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

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URL	https://fra.repo.nii.ac.jp/records/2013226

(a) AR1 (b) AR2

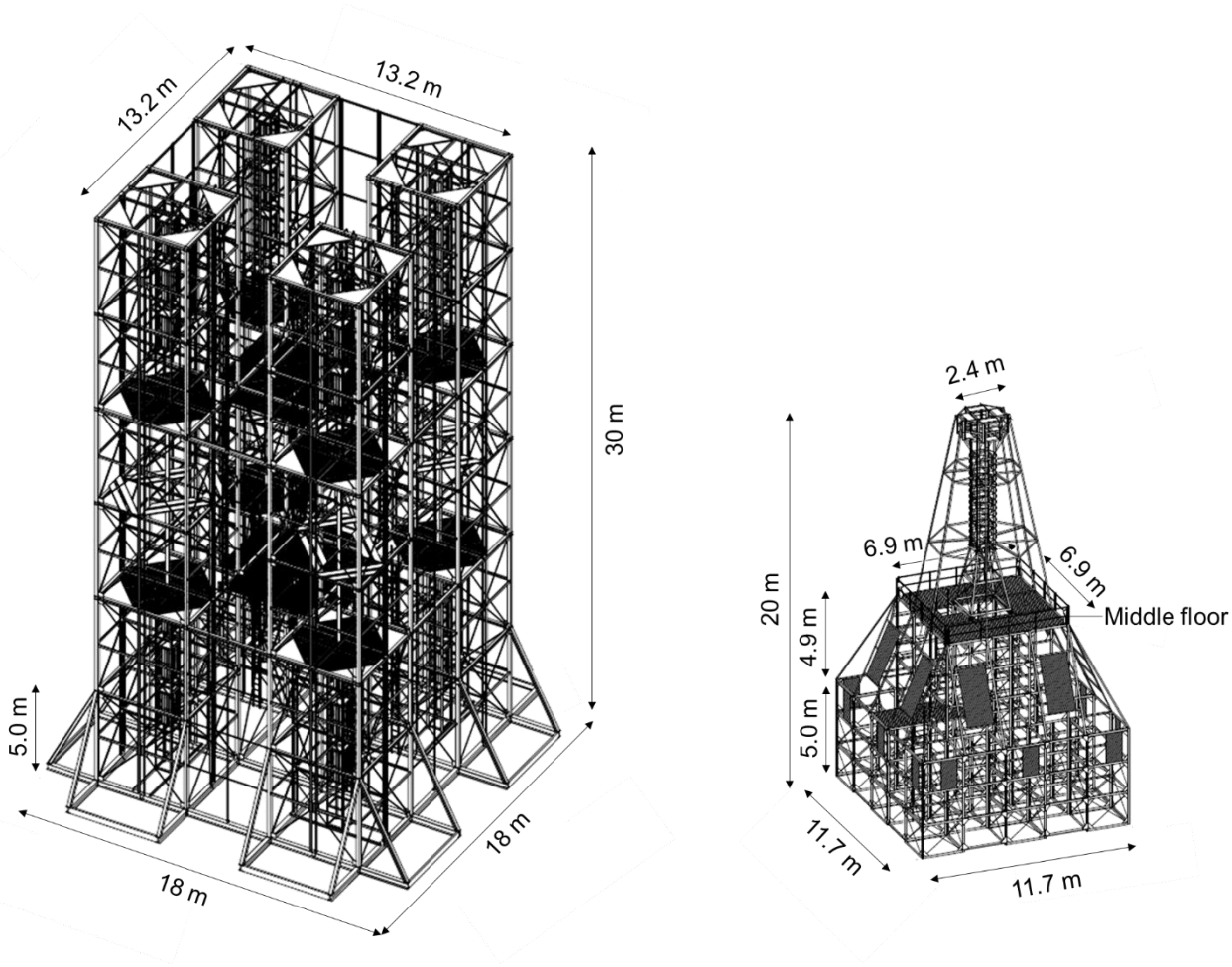


Fig. S1 Schematic drawing of artificial reef (AR) 1 (a) and AR2 (b).

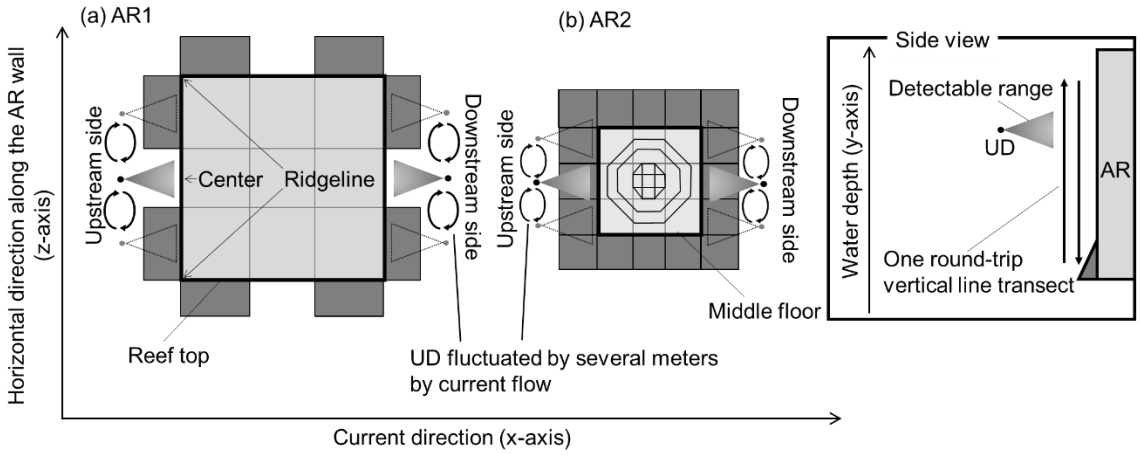


Fig. S2 Schematic drawing of the top view showing the state of the video recording with the underwater drone (UD). One round-trip vertical line transects were conducted upstream and downstream of artificial reef (AR) 1 (a) and AR2 (b). The position of the UD fluctuated by several meters in the horizontal direction along the AR wall (z-axis) or the current direction (x-azis) caused by the flow environment around ARs (x-axis).

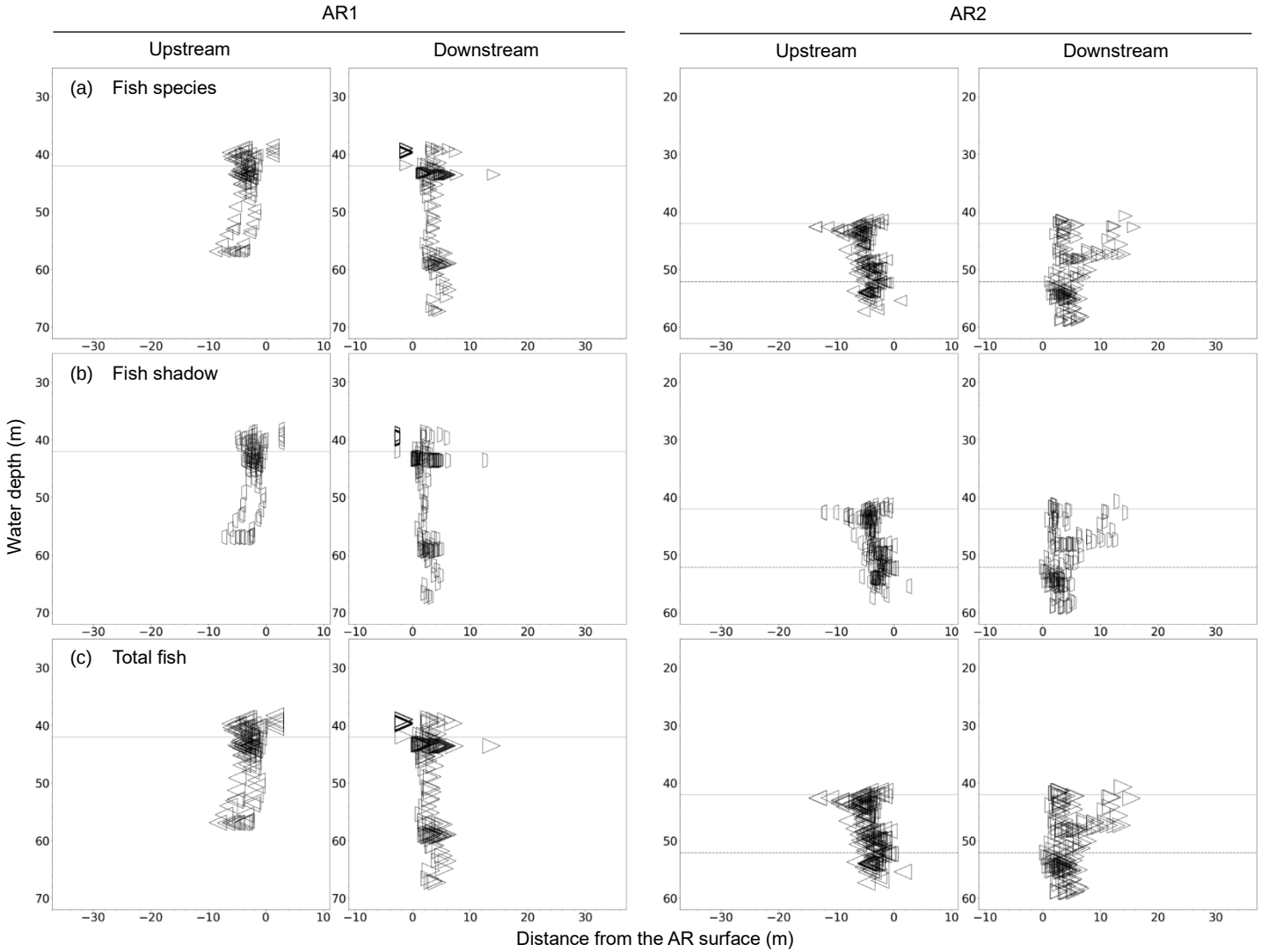
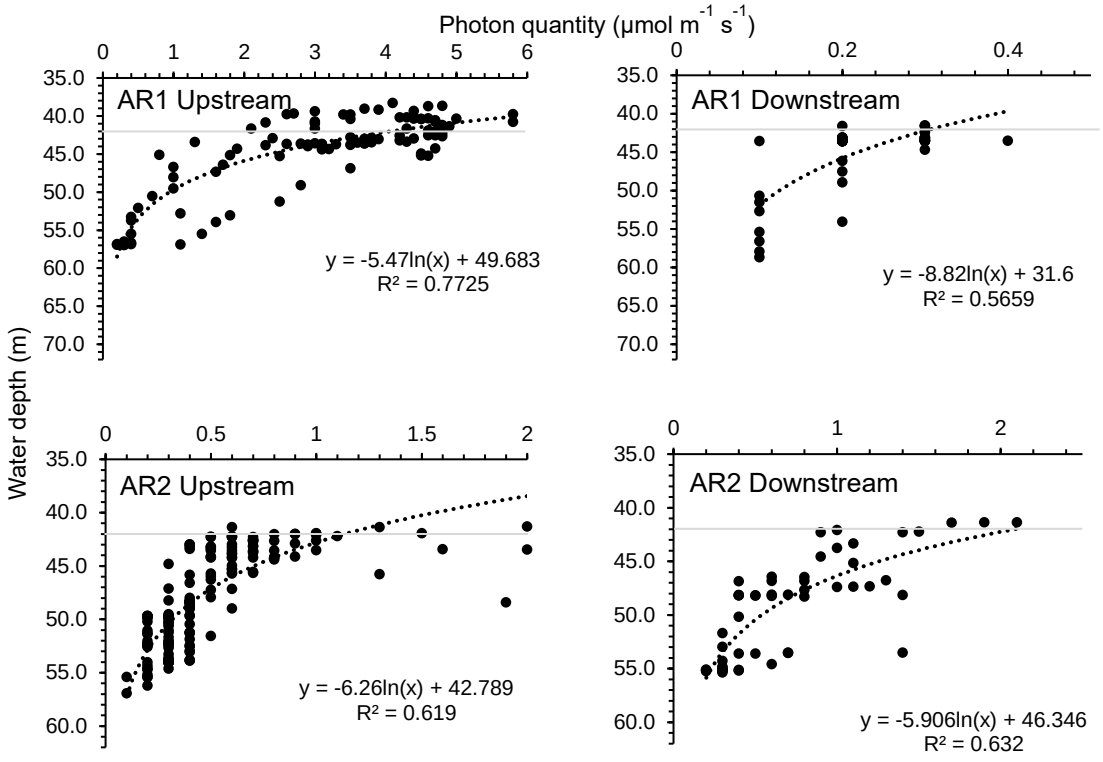


Fig. S3 Estimated detectable ranges of fish species (a), fish shadows (b), and total fish (c) of all analyzed images upstream and downstream of artificial reef (AR) 1 and AR2. The detectable ranges of each image are displayed on a vertical two-dimensional plane, assuming that the UD was on the same vertical plane at the

time of image recording. The horizontal distance from the AR surface is expressed as a negative or positive value for images recorded upstream or downstream of the AR, respectively. The nearest surface of the AR from the underwater drone was set as 0 m on the horizontal axis. The water depth at the top of the reef in both ARs was 42 m (gray lines). The dashed gray lines indicate the depth of the middle floor in AR2.

(a) Photon quantity



(b) Turbidity

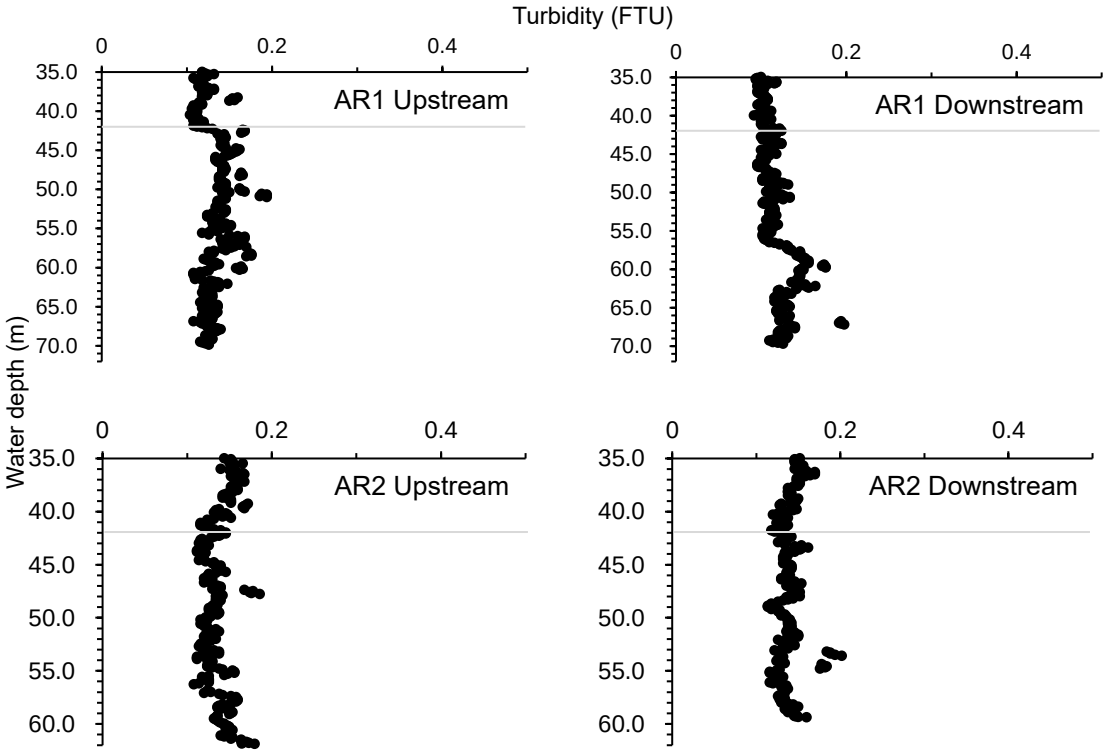


Fig. S4 Photon quantity at the time of image recording (a) and moving average turbidity every 0.5 m of water depth (b) upstream or downstream of artificial reef (AR) 1 and AR2. The water depth at the top of the reef in both ARs was 42 m (gray lines). The dashed black lines indicate the fitted curve of the logarithmic function.

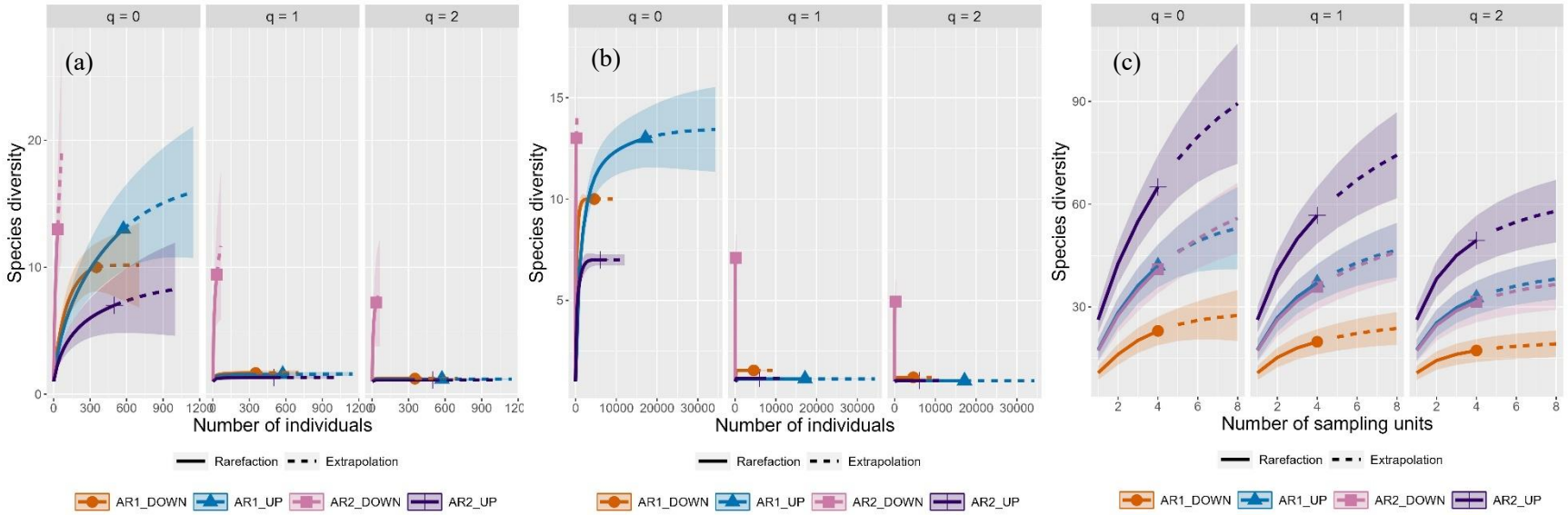
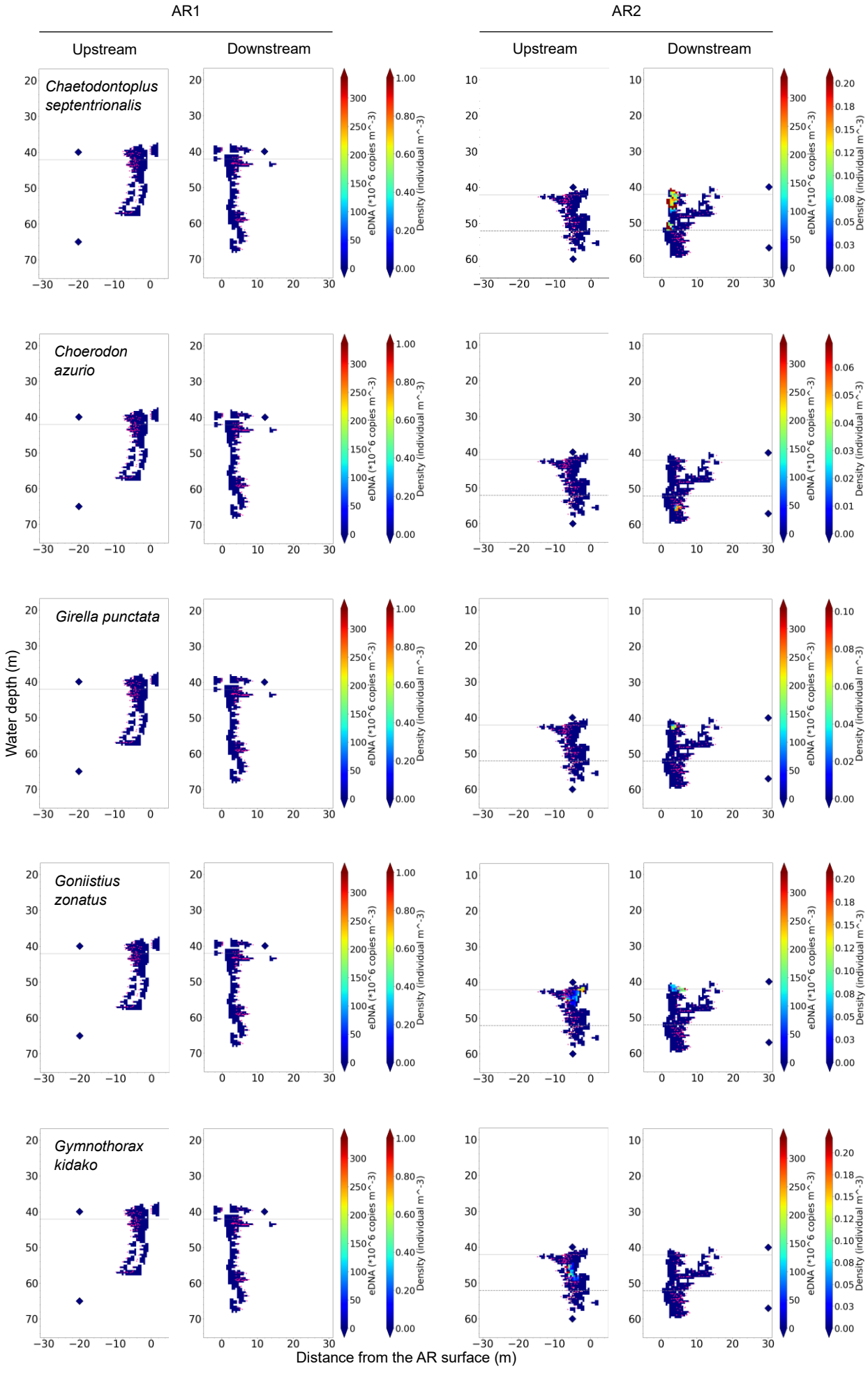
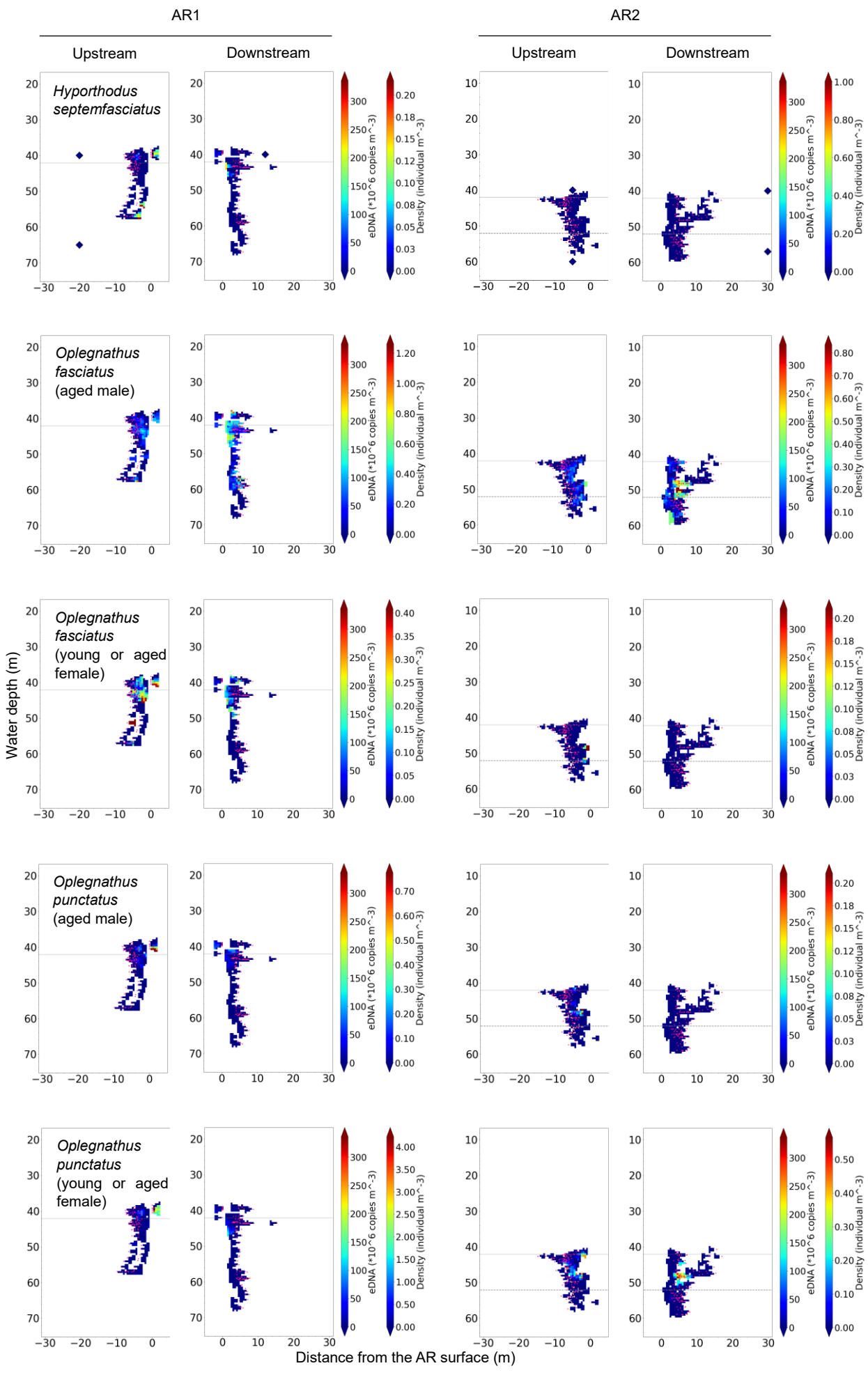
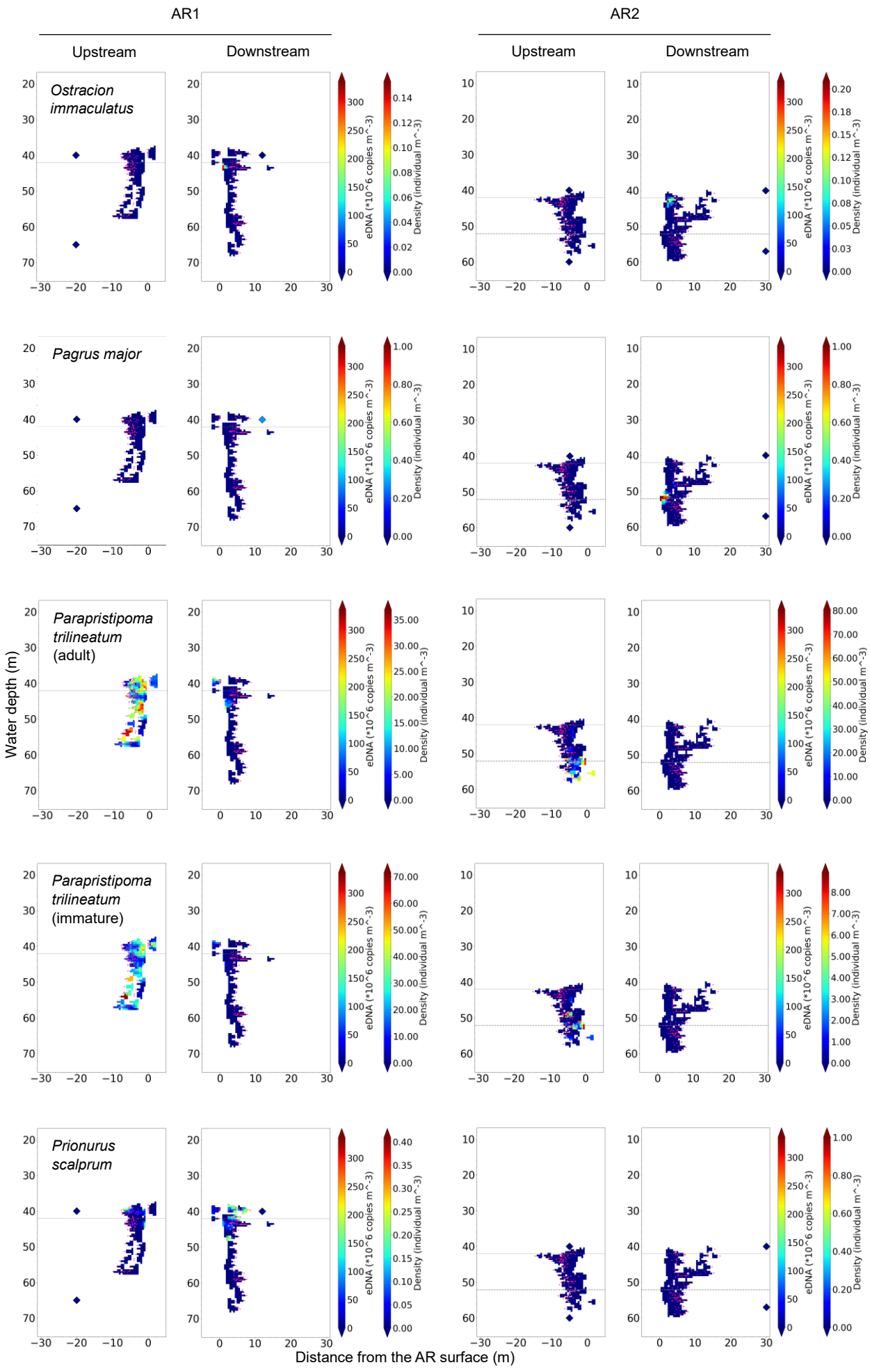


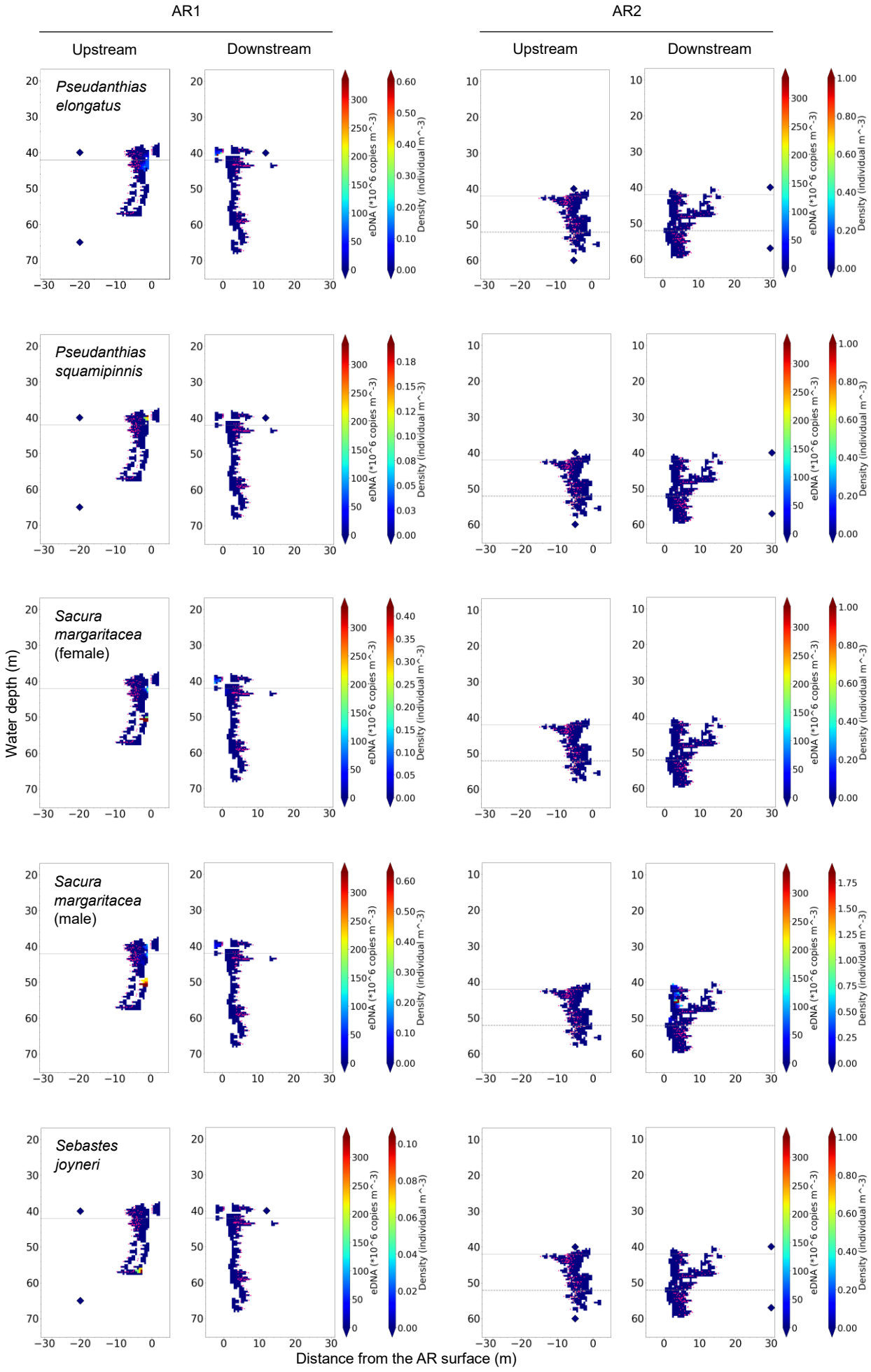
Fig. S5 Sample size-based rarefaction (solid line) and extrapolation (dotted line) sampling curves with 95% confidence intervals (shaded area) for the MaxN data of the video surveys (a), total fish count data of the video survey (b), or presence or absence of species data in qMiSeq (c) for each study station, separated by diversity order: q = 0 (species richness, left panel), q = 1 (exponential Shannon diversity, middle panel), and q = 2 (inverse Simpson diversity, right panel). The solid marks represent the reference samples. Sampling units in (c) indicate the replicates of environmental DNA samples.

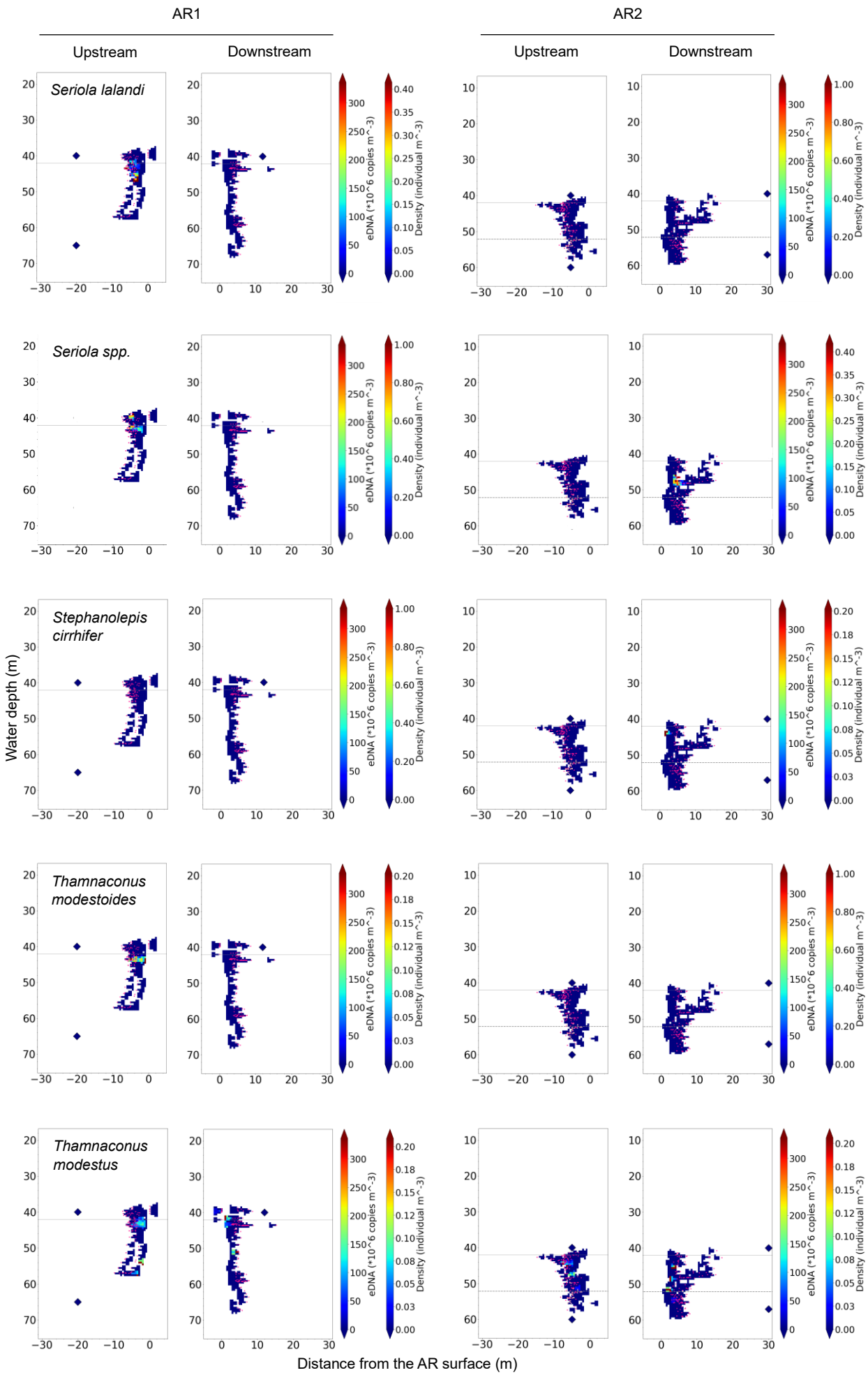
Fig. S6 High-density school image of *Parapristipoma trilineatum* recorded by the underwater drone (UD) video survey upstream of artificial reef 2. Individuals in the foreground hid individuals far from the UD.


AR1
AR2
Upstream
Downstream
Upstream
Downstream
Chaetodontoplus septentrionalis
Choerodon azurio
Girella punctata
Goniistius zonatus
Gymnothorax kidako
Water depth (m)
Distance from the AR surface (m)
eDNA (*10^6 copies m^-3)
Density (individual m^-3)


AR1
AR2
Upstream
Downstream
Upstream
Downstream
Hyporthodus septemfasciatus
Oplegnathus fasciatus (aged male)
Oplegnathus fasciatus (young or aged female)
Oplegnathus punctatus (aged male)
Oplegnathus punctatus (young or aged female)
Water depth (m)
Distance from the AR surface (m)
eDNA (*10^6 copies m^-3)
Density (individual m^-3)


AR1
AR2
Upstream
Downstream
Upstream
Downstream
Ostracion immaculatus
Pagrus major
Parapristipoma trilineatum (adult)
Parapristipoma trilineatum (immature)
Prionurus scalprum
Water depth (m)
Distance from the AR surface (m)
eDNA (*10^6 copies m^-3)
Density (individual m^-3)


AR1
AR2
Upstream
Downstream
Upstream
Downstream
Pseudanthias elongatus
Pseudanthias squamipinnis
Sacura margaritacea (female)
Sacura margaritacea (male)
Sebastes joyneri
Water depth (m)
Distance from the AR surface (m)
eDNA (*10^6 copies m^-3)
Density (individual m^-3)


AR1
AR2
Upstream
Downstream
Upstream
Downstream
Seriola lalandi
Seriola spp.
Stephanolepis cirrhifer
Thamnaconus modestoides
Thamnaconus modestus
Water depth (m)
Distance from the AR surface (m)
eDNA (*10^6 copies m^-3)
Density (individual m^-3)

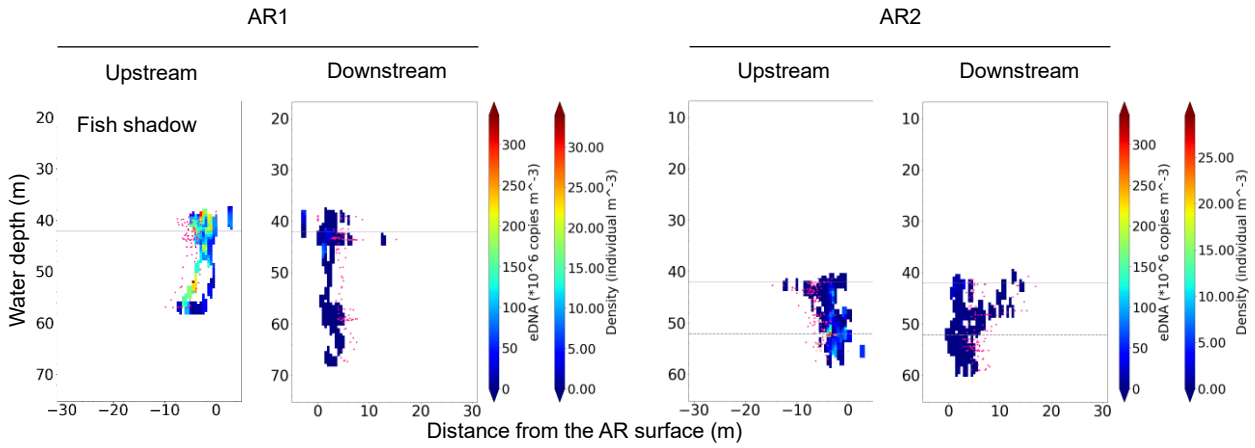


Fig. S7 Two-dimensional spatial distributions of the density of identified fish species (including one genus) other than total fish, total *Parapristipoma trilineatum*, total *Oplegnathus punctatus*, total *Oplegnathus fasciatus*, total *Sacura margaritacea*, or *Microcanthus strigatus* upstream or downstream of artificial reef (AR) 1 or AR2. Diamonds indicate the quantitative MiSeq sequencing approach (qMiSeq) results for the number of species and concentration of environmental DNA (eDNA) copies of total fish and corresponding species in the middle and bottom layers. The horizontal distance from the AR surface is expressed as a negative or positive value for images recorded at the upstream or downstream side of the ARs, respectively. The nearest surface of the ARs from the underwater drone (UD) was set as 0 m on the horizontal axis. Dark pink circles indicate the position of the UD when the images were recorded; light pink circles indicate that individuals were overlaid and the density was underestimated. The water depth at the top of the reef in both ARs was 42 m (gray lines). The dashed gray lines indicate the depth of the middle floor in AR2.

Table S1. Recording conditions of the underwater drone (UD) at video surveys

	Recording			Number of analyzed images	Velocity[a] (m s^{-1})	Water temperature [a] (°C)	Salinity[a]	Photon quantity [b] (µmol m^{-2} s^{-1})	Turbidity[b] (FTU)	Predicted detectable distance[a] (m)		Horizontal distance from the AR surface to the UD[c] (Absolute value, m)	Water depth of the UD[d] (m)
	Start time	End time	Recording duration (m:s)							Fish species	Fish shadows		
AR1[e]													
Upstream	11:49:02	11:59:33	10:31	101	0.08 ± 0.05	21.6 ± 1.4	34.1 ± 0.2	3.3 (0.2–5.8)	0.13 (0.10–0.28)	2.3 ± 0.0	3.1 ± 0.0	4.9 ± 1.2 (0.0–9.9)	38.3–57.0
Downstream	9:50:35	10:08:12	17:37	169	0.10 ± 0.03	22.6 ± 1.3	34.0 ± 0.1	0.2 (0.0-0.4)	0.11 (0.09–0.49)	2.2 ± 0.0	3.1 ± 0.0	3.8 ± 2.6 (0.0–15.2)	38.8–67.3
AR2[e]													
Upstream	14:38:03	14:56:46	18:43	172	0.07 ± 0.04	19.4 ± 0.2	34.4 ± 0.0	0.4 (0.9–2.0)	0.13 (0.09–0.20)	2.2 ± 0.0	3.1 ± 0.0	6.1 ±2.0 (0.0–14.8)	41.3–57.3
Downstream	13:45:24	13:58:49	13:25	133	0.09 ± 0.04	19.3 ± 0.1	34.4 ± 0.0	0.5 (0.2–3.3)	0.13 (0.10–0.46)	2.2 ± 0.0	3.1 ± 0.0	6.4 ± 2.9 (2.5–16.9)	40.7–59.0

[a] Mean ± standard deviation

[b] Median (minimum–maximum)

[c] Mean ± standard deviation (minimum–maximum)

[d] Range between the minimum and maximum water depths among the images

[e] Artificial reef

Table S2. Details of environmental DNA sampling

Sample ID	Site	Distance from AR[a] (m)	eDNA[b] sampling Time	Latitude (N)	Longitude (E)	Depth (m)	Depth layer	eDNA sampling depth (m)	Filtered volume (L)
1	AR1 downstream	12	10:33	34.99566667	139.7918333	71	Bottom	65	2.0
2	AR1 downstream	12	10:33	34.99566667	139.7918333	71	Bottom	65	2.0
3	AR1 downstream	12	10:45	34.99566667	139.7918333	71	Middle	40	2.0
4	AR1 downstream	12	10:45	34.99566667	139.7918333	71	Middle	40	2.0
5	AR1 upstream	20	11:40	34.99581667	139.7915333	72	Bottom	65	2.0
6	AR1 upstream	20	11:40	34.99581667	139.7915333	72	Bottom	65	2.0
7	AR1 upstream	20	11:40	34.99581667	139.7915333	72	Middle	40	2.0
8	AR1 upstream	20	11:40	34.99581667	139.7915333	72	Middle	40	2.0
9	AR2 downstream	30	13:29	34.9967	139.8032167	61	Bottom	57	2.0
10	AR2 downstream	30	13:29	34.9967	139.8032167	61	Bottom	57	2.0
11	AR2 downstream	30	13:35	34.9967	139.8032167	61	Middle	40	2.0
12	AR2 downstream	30	13:35	34.9967	139.8032167	61	Middle	40	2.0
13	AR2 upstream	5	14:20	34.99691667	139.80295	63	Bottom	60	2.0
14	AR2 upstream	5	14:20	34.99691667	139.80295	63	Bottom	60	2.0
15	AR2 upstream	5	14:28	34.99691667	139.80295	63	Middle	40	2.0
16	AR2 upstream	5	14:28	34.99691667	139.80295	63	Middle	40	2.0
17	Negative control (DW[c])	NA[d]	15:59	NA	NA	NA	NA	NA	2.0
18	Negative control (DW)	NA	15:59	NA	NA	NA	NA	NA	2.0

[a]AR: artificial reef
[b]eDNA: environmental DNA
[c]DW: distilled water
[d]NA: not applicable

Table S3. Experimental conditions and the detectable distance of fish species or shadows using the five full-scale fish models

Experimental site	Date	Lighting condition	Water condition	Photon quantity[a]	Turbidity[b]	Detectable distance[c] (m)	
						Fish species	Fish shadow
Indoor water tank	December 20, 2021	Mercury lamp	Freshwater	1.53	0.50[d]	2.2 ± 0.4	4.0 ± 0.0
Height: 0.8–1 m				0.8	0.50[d]	2.2 ± 0.4	3.6 ± 0.2
Width: 40 m				0.61	0.50[d]	2.2 ± 0.4	3.6 ± 0.2
Depth: 61.5 m				0.46	0.50[d]	2.2 ± 0.4	3.2 ± 0.3
				0.18	0.50[d]	2.6 ± 0.2	3.0 ± 0.0
				0.09	0.50[d]	0.0 ± 0.0	0.0 ± 0.0
Indoor water tank	December 20, 2021	LED	Freshwater	2.3	2.82[d]	0.5 ± 0.0	1.0 ± 0.0
Height: 1.0 m			+ cellulose	1.91	0.50[d]	2.1 ± 0.1	3.0 ± 0.0
Width: 12 m				1.81	2.82[d]	0.5 ± 0.0	1.0 ± 0.0
Depth: 0.5 m				1.76	1.20[d]	1.6 ± 0.2	2.5 ± 0.0
				1.34	0.69[d]	3.1 ± 0.2	4.5 ± 0.0
				0.99	0.69[d]	2.6 ± 0.2	3.8 ± 0.3
				0.99	1.20[d]	1.5 ± 0.0	2.0 ± 0.0
				0.94	0.91[d]	2.1 ± 0.2	3.1 ± 0.2
				0.63	0.50[d]	2.5 ± 0.0	3.0 ± 0.0
				0.58	0.91[d]	2.2 ± 0.4	3.2 ± 0.3
Indoor water tank	February 1, 2022	LED	Freshwater	0.14	0.70[e]	0.0 ± 0.0	0.0 ± 0.0
Height: 1.4 m				0.12	0.70[e]	2.6 ± 0.2	3.2 ± 0.4
Width: 8 m				0.1	0.70[e]	0.0 ± 0.0	0.0 ± 0.0
Depth: 3 m				0.09	0.70[e]	0.0 ± 0.0	0.0 ± 0.0
Outdoor pier at Marine Biosystems Research Center, Chiba University	August 19, 2022	Natural light	Natural seawater	143.67	0.66[f]	5.6 ± 0.2	6.6 ± 0.2
	November 2, 2022			90.25	0.72[f]	4.4 ± 0.2	4.6 ± 0.2
Taka-maru pier at Tateyama Bay	October 27, 2022	Natural light	Natural seawater	356.47	0.41[f]	4.5 ± 0.0	5.5 ± 0.0

[a] μmol m^{-2} s^{-1}
[b] Formazine turbidity unit (FTU), standardized to the value of the conductivity temperature depth profiler (CTD, RINKO-Profiler)
[c] Mean ± standard deviation of five full-scale fish models
[d] Data from the portable ss/turbidity meter (SSTR-5Z)
[e] Data from the CTD
[f] Data from the memory chlorophyll turbidity meter (INFINITY-CLW ACLW2-USB)

Table S4. Analysis of the selected general linear models to estimate the detectable distance of fish species and shadows

Detectable distance of fish species (m)		
	Estimate	Standard Error
(Intercept)	2.30	0.19
Photon quantity (μmol m^{-2} s^{-1})	0.011	0.0014
Turbidity (FTU)	-0.61	0.17
Residual standard error: 1.12 on 112 degrees of freedom, adjusted R^2: 0.42, F-statistic: 42.38 on 2 and 112 degrees of freedom, AIC[a]: 29.9		

Detectable distance of fish shadow (m)		
	Estimate	Standard Error
(Intercept)	3.16	0.24
Photon quantity (μmol m^{-2} s^{-1})	0.011	0.0017
Turbidity (FTU)	-0.75	0.21
Residual standard error: 1.44 on 112 degrees of freedom, adjusted R^2: 0.34, F-statistic: 30.18 on 2 and 112 degrees of freedom, AIC[a]: 86.67		

[a] Akaike's Information Criterion

A significant difference was observed between the selected and null models by the likelihood ratio test ($p < 0.05$)

Table S5. Primer sets used in this study

Primer ID	Primer sequence (5' − > 3')
MiFish U-F	NNNNNNGTCGGTAAAACTCGTGCCAGC
MiFish U-R	NNNNNNCATAGTGGGGTATCTAATCCCAGTTTG
MiFish Ev2-F	NNNNNNRGTTGGTAAATCTCGTGCCAGC
MiFish Ev2-R	NNNNNNGCATAGTGGGGTATCTAATCCTAGTTTG
MiFish U2-F	NNNNNNGCCGGTAAAACTCGTGCCAGC
MiFish U2-R	NNNNNCATAGGAGGGTGTCTAATCCCCGTTTG
Second PCR primer-F	CAAGCAGAAGACGGCATACGAGATXXXXXXXGTCTCGTGGCTCGG
Second PCR primer-R	AATGATACGGCGACCACCGAGATCTACACXXXXXXXTCGTCGGCAGCGTC

Table S6. The mean density of identified fish species, total fish, or fish shadows at each study station [upstream or downstream side of each artificial reef (AR)] in video surveys

Ranking[a]	Fish species	eDNA detection[b]	Density[c]
AR1			
Upstream	Total		20.376 ± 11.142
	Fish shadow		10.251 ± 6.589
1	*Parapristipoma trilineatum*	1	35.632 ± 21.595
	(Immature)		(21.129 ± 14.375)
	(Adult)		(14.503 ± 9.112)
2	*Oplegnathus punctatus*	0	0.222 ± 0.645
	(Young or aged female)		(0.189 ± 0.568)
	(Aged male)		(0.032 ± 0.115)
3	*Oplegnathus fasciatus*	1	0.182 ± 0.231
	(Young or aged female)		(0.074 ± 0.117)
	(Aged male)		(0.108 ± 0.140)
4	*Sacura margaritacea*	1	0.048 ± 0.183
	(Female)		(0.014 ± 0.069)
	(Male)		(0.034 ± 0.121)
5	*Seriola lalandi*	1	0.024 ± 0.076
6	*Pseudanthias elongatus*	0	0.015 ± 0.046
7	*Hyporthodus septemfasciatus*	0	0.014 ± 0.041
8	*Prionurus scalprum*	1	0.012 ± 0.036
9	*Thamnaconus modestus*	1	0.011 ± 0.032
10	*Thamnaconus modestoides*	0	0.010 ± 0.033
11	*Seriola* spp.	1	0.004 ± 0.013
12	*Pseudanthias squamipinnis*	0	0.002 ± 0.018
13	*Sebastes joyneri*	1	0.002 ± 0.018
Downstream	Total		1.081 ± 2.909
	Fish shadow		0.476 ± 1.328
1	*Parapristipoma trilineatum*	1	1.612 ± 5.592
	(Immature)		(0.546 ± 2.372)
	(Adult)		(1.067 ± 3.476)
2	*Oplegnathus fasciatus*	0	0.212 ± 0.255
	(Young or aged female)		(0.024 ± 0.056)
	(Aged male)		(0.188 ± 0.226)
3	*Oplegnathus punctatus*	0	0.068 ± 0.212
	(Young or aged female)		(0.054 ± 0.198)
	(Aged male)		(0.014 ± 0.039)
4	*Prionurus scalprum*	0	0.031 ± 0.064
5	*Microcanthus strigatus*	1	0.008 ± 0.036
6	*Thamnaconus modestus*	1	0.007 ± 0.028
7	*Pseudanthias elongatus*	0	0.004 ± 0.037
8	*Sacura margaritacea*	1	0.004 ± 0.024
	(Female)		(0.002 ± 0.013)
	(Male)		(0.002 ± 0.011)
9	*Hyporthodus septemfasciatus*	0	0.004 ± 0.018
10	*Ostracion immaculatus*	0	0.002 ± 0.014
AR2			

Upstream	Total		4.876 ± 7.358
	Fish shadow		2.038 ± 3.398
1	*Parapristipoma trilineatum*	1	8.427 ± 16.657
	(Immature)		(0.587 ± 1.551)
	(Adult)		(7.840 ± 15.663)
2	*Oplegnathus fasciatus*	1	0.063 ± 0.108
	(Young or aged female)		(0.006 ± 0.030)
	(Aged male)		(0.058 ± 0.088)
3	*Microcanthus strigatus*	0	0.053 ± 0.260
4	*Oplegnathus punctatus*	0	0.033 ± 0.089
	(Young or aged female)		(0.028 ± 0.074)
	(Aged male)		(0.005 ± 0.023)
5	*Goniistius zonatus*	1	0.009 ± 0.029
6	*Gymnothorax kidako*	0	0.007 ± 0.027
7	*Thamnaconus modestus*	1	0.005 ± 0.021
Downstream	Total		0.218 ± 0.300
	Fish shadow		0.185 ± 0.478
1	*Oplegnathus fasciatus*	0	0.112 ± 0.181
	(Young or aged female)		(0.000 ± 0.000)
	(Aged male)		(0.112 ± 0.181)
2	*Sacura margaritacea*	1	0.039 ± 0.194
	(Female)		(0.000 ± 0.000)
	(Male)		(0.039 ± 0.194)
3	*Pagrus major*	1	0.038 ± 0.160
4	*Oplegnathus punctatus*	0	0.028 ± 0.086
	(Young or aged female)		(0.0280 ± 0.086)
	(Aged male)		(0.000 ± 0.000)
5	*Chaetodontoplus septentrionalis*	1	0.022 ± 0.057
6	*Microcanthus strigatus*	0	0.022 ± 0.056
7	*Seriola* spp.	1	0.012 ± 0.057
8	*Thamnaconus modestus*	1	0.007 ± 0.033
9	*Goniistius zonatus*	1	0.004 ± 0.020
10	*Ostracion immaculatus*	0	0.003 ± 0.018
11	*Stephanolepis cirrhifer*	0	0.002 ± 0.020
12	*Girella punctata*	0	0.001 ± 0.008
13	*Choerodon azurio*	1	0.001 ± 0.007

[a] Descending order of the predicted density at each study station

[b] "1" and "0" indicate that the fish species were also detected or undetected by qMiSeq at each study station, respectively

[c] Observed volumetric density within the two-dimensional observation range of all analyzed images from each study station (individual m^{-3}, mean ± standard deviation)

Table S7. Sequence reads were assigned to fish species in the 16 field and two negative control samples collected on October 27, 2021.

Separate Excel file name: Table Supplements.xlsx

Table S8. R^2 values of the relationships between the copy numbers and sequence reads of standard DNA

Sample	R^2
1	0.753
2	0.252
3	0.241
4	0.895
5	0.766
6	0.957
7	0.887
8	0.941
9	0.936
10	0.896
11	0.921
12	0.926
13	0.764
14	0.797
15	0.052
16	0.753
17	0.970
18	0.981

Table S9. Environmental DNA concentration (copies (mL water)$^{-1}$) of the detected species in the 12 field and two negative control samples collected on October 27, 2021

Separate Excel file name: Table Supplements.xlsx

Table S10. Ranking of the mean environmental DNA (eDNA) concentration for the 10 dominant species in the 12 field samples

Rank	Fish species	Observed by video survey[a]	Mean eDNA concentration [copies (mL water)$^{-1}$]
1	*Parapristipoma trilineatum*	1	71.94
2	*Pagrus major*	1	8.05
3	*Spratelloides gracilis*	0	5.46
4	*Katsuwonus pelamis*	0	5.05
5	*Sacura margaritacea*	1	2.67
6	*Gnathophis heterognathos*	0	2.63
7	*Arothron firmamentum*	0	1.79
8	*Maurolicus japonicus*	0	1.51
9	*Dentex* spp.	0	1.48
10	*Platycephalus indicus*	0	1.18

[a] "1" and "0" indicate the fish species observed or not observed by video survey, respectively

Table S11. Comparison of MiFish primer sequences with sequences in the corresponding region of the observed species using an underwater drone and dominant species in environmental DNA analysis (downloaded data from NCBI).

Separate Excel file name: Table Supplements.xlsx

Detailed Materials & Methods

2.1 Water sampling for environmental DNA analysis

Water sampling for qMiSeq upstream and downstream of AR1 and AR2 was conducted on October 27, 2021 (Table S2). Water was sampled immediately after the video survey of each study station. Ten liters of seawater was collected from the middle (40 m depth) and bottom (5 m above the bottom) layers using one cast of two Niskin water samplers (5 L × 2 samples) at each study station. Two liters of seawater were subsampled from the 5 L seawater of the Niskin sampler using a measuring bottle, and the remaining 3 L of seawater was used to pre-wash the measuring bottle and filtration devices. Two 2 L samples were collected from 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) through an aspirator (the two filters were subsets of a single water collection) in a laboratory on *Taka-Maru* (Sato et al. 2021). After filtration for an average time of 15 min, an outlet port of the filter cartridge was sealed with an outlet Luer cap, and 1.5 mL RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) was injected into the cartridge to prevent environmental (eDNA) degradation, and the inlet port was sealed with an inlet Luer cap. The Niskin water samplers were bleached before each water collection using a commercial bleach solution, whereas the filtering devices were

bleached after every filtration. Two liters of MilliQ water were filtered with the filtering devices as a negative control to test for possible contamination. The filter cartridges were placed in a freezer immediately after filtration and stored at -20 °C until DNA extraction. A total of 18 eDNA samples (16 field samples [four stations × two depth layers × two replicates] + two negative controls) were collected and filtered. Disposable latex or nitrile gloves were worn during sampling and replaced between sampling stations.

2.2.1. Estimation of fish density

Creating the statistical models to predict the detectable distance of fish

Angling fishing surveys were conducted near AR1 and AR2 on the *Taka-maru* deck to determine the body size of the distributed fish species on June 10, 2021. The total length (TL) of rockfish (*Sebastes joyneri*), black scraper (*Thamnaconus modestus*), and Japanese jack mackerel (*Trachurus japonicus*) was measured. The TLs of purchased adult and immature chicken grunt (*Parapristipoma trilineatum*) caught by the Tomiura Fishery Cooperative and Kyonan Katsuyama Fishery Cooperative near Tateyama Bay were also measured on the same day. Color photographs of these species were taken from the side of the fish bodies. The photographs were waterproofed and fixed with sticks to create full-scale fish models. The full-scale fish models of *P. trilineatum* were created for both

growth stages because immature *P. trilineatum* has a striped pattern on its body, which is absent in adults (Fig. 2a). The TL of each full-scale fish model was referenced from the mean value of the collected or purchased individuals (*S. joyneri*: 20.7 cm, n = 7; *T. modestus*: 34.1 cm, n = 5; *T. japonicus*: 25.2 cm, n = 11; *P. trilineatum* (immature or adult): 23.6 cm, n = 4).

The detectable distance of fish species or shadows was investigated in 23 different turbidity–photon quantity conditions using the created full-scale fish models from December 20, 2021, to November 2, 2022. Twenty conditions existed in three indoor water tanks installed in the Kamisu office of the Fisheries Technology Institute. Two conditions were in the outdoor environment at the pier in front of the Marine Biosystems Research Center, Chiba University, and one condition was in an outdoor environment at *Taka-maru* Pier in Tateyama Bay. Tap water was used in the indoor water tanks, and the turbidity and photon quantity were changed by adding cellulose particles and turning the lighting equipment on or off, respectively. Turbidity and photon quantity depend on natural seawater and light in outdoor environments, respectively. The experimental ranges of turbidity and photon quantity were 0.41–2.82 formazine turbidity units (FTU) and 0.09–356.47 μmol m^{-2} s^{-1}, respectively. The turbidity of tap water was at least 0.41 FTU, which was challenging to reduce further. The detailed experimental conditions are

presented in Table S3.

The underwater drone (UD) was placed in a water tank or natural water and maintained in a horizontal position. The full-scale fish models were moved away from the UD in increments of 0.5 m from a distance of 0.5 m until they became unrecognizable when recorded with the video camera (0.5 m to 6.5 m from the UD). The recording settings were the same as those used in the field surveys. Turbidity at the UD position was measured using three turbidity meters: a conductivity temperature depth profiler (CTD) instrument, a memory chlorophyll turbidity meter (INFINITY-CLW ACLW2-USB, JFE Advantech, Hyogo, Japan), or a portable ss/turbidity meter (SSTR-5Z, Kasahara Chemical Instruments, Saitama, Japan). A calibration curve was drawn between the three turbidity meters in advance, and the FTU was standardized to the CTD value. A small memory photon recorder was used to measure the photon quantity at the UD position. Images were clipped and resized every 0.5 m from the UD to the full-scale fish model from the recorded videos using the same method as that for the image analysis of video surveys. The detectable distance of fish species or shadows of each full-scale fish model for each environmental condition was determined by visual assessment of these images by the first author.

Subsequently, a general linear model was constructed to estimate the detectable

distance of fish species or shadows from the recording environment. The response variable was set at a detectable distance of fish species or shadows. The explanatory variables were photon quantity, turbidity, and TL. Although TL is not an environmental factor, it was added as an explanatory variable to examine its effect on detectable distance. Variable selection was performed using Akaike's Information Criterion (AIC) among the initial models to predict the detectable distances based on these explanatory variables, and the best-fitting model (minimum AIC) for the goodness of prediction was selected. A likelihood ratio test was used to compare the selected and null models (the model with only intercept as a predictor). The general linear model was analyzed using the lm function in R. The normality of the response variables was confirmed with a q-q plot using the qqnorm and qqline functions.

General linear models with turbidity and photon quantity as explanatory variables were selected for the detectable distances of fish species and shadows as a result of variable selection by AIC (Table S4). Turbidity and photon quantity had significantly negative and positive effects on the detectable distance, respectively. The TL was not selected as an explanatory variable for either of the models. The adjusted R^2 values, which indicate the goodness of fit of the model, were 0.42 and 0.34 for fish species and shadows, respectively. A significant difference was observed between the selected and null models of fish species

or shadows using the likelihood ratio test.

Determination of the shape of the UD field of view

The shape of the field of view of the UD video camera was confirmed in an indoor 2 t tank. A ruler with a known distance to the lens was recorded, and the actual vertical and horizontal size (m) of the video frame was confirmed at the ruler position. The shape of the field of view of the camera in the water was a rectangular pyramid with a height (distance to the subject from the lens of UD): base area (recorded width × height) of 1:1.53 × 0.86.

Estimation of fish density

Fish density for each image was calculated using the selected model in section 2.2.1. The best-fitting models (Formula 1 and 2), turbidity at the water depth of each image recording, and photon quantity at the time of recording were used to predict A (m) and B (m) for each image recorded by the UD in the field using the predict function in R. The spatial volume where fish species or shadows can be detected (hereafter referred to as the detectable spatial volume of fish species or shadows) was $0.44A^3$ (m^3) as a rectangular pyramid shape (height: base area = 1:1.53 × 0.86) with height A (m) or 0.44 (B^3-A^3) (m^3)

as a rectangular frustum shape (rectangular pyramid with height B (m) - rectangular pyramid with height A (m)), respectively. The density of each fish species or shadow was calculated by dividing the number of identified individuals by $0.44A^3$ or 0.44 (B^3-A^3), respectively. The density of total fish in each image was calculated by dividing the total number of individuals by $0.44B^3$ (m^3).

2.2.2. Estimation of the fish community spatial distribution

Estimation of the recording position

The distance from the AR surface to the UD was estimated using ImageJ software version 1.51J8 (National Institutes of Health, Bethesda, MD, USA) to estimate the coordinate range within which the fishes pictured existed. First, information was obtained on the size of the ARs from the manufacturers (Fig. S1). The pixel length was measured on the constituent column material of the ARs in the image nearest to the UD according to ImageJ software, and the ratio of pixels to meters was calculated. The meter length corresponding to 627.5 pixels [1.53^{-1} × 960 pixels (= frame width)] was taken as the distance from the AR surface to the UD. AR1 was also recorded immediately over the top of the reef. In this case, an accurate estimation of the horizontal distance from the AR surface was challenging; therefore, it was assumed to be 0 m. The actual horizontal

position of the UD was between 2 and 6.6 m from the center of AR1. The distance from the AR surface was expressed as a negative or positive value for AR images recorded at the upstream and downstream sides, respectively.

2.3. DNA extraction and quantitative metabarcoding with qMiSeq

The eDNA was extracted and purified as previously described (Miya et al. 2016). After removing RNAlater inside the cartridge by centrifugation at 3,500 × *g* for 2 min, a mixture of 20 μL proteinase-K solution, 220 μL phosphate-buffered saline, and 200 μL buffer AL was injected into the cartridge and incubated at 56 °C for 20 min. The eDNA extracts were transferred to a 2-mL tube from the inlet of the filter cartridges by centrifugation at 5500 × *g* for 1 min. The collected DNA was purified using a DNeasy Blood and Tissue kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. DNA was eluted in 100 μL AE buffer and frozen at -20 °C.

qMiSeq was performed with MiFish primers (Miya et al. 2015) to identify the fish taxa and quantify their eDNA concentrations. Five artificially designed internal standard DNAs were used to calculate standard curves to estimate the DNA copy numbers (Ushio et al. 2018; Sato et al. 2021). This standard curve enabled us to convert the number of sequence reads into DNA copies without being affected by differences in PCR efficiency

among samples. Paired-end library preparation with a two-step PCR was performed in a 12 μL reaction mixture as previously described (Sato et al. 2021). Briefly, first-round PCR was performed with MiFish primers of actinopterygian (MiFish-U), elasmobranch (MiFish-Ev2), and sea sculpins (MiFish-U2) versions (Table S5), which can amplify fish DNA and five internal standard DNAs (2.5, 12.5, 25, 50, and 100 copies/reaction). Eight replicates were performed for the first PCR, and the products were pooled in a single 1.5-mL tube. The first PCR products were sent to Environmental Research & Solutions to outsource the subsequent MiSeq sequencing processes. Pooled products were purified using Agencourt AMPure XP beads. DNA concentrations were quantified using the QuantiFluor ONE dsDNA System (Promega, Waltham, MA, USA) and adjusted to 0.1 ng μL^{-1}. A second-round PCR was performed to add the index sequence and adapter sequence for the Illumina sequencing platform (Table S5). The indexed products of the second-round PCR were pooled, and the target bands (~ 370 bp) were excised using E-Gel SizeSelect agarose gels (Thermo Fisher Scientific).

The concentration of each second PCR product was measured using the QuantiFluor ONE dsDNA system; each sample was adjusted to 9 pM and combined. The prepared DNA libraries were sequenced on a MiSeq platform using the MiSeq v2 reagent kit (Illumina, San Diego, CA, USA). Raw MiSeq data were converted into FASTQ files using

the bcl2fastq program provided by Illumina (bcl2fastq v2.18). The FASTQ files were demultiplexed using the *clsplitseq* command implemented in Claident (Tanabe & Toju 2013) and filtered when having a quality score > 30, and classified to each sample based on adapter sequences. The paired-end reads were merged and underwent quality control to remove reads with ambiguous sites, sequence length < 100 bp, sequence length > 250 bp, and error rate > 2.0%. The erroneous sequences were denoised, and PCR artifacts (chimeras) were removed using the functions of DADA2 and VSEARCH implemented in Claident (Tanabe & Toju 2013). The processed reads were subjected to a BLASTn search against the fish DNA sequences in the NCBI database. The top BLAST hits with a percent identity ≥ 98.5 % and a query coverage of 100% was applied and used in further analyses. Assembled sequences assigned to the same species were clustered. To remove possible contaminants, species whose sequence reads were < 0.05% of the total reads, and singletons were removed, which was considered as noise for each sample (Sato et al. 2021). After taxonomic assignment, the taxonomic assignments were modified because the program may assign a single species name, whereas closely related species cannot be distinguished using the 12S rRNA gene. In such cases, a genus or a higher taxonomic rank was assigned to the closeted reads. For example, some query sequences were aligned to two closely related species: *Scomber japonicus* and *S. australasicus*. In this case,

Scomber spp. was assigned to this sequence. Two species (*Oncorhynchus keta* and *Gobio gobio*) were detected in this study; however, they do not exist in Tateyama Bay. Therefore, these species were considered contaminations and removed. Primer–template mismatch of the observed species using UD and dominant species in eDNA analysis was verified using the sequences of the mitochondrial 12S rRNA gene in the NCBI Nucleotide Database. For species whose priming region sequence was unknown, the degree of mismatches for closely related species was determined and used.

The number of DNA copies in each sample was estimated as previously described (Ushio et al. 2018, Sato et al. 2021). The number of DNA copies in each sample was estimated using standard curves of the internal standard DNAs—Std. A (100 copies μL^{-1}), Std. B (50 copies μL^{-1}), Std. C (25 copies μL^{-1}), Std. D (12.5 copies μL^{-1}), and Std. E (2.5 copies μL^{-1})—using linear regression without intercept because the calculated eDNA copies should be 0 or more. The regression equation was: MiSeq sequence reads = regression slope × the number of standard DNA copies [μL^{-1}]. Because the R^2 values of the standard curves in the three field samples were considerably low (No. 2, 3, and 15 in Table S2, R^2 values = 0.052−0.252) or only one out of the five internal standard DNAs in the one field sample was detected (No. 1 in Table S8), these samples were not used for the conversions to eDNA concentration. The read numbers of each species in the

remaining 12 field samples were converted to a DNA copy number (R^2 values > 0.753) (Table S9). The number of non-standard fish DNA copies was converted by dividing the number of MiSeq sequence reads by a sample-specific regression slope (i.e., the number of DNA copies = MiSeq sequence reads regression slope^{-1}).

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