

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

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Fig. 1 Survey area of the underwater drone showing examples of images and a schematic of observations;
 maps showing the wider sampling area encompassing Tokyo Bay, Japan, study stations, and current direction

and velocity at a depth of 35 m at every 1 min interval (red arrows) (a); school of *Parapristipoma trilineatum*

4	gathered at the top of the reef on the upstream side of the artifical reef (AR) 1 (b, left image) or gathered at
5	the middle floor of the upstream side of AR2 (b, right image); schematic diagram of the recording methods
6	used by the underwater drone and water sampling and conductivity temperature depth (CTD) measurements
7	(c). The maps were created using ArcGIS Sofware 10.6.0.8321 by ESRI (https://www.esri.com/) based on the
8	municipal boundary data of Japan (Esri Japan) and Global Map Japan (Geospatial information Authority of
9	Japan) as well as the M7000-series isobath data set (Japan Hydrographic Association).





13 morphology: immature (a) or adult (b, individual in the center of the image) Parapristipoma trilineatum;

- 14 young fish or aged female (c) or aged male (d) *Oplegnathus punctatus*; young fish or aged female (e) or aged
- 15 male (f) *Oplegnathus fasciatus*; female (g) or male (h) *Sacura margaritacea*.
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Fig. 3 Observed two-dimensional spatial distributions of the number of fish species and the density of total 21 22 fish, Parapristipoma trilineatum, Oplegnathus punctatus, Oplegnathus fasciatus, Sacura margaritacea, and Microcanthus strigatus upstream and downstream of artificial reef (AR) 1 or AR2. Diamonds indicate the 23 24 quantitative MiSeq sequencing approach (qMiSeq) results for the number of species and concentration of environmental DNA (eDNA) copies of total fish and these species at the middle and bottom layers. The 25 horizontal distance from the AR surface is expressed as a negative or positive value for images recorded 26 upstream or downstream of the ARs, respectively. The nearest AR surface from the underwater drone (UD) 27 was set at 0 m on the horizontal axis. Dark pink circles indicate the positions of the UD when the images were 28 recorded; light pink circles indicate that individuals were overlaid and the density was underestimated. The 29 water depth of the top of the reef in both ARs was 42 m (gray lines). The dashed gray lines indicate the depth 30 of the middle floor of AR2. 31

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Fig. 4 Predicted relationship between the horizontal distance from the artificial reef (AR) surface and the number of fish species and the densities of total fish, *Parapristipoma trilineatum, Oplegnathus punctatus, Oplegnathus fasciatus, Sacura margaritacea,* and *Microcanthus strigatus* at a depth of reef top (42 m deep) on the upstream and downstream sides of AR1 and AR2 using the selected zero-inflated negative binomial or generalized linear models. The horizontal distance from the AR surface was expressed as a negative or positive value for images recorded from the upstream or downstream side of the ARs, respectively. The nearest surface of the ARs from the underwater drone (UD) was set at 0 m on the horizontal axis.

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Fig. 5 Number of fish species detected using environmental DNA (eDNA) metabarcoding with the quantitative
MiSeq sequencing approach (qMiSeq, blue), video surveys by the underwater drone (pink), or both methods
(grey) at each study station [upstream (Up) or downstream (Dw) side of artificial reef (AR) 1 or AR2].



Fig. 6 Number of fish species, environmental DNA (eDNA) concentration of total fish, *Parapristipoma trilineatum*, *Pagrus major*, *Katsuwonus pelamis*, *Spratelloides gracilis*, and *Sacura margaritacea*, at each

52 study station [upstream (Up) or downstream (Dw) side of artificial reef (AR) 1 or AR2].