Fine-scale spatial distribution of a fish community in artificial reefs investigated using an underwater drone and environmental DNA analysis

Fine-scale spatial distribution of fish community in high-rise artificial reefs using an underwater drone and quantitative environmental DNA metabarcoding

Running page head: Underwater drone estimates artificial reef effects

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Abstract

Although artificial reef (AR) effect evaluation is useful for planning the installation of high-rise ARs and their management, few studies have investigated them quantitatively. The fine-scale two-dimensional fish distribution in ARs was estimated regarding current fields and vertical structures of two high-rise ARs (20 and 30 m high at 62 and 72 m depths, respectively) in Tateyama Bay, central Japan, using underwater drone recordings with vertical line transects and environmental DNA (eDNA) metabarcoding. The species detected by video surveys (21 organisms were identified to species, and one to genus) were fewer than by eDNA analysis (103 species and 6 genera), especially in pelagic, small-sized, and cryptic fish. Video surveys revealed the demersal fish distribution increased with decreasing horizontal distance from the AR surface within 20 m, and the richness and total fish density were significantly higher upstream of the ARs. Conversely, the fish eDNA concentration showed different patterns with significantly higher concentrations downstream of the ARs. The richness peaked at horizontal AR surfaces (e.g., reef top) but density of the dominant species peaked near the bottom by video survey. In comparison, eDNA analysis indicated lower richness and higher eDNA concentration of the dominant species at the reef top. Such discrepancies may be explained by the influence of eDNA transport or its specific behavior or buoyancy. Video surveys indicated

35 the growth stage and sex information of four species from their morphology, which is not
36 possible using eDNA analysis. This study shows the advantages of each evaluation
37 method can complement each other.
38
39 Keywords: artificial reef, underwater drone, environmental DNA, distribution, species
40 richness, remotely operated vehicle
41

1. INTRODUCTION

Creating fishing grounds by deploying artificial reefs (ARs) has been actively promoted worldwide since the 1960s (Lima et al. 2019). National projects of the Japanese government began installing ARs in Japanese coastal waters in 1971 to create fishing grounds and maintain and develop commercial fisheries (Sato et al. 2021). ARs are feeding grounds and nursery areas that cause the aggregation and stock enhancement of fish and form rich communities comprising various aquatic organisms (Bohnsack & Sutherland 1985). Therefore, ARs can increase fishery stocks in terms of abundance and richness (Bohnsack & Sutherland 1985).

Fish distribution around ARs has been studied using various methods such as underwater visual census, surveys using fishing nets, fisheries-based observations, and echo sounder surveys (Polovina & Sakai 1989, Kakimoto 1993, Tessier et al. 2005, Brotto et al. 2007, Kang et al. 2011). These monitoring methods can provide valuable, comparable quantitative survey data on fish aggregation to a point. Dense schools of fish are more abundant upstream of ARs which was confirmed by bottom gillnets (3822 ARs with a height of 1 m installed at a depth of 58 m in Japan; Kakimoto 1967), stationary underwater cameras (139 ARs with a volume of 1.5 m^3 installed at a depth of 20 m in Japan; Okamoto et al. 1979), multibeam echosounders (ARs with a height of 8–12 m

60 installed at a depth of 36–42 m in Australia; Holland et al. 2021), and environmental DNA
61 (eDNA, which is DNA derived from environmental samples such as water) analysis (ARs
62 with a height of 30 m installed at a depth of 75 m in Tateyama Bay, Japan; Inoue et al.
63 2022). The impact of the flow field on fish aggregation has been focused upon. By taking
64 advantage of fish aggregation upstream of the reefs, local fishermen can efficiently catch
65 fish by anchoring their ships upstream of the AR and directing their fishing gear toward
66 the AR (Inoue et al. 2018, 2020, 2022). Moreover, predicting the fine-scale fish
67 distribution in relation to AR structure or special flow fields formed by ARs (Liu & Su
68 2013, Li et al. 2021) is useful for managing fishing grounds formed by ARs, selecting
69 installation sites, and designing layouts and structures. However, few studies have
70 investigated the quantitative evaluation of fish school formation in ARs and its
71 relationship with current fields, especially in high-rise ARs (Holland et al. 2021, Inoue et
72 al. 2022).
73 Recently, an eDNA approach, especially metabarcoding using a high-throughput
74 sequencer and universal primer sets, was used to assess coastal fish species diversity
75 (Thomsen et al. 2012, Miya et al. 2015, Port et al. 2016, Yamamoto et al. 2017, Polanco
76 Fernández et al. 2021). The quantitative MiSeq sequencing approach (qMiSeq) could
77 evaluate the eDNA concentrations of multiple species simultaneously (Ushio et al. 2018).

78 Compared to the underwater visual census, eDNA analysis is quick, easy, involves non-
79 invasive sampling, and has a high detection sensitivity for fish diversity, especially for
80 pelagic species (Port et al. 2016, Yamamoto et al. 2017, Polanco Fernández et al. 2021).
81 Therefore, this method was used for the first time to evaluate fish aggregation on a high-
82 rise AR in the open ocean. Our previous studies revealed that the fish eDNA concentration
83 increased sharply with decreasing distance from the AR (Sato et al. 2021) and a
84 significantly higher fish eDNA concentration upstream side of the ARs (Inoue et al. 2022).
85 However, eDNA analysis has some limitations when evaluating fish distributions. First,
86 detecting the fish abundance distribution in the area surrounding ARs (Sato et al. 2021,
87 Inoue et al. 2022) is simultaneously influenced by eDNA production, transport, and
88 degradation (Goldberg et al. 2015). Second, the eDNA concentration can be positively
89 correlated with the density and biomass of fish species (van Bleijswijk et al. 2020, Maes
90 et al. 2023); however, eDNA concentration alone cannot determine whether many small
91 individuals (high density) or few big ones (low density) are present with the same biomass.
92 Namely, eDNA analysis cannot determine the growth stage or size of the detected fish.
93 Recently, underwater drones (UDs, small remotely operated vehicles (ROVs)) that
94 observe aquatic organisms have been used as research tools (Sward et al. 2019). UDs are
95 relatively inexpensive and small compared to conventional ROVs and have advanced

96 attitude control functions and a rich interface; thus, they are easy to operate. The
97 information acquired by UDs is similar to that of an underwater visual census (Hellmrich
98 et al. 2023), such as species richness, categorical growth stage information from
99 morphological features, and fish counts. Furthermore, UDs can be used in deeper waters
100 with little effort, unlike underwater visual census (Andaloro et al. 2013, Sward et al. 2019).
101 The natural or AR effects on aquatic organism aggregation estimated by conventional
102 ROVs primarily focus on detailed benthic organism distribution on the horizontal two-
103 dimensional plane of the seafloor. However, few studies have investigated the vertical
104 fish distribution along these structures (Ajemian et al. 2015). Thus, video surveys using
105 UDs will be able to quantitatively determine the fine-scale spatial fish distribution around
106 ARs related to current fields and their vertical structure. Moreover, these relatively novel
107 methods, UDs and eDNA analysis, can be used simultaneously to monitor fish
108 distributions around ARs, such as richness and abundance distributions of aggregating
109 fishes around ARs with growth stage information. Therefore, using UDs could
110 compensate for the disadvantages of eDNA, such as the ambiguity in fish eDNA
111 distribution owing to transport and degradation and lack of growth stage information.
112 However, UDs have limitations; video surveys underestimate fish abundance when
113 compared with underwater visual census (Bortone et al. 2000, Tessier et al. 2005,

Andaloro et al. 2013), likely owing to their narrow field of view and low resolution (Tessier et al. 2005, Andaloro et al. 2013). Thus, using an absolute abundance index such as volumetric density (hereafter, density) is essential to assess fish abundance more accurately (Williams et al. 2018). Therefore, estimating the field of view volume of the camera (sampling volume) is crucial to converting count data into an absolute abundance index that is easier to compare with other monitoring methods. A method for estimating the sampling volume has been developed for stereo camera observations (Rand et al. 2006, Williams et al. 2018); however, no similar method exists for monaural camera observation.

Therefore, this study developed a video survey method using UD and applied quantitative eDNA metabarcoding (qMiseq) to estimate the spatial distribution of fish communities around ARs in vertical two-dimensional planes to explore fine-scale fish distribution in relation to AR structure and flow fields formed by ARs. Namely, to evaluate using volumetric density, the sampling volume of monaural camera equipped in UD was estimated. The survey results of ARs were compared using video surveys and qMiSeq, and the characteristics of the data generated from the video surveys were organized. Specifically, this study aimed to (1) characterize the fish communities of two high-rise ARs by vertical line transects using the UD to calculate species composition and density concerning local currents and AR shape and (2) to compare the detected fish

132 communities and their spatial distributions resulting from video surveys and eDNA
133 analysis.
134
135 **2. MATERIALS & METHODS**
136 **2.1. Field survey**
137 **2.1.1. Study site**
138 Field surveys were performed at two high-rise ARs installed in Tateyama Bay, central
139 Japan, near the Kuroshio warm current facing the Pacific Ocean on October 27, 2021 (Fig.
140 1a). Many ARs were implemented in this area to create fishing grounds; 9391 small-scale
141 ARs exist, such as tire reefs, established in the shallow waters by local fishing
142 cooperatives, Tateyama city of Chiba Prefecture, and Chiba Prefecture during 1982–1998
143 and 240 relatively large ARs (Fig. 1a) were established by Chiba Prefecture during 2006–
144 2010. This study focused on the highest steel-framed AR (30 m high) deployed at a water
145 depth of 72 m by Chiba Prefecture in 2010 (AR1, SKS Reef UT-304, Nippon Steel
146 Kobelco Metal Products, Tokyo, Japan, Fig. 1a, b, c) and the steel-framed AR (20 m high)
147 deployed at a distance of 1000 m from AR1 at a depth of 62 m by Chiba Prefecture in
148 2008 (AR2, Three Star Reef I- 2SND-13V, Nakayama Steel Works, Osaka, Japan, Fig. 1a,
149 b, c). Detailed size information of the ARs is shown in Fig. S1. Previous eDNA surveys

150 of fish communities were conducted at AR1 (Sato et al. 2021, Inoue et al. 2022). Set-net
151 fishing was performed approximately 1.7 km away from AR1 (Fig. 1a).
152
153 **2.1.2. Video surveys using the UD**
154 The research vessel *Taka-maru* (Japan Fisheries Research and Education Agency:
155 FRA) was located upstream and downstream of each AR (four study stations, Fig. 1a, c).
156 The UD (FIFISH V6 Plus, QYSEA, Guangdong, China) was equipped with a depth
157 recorder (DEFI2-D20HG, JFE Advantech, Hyogo, Japan) and a photon recorder (DEFI2-
158 D20HG, JFE Advantech) was placed from *Taka-maru* and operated toward the ARs (Fig.
159 1c). The video camera of the UD was aimed at the AR from near the AR while maintaining
160 a horizontal posture by setting the control system. One round-trip vertical line transect
161 was conducted from the sea surface to the middle height of the AR at each study station,
162 and videos were taken using the UD built-in camera along the transects (Fig. 1c).
163 However, the transects did not proceed smoothly owing to the current flow; hence, the
164 same depth zone was recorded repeatedly during the course maintenance process (Fig. S2
165 and S3). No fish were observed in water depths shallower than the reef top (personal
166 observation, Y. Miyajima-Taga); therefore, shallow depth zones with no AR structure in
167 the video frame were excluded from subsequent analyses. The vertical speed during the

168 transects at depth zones above and below the reef top was 0.18 ± 0.07 and 0.11 ± 0.01 m
169 s^{-1} (mean ± SD), respectively. Recordings were performed with a video resolution of 4k
170 UHD 25 fps. No artificial lighting was used because the study stations were at a low
171 turbidity area where the Kuroshio Current flows in, and sufficient light intensity was
172 present for observation even at the depth of the survey. The visibility when the UD built-
173 in lighting was turned on was checked in advance at the study stations; however, the
174 lighting did not illuminate the video field of view evenly, resulting in uneven visibility
175 and no improved visibility. The total recording time was 1 h 0 m 16 s, between 09:50:35
176 and 14:56:46. The detailed recording conditions are listed in Table S1.
177 The current field during the field surveys was measured at multiple layers using a ship-
178 mounted 300-kHz acoustic Doppler current profiler (ADCP, Teledyne RD Instruments,
179 Poway, CA, USA, Fig. 1a). Ten second-averaged current field data were used every 4 m
180 depths between 39–55 m (AR1) or 39–50 m (AR2) as the current velocity of each study
181 station. Whether the UD was located appropriately upstream or downstream of the ARs
182 during the video recording was confirmed from the ADCP data and the dynamics of
183 floating objects in the water column by visual observation of the videos. To ensure that
184 fish behavior during the video survey was not significantly affected, the vertical profiles
185 of turbidity, salinity, and water temperature were measured 19 min 43 s to 34 min 8 s

before the video survey from the sea surface to just above the seabed near each video survey point except upstream of AR2 (Fig. 1c) using a conductivity temperature depth profiler (CTD, RINKO-Profiler, JFE Advantech).

2.1.3. Water sampling for eDNA analysis

Water sampling upstream and downstream of AR1 and AR2 for eDNA analysis was conducted on the same date as the video surveys (October 27, 2021). The water samplings were conducted at the same points of CTD measurement immediately after the video survey of each study station (Fig. 1c). Ten liters of seawater was collected from the middle (40 m deep) and bottom (5 m above the sea bottom) layers at each study station in the immediate vicinity of the ARs (20 m upstream and 12 m downstream of AR1; 5 m upstream and 30 m downstream of AR2) using one cast of two Niskin water samplers (5 L × 2 samples). Two 2 L samples were subsampled from the two Niskin water samples and immediately filtered using a combination of Sterivex filter cartridges (nominal pore size = 0.45 μm; Merck Millipore, Burlington, MA, USA) using an aspirator (the two filters were subsets of a single water collection) in a laboratory on *Taka-Maru* (Sato et al. 2021) (Supplementary Materials 2.1). The filter cartridges were stored at -20 °C until DNA extraction. In total, 18 eDNA samples (16 field samples [four stations × two depth

204 layers × two replicates] plus two negative controls) were collected and filtered (Table S2).

205

206 **2.2. Image analysis of the recorded video data**

207 Quantifying the spatial volume and position of a detectable range of fish in the recorded
208 videos is necessary to estimate the two-dimensional fish distributions based on volumetric
209 density. The images were clipped and resized (960 × 960 pixels) every 6 s in the recorded
210 videos to avoid excessive thinning out or overlapping information and used for the
211 subsequent analyses.

212

213 **2.2.1. Estimation of fish density**

214 Fish counting and species identification in each image were visually performed by the
215 first author with reference to the "Encyclopedia of Japanese Marine Fishes" (Yoshino
216 2019). Fish were identified using the preceding and following videos when identifying
217 species was challenging owing to the posture of the fish. Only species whose body
218 morphology differs according to sex and growth stage (e.g., immature, adult, or aged)
219 were further subclassified. Fish that were difficult to classify were counted as "unknown
220 species" (hereafter referred to as fish shadows). Fish shadows were defined as fish body
221 images wherein the shape of the caudal fin was confirmed. Among all identified fish

222 species in the video surveys, the only species that did not correspond to the homocercal
223 tail type (tail type of most teleost fish) was *Gymnothorax kidako* (isocercal tail type),
224 which appeared less frequently than other species. Therefore, the homocercal tail was
225 used to define fish shadows.

226 Species could be identified when individuals were located near the UD if they were
227 recorded horizontally to the ARs. Identifying fish species becomes impossible when the
228 distance of the fish increases from the UD; however, identifying it as a fish shadow is still
229 feasible. As the distance increases, the fish shadow becomes harder to identify and begins
230 to appear like a spindle-shaped object. This phenomenon was likely due to the recording
231 environment, such as the turbidity or photon quantity. Therefore, best-fitting general
232 linear models were created to predict the maximum detectable distance of fish species or
233 fish shadows from the UD considering the turbidity and photon quantity using full-scale
234 fish models with 20.7–34.1 cm of total length (Supplementary Materials 2.2.1. and Table
235 S3 and S4). All statistical analyses in this study were performed using R version 3.6.0 and
236 4.3.1 (R Core Team 2018). Turbidity and photon quantity had negative and positive
237 effects, respectively, on the detectable distances of fish. The selected models were as
238 follows:

239 $$A = 2.30 + 0.011P - 0.61T \qquad (1)$$

240 $$B = 3.16 + 0.011P - 0.75T \qquad (2)$$

241 where A and B (m) are the detectable distance of fish species and shadows, respectively,
242 and P (μmol m^{-2} s^{-1}) and T (FTU) are the photon quantity and turbidity of each image,
243 respectively. The density of each species, fish shadow, and total fish in each image was
244 calculated by dividing the number of each species, fish shadow, or total fish by $0.44A^3$,
245 0.44 (B^3–A^3), or $0.44B^3$ (m^3), respectively (Supplementary Materials 2.2.1.). Therefore,
246 the fish density within the detectable range of each image was assumed to be uniform.
247 The shape of the field of view of the UD is a rectangular pyramid with a height (distance
248 to the subject from the lens): base area (recorded width × height) of 1:1.53 × 0.86.
249
250 **2.2.2. Estimation of the fish community spatial distribution**
251 The position of the UD relative to the AR at the time each image was recorded was first
252 estimated to determine the position of the detectable range of each image. For each image,
253 the water depth of the UD was logged using a depth recorder, and the horizontal distance
254 from the UD to the nearest constituent material of the AR was estimated (hereafter
255 referred to as the distance from the AR surface, Fig. 1c) based on the size information of
256 the ARs and calculation of the pixel length from the video frame using ImageJ software
257 version 1.51J8 (National Institutes of Health, Bethesda, MD, USA; Supplementary

Materials 2.2.2.).

To estimate the fish distribution, a point cloud on the vertical two-dimensional plane with the x-axis as the horizontal distance from the AR surface and the y-axis as the water depth was set at intervals of 0.5 m. The x-coordinate ranged from -30 to 30 m (AR1) and from -20 to 20 m (AR2), whereas the y-coordinate ranged from the seabed (= 72 m) to 25 m (AR1) and the seabed (= 62 m) to 22 m (AR2). The distance from the AR surface was expressed as a negative or positive value for AR images recorded upstream or downstream of the ARs, respectively. Assuming that all UD positions during recording were on the same vertical two-dimensional plane (Fig. S3), two-dimensional coordinates of the detectable range of fish per image projected onto the vertical plane were obtained from the coordinates of the UD and the shape of the detectable range of fish estimated in section 2.2.1.

Of the point cloud, all points (retaining two-dimensional coordinates) within the two-dimensional detectable range—including those on the boundary—of each species, fish shadow, or total fish of each image were linked with the following data corresponding to each image: the number of species, the numbers and densities of each species, fish shadows and total fish, the recorded study station, and the detectable spatial volume. The genus was regarded and counted as one species if only the genus could be identified.

276 At points where the two-dimensional detectable range of each image overlapped, the
277 data of multiple images were linked. The mean value of these multiple data was also
278 linked at these points. MeanCount (the average number of fish occurrences in multiple
279 video frames) is used as a relative index of fish abundance primarily at stationary
280 underwater camera monitoring (Conn 2011) and was applied in this study. The maximum
281 number of relative abundance (MaxN) is the most commonly used for video survey data,
282 especially along artificial structures (Ajemian et al. 2015, Sward et al. 2019). MaxN is
283 the greatest number of individuals observed within one video frame of multiple video
284 frames during the transects and a conservative abundance value (Watson et al. 2005). This
285 conservative nature is a great advantage to avoid overestimation and to evaluate ARs as
286 a marine protected area. However, assessing ARs as a fishing ground requires a more
287 accurate reflection of the true fish abundance to estimate economic benefits. Therefore,
288 this study used the mean value as an index of fish abundance because the MeanCount
289 scale is linearly related to true fish abundance, and MaxN scales are nonlinearly related
290 (Conn 2011, Bacheler et al. 2013, Schobernd et al. 2013).
291 Points without linked data were excluded from the point cloud. Therefore, the point
292 cloud dataset for each study station had two-dimensional coordinates, multiple and mean
293 data of the number and density of fish, and recording environment information (hereafter

294 referred to as point cloud data). A heat map of the two-dimensional spatial distribution of
295 the mean number of species or density was constructed from the point cloud data without
296 interpolation. Matplotlib version 3.3.4, numpy version 1.19.2, pandas version 1.2.3, and
297 scipy version 1.6.1 of Python version 3.7.10 libraries were used for the point cloud data
298 analysis.
299
300 **2.3. DNA extraction and quantitative metabarcoding with qMiSeq**
301 eDNA was extracted and purified (Sato et al. 2021) by modifying a previous method
302 (Miya et al. 2016) (Supplementary Materials 2.3). qMiSeq was performed with MiFish
303 primers of actinopterygian (MiFish-U), elasmobranch (MiFish-Ev2), and sea sculpins
304 (MiFish-U2) versions (Table S5) to identify the fish taxa and quantify their eDNA
305 concentrations. MiFish primers were selected because of their detection ability and
306 congruence with the dataset of capture-based surveys for fish (Collins et al. 2019, Miya
307 et al. 2020). Five artificially designed internal standard DNAs were combined with a
308 template eDNA sample in the first PCR to calculate standard curves and estimate DNA
309 copy numbers (Ushio et al. 2018, Sato et al. 2021). Paired-end library preparation with
310 two-step PCR was performed (Supplementary Materials 2.3). The prepared DNA libraries
311 were sequenced on a MiSeq platform using the MiSeq v2 reagent kit (Illumina, San Diego,

312 CA, USA). Environmental Research & Solutions, Kyoto, Japan, performed library
313 preparation and sequencing.

314 Raw MiSeq data was converted into FASTQ files using the bcl2fastq program provided
315 by Illumina (bcl2fastq version 2.18). The FASTQ files were then demultiplexed using the
316 command implemented in Claident (Tanabe & Toju 2013), trimmed to sequences with a
317 quality score > 30, and classified to each sample. The paired-end reads were merged and
318 underwent quality filtering to remove reads with ambiguous sites, sequence length < 100
319 bp, sequence length > 250 bp, and error rate > 2.0%. The processed reads were subjected
320 to a BLASTn search against the fish DNA sequences from the NCBI database. The top
321 BLAST hits with an identity ≥ 98.5%, a query coverage of 100% was applied, and species
322 (or genus) names were assigned to the reads. The read number of the negative controls
323 was not used as the cut-off because the read number can vary among samples owing to
324 differences in PCR inhibition (Ushio et al. 2018). The detected species were compared
325 with local set-net catch records (Fig. 1a) (Sato et al. 2021) and distribution ranges from
326 FishBase (Froese & Pauly 2022). Two species (*Oncorhynchus keta* and *Gobio gobio*)
327 were detected in this study; however, they and their closely related species (sequence
328 identity ≥ 98%: *O. nerka* and *O. kisutch* for *O. keta,* and no species for *G. gobio*) do not
329 exist in Tateyama Bay. Therefore, these species were considered contamination and

330 excluded. Because primer–template mismatches can reduce the detection probability of
331 species with low primer specificity (Piñol et al. 2015), the mismatches for the observed
332 species were checked using the UD, and dominant species were verified through eDNA
333 analysis. The number of DNA copies in each sample was calculated using standard curves
334 of the internal standard DNAs—Std. A (100 copies μL^{1}), Std. B (50 copies μL^{-1}), Std. C
335 (25 copies μL^{-1}), Std. D (12.5 copies μL^{-1}), and Std. E (2.5 copies μL^{-1})—using linear
336 regression without intercept because the calculated eDNA copies should be ≥ 0 (Ushio et
337 al. 2018, Sato et al. 2021). If a fish species was detected from the negative controls, we
338 performed a background correction of eDNA concentration by subtracting the maximum
339 concentration of fish species DNA (copies/ml water) in the negative controls from the
340 concentration of DNA in the field sample. Details of the MiSeq metabarcoding and
341 qMiseq procedures are described in the Supplementary Materials 2.3.
342
343 **2.4. Data analysis**
344 Factors affecting the spatial distribution of the number of species or the densities of
345 total fish and dominant species were confirmed using the point cloud data of all study
346 stations. Five species with the highest appearance frequency in each study station in the
347 video surveys (chicken grunt *Parapristipoma trilineatum*, spotted knifejaw *Oplegnathus*

348 *punctatus*, striped beakfish *O. fasciatus*, Pacific anthiinae *Sacura margaritacea*, and
349 Stripey *Microcanthus strigatus*) were selected as dominant species (Table S6). Multiple
350 regression analyses were performed to test the effects of the AR type (AR1 or AR2),
351 horizontal position relative to the current direction (upstream or downstream), the
352 absolute value of the horizontal distance from the AR surface, vertical position relative to
353 the top of the reef (upper or lower, Fig. 1c), and the absolute value of the vertical distance
354 from the top of the reef on the number of species, total fish, and five dominant species.
355 The distribution of fish, upstream and downstream of the ARs, was approximately more
356 abundant closer to the AR surface and reef top. Therefore, the explanatory variables
357 related to the horizontal or vertical position were divided into the direction and the
358 absolute distance from the ARs to elucidate the distribution pattern of fish accurately. The
359 offset term for the analysis of the number of total fish or five dominant species was the
360 log-based detectable spatial volume. Interaction terms were not included in the model.
361 For the number of species data, the generalized linear model (GLM) of the Poisson
362 distribution with a log link function was used. The density of the *M. strigatus* data with
363 overdispersion but not zero inflation was analyzed with the GLM of negative binomial
364 distribution with a log link function. For other data, the zero-inflated negative binomial
365 model (ZINB) was used as overdispersion and zero inflation were confirmed

366 (Tlhaloganyang & Sakia 2020). ZINB is a two-component mixture model with a count
367 model part (a negative binomial distribution with a log link function) and a zero-inflated
368 model part (a binomial distribution with a logit link function) to predict excess zeros. The
369 relationship between the horizontal distance from the AR surface and the number of
370 species or the density of total fish or dominant species at the depth of the reef top (42 m
371 deep) was predicted using the selected ZINB or GLM; the horizontal distance from the
372 AR surface used for prediction was extrapolated up to 25 m, exceeding the recorded range
373 of the video surveys.

374 Multiple regression analysis was performed to test the effects of the AR type (AR1 or
375 AR2), the water collection position relative to the current direction (upstream or
376 downstream), and the depth layer (middle or bottom) on the number of species and the
377 eDNA copy numbers of total fish and five dominant species in qMiSeq using GLMs.
378 Interaction terms were not included in the model. The dominant species in qMiSeq were
379 *P. trilineatum*, Japanese sea bream *Pagrus major*, *S. margaritacea*, Silver-stripe round
380 herring *Spratelloides gracilis,* and skipjack tuna *Katsuwonus pelamis*. The models
381 assumed a Poisson distribution with a log link function for the number of species or a
382 normal distribution with an identity link function for a log-based eDNA density [log
383 (eDNA + 0.1)].

384 The ZINB, GLM of the Poisson or normal distribution, and GLM of the negative
385 binomial distribution were analyzed using zeroinfl and predict functions of the pscl
386 package, glm and predict functions of the stats package, and glm.nb and predict functions
387 of the MASS package in R, respectively. The r2 function of the sjmisc package in R was
388 used to calculate the R^2 value, which indicates the explanatory power of selected models.
389 Bayesian information criterion (BIC) was used to select an appropriate model (minimum
390 BIC) among the candidate models (Aho et al. 2014). The explanatory variables of the
391 selected model were confirmed as significant in model construction by comparing the
392 selected best minimum BIC and null models (the model with only intercept as predictor)
393 using the likelihood ratio test with a statistical significance level of 0.05. The lrtest
394 function of the lmtest package was used to perform likelihood ratio tests. By calculating
395 the variance inflation factor (VIF), we confirmed that there was a low multicollinearity
396 in the count model part of each selected model using the check_collinearity function of
397 the performance package in R (VIF = 1.00 ~ 3.24).
398 The effect of the sample size (i.e., the sampling fish number of the video surveys or the
399 qMiSeq replications) on observed species diversity was evaluated using the iNEXT
400 function of the iNEXT package in R (Chao et al. 2014). Sample size-based rarefaction
401 and extrapolated sampling curves for species diversity were estimated (i.e., species

richness, exponential Shannon diversity, and inverse Simpson diversity). Species richness, Shannon diversity, and Simpson diversity are indices that place more weight on the frequencies of all, rare, and abundant species, respectively (Chao et al. 2014). Since the true recorded fish number may be between the maximum number of fish counted in one image among all analyzed images (= MaxN) and total fish counts (including double counting), both index values per study station were used as the sample size for the video surveys in iNEXT analysis. The presence or absence of data on species per replicate was used as the sample size for qMiSeq in the iNEXT analysis.

3. RESULTS

3.1. Video surveys

Turbidity did not show a clear trend with water depth, whereas photon quantity attenuated logarithmically with increasing water depth (Fig. S4); detailed recording conditions are listed in Table S1. The range of the average predicted detectable distances of fish species and shadow were 2.2–2.3 m and 3.1 m, respectively (Table S1). The recorded range (overall detectable range of all analyzed images) was from 16.9 m outside to 3.1 m inside horizontally from the AR surface at maximum (Fig. S3).

From the visual identification of 575 images, 21 organisms were identified to species,

and one to genus from 27,965 fishes, whereas the other 13,456 fishes were fish shadows (Tables S1 and S6). However, these estimates may include double counting. All identified species were demersal (Froese & Pauly 2022), and the sex and growth stage of four species, *Parapristipoma trilineatum*, *Oplegnathus punctatus*, *Oplegnathus fasciatus,* and *Sacura margaritacea*, were identified (Fig. 2 and Table S6). iNEXT analysis indicated that in both sample size indexes, each diversity index at each study station except downstream of AR2 was not predicted to increase considerably even with larger sample sizes than those in this study. Species richness was predicted to be higher in the order of upstream of AR1, downstream of AR1, and upstream of AR2 (Fig. S5). The extrapolated curves tended toward infinity regarding the richness downstream of AR2 and may not be predicted correctly, probably due to the small number of observed fish compared to other sites.

The most abundant species was *P. trilineatum* (Table S6). Owing to the high density of *P. trilineatum* upstream of AR2, individuals far from the UD may have been hidden by individuals in the foreground, resulting in density underestimation in some images (Fig. S6). The spatial distribution of the number of species and the densities of total fish and the five dominant species are shown in Fig. 3. The densities of other species and subclassifications and fish shadows in the video surveys are shown in Fig. S7. The

438 number of species and densities of some species (e.g., *O. punctatus, Microcanthus*
439 *strigatus, Ostracion immaculatu*) peaked at the reef top of ARs and the middle floor of
440 AR2 (Fig. 1c, S1). The distribution trends of total fish were similar to those of *P.*
441 *trilineatum* and primarily distributed on the upstream side of both ARs, with the
442 maximum peak of distribution in 50-55 m depth, followed by the second peak at the reef
443 top (40–50m deep).

444 The GLM and ZINB results of the video surveys are shown in Table 1. In the ZINB,
445 the presence probability and abundance were evaluated using zero-inflation and count
446 models, respectively. The presence probability increases if the coefficient sign of the
447 explanatory variable in the zero-inflation model is negative. If the coefficients in the count
448 and zero-inflation models are positive and negative, respectively, the explanatory variable
449 has a positive effect on the fish distribution. In contrast, an example of a distribution
450 pattern when the coefficients of both models are positive is when schools of fish are
451 concentrated in a narrow area. All explanatory variables were selected in each minimum
452 BIC model. A significant difference was observed between each selected and null model
453 according to the likelihood ratio test. The results of count models indicated that AR1 had
454 a positive effect on all response variables except in *S. margaritacea* and *M. strigatus,* with
455 higher species richness and density in AR1 than in AR2. The upstream side of both ARs

had a positive effect on all response variables except for *M. strigatus*, indicating higher species richness and density upstream than downstream. All response variables significantly decreased with increasing horizontal distance from the AR surface except for *O. fasciatus*. The upper side of the reef top had a positive effect on all response variables except for the number of species and *O. fasciatus* and *M. strigatus*. The number of species and the densities of total fish, *O. fasciatus*, and *M. strigatus* decreased with increased vertical distance from the reef top.

At a reef top depth of 42 m, it was predicted that fish would hardly be distributed at a distance of approximately 20 m or more horizontally from the AR surface (Fig. 4). The appearance range of *O. punctatus* and *O. fasciatus* was within approximately 15 m from the AR surface, whereas that of *S. margaritacea* and *M. strigatu* was even narrower, approximately within 5 m. The appearance range of *P. trilineatum* upstream or downstream of the ARs was within approximately 15 or 8 m, respectively. While the fish density was assumed to be uniform within the detectable range of each image in section 2.2.1., sometimes the fish within the detectable range did not distribute close to the UD (Fig. 1b); however, sometimes they did (Fig. S6).

3.2. eDNA analysis

474 **3.2.1. Sequence read, fish fauna, and eDNA concentrations evaluation using qMiSeq**

475 MiSeq paired-end sequencing of the 18 libraries comprised 16 field and two negative
476 field samples, yielding a total of 1,215,827 reads. The final list of the field samples
477 included 103 fish species and six genera (Table S7). The proportions of demersal and
478 pelagic fish in all species were 79.8 and 20.2%, respectively. In this MiSeq run, the
479 sequence reads of the field and negative field samples were 851–132,894 (0.09–14.66%
480 of non-standard fish reads) and 2–610 (0.00–0.07% of non-standard fish reads),
481 respectively, excluding the standard DNA reads. In the negative controls, 1–597 reads
482 were contaminants for each species (e.g., *Seriola quinqueradiata* and *Siganus fuscescens*)
483 (Table S7). The read numbers of the four field samples were not converted to an eDNA
484 concentration because four out of the five internal standard DNAs were not detected for
485 sample No. 1 or R^2 values of the regression lines between the sequence reads and copy
486 numbers of standard DNAs were low for three other samples (No. 2, 3, and 15 in Table
487 S2) (R^2 values: 0.052−0.253, Table S8). For the remaining 12 field and two negative field
488 samples, the sequence reads of internal standard DNA had a positive relationship with
489 copy numbers based on a linear regression without intercept (R^2 values > 0.753, Table
490 S8). The read numbers of each species in these 14 samples were converted to an eDNA
491 concentration (Table S9) using the internal standard DNAs (Ushio et al. 2018).

492 Contamination levels were examined using the field negative controls (Ushio et al. 2019).
493 The total eDNA concentration of the two field negative controls was < 0.12% of the mean
494 eDNA concentration of field-positive samples (Table S9), indicating only a small amount
495 of contamination during field sampling, filtration, and library preparation. Nevertheless,
496 we subtracted the maximum concentration of fish species DNA (copies/ml water) in the
497 negative controls from the concentration of DNA in the field sample.

498 The top ten species with eDNA concentrations are shown in Table S10. This study
499 focused on five dominant species accounting for 88.1% of the total number of DNA
500 copies: 63.6% for *P. trilineatum*, 7.1% for *Pagrus major*, 4.8% for *Spratelloides gracilis*,
501 4.5% for *Katsuwonus pelamis,* and 2.4% for *S. margaritacea.* qMiSeq consistently
502 detected more species than video surveys (Fig. 5). Only 17.2% demersal, 0.0% pelagic,
503 or 13.8% total fish species detected using qMiSeq were confirmed by video surveys
504 (Table S6 and S7). Furthermore, several species detected by video surveys were
505 undetected by qMiSeq (8/22 species, Table S6). The number of primer–template
506 mismatches for the species observed by the UD and dominant species in eDNA analysis
507 varied from 0 to 2 (Table S11). Although no significant differences in primer–template
508 mismatches were observed between the detected and non-detected species using eDNA
509 analysis (Welch Two Sample *t*-test, $p = 0.138$), the average number of the mismatches

510 was slightly higher for the non-detected species than for the detected species (1.50 vs.
511 1.06). Each diversity index of each study station was predicted to increase moderately as
512 the replicates increase, especially upstream of AR2 (Fig. S5). Each diversity index was
513 the highest upstream of AR2, followed by downstream of AR2 and upstream of AR1.
514
515 **3.2.2. Factors affecting fish eDNA concentrations**
516 The selected model for the number of species, *P. major* and *K. pelamis* eDNA contained
517 the AR type (Table 2). The estimated parameters indicated that the number of species was
518 higher in AR2 than in AR1, whereas the eDNA concentrations of *P. major* and *K. pelamis*
519 were higher in AR1 than in AR2 (Fig. 6 and Table 2). The horizontal position relative to
520 the current direction was included in all the selected models except for *S. gracilis*. The
521 estimated parameters indicated that the number of species was higher upstream, whereas
522 the eDNA quantities were higher downstream. The depth layer was included in the
523 selected models for the number of species, total fish, and *P. trilineatum*. The number of
524 species was higher in the bottom layer than in the middle layer, whereas total fish and *P.*
525 *trilineatum* were higher in the middle layer. A null model was selected for *S. gracilis*
526 eDNA concentration. Significant differences were observed between all selected and null
527 models according to the likelihood ratio test except those of *S. gracilis* (Table 2).

528

529 **4. DISCUSSION**

530 **4.1. Development of the image analysis method**

531 Quantitative evaluation of fish distribution around ARs is crucial for the installation
532 planning of ARs or resource management in fisheries, and combining multiple monitoring
533 methods is effective for advancing quantitative evaluation (Bacheler et al. 2013). This
534 study revealed the fish distribution in ARs along the vertical line transects using a built-
535 in UD monaural camera. This quantification of the fish species richness and density was
536 based on laboratory and field experiments in multiple turbidity and light conditions to
537 obtain the detectable distance of fish by the camera. For image analysis in the video
538 surveys, the estimated absolute abundance index, i.e., volumetric density, can be used to
539 compare these results to those of eDNA analysis. However, the video survey method has
540 some limitations.

541 First, the turbidity ranges in the field video surveys were low (FTU < 0.49) and could
542 not be completely covered in the experimental conditions (FTU: 0.50−2,82), resulting in
543 an extrapolate prediction of the detectable distance. Therefore, the clearer the water
544 turbidity, the wider the detectable range; however, uncertainty existed in the lower
545 turbidity conditions of the field. Nonetheless, the turbidity was relatively constant during

546 the field survey (0.09–0.49), indicating a small effect on the detectable distances.
547 Therefore, the influence of this uncertainty was negligible when comparing species
548 richness and density among the images. Furthermore, differences in the predicted
549 detectable ranges among images were primarily attributed to photon quantity; therefore,
550 the shallower the water depth, the wider the detectable range was. The ranges predicted
551 by photon quantity were reliable because the field photon values were covered in the
552 experimental conditions.

553 Second, although the video surveys were performed along a vertical line transect, the
554 UD position fluctuated by several meters. Furthermore, position information on the z-axis
555 (horizontal direction along the AR wall, Fig. S2) could not be obtained. Therefore,
556 aggregated fish may have specific distribution patterns near the ridgeline or in the center
557 of the AR side. Future research with the three-dimensional position information (i.e.,
558 latitude, longitude, and water depth) through the introduction of an underwater position
559 system would allow for the creation of a 3D image of fish distribution around ARs.

560 Finally, although the image analysis was performed based on the assumption that the
561 fish density was uniform within the detectable spatial range, sometimes fish distribution
562 bias was observed within the detectable range even though the range was relatively
563 narrow, such as that the fish were not distributed close to the UD. In this case, the actual

564 fish distribution may have been closer to the AR surface by, at most, a detectable distance
565 (fish species: 2.1–2.2 m, fish shadow: 3.1 m, on average), or the local fish density may
566 have been higher. Furthermore, the estimated density may be affected by changes in the
567 detectable range depending on fish size. For example, when the small- and large-sized
568 fishes are located at the same distance from ROVs, identifying the morphological features
569 of small-sized fish whose constituting pixels are fewer is more challenging than that of
570 large-sized fish (Andaloro et al. 2013). Thus, the true detectable range of fish smaller than
571 those used in the full-scale fish models in the experiment (20.7–34.1 cm of total length)
572 may be smaller than those estimated in this study. The sampling volume estimation
573 method using a monaural camera is applicable without increasing research equipment and
574 complicated analysis. In contrast, Rand et al. (2006) estimated all identified fish positions
575 to define the sampling space using stereo cameras. This method using stereo cameras
576 could overcome the limitations of the present study and, therefore, allow more accurate
577 density estimation.
578
579 **4.2. Detected fish communities using UDs and eDNA surveys**
580 The detected fish communities were compared between the two methods used. On the
581 day of water sample collection, eDNA metabarcoding detected more species than the

582 video surveys conducted on the same day as sample collection. This finding is similar to
583 that of previous studies comparing eDNA metabarcoding and underwater visuals census,
584 i.e., higher detection ability for cryptic, pelagic, tiny, or rare fish species using eDNA
585 metabarcoding even without a complete reference database (Port et al. 2016, Yamamoto
586 et al. 2017, Polanco Fernández et al. 2021). Most species only detected by our eDNA
587 analysis had the following characteristics that could cause false-negative results in the
588 video surveys: small-sized species (*Maurolicus japonicus*, *Spratelloides gracilis*, and
589 *Ostorhinchus semilineatus*), pelagic strong swimmers (*Katsuwonus pelamis*, *S. gracilis*,
590 and *Scomber* spp.), cryptic fish inhabiting AR structures (*Paralichthys olivaceus*,
591 *Sebastiscus marmoratus*, and *Hime japonica*), or species which may disperse as pelagic
592 egg or larvae in autumn (*Acanthopagrus latus*, *Arothron firmamentum*, and *Dentex* spp.)
593 (Abol-Munafi and Umeda 1994,). Owing to the survey design of this study, the UD only
594 recorded demersal fish that were likely strongly localized in ARs. ROVs do not allow for
595 a complete description of the fish community but are an appropriate method to census
596 high abundance and low mobility fish, both from a qualitative and quantitative point of
597 view (Smith 1988, Willis 2001, Tessier et al. 2005, Andaloro et al. 2013).

598 The differences in species composition between the two methods may be attributed to
599 several causes. First, the video survey results were a snapshot of the short temporal range

and may have a bias related to fish activity time. In contrast, the qMiSeq results reflect temporally accumulated eDNA distributions. The eDNA result has a few hours of temporal information until the eDNA is dispersed and degraded (Murakami et al. 2019).

Second, the qMiSeq results reflect spatially accumulated eDNA distributions, namely spatial influence on eDNA inflow from outside the study stations (Murakami et al. 2019). Because several ARs and one set-net are in the surrounding area of the target ARs (Fig. 1a), eDNA transport from these ARs may increase the detected species even at low eDNA concentrations. Furthermore, eDNA derived from pelagic fish at shallow depths (not observed close to the ARs by the UD) could have sunk to AR depths and was sampled there. In contrast, the detectable range of fish in each image or the whole recorded range in the video surveys was narrow; highly mobile pelagic fish migrate over a wider range around ARs, and cryptic fish may have few opportunities to swim within the narrow-recorded range. In addition, the detectable range of small-sized fish may be smaller than that of large-sized fish, resulting in small-sized fish being harder to detect.

Third, a bias associated with the avoidance or attraction behavior of fish is also conceivable. Although UDs are less likely to induce fish avoidance behavior than divers (Hellmrich et al. 2023), the possibility cannot be excluded that some species avoid or are attracted to UDs. Fourth, several species (3–6 species per study station) detected by the

video surveys were undetected by qMiSeq (Fig. 5). This finding could be due to the incomplete species detection power of the universal primer set MiFish for certain species via primer–template mismatches or an incomplete reference database (e.g., *Hyporthodus septemfasciatus, Pseudanthias squamipinnis,* and *Stephanolepis cirrhifer*). Additionally, a low amount of eDNA discharged from these species can be another reason. Because species diversity was estimated to increase as the number of replicates increased in eDNA analysis, such species could be detected if the sample size is increased.

Information was obtained on the growth stages and sexes of *Parapristipoma trilineatum*, *Oplegnathus punctatus*, *Oplegnathus fasciatus,* and *Sacura margaritacea* through the video surveys. Growth stage information from video surveys may be useful as complementary information to eDNA analysis. For example, Sato et al. (2021) inferred that the reason why splendid alfonsino (*Beryx splendens*) was detected in waters evidently shallower than the habitat depth of adults by qMiSeq was the presence of juveniles. In such case, video surveys are useful because they can confirm the growth stage. Furthermore, although qMiSeq provided quantitative values of eDNA concentrations that cannot directly be converted into density, the video surveys could obtain the density information. The volumetric biomass can be roughly estimated using the body weight information of individuals at the same growth stage caught nearby with these obtained

636 data. Furthermore, detailed fish size measurements can be obtained by installing stereo
637 body length measurement technology on UDs (Harvey et al. 2002, Garner et al. 2021).
638
639 **4.3. Fine-scale spatial distribution of fish communities**
640 The spatial distribution trends in the video surveys differed depending on species.
641 However, the shorter the horizontal distance from the AR surface, the higher the predicted
642 number of appearance species and total fish density were, and the effective range of ARs
643 on fish at the depth of reef top (42 m deep) was predicted within approximately 20 m at
644 most. Therefore, the aggregation effect of ARs on fishes was revealed in terms of richness
645 and abundance. However, the video survey area was limited to the immediate vicinity of
646 the ARs; therefore, a wider survey may change the fish distribution at the extrapolation
647 area (16.9–25 m from the AR surface) predicted in this study. These results were similar
648 to those of previous studies showing a higher fish density or fish eDNA near ARs (Noh
649 et al. 2017, Inoue et al. 2018, 2020, Sato et al. 2021). Fish eDNA concentration had the
650 highest peak near the ARs and sharply decreased at a distance of 150 m from the ARs,
651 indicating that the spatial scale of fish aggregation is within a range of 150 m from the
652 AR (Sato et al. 2021, Inoue et al. 2022). However, these fish aggregation ranges observed
653 using eDNA analysis only represent the detectable range of eDNA generated and

654 transported from ARs and may not necessarily reflect actual fish distributions. Therefore,
655 the spatial range of demersal fish aggregation to ARs observed by the video surveys was
656 narrow compared to that by eDNA analysis and is more reliable.

657 Detailed differences were observed in the spatial distribution for each species
658 associated with current flow in the video surveys. Specifically, a significantly higher
659 number of species, total fish density, and the density of *P. trilineatum* and *S. margaritacea*,
660 which were commonly detected as the dominant species by both methods, were observed
661 upstream than downstream of the ARs in the video surveys. In contrast, the eDNA
662 statistical analysis showed that the total fish density and the density of both species were
663 significantly higher downstream than upstream of ARs. The discrepancy in the
664 distribution trends between the two methods in this study may be due to the transport of
665 eDNA by the current flow. Therefore, eDNAs released upstream may be carried
666 downstream. In addition, a slight spatial lag was observed between the video surveys and
667 the water samplings; therefore, the induced differences in the unmeasured fine-scaled
668 flows may lead to the discrepancy between them.

669 Our previous study conducted in the same study site using qMiseq showed that
670 dominant species, including *P. trilineatum* assembled upstream of ARs (Inoue et al. 2022).
671 The same qMiSeq technique was used in this study and by Inoue et al. (2022); however,

672 the results differed, likely owing to this study sampling eDNA in the immediate vicinity
673 of the ARs, whereas the other study covered a larger area of 1.48 km^2 per AR. Therefore,
674 the eDNA approach may require a larger survey area to determine the spatial distribution
675 pattern of fishes around ARs than that used in this study.

676 According to the video surveys, AR1 had significantly higher richness and total
677 abundance than those of AR2. This finding may be because of the difference in the size
678 and shape of the ARs: the area of the AR1 reef top is considerably larger than that of AR2.
679 Many species were located relatively closer to the AR reef top and the middle floor of
680 AR2. Thus, the large horizontal surface of AR1 may affect fish distribution. A previous
681 study using ROV observations showed the same distribution pattern, wherein fish
682 abundance was concentrated at the reef top (Ajemian et al. 2015). In the present study,
683 the fish density in the video surveys was corrected using the detectable volume
684 considering photon quantity and turbidity. In contrast, as the water becomes shallower
685 and brighter, the range in which fish can be identified becomes wider, potentially
686 increasing the number of identified species. The upstream side of AR1 was brighter than
687 that of other study stations; therefore, the number of identified species may be increased
688 because of this environmental bias. qMiSeq results showed an opposite trend in diversity
689 to that of the video surveys and no difference in total eDNA concentrations between the

690 two ARs. The discrepancy in the number of detected fish species is possibly due to the
691 higher eDNA detection in AR2 for pelagic fish. However, the discrepancy in the density
692 pattern between the two methods may be due to the fact that fish were distributed to a
693 greater degree outside the range recorded by the videos or subtle differences in the eDNA
694 sampling points between the ARs. The influence of eDNA transport from other ARs and
695 the set-net was also considered; however, its effect was likely negligible on the eDNA
696 concentrations of the dominant species. The first reason is that previous studies showed
697 that the eDNA concentration sharply decreases at a distance of 150 m from the ARs, and
698 other ARs are not located within a distance of 200 m from the target ARs (Sato et al. 2021,
699 Inoue et al. 2022). Secondly, one-tenth to one thousand levels of eDNA concentration
700 were reported at 300 and 600 m from the source (Murakami et al. 2019); we assume that,
701 this level of transported eDNA unlikely affects fish eDNA concentration around the target
702 ARs.

703 The eDNA concentrations of *P. trilineatum* were much higher in the middle layer (40
704 m depth) than those in the bottom layer (67 and 57 m deep at AR1 and 2, respectively),
705 whereas the species richness were higher in the bottom layer. However, according to the
706 video surveys, the maximum vertical peaks of these densities were at 50–55 m depth and
707 those of species richness were near the reef top (40–50m deep) for both ARs; therefore,

708 the eDNA concentration of *P. trilineatum* and eDNA species richness should be most
709 abundant near the bottom and middle layers, respectively. Possible reasons for this
710 discrepancy are the specific behavior or buoyancy of eDNA of each species and its
711 interaction with vertical currents, which may move eDNA vertically. Furthermore,
712 because the study period (late October) does not match with the spawning and dispersal
713 season (June to August) (Nunobe et al. 2008), it is unlikely that the eDNA method
714 detected eggs and larvae.

715 In conclusion, this study quantitatively evaluated fish aggregation around ARs on a
716 fine spatial scale using video surveys from UDs over a relatively short field time, though
717 the detection probability of fish using ROVs generally increases with longer observation
718 time or the number of censuses (Pita et al. 2014). However, the fish counting and
719 identification procedures in this study took several months. Therefore, automatic fish
720 recognition based on deep learning should be applied in the future (Meng et al. 2018,
721 Villon et al. 2018, Li et al. 2022). Quantitative evaluation using video surveys was likely
722 inferior to eDNA analysis in species detection sensitivity in small-sized, pelagic, and
723 cryptic fish. Conversely, eDNA metabarcoding could have detected some species by
724 transporting eDNA from other ARs. Therefore, the video survey was more suitable for
725 observation of a narrow area around ARs than eDNA analysis because of the effects of

726 the current transportation of eDNA. Furthermore, the video survey could obtain the
727 spatial distribution of low-mobility demersal fishes based on the richness, density, and
728 information on the growth stages and sexes of fish based on their morphology. Therefore,
729 this study provides an understanding of the characteristics of both novel methods for the
730 quantitative assessment of fish assemblages in ARs and shows that the disadvantages of
731 each method may complement each other to a certain extent.

732

733 **SEQUENCE DATA**

734 DDBJ accession numbers of the DNA sequences analyzed in this study are
735 PRJDB16358 (BioProject ID) and DRR500425-DRR500450 (DDBJ Sequence Read
736 Archive).

737

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748

749 **LITERATURE CITED**

750 Aho K, Derryberry D, Peterson T (2014) Model selection for ecologists: the worldviews
751 of AIC and BIC. Ecology 95:631–636

752 Ajemian MJ, Wetz JJ, Shipley-Lozano B, Stunz GW (2015) Rapid assessment of fish
753 communities on submerged oil and gas platform reefs using remotely operated
754 vehicles. Fish Res 167:143–155

755 Andaloro F, Ferraro M, Mostarda E, Romeo T, Consoli P (2013) Assessing the suitability
756 of a remotely operated vehicle (ROV) to study the fish community associated with
757 offshore gas platforms in the Ionian Sea: a comparative analysis with underwater visual
758 censuses (UVCs). Helgol Mar Res 67:241–250

759 Bacheler NM, Schobernd CM, Schobernd ZH, Mitchell WA, Berrane DJ, Kellison GT,
760 Reichert MJM (2013) Comparison of trap and underwater video gears for indexing reef
761 fish presence and abundance in the southeast United States. Fish Res 143:81–88

762 Bohnsack JA, Sutherland DL (1985) Artificial reef research: A review with
763 recommendations for future priorities. Bull Mar Sci 37:11–39
764 Brotto DS, Krohling W, Zalmon IR (2007) Comparative evaluation of fish assemblages
765 census on an artificial reef. Rev Bras Zool 24:1157–1162
766 Bortone SA, Samoilys MA, Francour P (2000) Fish and macroinvertebrate evaluation. In:
767 Seaman W Jr (ed) Artificial reef evaluation with application to natural marine habitats.
768 CRC Press, Boca Raton, p 127–164
769 Chao A, Gotelli NJ, Hsieh TC, Sander EL, Ma KH, Colwell RK, Ellison AM (2014)
770 Rarefaction and extrapolation with Hill numbers: a framework for sampling and
771 estimation in species diversity studies. Ecol. Monog 84:45–67
772 Collins RA, Bakker J, Wangensteen OS, Soto AZ and others (2019) Non-specific
773 amplification compromises environmental DNA metabarcoding with COI. Methods
774 Ecol Evol 10:1985−2001
775 Conn PB (2011) An evaluation and power analysis of fishery independent reef fish
776 sampling in the Gulf of Mexico and U.S. South Atlantic. NOAA Technical
777 Memorandum NMFS-SEFSC-610
778 Froese R, Pauly D (2022) FishBase. www.fishbase.org (accessed 17 Jan 2023)
779 Garner SB, Olsen AM, Caillouet R, Campbell MD, Patterson WF III (2021) Estimating

780 reef fish size distributions with a mini remotely operated vehicle-integrated stereo
781 camera system. PLOS ONE 16:e0247985
782 Goldberg CS, Strickler KM, Pilliod DS (2015) Moving environmental DNA methods
783 from concept to practice for monitoring aquatic macroorganisms. Biol Conserv 183:1–
784 3
785 Harvey E, Fletcher D, Shortis M (2002) Estimation of reef fish length by divers and by
786 stereo-video: a first comparison on the accuracy and precision in the field on living fish
787 under operational conditions. Fish Res 57:255–263
788 Hellmrich LS, Saunders BJ, Parker JRC, Goetze JS, Harvey ES (2023) Stereo-ROV
789 surveys of tropical reef fishes are comparable to stereo-DOVs with reduced
790 behavioural biases. Estuar Coast Shelf Sci 281:108210
791 Holland MM, Becker A, Smith JA, Everett JD, Suthers IM (2021) Fine-scale spatial and
792 diel dynamics of zooplanktivorous fish on temperate rocky and artificial reefs. Mar
793 Ecol Prog Ser 674:221–239
794 Inoue N, Nambu R, Kuwahara H, Kuwamoto J, Yokoyama J, Kanaiwa M (2018) The
795 present situation of utilization and the effects of artificial reefs on the resource density
796 of Japanese butterfish *Hyperoglyphe japonica* and red sea bream *Pagrus major* in the
797 waters of the Iki Islands, Nagasaki, Japan. Nippon Suisan Gakkaishi 84:1010–1016

798 (article written in Japanese with English abstract)
799 Inoue N, Ishimaru S, Hashimoto K, Kuwamoto J, Masubuchi T, Kanaiwa M (2020) Using
800 standardized CPUE to estimate the effect of artificial reefs on fish abundance. In:
801 Bortone AS, Otake S (eds) Modern Fisheries Engineering: Realizing a Healthy and
802 Sustainable Marine Ecosystem, CRC Press, Florida, US, p 75–84
803 Inoue N, Sato M, Furuichi N, Imaizumi T, Ushio M (2022) The relationship between
804 eDNA density distribution and current fields around an artificial reef in the waters of
805 Tateyama Bay, Japan. Metabarcoding Metagenom 6:e87415
806 Kakimoto H (1967) On the effective areas of the artificial reefs. Suisan Zoshoku 14:181–
807 198 (article written in Japanese)
808 Kakimoto H (1993) Control of fish behavior by the artificial reef. Fish Eng (Jpn) 30:59–
809 67
810 Kang M, Nakamura T, Hamano A (2011) A methodology for acoustic and geospatial
811 analysis of diverse artificial-reef datasets. ICES J Mar Sci 68:2210–2221
812 Li J, Li J, Gong P, Guan C (2021) Effects of the artificial reef and flow field environment
813 on the habitat selection behavior of *Sebastes schlegelii* juveniles. Appl Anim Behav Sci
814 245:105492
815 Li L, Shi F, Wang C (2022) Fish image recognition method based on multi-layer feature

816 fusion convolutional network. Ecol Inf 72:101873

817 Lima JS, Zalmon IR, Love M (2019) Overview and trends of ecological and

818 socioeconomic research on artificial reefs. Mar Environ Res 145:81–96

819 Liu TL, Su DT (2013) Numerical analysis of the influence of reef arrangements on

820 artificial reef flow fields. Ocea Engi 74:81–89

821 Maes SM, Desmet S, Brys R, Sys K, Ruttink T, Maes S, Hostens K, Vansteenbrugge L,

822 Derycke S (2023) Detection and quantification of two commercial flatfishes (*Solea*

823 *solea* and *Pleuronectes platessa*) in the North Sea using environmental DNA. Environ

824 DNA 00: 1–15. https://doi.org/10.1002/edn3.426

825 Meng L, Hirayama T, Oyanagi S (2018) Underwater-drone with panoramic camera for

826 automatic fish recognition based on deep learning. IEE Access 6:17880–17886

827 Miya M, Minamoto T, Yamanaka H, Oka S, Sato K, Yamamoto S, Sado T, Doi H (2016)

828 Use of a filter cartridge for filtration of water samples and extraction of

829 environmental DNA. J Visual Exper 117:54741

830 Miya M, Sato Y, Fukunaga T, Sado T, Poulsen JY, Sato K, Minamoto T, Yamamoto S,

831 Yamanaka H, Araki H, Kondoh M, Iwasaki W (2015) MiFish, a set of universal PCR

832 primers for metabarcoding environmental DNA from fishes: detection of more than

833 230 subtropical marine species. R Soc Open Sci 2:150088

834 Miya M, Gotoh RO, Sado T (2020) MiFish metabarcoding: a high-throughput approach
835 for simultaneous detection of multiple fish species from environmental DNA and other
836 samples. Fish Sci 86:939–970
837 Murakami H, Yoon S, Kasai A, Minamoto T, Yamamoto S, Sakata MK, Horiuchi T,
838 Sawada H, Kondoh M, Yamashita Y, Masuda R (2019) Dispersion and degradation of
839 environmental DNA from caged fish in a marine environment. Fish Sci 85:327–337
840 Noh J, Ryu J, Lee D, Khim JS (2017) Distribution characteristics of the fish assemblages
841 to varying environmental conditions in artificial reefs of the Jeju Island, Korea. Mar
842 Pollut Bull 118:388–396
843 Nunobe J, Kinoshita I, Sashida M, Murata O (2008) Distribution of larvae of
844 *Parapristipoma trilineatum* (Haemulidae) in Tosa Bay, Japan. Bull Jpn Soc Fish
845 Oceanogr 72:83–91 (article written in Japanese with English abstract)
846 Okamoto M, Kuroki T, Murai T (1979) Fundamental studies on the ecology of fishes near
847 artificial reefs 1. Preparatory observation of fish populations. Nippon Suisan Gakkaishi
848 45:1085–1090 (article written in Japanese with English abstract)
849 Piñol J, Mir G, Gomez-Polo P, Agustí N (2015) Universal and blocking primer
850 mismatches limit the use of high-throughput DNA sequencing for the quantitative
851 metabarcoding of arthropods. Mol Ecol Resour 15:819– 830

852 Pita P, Fernández-Márquez D, Freire J (2014) Short-term performance of three
853 underwater sampling techniques for assessing differences in the absolute abundances
854 and in the inventories of the coastal fish communities of the Northeast Atlantic Ocean.
855 Mar Freshw Res 65:105–113
856 Polanco Fernández A, Marques V, Fopp F, Juhel J-B, Borrero-Pérez GH, Cheutin M-C,
857 Dejean T, González Corredor JD, Acosta-Chaparro A, Hocdé R, Eme D, Maire E,
858 Spescha, M, Valentini A, Manel S, Mouillot D, Albouy C, Pellissier L (2021)
859 Comparing environmental DNA metabarcoding and underwater visual census to
860 monitor tropical reef fishes. Environ DNA 3:142–156
861 Polovina JJ, Sakai I (1989) Impacts of artificial reefs on fishery production in Shimamaki,
862 Japan. Bull Mar Sci 44:997–1003
863 Port JA, O'Donnell JL, Romero-Maraccini OC, Leary PR, Litvin SY, Nickols KJ,
864 Yamahara KM, Kelly RP (2016) Assessing vertebrate biodiversity in a kelp forest
865 ecosystem using environmental DNA. Mol Ecol 25:527–541
866 R Core Team (2018) R: A language and environment for statistical computing. R
867 Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org
868 (accessed November 2023)
869 Rand PS, Taylor JC, Eggleston DB (2006) A video method for quantifying size

870 distribution, density, and three-dimensional spatial structure of reef fish spawning
871 aggregations. AquaDocs 124:4–9
872 Sato M, Inoue N, Nambu R, Furuichi N, Imaizumi T, Ushio M (2021) Quantitative
873 assessment of multiple fish species around artificial reefs combining environmental
874 DNA metabarcoding and acoustic survey. Sci Rep 11:19477
875 Schobernd ZH, Bacheler NM, Conn PB (2013) Examining the utility of alternative video
876 monitoring metrics for indexing reef fish abundance. Can J Aquat Sci 71)
877 Smith MPL (1988) Effects of observer swimming speed on sample counts of temperate
878 rocky reef fish assemblages. Mar Ecol Prog Ser 43:223–231
879 Sward D, Monk J, Barrett N (2019) A systematic review of remotely operated vehicle
880 surveys for visually assessing fish assemblages. Front Mar Sci 6:134
881 Tanabe AS, Toju H. (2013) Two new computational methods for universal DNA
882 barcoding: A benchmark using barcode sequences of bacteria, Archaea, animals, fungi,
883 and land plants. PLOS ONE 8:e76910
884 Tessier E, Chabanet P, Pothin K, Soria M, Lasserre G (2005) Visual censuses of tropical
885 fish aggregations on artificial reefs: slate versus video recording techniques. J Exp Mar
886 Biol Ecol 315:17–30
887 Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E (2012)

888 Detection of a diverse marine fish fauna using environmental DNA from seawater
889 samples. PLOS ONE 7:e41732
890 Tlhaloganyang BP, Sakia RM (2020) Zero inflated Poisson distribution in equidispersed
891 data with excessive zeros. Res J Mathematical and Statistical Sci 8:31–34
892 Ushio M, Murakami H, Masuda R, Sado T, Miya M, Sakurai S, Yamanaka H, Minamoto
893 T, Kondoh M (2018) Quantitative monitoring of multispecies fish environmental DNA
894 using high-throughput sequencing. Metabarcoding Metagenom 2:e23297
895 Ushio M (2019) Use of a filter cartridge combined with intra-cartridge bead-beating
896 improves detection of microbial DNA from water samples. Methods Ecol Evol
897 10:1142–1156
898 van Bleijswijk JDL, Engelmann JC, Klunder L, Witte H, Witte JIJ, van der Veer HW
899 (2020) Analysis of a coastal North Sea fish community: Comparison of aquatic
900 environmental DNA concentrations to fish catches. Environ DNA 2:429–445
901 Villon S, Mouillot D, Chaumont M, Darling ES, Subsol G, Claverie T, Villéger S (2018)
902 A deep learning method for accurate and fast identification of coral reef fishes in
903 underwater images. Ecol Inf 48:238–244
904 Watson DL, Harvey ES, Anderson MJ, Kendrick GA (2005) A comparison of temperate
905 reef fish assemblages recorded by three underwater stereo-video techniques. Mar Biol

906 148:415–425

907 Williams K, Rooper CN, Robertis AD, Levine M, Tower R (2018) A method for
908 computing volumetric fish density using stereo cameras. J Exp Mar Biol Ecol 508:21–
909 26

910 Willis TJ (2001) Visual census methods underestimate density and diversity of cryptic
911 reef fishes. J Fish Biol 59:1401–1408

912 Yamamoto S, Masuda R, Sato Y, Sado T, Araki H, Kondoh M, Minamoto T, Miya M
913 (2017) Environmental DNA metabarcoding reveals local fish communities in a species-
914 rich coastal sea. Sci Rep 7:40368

915 Yoshino Y (2018) Yamakei handy illustrated book revised edition Japanese marine fishes.
916 Yamatokeikokusya, Tokyo (written in Japanese)

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Tables

Table 1. Selected (minimum Bayesian information criterion) models (= models that included all explanatory variables considered, i.e., first models) for estimating the spatial distribution of the number of species and densities of total fish and five dominant species in the video surveys

Response variable	Explanatory variable	Coefficient		R^2
		Count model	Zero-inflation model	
Number of species	Intercept	1.448	-	0.471
(GLM with Poisson distribution)	AR type: AR2	-0.414	-	
	Horizontal position: upstream	0.207	-	
	Horizontal distance (m)	-0.214	-	
	Vertical position: upper	-0.266	-	
	Vertical distance (m)	-0.065	-	
	Likelihood ratio test (selected model vs null model): Chisq = 2127.4, $p < 0.001$			
Density (individual m^{-3})				
Total fish	Intercept	1.060	-23.618	1.000
(ZINB)	AR type: AR2	-1.120	16.641	
	Horizontal position: upstream	3.033	2.516	
	Horizontal distance (m)	-0.267	0.752	
	Vertical position: upper	1.102	0.811	
	Vertical distance (m)	-0.054	-0.061	
	Log (theta)	-0.911	-	
	Likelihood ratio test (selected model vs null model): Chisq = 5731.4, $p < 0.001$			
P*arapristipoma trilineatum*	Intercept	2.391	-0.777	1.000
(ZINB)	AR type: AR2	-0.499	3.999	
	Horizontal position: upstream	1.038	-5.335	
	Horizontal distance (m)	-0.073	0.758	
	Vertical position: upper	0.588	-0.505	

	Vertical distance (m)	0.046	-0.019	
	Log (theta)	-0.393	-	
	Likelihood ratio test (selected model vs null model): Chisq = 3119.5, $p < 0.001$			
Oplegnathus punctatus (ZINB)	Intercept	-1.433	-3.078	0.360
	AR type: AR2	-0.508	0.338	
	Horizontal position: upstream	0.604	1.450	
	Horizontal distance (m)	-0.478	-0.399	
	Vertical position: upper	0.887	1.951	
	Vertical distance (m)	0.377	0.641	
	Log (theta)	-0.924	-	
	Likelihood ratio test (selected model vs null model): Chisq = 905.43, $p < 0.001$			
Oplegnathus fasciatus (ZINB)	Intercept	-1.088	-6.472	0.231
	AR type: AR2	-0.895	-0.186	
	Horizontal position: upstream	0.188	1.986	
	Horizontal distance (m)	0.034	0.708	
	Vertical position: upper	-0.569	1.399	
	Vertical distance (m)	-0.012	0.182	
	Likelihood ratio test (selected model vs null model): Chisq = 866.45, $p < 0.001$			
	Log (theta)	0.414		
Sacura margaritacea (ZINB)	Intercept	-2.394	-1117.09	0.659
	AR type: AR2	3.748	494.83	
	Horizontal position: upstream	1.270	508.57	
	Horizontal distance (m)	-1.052	44.49	
	Vertical position: upper	1.259	403.80	
	Vertical distance (m)	0.029	57.29	
	Log (theta)	-2.614	-	
	Likelihood ratio test (selected model vs null model): Chisq = 410.52, $p < 0.001$			
Microcanthus strigatus (GLM with negative binomial distribution)	Intercept	0.122	-	0.454
	AR type: AR2	3.710	-	
	Horizontal position: upstream	-2.271	-	
	Horizontal distance (m)	-0.810	-	
	Vertical position: upper	-0.127	-	
	Vertical distance (m)	-0.261	-	

Likelihood ratio test (selected model vs null model): Chisq = 482.94, $p < 0.001$

AR: artificial reef
GLM: generalized linear model
ZINB: zero-inflated negative binomial model

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924 Table 2. Selected [minimum Bayesian information criterion (BIC)] and full models for estimating the spatial distribution of the number

925 of species and environmental DNA (eDNA) concentration of total fish and five dominant species in eDNA analysis

Response variable	Explanatory variable	Selected model			Full model		
		Coefficient	BIC	R^2	Coefficient	BIC	R^2
Number of species	Intercept	2. 981	100.6	0. 884	2. 981	100.6	0. 884
	AR type (AR1)	-0.443			-0.443		
	Direction (upstream)	0. 457			0. 457		
	Depth layer (middle)	-0.274			-0. 274		
	Likelihood ratio test (selected model vs null model): Chisq = 34.45, $p < 0.001$						
eDNA concentration [copies (mL water)$^{-1}$]							
Total fish	Intercept	3.730	33.5	0.769	3.754	35.0	0.788
	AR type (AR1)	-			-0.413		
	Direction (upstream)	-1.265			-1.095		
	Depth layer (middle)	1.803			1.900		
	Likelihood ratio test (selected model vs null model): Chisq = 17.60, $p < 0.001$						
Parapristipoma trilineatum	Intercept	0.560	39.6	0.889	0.895	40.6	0.902
	AR type (AR1)	-			-1.260		
	Direction (upstream)	-1.521			-0.633		
	Depth layer (middle)	4.738			4.589		
	Likelihood ratio test (selected model vs null model): Chisq = 26.41, $p < 0.001$						
Pagrus major	Intercept	-0.715	52.9	0.450	-0.886	55.2	0.456
	AR type (AR1)	2.576			2.490		

	Direction (upstream)	-2.107			-2.021		
	Depth layer (middle)	-			0.314		
	Likelihood ratio test (selected model vs null model): Chisq = 7.168 p = 0.028						
Spratelloides gracilis	Intercept	-0.249	57.8	0	-0.771	63.5	0.135
	AR type (AR1)	-			1.663		
	Direction (upstream)	-			-0.508		
	Depth layer (middle)	-			0.250		
	Likelihood ratio test (selected model vs null model): Chisq = 0, p = 1.000						
Katsuwonus pelamis	Intercept	-1.459	50.8	0.429	-1.730	56.4	0.550
	AR type (AR1)	2.188			2.053		
	Direction (upstream)	-1.969			-1.833		
	Depth layer (middle)	-			0.496		
	Likelihood ratio test (selected model vs null model): Chisq = 6.732, p = 0.035						
Sacura margaritacea	Intercept	0.518	50.3	0.251	0.954	54.3	0.309
	AR type (AR1)	-			0.325		
	Direction (upstream)	-1.691			-1.955		
	Depth layer (middle)	-			-0.836		
	Likelihood ratio test (selected model vs null model): Chisq =3.471, p = 0.062						

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