos were analyzed using the free software VLC (www.videolan.org). For each video replicate, we analyzed the fish assemblage by estimating species richness and the maximum number of individuals of a single species in a frame (MaxN), representing a conservative measure of the relative abundance (Whitmarsh et al., 2017). In all videos, fish were identified to the lowest possible taxonomic level. If identification to the species level was not possible, the fish was identified to the family level.

eDNA Metabarcoding

Water sampling and filtration

Seawater samples were collected from Bouraké and Control reef (Figure 1) for four consecutive days from June 20 to 23, 2022, between 09:30 and 12:00 from a boat. All sampling materials used for water collection were single-use containers UV-sterilized before sampling and separated by sampling site and days in individual bags to prevent contamination.

Three 1 L samples of surface water were collected with a disposable 1 L beaker at each site and transferred to 1.5 L plastic bags with a screw cap. Water samples were collected on an outgoing tide up to two hours before low tide from Bouraké and immediately after at the control reef. A bag containing 1 L MiliQ water was opened at the control site and exposed to the conditions as a field blank to detect contamination during the water collection. All samples were immediately placed in a dark cooler with ice and filtered within 2 hours of water collection.

Water samples were filtered following a published protocol [46], with a modification for filtering multiple samples similar to the protocol described by Açıkbaş *et al.* [39]. Samples were filtered onto Sterivex cartridge filters (pore size 0.45 μm; Merck Millipore) with an addition of a filter

blank per filtering day to detect contamination during the filtering protocol. Filters were filled with RNAlater, sealed with a lure lock cap, and stored at 4°C for up to 10 days during transportation and then at -80°C until extraction.

All eDNA samples were extracted at the Marine Climate Change Unit eDNA facility at the Okinawa Institute of Science and Technology Graduate School (OIST) in a dedicated room where only eDNA sample extraction is performed. The room was decontaminated with a 20% bleach solution after every use, and samples were extracted from a clean bench with all equipment bleached and UV-sterilized before and after every use.

Environmental DNA was extracted following the protocol with modifications detailed in Açıkbaş *et al.* [39] using the Qiagen DNeasy Blood and Tissue kit and QIAvac (Qiagen); an extraction blank was included with each batch of extraction (total of two). All samples were cleaned using a DNeasy PowerClean Pro Cleanup Kit (Qiagen), following the manufacturer's protocol to remove inhibitors. Library prep was conducted in a separate room at an eDNA-dedicated clean bench using the primer set of MiFish-U-forward (5′-ACA CTC TTT CCC TAC ACG ACG CTC TTC CGA TCT NNN NNN GTC GGT AAA ACT CGT GCC AGC-3′), MiFish-U-reverse (5′-GTG ACT GGA GTT CAG ACG TGT GCT CTT CCG ATC TNN NNN NCA TAG TGG GGT ATC TAA TCC CAG TTT G-3′), [47] in a two-step PCR following the guidelines of Minamoto *et al.* [48]. The first PCR step consisted of 35 cycles of a 12 µl reaction volume containing 6.0 µl Q5® High-Fidelity 2X Master Mix (New England Biolabs), 0.7 µl of each MiFish -U primer (10 µM primer F/R), 2.6 µl sterile distilled H2O, and 2.0 µl eDNA template. Eight technical replicates were performed per sample to minimize PCR dropouts using a 0.2 ml 8-strip tube. The thermal cycle conditions were initial 3-min denaturation at 95°C, followed by 35 cycles of denaturation at 98°C for 20 s, annealing at 65°C for 15 s, and extension at 72°C for 15 s, with the final extension at the

same temperature for 5 min. One PCR blank was added per 11 samples on each thermal cycle run. The eight technical replications were pooled into one well per sample, and the second PCR and library preparation was performed at the Sequencing Section (SQC) at OIST following the protocol from Miya *et al.* [47]. Samples were size-selected using the GeneRead Size Selection Kit (Qiagen) diluted to 0.1 ng/µl, and a second round PCR was performed using dual-index sequences [47]. A total of 40 samples (24 samples, four field blanks, four filter blanks, two extraction blanks, and six PCR blanks (four first PCR, two second PCR) were sequenced on a shared run on the MiSeq v3 600 cycles with a PhiX Control library (v3) spike-in (expected at 20%) following the manufacturer's protocol. The data have been deposited with links to BioProject accession number PRJDB17980 in the DDBJ BioProject database.

Data processing and taxonomic assignment

MiSeq raw reads were quality checked using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) and MultiQC [49], and the adapter sequence was removed using Cutadapt version 4.2-1 [50]. Reads were processed using the pooled option to detect rare reads in DADA2 [51] to generate amplicon sequence variants (ASV) in R version 4.3.1 [52] and RStudio version 2023.09.1+494 [53].

Taxonomic assignment of ASVs was conducted using Taxy, a new tool developed by our laboratory (available at https://github.com/ndierckx/Taxy, unpublished). Taxy aligns each ASV against a locally stored nucleotide database from NCBI [54] using BLAST [55], then determines the most plausible taxonomic assignment based on the top 10 BLAST hits and collapses taxa in cases of equally good hits. We applied a minimum threshold of 97% sequence identity to define

184 taxa at the species level. The assigned taxa were manually checked for correct scientific names
185 and phylogenetic assignments using the World Register of Marine Species (WoRMS) database
186 [56]. We verified the species occurrence in New Caledonia and habitat preference for each species
187 using the *rfishbase* package [57]. To refine taxonomic assignments, family-level phylogenetic
188 trees were created in Geneious prime version 2023.2.1 (https://www.geneious.com) using the
189 Geneious Tree Builder from the sequences of all ASVs. Trees were visually verified, and if a
190 monophyletic group was formed within ASVs, that group was assigned to the highest taxonomic
191 classification with a unique group ID and considered a species based on monophyletic groups (e.g.,
192 *Genus* sp. 1, *Genus* sp. 2, Family sp. 1, etc.) similar to the process described by Oka *et al.* [41],
193 which allows unique ASVs that lack reference sequences to be assigned as a species.

194 The MiSeq run of the 40 samples produced a total of 19,183,919 reads. After adapter trimming,
195 quality filtering, denoising, and chimera removal, we retained 15,415,168 reads. The reads were
196 assigned to 712 ASVs. Our goal was to detect the presence or absence of species between sites
197 and, therefore, implement a vigorous screening for contamination. Custom R scripts were run to
198 filter each sample according to various blanks in reverse chronological order of sample contact
199 (2nd PCR blank, 1st PCR blank, extraction blank, filter blank, and field blank). All samples
200 associated with a blank were examined, and a conservative threshold for an ASV to be removed
201 as contamination was determined based on the number of reads across all samples. This resulted
202 in dropping ASVs with read counts above 4 – 61 across the blank, resulting in 43 ASVs being
203 removed from the total samples. Finally, ASVs with read counts below ten were removed from all
204 samples. All ASVs dropped were examined, and none of the dropped ASVs coincided with a
205 sample site (i.e., Bouraké or control), creating a false signal. After vigorous filtering, ASVs were
206 merged into species, resulting in 384 unique ASV/species (i.e., *Genus* sp.1, etc.; from here on, will

refer to as species). Of the 384 species detected, seven deep-sea species and one freshwater cyprinid were removed from subsequent analyses. We also detected and removed four species of fishes from Japan, which were target species collected from our previous work. We hypothesize that the sampling gear was likely contaminated by these species that divers had collected seven months prior during a different research project. This resulted in a total of 372 species detected from our analyses, of which 218 (59%) were identified to the species.

Analyses of fish community structure

Sample-based species accumulation curves and estimated total number of species (S_{max}) were calculated using the iNEXT function in the iNEXT R package version 3.0.0 [58]. A subsequent resemblance matrix was calculated on multivariate data using the Jaccard coefficient [59]. Nonmetric Multidimensional Scaling (nMDS) was run to visualize, in bi-dimensional space, changes in fish assemblages as a result of the main considered factors using the metaMDS function, and Permutational Analysis of Variance, PERMANOVA [60] in adonis2 with 9999 permutations in vegan R package version 2.6-4 [61]. For both eDNA and UVC, we ran PERMANOVA analysis with a fixed factor: Site with two levels (Bouraké and control site), and an additional independent analysis to determine the effect of tide on species composition in Bouraké; we tested Bouraké as a fixed factor with two levels: low and high tide on the fish community. An Indicator Species Analysis (ISA) [62] was performed using the *indicspecies* R package version 1.7.14 [63] to identify species associated with a specific site or condition to determine if a fish species was associated with a site (control or Bouraké) or condition (low or high tide in Bouraké). A simple ISA analysis comparing control and Bouraké for both eDNA and UVC was run on presence-absence data [64] for eDNA and abundance data for UVC. We also investigated the effects of the

tide in Bouraké by splitting the UVC survey into three conditions: control (CTL) Bouraké high tide (HT) and Bouraké low tide (LT), using a combined multiple group ISA [65] where a combination of groups (i.e. CTL + HT or CTL + LT) was possible. We speculated that some species associated with two groups, such as control and high tide, could be interpreted as transient species that avoid low pH, and control and low tide species as common species that occurred at both sites and may tolerate low pH levels.

Results

Water parameters

Temperature, $pH_{T,}$ and dissolved oxygen levels were measured and plotted against depth from the YSI 600 OMS-M (Figure 2). Water parameter changes in Bouraké strongly correlated with the tidal cycles (Figure 2). The average pH in Bouraké was 7.56 ± 0.055 pH_T units at low tide and 8.03 ± 0.056 pH_T units at high tide, with tidal pH swings of 0.474 ± 0.054 pH_T (Supplemental Table 1). Both sites experienced a drop in water temperature at night from June 20, but overall, daily temperature oscillated with the tide at Bouraké (Figure 2). Dissolved oxygen was also affected by the tide at Bouraké, with drops of DO of up to 1.80 ± 0.39 mg O_2 L^{-1} at low tide (Supplemental Table 1); at the control site, dissolved oxygen dropped during low tide at night and peaked during low tides at day.

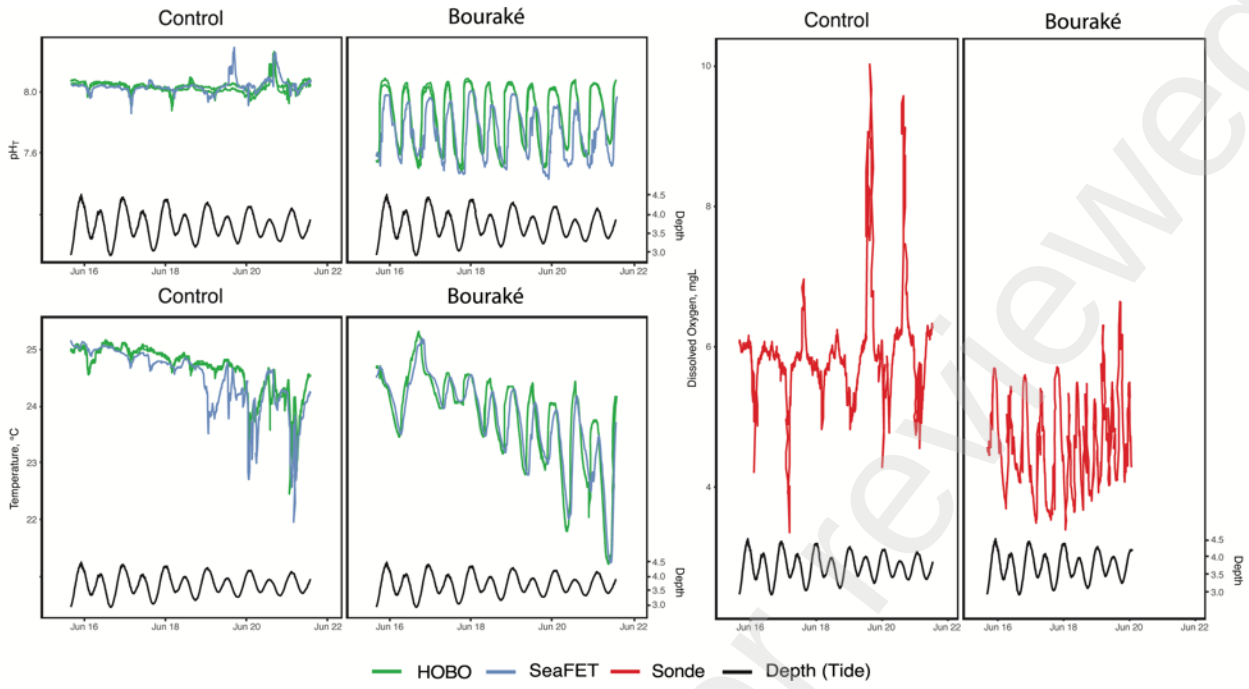


Figure 2. Plot of pH_T, seawater temperature, and dissolved oxygen against depth (a proxy for tide) at control reef and Bouraké. Each colored line represents an instrument used to measure the parameter. Black lines represent depth, a proxy for the tide.

eDNA

The 372 species detected belonged to 74 families and 30 orders (Figure 3, Supplemental Table 2). Of the 372 species, 112 species (30%) were species unique to Bouraké, 169 species (45%) were only found in the control reef, and 92 species (25%) were found at both sites (Figure 4). A total of 203 species were detected in Bouraké, with the species accumulation cure calculating a S_{max} of 239 (95% CI: 221- 274), and 260 species were detected in control with a S_{max} of 399 (95% CI: 378- 481, Figure 4). The species accumulation curve suggested that with 30 sampling efforts, we would have reached S_{max} for the control site and 13 sampling efforts to reach S_{max} at Bouraké (Figure 4).

The NMDS ordination resulted in two distinct clusters, one each associated with Bouraké and control (Figure 5, NMDS stress = 0.114). PERMANOVA also revealed significant differences in community compositions between Bouraké and control groups (Supplemental Table 3; $F = 4.65$, $p < 0.0001$, $R^2 = 0.17$).

An Indicator Species Analysis (ISA) was performed to determine species strongly associated with sites. An indicator value (IndVal) with a significance level of $p = 0.05$ was tested. Seventy-nine species were strongly associated with a specific site, with 17 species associated with control and 62 with Bouraké (Table 1, Supplemental Table 4). These species were from 18 orders and 38 families, with Gobiiformes having the greatest number of species (24), followed by Mugilidae (5 species), Lutjanidae (4 species), and Apogonidae (4 species, Table 1, Figure 6).

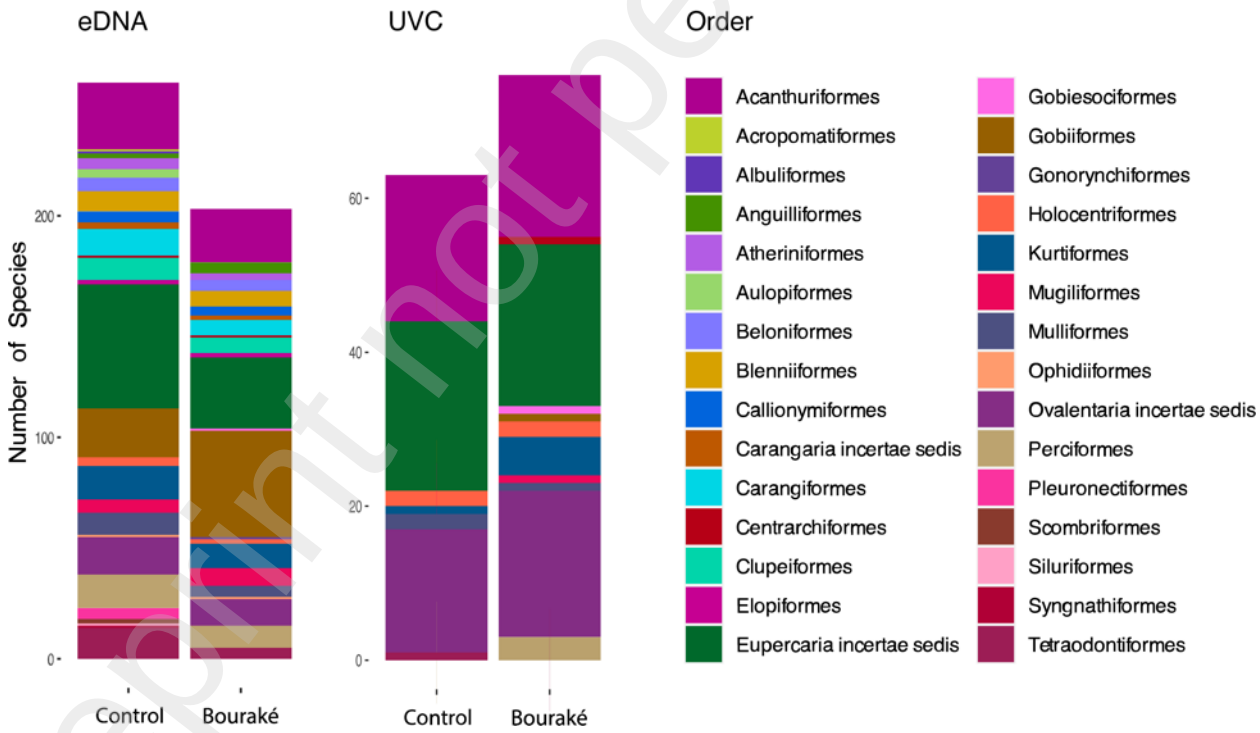
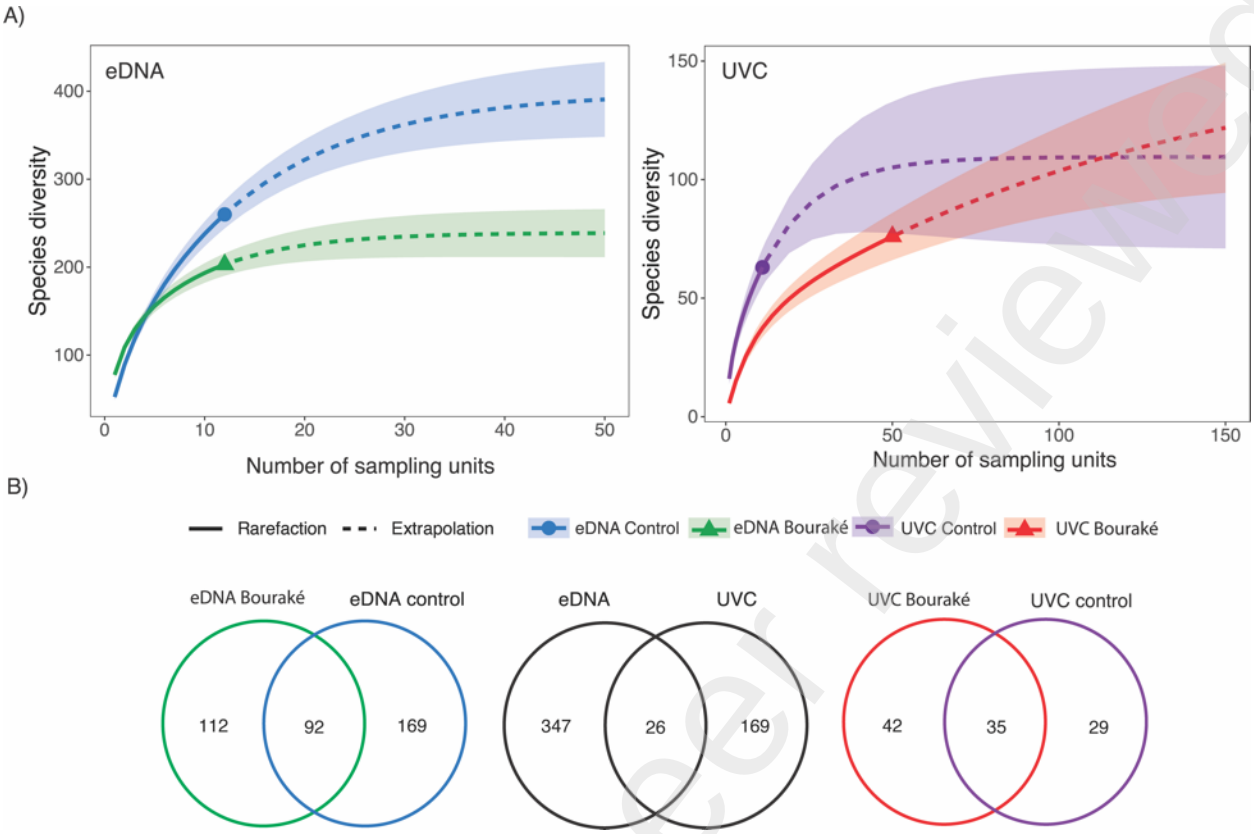


Figure 3. Number of species detected by both eDNA and UVC. Species are grouped by order.



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275

276 Figure 4. A) Species accumulation curves and 95% confidence interval of species detected with

277 both methods at each site. The dotted lines are extrapolated plots. B) Venn diagram of the number

278 of species detected by each method at each site.

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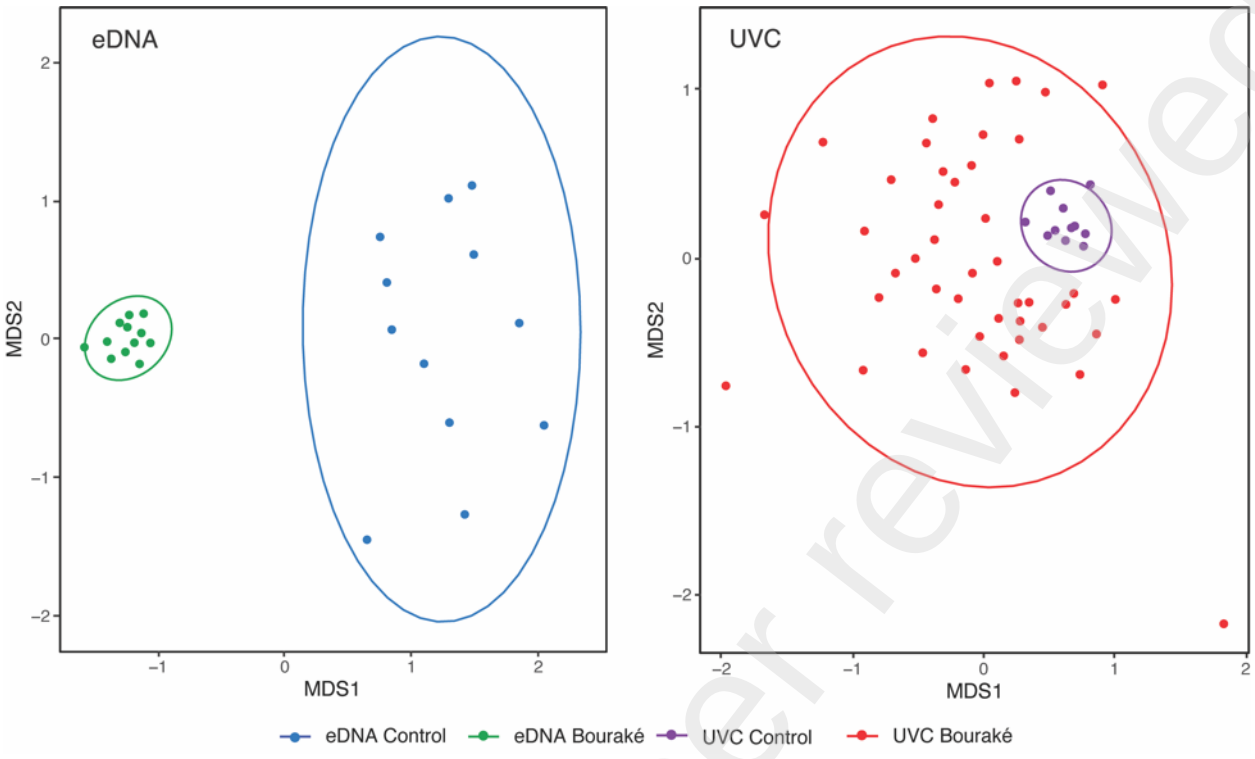

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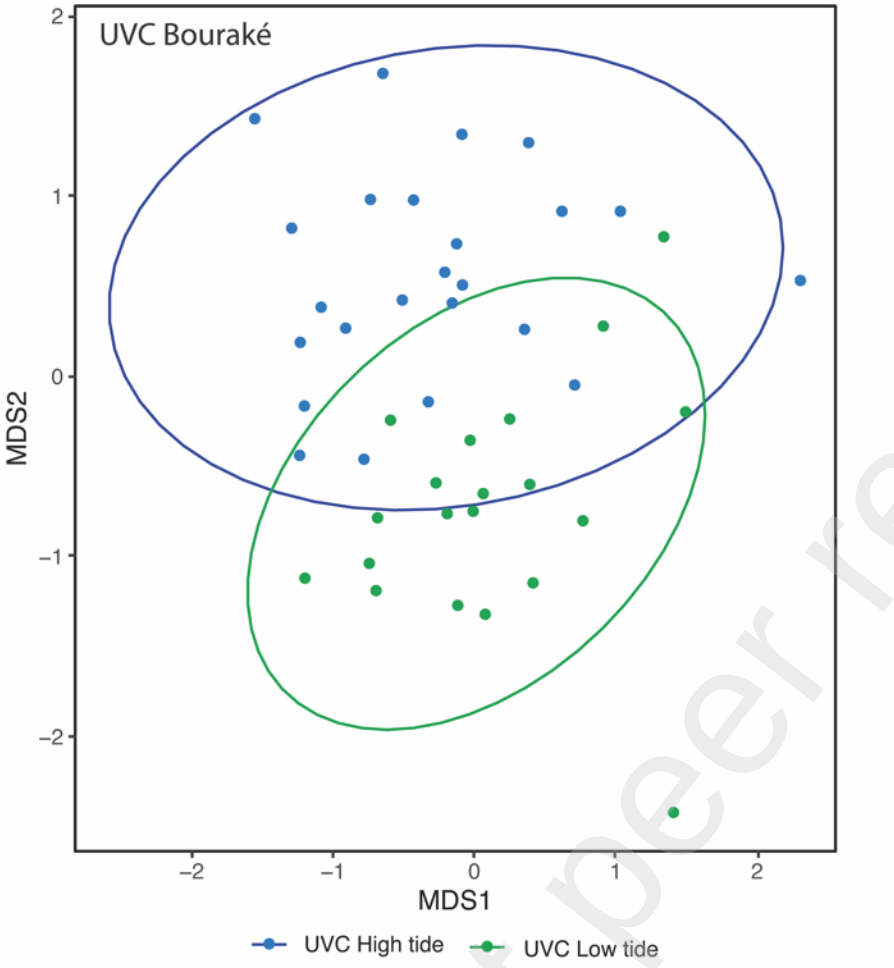
281 Figure 5. Nonmetric multidimensional scaling (NMDS) plot of fish communities detected at each

282 site and surveying method.



283

284 Supplementary Figure 1. Nonmetric multidimensional scaling (NMDS) plot of fish communities
285 detected at Bouraké in the UVC survey by tide

286

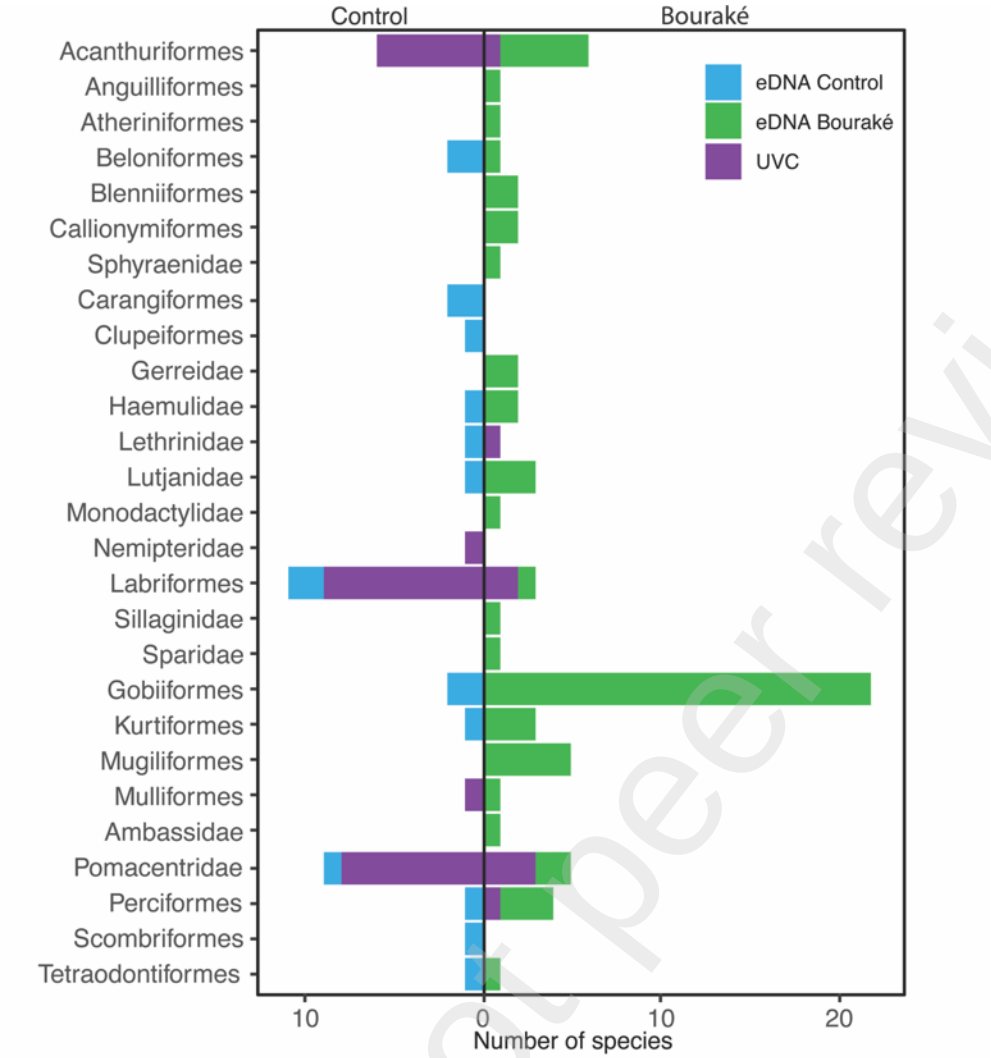
287 **UVC surveys**

288 We detected 105 species belonging to 22 families and 12 orders (Figure 3, Supplemental Table 2);
289 of the 105 species, 42 species (=40%) were unique to Bouraké, 29 species (=28%) were only found
290 in the control reef, and 35 species (=33%) were found at both sites (Figure 4). A total of 76 species
291 were detected in Bouraké, with the species accumulation curve calculating a S_{max} of 157 (95% CI:
292 107- 288), and 63 species were detected in control with a S_{max} of 110 (95% CI: 82- 180, Figure 4).

The species accumulation curve suggested that with 33 sampling efforts, we would have reached S_{max} for control and 150 sampling efforts at Bouraké (Figure 4). The NMDS ordination resulted in two clusters, one associated with each site (Figure 5, NMDS stress = 0.159). PERMANOVA revealed significant differences in community composition between Bouraké and control groups (Supplemental Table 3; $F = 4.83$, $p < 0.0001$, $R^2 = 0.08$). Indicator Species Analysis (ISA) found 24 species strongly associated with the control site. These species belonged to four orders and eight families, with most of the species belonging to three groups: Acanthuriformes (six species), Labriformes (Labridae and Scaridae, nine species), and Pomacentradae (seven species, Table 1, Figure 6).

When dividing the UVC surveys by tide, the NDMS ordination detected statistically significant differences in community composition within Bouraké between low and high tides (Supplemental Figure 1, NMDS stress = 0.145). PERMANOVA revealed significant differences in community composition between Bouraké and control groups (Supplemental Table 3; $F = 4.06$, $p < 0.0001$, $R^2 = 0.08$).

We ran an ISA by splitting Bouraké by tide and allowing for multiple group assignments. We found nine additional species associated with a specific tide or site (Table 1). Eight of the species that were additionally detected were associated with Bouraké (three at high tide, five at low tide), and one species, *Lethrinus harak*, was found to be associated with both control and high tide. The ISA also reassigned two species, *Acanthurus* sp. and *Chaetodon* sp. to both control and low tide (Table 1).



313

314 Figure 6. Indicator Species Analysis (ISA) plot of species associated with each site. Plots to the
315 left of the line represent species associated with the control site and species on the right represent
316 species found in Bouraké. Blue and green represent species detected by eDNA associated with
317 each site, and purple represents species detected by UVC associated with each site.

318 **Combining results**

319 A total of 452 species were identified with both survey methods, with 26 species overlapping
320 between the two methods. The total number of species detected was likely an overestimate due to

the inability of eDNA to identify 218 (59%) of the unique ASVs to the species level. When combining the results of ISA from both survey methods, 103 species belonging to 40 families and 18 orders were identified as strongly associated with at least one site. Of these 103 species, 62 were associated with Bouraké, and 41 were associated with the control reef.

Four major groups accounted for 55% of the species detected by the ISA. These groups were Gobiiformes, with 24 species, 22 species associated with Bouraké, and two with the control; Labriformes (Labridae and Scaridae), with 14 species, of which 11 species were associated with control and three with Bouraké; Pomacentridae with 12 species of which eight species were associated with control and four with Bouraké; and Acanthuriformes with 12 species of which six species were associated with control and six with Bouraké (Figure 6).

Discussion

The use of natural analogues, such as Bouraké, to predict the future of marine communities under climate change has only recently attracted much research attention. Our study detected changes in fish species composition between Bouraké and a nearby reference site. Our results suggest that factors such as the change in habitat and the condition within Bouraké, as well as the direct effects of multiple environmental stressors (e.g., low pH levels, temperature fluctuations, low oxygen levels, as well as strong tidal fluctuations, and high nutrient levels) between the two sites, likely drove changes in fish species composition.

Fishes from Bouraké natural analogue

The indicator species analysis (ISA) results of both UVC and eDNA revealed 69 species from 29 families and 15 orders strongly associated with the natural analogue of Bouraké. Unsurprisingly, when examining habitat preferences for the species associated with the mangrove reef of Bouraké,

many were found to be associated with mangroves. These included species in the mullets (Mugilidae) and snappers (Lutjanidae), such as the mangrove red snapper (*Lutjanus argentimaculatus*). Mangroves are an important nursery for juveniles of many fish species [66]. Previous work from Bouraké by Dubuc *et al.* [37] found juveniles of several species of groupers (Serranidae) detected in our ISA in Bouraké. Juveniles of these Serranidae species are known to utilize mangroves, and the species we detected were likely juveniles utilizing this environment.

Eight species of tangs (Acanthuridae), butterflyfish (Chaetodontidae), and damselfish (Pomacentridae) families were found in Bouraké, and their occurrence could not be explained solely by habitat. *Acanthurus blochii* was detected with eDNA and at high tide in Bouraké by UVC, which could indicate that this species travels into Bouraké at high tide to utilize the habitat. Bouraké has a highly diverse and high coverage of macroalgae [35], which could be a potential food source for this species. Similarly, Dubuc *et al.* [37] found other Acanthuridae to be common in Bouraké, with most of the species at high or intermediate tide. This would support the notion that these species travel into Bouraké and forage on abundant food. Tides are also known to influence fish communities on fringing mangroves [67], which supports our hypothesis that these species are moving in and out of Bouraké with the tides.

Two species of Chaetodontidae, *Chaetodon auriga* and *C. lineolatus,* are corallivores that eat sea anemones and algae [68]. Work from natural analogues, including Bouraké, has found a high abundance of zoantharians [69], which may be a potential resource these species utilize [70]. In previous work, Chaetodontidae species, including these two, were commonly found during intermediate tides in Bouraké [37]. Furthermore, Dubuc et al. [36] measured dissolved oxygen (DO) and temperature at Bouraké and found some species, including *Chaetodon auriga* and *C. lineolatus*, are more tolerant of the extreme conditions at Bouraké. This concept can also be applied

366 to pH, which also follows the same pattern as DO at Bouraké, suggesting that some species are
367 more tolerant to the combination of extreme conditions and could be taking advantage of the
368 resources available in Bouraké.

369 Five species of Pomacentridae were associated with Bouraké. *Neopomacentrus taeniurus* and
370 *Neopomacentrus* sp. were identified with both UVC and eDNA methods and are likely one species,
371 a freshwater demoiselle found in mangroves. The other Pomacentridae found at Bouraké were
372 *Chromis* sp., *Dascyllus aruanus,* and *Stegastes lividu. Stegastes lividus* preferentially favors red
373 algae [71]. The availability of algae for food may be one factor leading to these Pomacentridae
374 being associated with Bouraké.

375 Availability of resources may explain the occurrence of Acanthuridae, Chaetodontidae, and
376 Pomacentridae found in Bouraké. Work at natural analogues has shown increases in algae [18-20],
377 sea anemones [72], and zoantharians [69] which are primary food sources for the species in the
378 three families. This suggests that climate-driven habitat shifts, such as an increased abundance of
379 algae, anemones, or zoantharians, may boost the occurrence of some fish species and drive changes
380 in community compositions under climate change. Similarly, Cattano *et al.* [33] showed an
381 increased abundance of herbivore species at a CO_2 seep due to the greater biomass of primary
382 producers associated with enhanced nutritional quality under elevated CO_2 conditions. Habitat
383 shifts under climate change and the redistribution of resources will likely play a role in future fish
384 community assemblages. Understanding which species will be able to tolerate future conditions
385 under climate change and utilize the resources available could provide insight into future fish
386 assemblage.

In contrast to previous studies in natural analogues [27, 32, 33], our study found a high number of gobies (Gobiiformes) at Bouraké. This highlights one of the strengths of eDNA, as it allows for the detection of small cryptic species that UVC surveys may miss. The high number of gobies at Bouraké may be associated with the unique habitat of mangroves. However, gobies have also shown a high tolerance for a wide range of habitats, including those affected by ocean acidification [28, 31]. Gobies play important roles in energy transfer in coral reefs due to their short lifespan and high abundance [73]. The limited information on the presence of small cryptic species at natural analogues warrants further investigation and could be crucial in understanding the trophic cascades under climate change.

Species absent from Bouraké

Acanthuriformes, Pomacentridae, and Labriformes (Labridae and Scaridae) were the three major fish groups strongly associated with the control reef (Figure 6). The detection of these species at the control reef is as expected; however, the absence from Bouraké may suggest some species are less tolerant of the extreme conditions at Bouraké. In our study, indicator species analysis found several butterflyfish (Chaetodontidae) associated with either Bouraké (*Chaetodon auriga* and *C. lineolatus* mentioned in the previous section) or the control site *C. vagabundus* was one of the species found to be associated with the control site. However, prior work by Dubuc *et al.* [36], which examined fish assemblages in relation to DO at Bouraké, found *C. vagabundus* present at high tide but absent at low tide, suggesting this species was avoiding Bouraké at low tide when conditions were less favorable. In our study, *C. vagabundus* was prevalent at the control site, and given that DO and pH are both associated with tide at Bouraké, the absence of *C. vagabundus*

supports the notion that some species are avoiding Bouraké, which may be due to tolerance to extreme conditions.

The factors determining why certain species are present at Bouraké while others are not remain an intriguing question. Certain species of Acanthuriformes may exhibit higher tolerance to the conditions at Bouraké, with specific reef resources dictating species composition. Further investigation is needed to clarify what drives these differences in tolerance."

All the Pomacentridae that were strongly associated with the control were species that feed on zooplankton, such as *Abudefduf sexfasciatus, Amblyglyphidodon curacao, Pomacentrus amboinensis, P. coelestis,* and *P. moluccensis*, which were more abundant at the control reef as opposed to at the semi-enclosed bay. These species are often found near coral and feed in the water column, which is a typical habitat at the control reef rather than the semi-enclosed bay in Bouraké.

Of the ten wrasses (Labridae) identified in the indicator species analysis, two were found to be associated with high tide, and the rest of the species were associated with the control site. These Labridae were mostly foraging species that feed on small benthic invertebrates, except for the cleaner wrasse *Labroides dimidiatus.* Foraging species such as Labridae are highly mobile, yet the lack of wrasses at low tide may suggest they avoid the extreme conditions in Bouraké. An alternative hypothesis to explain the lack of Labridae could be due to the turbidity caused by high organic matter drifting from the mangrove mud throughout Bouraké during ebb tide. Labridae have high visual sensitivities with duplication of vision genes [74], and thus, increased turbidity at Bouraké may be a factor limiting their distribution. Turbidity is another major challenge facing our environment in the Anthropocene [75], and many studies have examined the negative impact of turbidity on fish behavior [76, 77]. Both the impacts from altered seawater chemistry due to

climate change, as well as the increase in sedimentation due to anthropogenetic activities, may be detrimental to wrasses.

Three of the four parrotfish (Scaridae) species were associated with the control reef. Scaridae are known foragers with large home ranges [78] and likely require larger territories to maintain ample food sources. Some Scaridae species may also avoid extreme conditions at Bouraké. Interestingly, eDNA data showed *Scarus ghobban* to be strongly associated with Bouraké. One factor that may explain this trend is that juvenile *S. ghobban* are known to form aggregations and enter silty and brackish environments [79]. Dubuc *et al.* [37] identified juvenile *Scarus* cf. *ghobban* at high tide, which is likely the same species. The inability to distinguish between the life cycle stages of fishes is one of the weaknesses of most genetic analyses. This poses challenges when analyzing eDNA data from an ecological context as habitat usage shifts at different life stages. Wright *et al.* [80] examined fish assemblages in mangrove-coral habitats and found a high abundance of juveniles, including Scaridae. Similarly, studies have shown that mangroves harbor a high density of juveniles due to the complexity of the habitat [81].

There was a low number of overlapping species between eDNA and UVC results, which could be associated with sampling efforts. Increasing the number of surveys may have clarified the relationship between life cycle stages and improved our ecological interpretation of some species found in Bouraké.

Limitation of survey methods

Both eDNA and UVC surveying methods are prone to biases. The use of eDNA can present difficulties when interpreting results due to the complex nature of the spatiotemporal scales

453 involved. Studies have reported eDNA detectability from hours to days [82, 83] and detection
454 scales ranging from a few hundred meters to kilometers [82, 84]. In our system, we utilized the
455 outgoing tide and a location where water funnels from the bay out (Figure 1) to collect eDNA of
456 species occupying Bouraké. However, false positives were likely due to the movement of mobile
457 fishes and varying persistence times of eDNA. Utilization of environmental RNA (eRNA), which
458 has a faster degradation time [83], may have promising applications, such as distinguishing false
459 positives [85], and should be considered in similar future work.

460 Primer choice and database availability are also challenges associated with eDNA analysis. We
461 used the MiFish-U primers [47], a universal fish primer for bony fishes, and did not consider
462 elasmobranchs or other primers available. We were able to assign species to 218 (59%) of the
463 ASVs detected. As database curation continues and new primers that can distinguish more species
464 emerge (see [86]), species-level assignment rates should increase. Environmental DNA studies
465 offer the unique opportunity to re-analyze datasets as methods improve, as long as DNA samples
466 are safely curated and available.

467 Only 26 species overlapped between our two survey methods. This can be attributed to several
468 factors, including false positive detections in eDNA and the comparatively lower sampling effort
469 of UVC surveys. The species accumulation curve showed that the number of UVC surveys needed
470 to be increased to detect the diversity of fish assemblage at both sites, likely contributing to the
471 lack of overlap between the two methods. Increasing the number of samples under limited time
472 and resources is a significant challenge for UVCs. The UVC method was also unable to detect
473 cryptic and highly mobile species, which is one of the biases of this method. Our study
474 demonstrates that combining both survey methods enhances the assessment of natural analogues
475 by highlighting the strengths of each method and compensating for their limitations.

What can natural analogues tell us about fish communities?

Previous work examining fish communities at natural analogues has found varying results between sites. In the CO_2 seeps of Papua New Guinea, Munday *et al.* [27] found little differences in the species composition between the seep and nearby control reefs. Work at two different CO_2 seeps from Nagelkerken and Connell [32] described how habitat shifts and predator reduction contributed to an increase of a few territorial fish species. Additional work by Nagelkerken *et al.* [87] at a CO_2 seep in New Zealand found a loss of fish diversity and homogenization of the fish community. Work by Cattano *et al.* [33] described the reduction in habitat complexity and a reduction of species richness. Overall, increased herbivores and a decrease in carnivores appear to be a trend in CO_2 vents for both fishes [33, 88] and invertebrates [89, 90]. Contrary to this, however, Munday *et al.* [27] detected a reduction of large predator fishes, which may have been due to the reduction of habitat complexity. Thus, the potential for predators to avoid elevated CO_2 needs to be examined further.

Shifts in trophic balance seem to be a trend at natural analogues, and the causes and effects of these patterns still need to be investigated. Many Lethrinidae, Lutjanidae, and Serranidae were present in Bouraké, with the mangrove habitat likely a factor contributing to the presence of these species. However, the relationship between carnivores' persistence and habitat complexity maintenance at natural analogue warrants further investigation in order to make stronger conclusions.

Natural analogues may be able to serve as a refuge for fish and corals and may also provide a 'head start' in adapting to future conditions of climate change, thus perhaps being key in the evolution and adaptation of fishes. In Bouraké, we found species of butterflyfish and damselfish that may opportunistically utilize Bouraké for resources. However, some species, such as Labridae and Scaridae, may also be at higher risk from the effects of climate change, with many species not

found in Bouraké but in nearby reefs. Indeed, work on Labridae at a CO_2 seep has shown that OA affects behavior such as spawning and nest-guarding [26, 29], and examining how Labridae may behave in Bouraké could further our understanding of the effects of climate change on fish behavior. Further investigation is warranted to determine the factors driving species diversity in natural analogues, as tolerance to climate change may not be the only factor structuring communities.

The current work utilized a unique natural analogue to examine how climate change may affect fish assemblages. Habitat availability is crucial for diverse fish communities, and anthropogenetic activities continue to threaten both habitats and fish populations. Utilizing natural analogues to understand the effects of climate change and potentially preserve these unique sites as refugia will be valuable in studying the impacts of coming climate change.

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Author Contribution

MI, DS, and TR conceived the study. MI, BM, and EK collected eDNA samples. DS collected UVC data. JDR, SA, BPH, SW, and RRM organized the sampling logistics in New Caledonia and deployed and collected various water parameter data. MI conducted lab work for eDNA extraction and library prep. MI conducted bioinformatic analysis with the help of ND and RH. DS and GT conducted UVC data analysis. MI wrote the manuscript with input from DS, GT, and TR. All authors read and approved the final manuscript.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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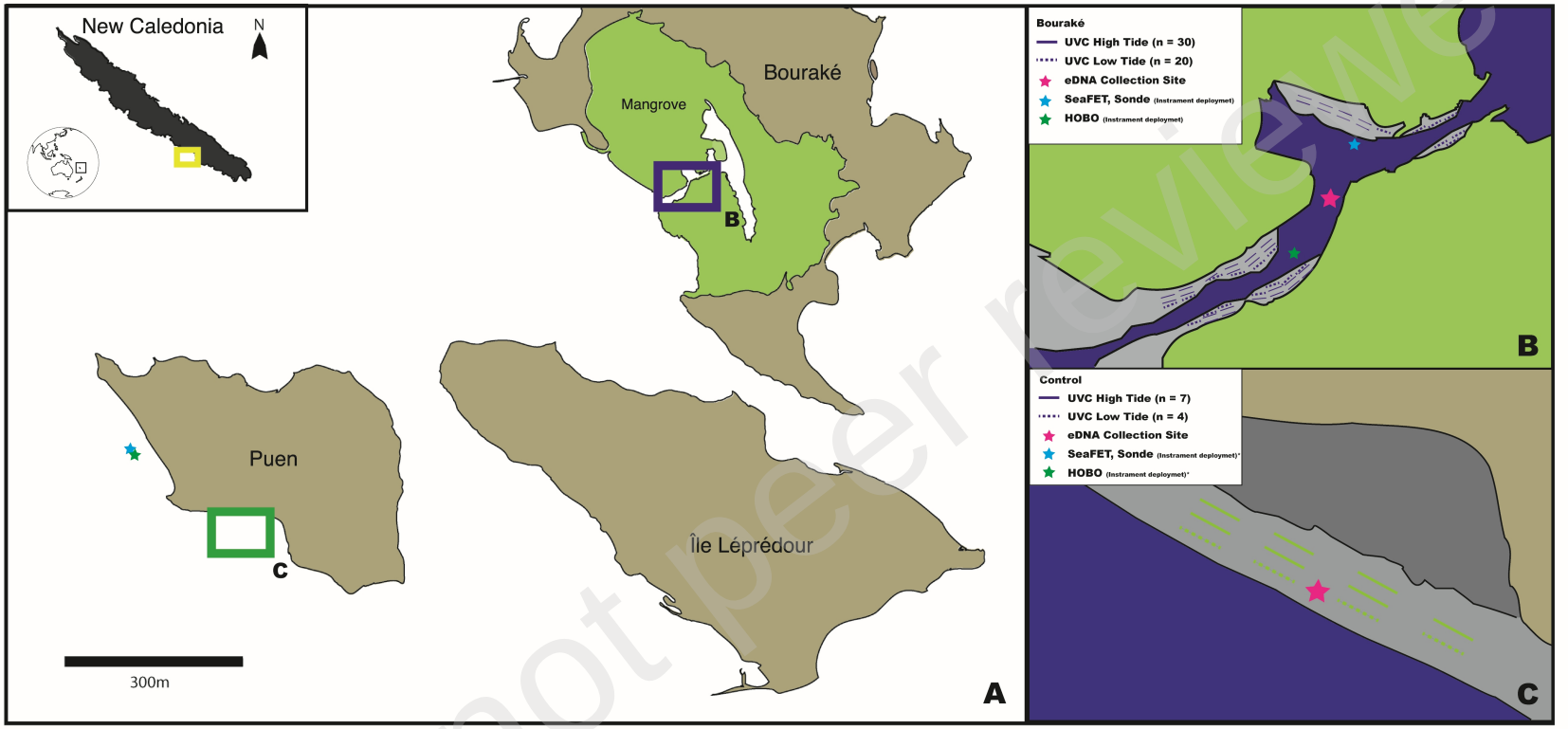

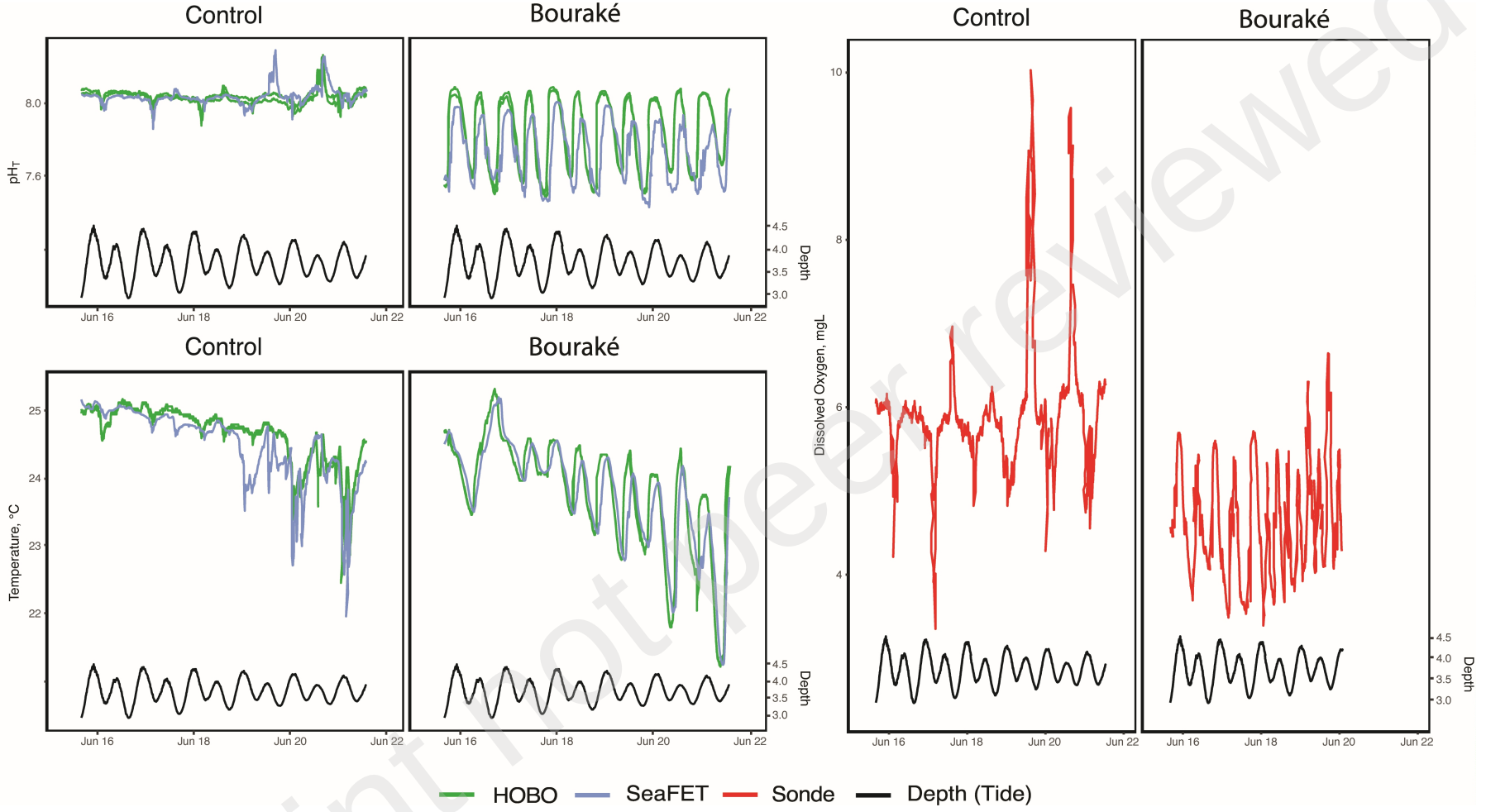

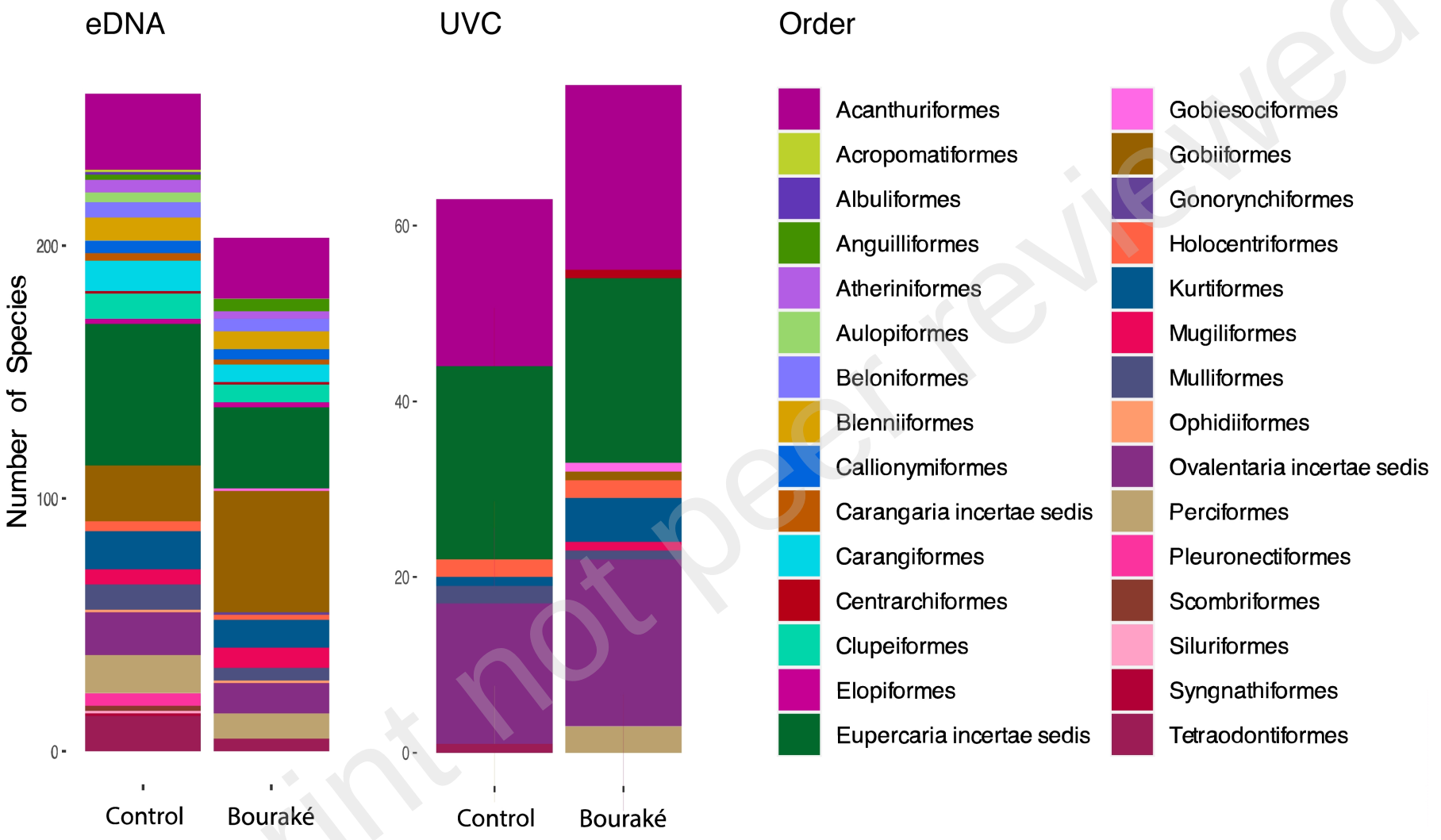

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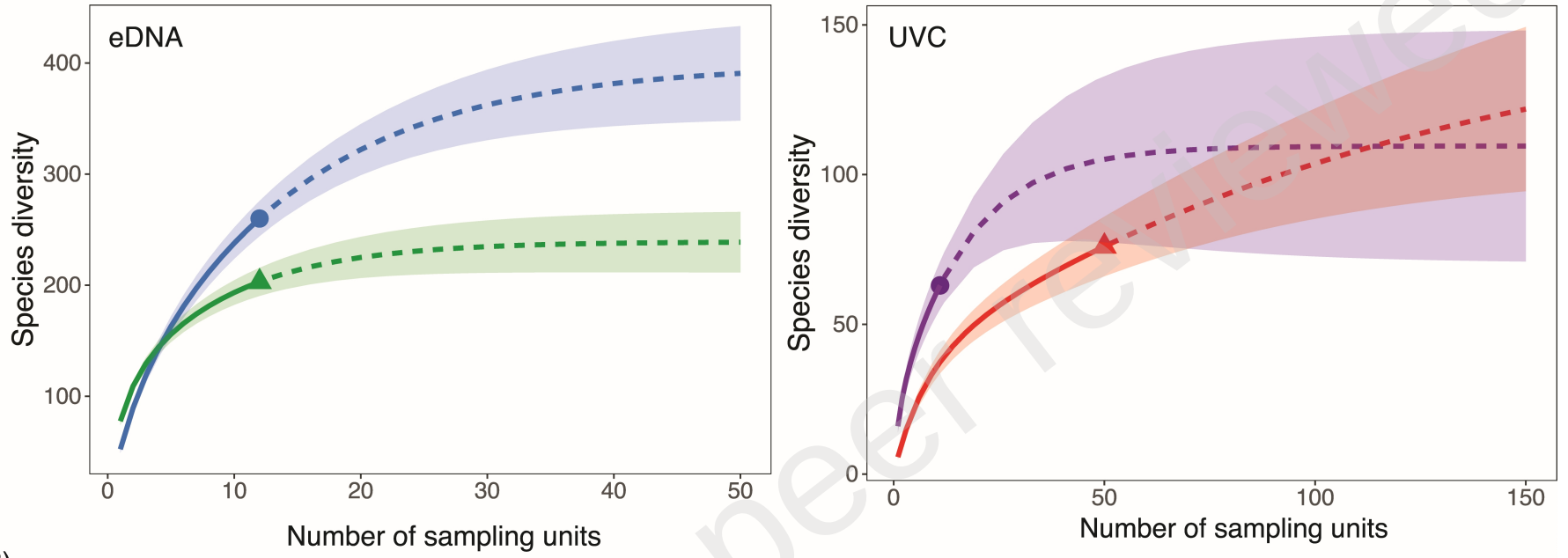

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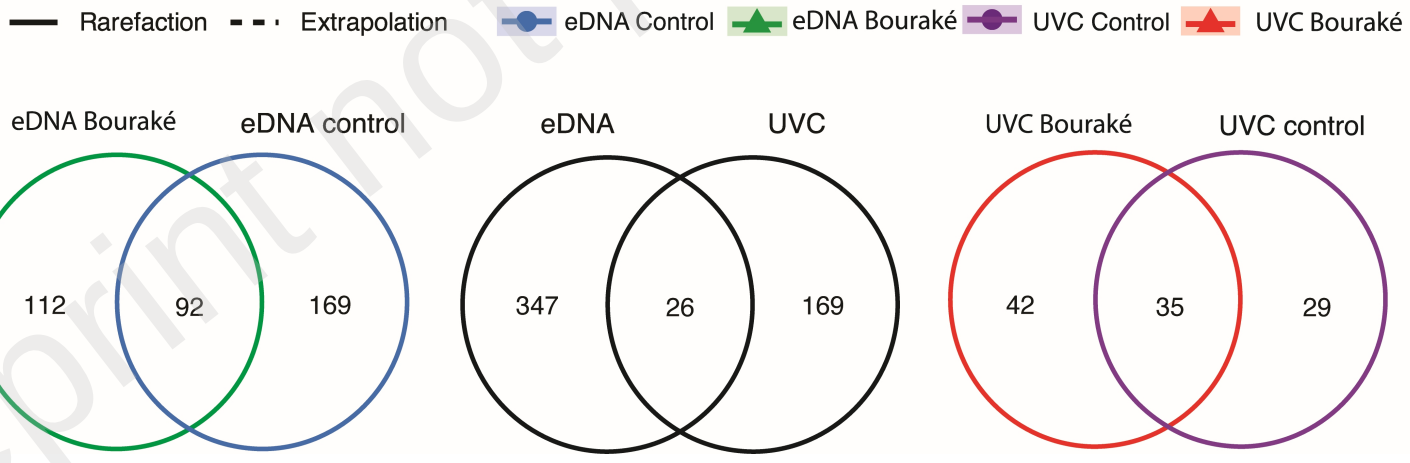

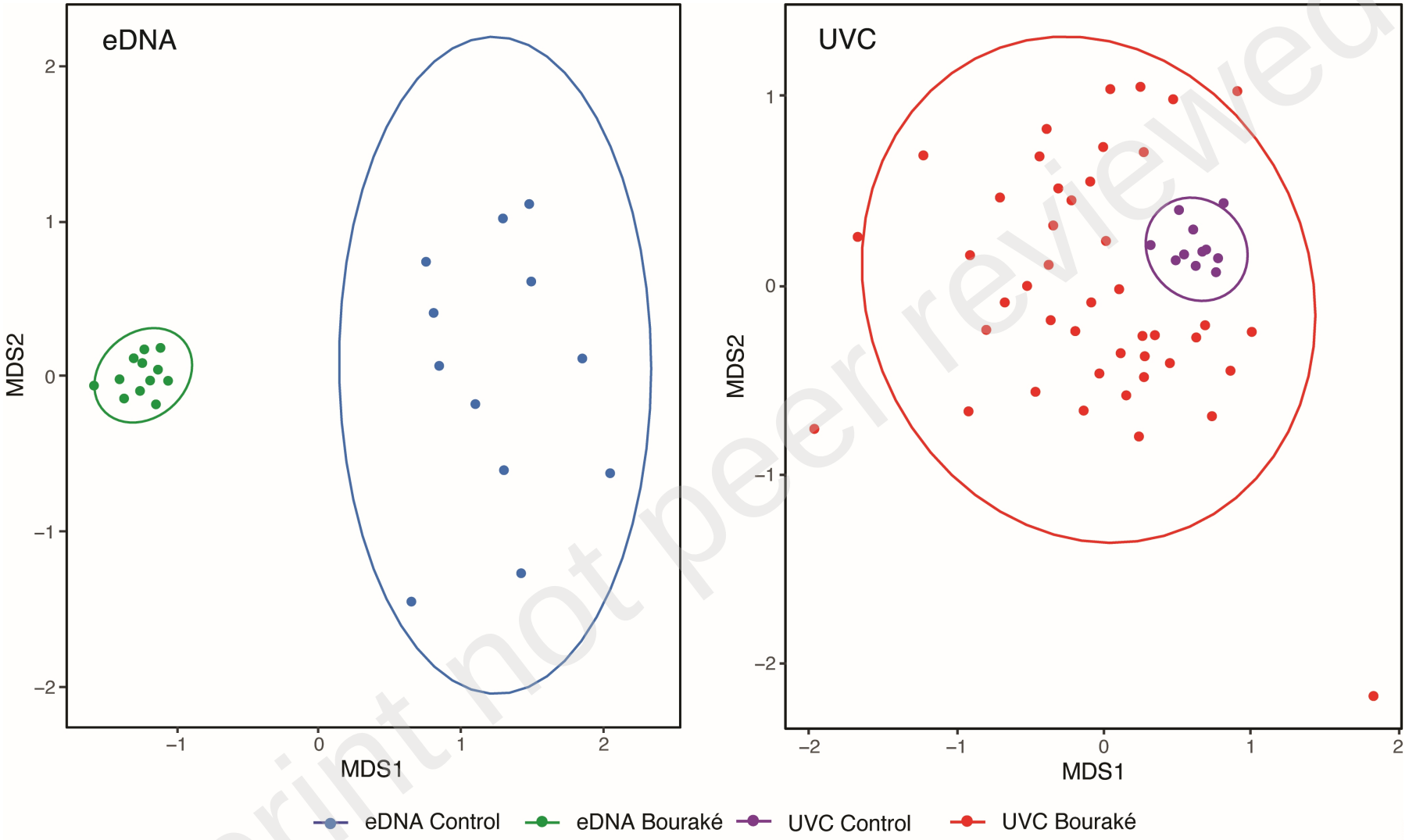

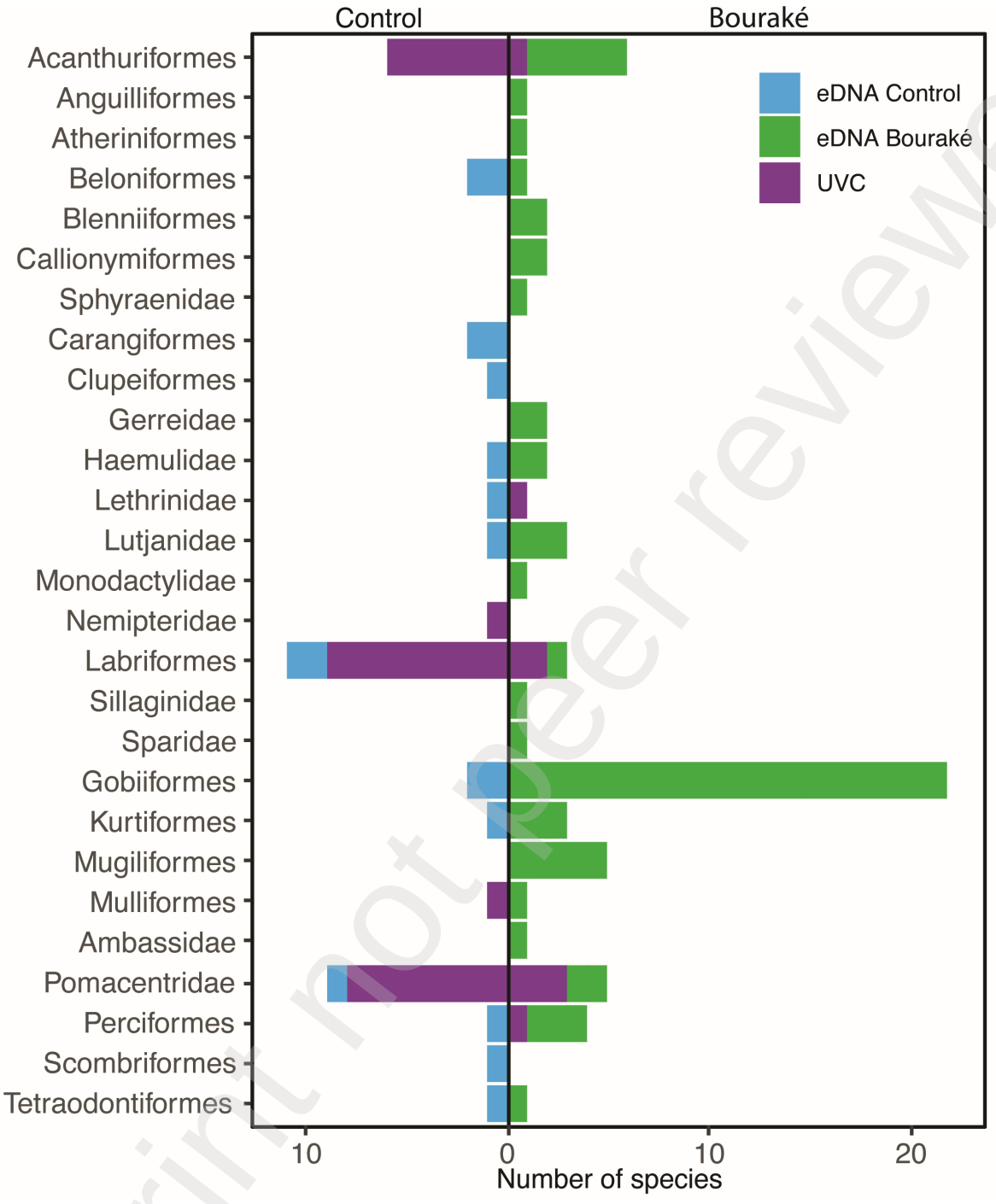

Order	Family	Species	eDNA	UVC	UVC (with tide)	Mangroves	Consensus
Acanthuriformes	Acanthuridae	*Acanthurus blochii*	Bouraké		HT		Bouraké
		Acanthurus sp.		Control	CTL+LT		Control
		Ctenochaetus sp.		Control	Control		Control
	Chaetodontidae	*Chaetodon auriga*			LT	X	Bouraké
		Chaetodon lineolatus	Bouraké				Bouraké
		Chaetodon lunulatus		Control	Control		Control
		Chaetodon sp.		Control	CTL+LT		Control
		Chaetodon vagabundus		Control	Control		Control
	Leiognathidae	*Aurigequula fasciata*	Bouraké			X	Bouraké
	Scatophagidae	*Scatophagus argus*	Bouraké			X	Bouraké
	Siganidae	*Siganus sp. 1*	Bouraké				Bouraké
	Zanclidae	*Zanclus cornutus*		Control	Control		Control
Anguilliformes	Muraenidae	*Strophidon sathete*	Bouraké				Bouraké
Atheriniformes	Atherinidae	*Atherinomorus sp. 2*	Bouraké				Bouraké
Beloniformes	Exocoetidae	*Parexocoetus sp. 1*	Control				Control
	Hemiramphidae	*Hemiramphus far*	Control			X	Control
	Zenarchopteridae	*Zenarchopterus dispar*	Bouraké			X	Bouraké
Blenniiformes	Blenniidae	*Omobranchus sp. 2*	Bouraké				Bouraké
	Tripterygiidae	*Enneapterygius sp. 2*	Bouraké				Bouraké
Callionymiformes	Callionymidae	*Callionymus enneactis*	Bouraké			X	Bouraké

		Synchiropus splendidus	Bouraké				Bouraké
Carangaria incertae sedis	Sphyraenidae	*Sphyraena sp. 1*	Bouraké				Bouraké
Carangiformes	Carangidae	*Gnathanodon speciosus*	Control			X	Control
	Echeneidae	*Echeneis naucrates*	Control				Control
Clupeiformes	Engraulidae	*Encrasicholina sp. 1*	Control				Control
Eupercaria incertae sedis	Gerreidae	*Gerres sp. 1*	Bouraké				Bouraké
		Gerres sp. 2	Bouraké				Bouraké
	Haemulidae	*Diagramma sp. 1*	Control				Control
		Haemulidae sp. 2	Bouraké				Bouraké
		Pomadasys sp. 1	Bouraké				Bouraké
	Labridae	*Cymolutes praetextatus*	Control				Control
		Gomphosus varius		Control	Control		Control
		Halichoeres richmondi		Control	Control		Control
		Halichoeres sp.		Control	Control		Control
		Halichoeres trimaculatus			HT	X	Bouraké
		Hemigymnus melapterus			HT	X	Bouraké
		Labroides dimidiatus		Control	Control		Control
		Thalassoma hardwicke		Control	Control		Control
		Thalassoma lunare		Control	Control		Control
		Thalassoma lutescens		Control	Control		Control
	Lethrinidae	*Lethrinus atkinsoni*	Control			X	Control

		Lethrinus harak			CTL + HT	X	Bouraké
	Lutjanidae	*Lutjanus argentimaculatus*	Bouraké			X	Bouraké
		Lutjanus fulvus	Bouraké		LT	X	Bouraké
		Lutjanus russellii	Bouraké			X	Bouraké
		Lutjanus sp. 1	Control				Control
	Monodactylidae	*Monodactylus argenteus*	Bouraké			X	Bouraké
	Nemipteridae	*Scolopsis bilineata*		Control	Control	X	Control
	Scaridae	*Chlorurus sordidus*		Control	Control		Control
		Scarus ghobban	Bouraké			X	Bouraké
		Scarus schlegeli	Control				Control
		Scarus sp.		Control	Control		Control
	Sillaginidae	*Sillago sp. 2*	Bouraké				Bouraké
	Sparidae	*Acanthopagrus sp. 1*	Bouraké				Bouraké
Gobiiformes	Eleotridae	*Eleotris melanosoma*	Bouraké				Bouraké
		Oxyeleotris sp. 1	Bouraké				Bouraké
	Gobiidae	*Acentrogobius sp. 3*	Bouraké				Bouraké
		Asterropteryx semipunctata	Bouraké			X	Bouraké
		Asterropteryx sp. 1	Bouraké				Bouraké
		Callogobius sp. 1	Bouraké				Bouraké
		Cristatogobius sp. 1	Bouraké				Bouraké
		Cryptocentrus sp. 3	Bouraké				Bouraké

		Drombus sp. 1	Bouraké		Bouraké
		Drombus sp. 3	Bouraké		Bouraké
		Exyrias sp. 1	Bouraké		Bouraké
		Favonigobius sp. 1	Bouraké		Bouraké
		Gobiidae sp. 1	Bouraké		Bouraké
		Gobiidae sp. 2	Bouraké		Bouraké
		Gobiidae sp. 3	Bouraké		Bouraké
		Microgobius sp. 1	Control		Control
		Mugilogobius sp. 1	Bouraké		Bouraké
		Oxyurichthys papuensis	Bouraké	X	Bouraké
		Oxyurichthys sp. 2	Bouraké		Bouraké
		Pandaka sp. 2	Bouraké		Bouraké
		Periophthalmus argentilineatus	Bouraké	X	Bouraké
		Redigobius balteatus	Bouraké	X	Bouraké
		Vanderhorstia sp. 1	Control		Control
		Yongeichthys sp. 1	Bouraké		Bouraké
Kurtiformes	Apogonidae	*Apogonidae sp. 1*	Bouraké		Bouraké
		Fibramia sp. 1	Bouraké		Bouraké
		Pristiapogon fraenatus	Control		Control
		Yarica hyalosoma	Bouraké	X	Bouraké
Mugiliformes	Mugilidae	*Crenimugil buchanani*	Bouraké	X	Bouraké

		Crenimugil sp. 2	Bouraké				Bouraké
		Mugil cephalus	Bouraké			X	Bouraké
		Planiliza macrolepis	Bouraké			X	Bouraké
		Planiliza melinoptera	Bouraké			X	Bouraké
Mulliformes	Mullidae	*Parupeneus barberinus*		Control	Control	X	Control
		Parupeneus indicus	Bouraké			X	Bouraké
Ovalentaria incertae sedis	Ambassidae	*Ambassis sp. 2*	Bouraké				Bouraké
	Pomacentridae	*Abudefduf sexfasciatus*		Control	Control		Control
		Abudefduf sp. 1	Control				Control
		Amblyglyphidodon curacao		Control	Control		Control
		Chromis sp.			LT		Bouraké
		Neopomacentrus sp.			LT		Bouraké
		Neopomacentrus taeniurus	Bouraké			X	Bouraké
		Plectroglyphidodon altus		Control	Control		Control
		Pomacentrus amboinensis		Control	Control		Control
		Pomacentrus coelestis		Control	Control		Control
		Pomacentrus moluccensis		Control	Control		Control
		Pomacentrus sp.		Control	Control		Control
		Stegastes lividus	Bouraké				Bouraké
Perciformes	Pinguipedidae	*Parapercis cylindrica*	Control				Control
	Serranidae	*Epinephelus coeruleopunctatus*	Bouraké				Bouraké

		Epinephelus lanceolatus	Bouraké		Bouraké
		Epinephelus sp.		LT	Bouraké
		Epinephelus sp. 1	Bouraké		Bouraké
Scombriformes	Scombridae	*Rastrelliger kanagurta*	Control		Control
Tetraodontiformes	Balistidae	*Pseudobalistes fuscus*	Control		Control
	Tetraodontidae	*Arothron sp. 1*	Bouraké		Bouraké

Table 1 Indicator Species Analysis (ISA) list of species associated with each site and the list of species associated with mangrove habitat. CTL (control) LT (low tide), and HT (high tide)