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ORIGINAL ARTICLE

Evaluation of community science monitoring with environmental DNA for marine fish species: "Fish survey project using environmental DNA"

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Abstract

Environmental DNA is a powerful tool for community science-based biodiversity surveys. However, the effectiveness of environmental DNA for environmental education and the time and physical costs perceived by volunteers for collecting environmental DNA remain unclear. Here, we evaluated a community science program for monitoring marine fish biodiversity using environmental DNA metabarcoding. This program aimed to investigate marine fish biodiversity in coastal areas along the Japanese archipelago. The participants were allowed to decide on the date and site to collect environmental DNA. They received a paper manual, a data sheet, and a sampling kit via a parcel delivery service. Before collecting environmental DNA, they watched a video manual for collecting environmental DNA and attended a webinar about the process and precautions for collecting environmental DNA provided by the scientists. At the sampling sites, they obtained environmental DNA samples from seawater themselves and sent the samples to the scientists via a refrigerated parcel delivery service. After collecting environmental DNA, they received fish name data from their samples and attended a webinar about survey results provided by the scientists. A cumulative total of 168 volunteers (84 pairs) participated in the program and detected 572 taxonomic groups of fish environmental DNA in the summer of 2020 and 2021. According to a questionnaire survey, more than 75% of the respondents answered that the project improved their understanding of biodiversity, marine environments, and environmental DNA. Approximately 95% of the respondents thought that environmental DNA collection work was meaningful to them. Some respondents commented on the difficulty of interpreting their fish name data, or the time and effort of selecting a sampling site. Therefore, improving the methods to communicate more information about fish name data and select sampling sites will further develop community science monitoring using environmental DNA.

KEYWORDS
biodiversity, citizen science, eDNA metabarcoding, MiFish

1 | INTRODUCTION

Citizen science, which scientists have begun to call "community science," has great potential for monitoring biodiversity and species distributions (Losey et al., 2012; Silvertown et al., 2015; Sullivan et al., 2009). The development of novel tools and technology has empowered community science and increased its potential. For example, the development of digital cameras and smartphones changed wildlife records from human observation to digital photographs in community science (Pocock et al., 2017). Photographs are simple and low-cost media to record and share wildlife information with online networks, such as iNaturalist (https://www.inaturalist.org). In addition to monitoring biodiversity and species distributions, sharing wildlife photographs is an important aspect of environmental education. Participants can understand the names of organisms by looking at others' photographs or by receiving comments for their photographs. However, there are several disadvantages with community science monitoring using photographs: the difficulty of species identification using photographs (Falk et al., 2019; Gardiner et al., 2012; Silvertown et al., 2015; Suzuki-Ohno et al., 2022) and the bias of the subject species in photographs due to human preferences (Marcenò et al., 2021).

Environmental DNA (eDNA) is currently attracting attention as a tool for community science monitoring (Biggs et al., 2015; Larson et al., 2017; Meyer et al., 2021; Miya et al., 2022; Tøttrup et al., 2021). The detection rate of target eDNA collected by participants is high, and the detection rate of the target is often higher than that of other traditional methods such as capture (Biggs et al., 2015; Goldberg et al., 2016). There are two types of community science monitoring using eDNA: detection of a specific species and detection of multiple species by eDNA metabarcoding. The development of a universal polymerase chain reaction (PCR) primer set for fish, MiFish (Miya et al., 2015), has promoted the use of eDNA metabarcoding to investigate fish biodiversity and distribution. Multiple species can be identified using eDNA, an open database, and computer programs, and their abundances may be estimated by quantifying eDNA concentrations (Fukaya et al., 2020; Ushio et al., 2018). Community science programs using eDNA metabarcoding can solve the problems of species identification and species bias caused by human preferences. However, the effectiveness of environmental education for participants and the time and physical costs perceived by participants for collecting eDNA remain unclear. The effectiveness of environmental education may be low because participants who collect eDNA cannot observe actual organisms. In addition, eDNA collection requires participants to concentrate on not contaminating samples or equipment for a long time (e.g., two hours) in the field, and it may be more difficult than other data collection methods such as taking photographs. This difficulty may include the time and physical costs for participants and reduce the number of participants and the samples of eDNA collected by the participants.

We focused on the community science program for marine fish biodiversity, "Fish survey project using eDNA." This program was initiated in 2020 by nine scientists who are co-authors of this article with the cooperation of Earthwatch Japan, a Japanese branch of the international nonprofit organization that connects volunteers with scientists for field investigation (https://www.earthwatch.jp). In this program, the participants were allowed to decide the date, time, and coastal sites to collect eDNA. They received a paper manual, a data sheet, and a sampling kit to collect eDNA via a parcel delivery service. Before eDNA collection, they watched a video manual and attended a webinar provided by the scientists. At the sampling sites, they obtained eDNA samples from seawater all by themselves and sent the samples to the scientists via a refrigerated parcel delivery service. The collected samples were analyzed by the scientists and the analyzed data were reported to the participants via a website and during a webinar after eDNA collection. After the webinar, a questionnaire survey was conducted to evaluate the program. It was expected that the webinar before eDNA collection and participants' collection work of eDNA would help the participants to understand eDNA and its characteristics. The list of fish names in their native language identified from eDNA would help the participants to understand the fish biodiversity at sampling sites. In addition, the webinar after eDNA collection would help the participants to understand the importance of fish biodiversity and the change in the marine environment. As there are only a few reports about community science programs using eDNA, it is important to discuss the effectiveness and costs for participants, to plan future community science and eDNA monitoring.

Here, we report on and evaluate the community science program for marine fish biodiversity. eDNA collection by participants was evaluated as the number of fish taxonomic groups and the comparison of eDNA data collected by the same participants at the same site in 2020 and 2021. The effectiveness of environmental education for participants was evaluated based on the results of the questionnaire survey. The costs perceived by participants for collecting eDNA and the issues of the program were also extracted from the questionnaire survey and the participants' personal reports.

2 | MATERIALS AND METHODS

2.1 | Fish survey project using eDNA

The project has three objectives: (1) to record the biodiversity of fishes along the coast of Japan at a high resolution with eDNA and the help of volunteers; (2) to create an open database of biodiversity based on eDNA; and (3) to create a foothold on how to conserve and use the coastal areas by learning about the state of the ecosystem from eDNA. To achieve these objectives, participants collected eDNA samples at coastal sites. Numerous fish species were detected using eDNA metabarcoding, and the eDNA concentrations of multiple fish species were quantified using the quantitative metabarcoding method (Ushio et al., 2018).

The nine scientists (co-authors of this article) recruited participants for the program with the cooperation of Earthwatch Japan (Figure 1). The participants received a paper manual (Appendix S1),

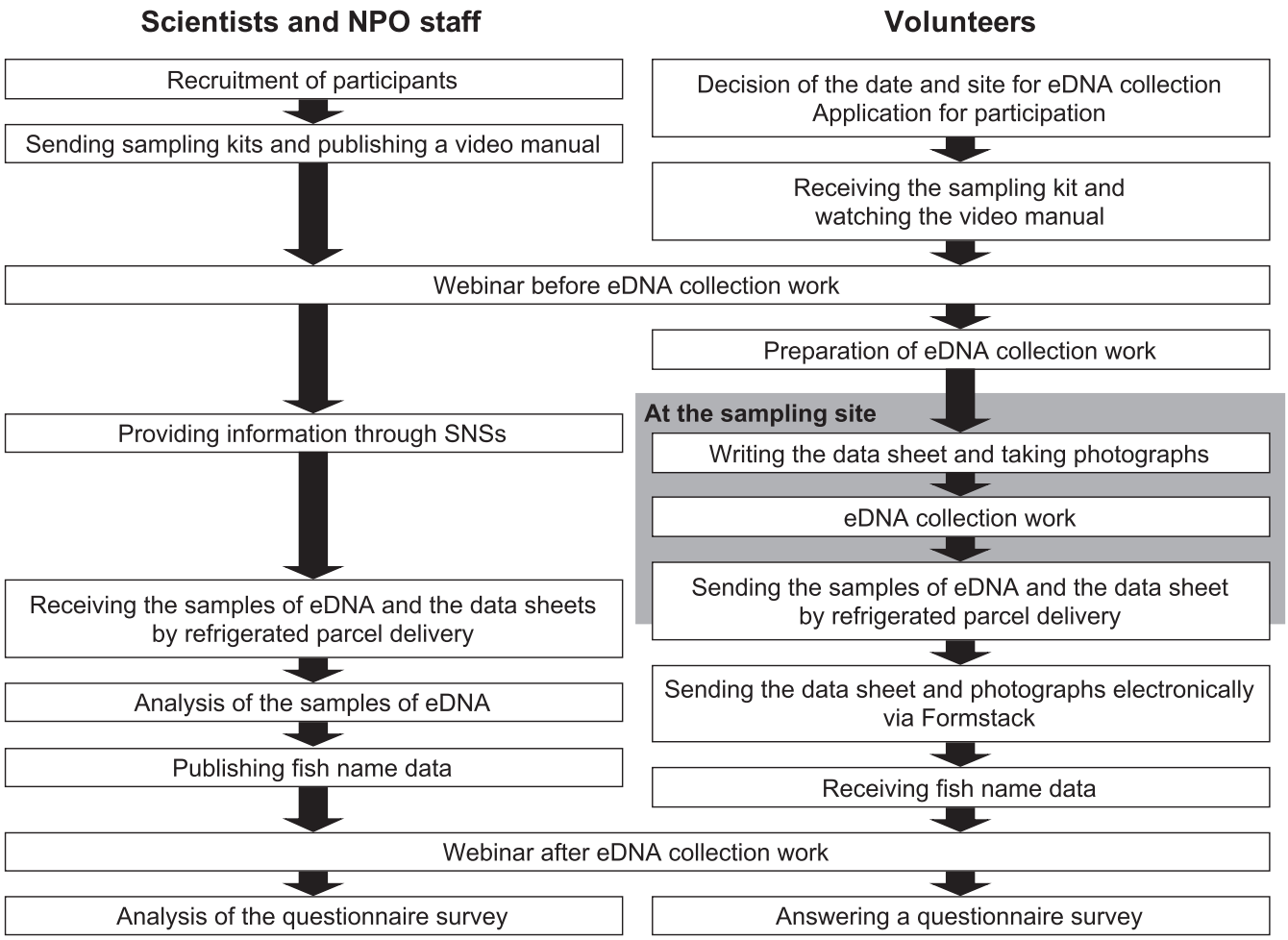

FIGURE 1 Flowchart of "Fish survey project using environmental DNA."

a data sheet (Appendix S2), and a sampling kit to collect eDNA (Figure 2). A video manual for eDNA collection was published on a website for the participants. Before eDNA collection, the main members organized a webinar to explain the outline of the program and the video manual. During the survey period, the main members provided information about eDNA collection and the weather (e.g., strong winds that cause high waves, heavy rain, and typhoons) through Facebook and Twitter. The participants prepared the items required for eDNA collection (Appendix S1). At the sampling points, the participants recorded field data on the data sheet and collected eDNA samples using the sampling kit according to the manual. The participants sent the data sheet and samples to scientists via a refrigerated parcel delivery service. During the refrigerated parcel delivery, the temperature was kept between 0 and 10°C. Field data and photographs of the survey sites were also collected electronically via Formstack by Earthwatch Japan. The samples were sent to a public institute for biological research, where the eDNA was extracted and the MiFish primer region (ca. 160–190 bp) of the mitochondrial 12S rRNA gene was amplified by PCR and sequenced using a high-throughput sequencer based on a public protocol (Minamoto et al., 2021). The fastq files obtained from sequencing were analyzed using the DNA barcoding system, Claident (Tanabe & Toju, 2013; available at http://www.claident.org) following the tutorial (https://github.com/astanabe/ClaidentTutorial), and the molecular identification results were summarized as a list of Japanese common name for detected fish species. The list was displayed on Google Maps for the participants to visualize. All the fish eDNA data obtained in the program were recorded and made available in an open database for eDNA metabarcoding survey, ANEMONE DB (https://db.anemone.bio). Some of the results were presented by the main members during the webinar after eDNA collection. After the webinar, the participants were asked to answer a questionnaire to evaluate the program (Appendix S3).

2.2 | Sampling kit for eDNA collection

The sampling kit to collect eDNA consisted of two cartridges (Sterivex-HV Filter, 0.45 um pore size, PVDF membrane, gamma irradiated, sterile), two syringes, cartridge caps, parafilm, two disposable pipettes, and RNAlater (Figure 2, upper left). The sampling kit, a paper manual, a data sheet, seals of the sample ID, rope (15 m long), paper towels, a garbage bag, a clear case, clips, ice packs, an armband of Earthwatch, safety pins (for the armband of Earthwatch),

FIGURE 2 Items sent to participants. The kit to collect environmental DNA consisted of two cartridges (Sterivex filter units), two syringes, cartridge caps, parafilm, two disposable pipettes, and RNAlater in the upper left of Figure 2.

TABLE 1 Basic information of participants in "Fish survey project using environmental DNA." The number of times that each participant had participated survey in Earthwatch Japan was counted excluding this project.

Sex				M									F	
2020				26									14	
2021				26									18	
Age structure	**Teens**	**20s**	**30s**	**40s**		**50s**		**60s**		**70s**			**80s**	
2020	2	11	2	9		7		6		2			1	
2021	2	9	4	12		8		8		1			0	
The number of times that each participant had participated survey in Earthwatch Japan				**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**≥10**	**≥20**	**≥30**
2020				26	1	6	1	1	0	0	0	1	0	2	1	1
2021				34	1	2	2	0	1	0	0	1	0	1	1	1

zipper bags (large, medium, and small size), a cool bag, and an invoice for refrigerated parcel delivery service were sent to the participants (Figure 2). In addition to the above items, participants were required to prepare a bucket, chlorine bleach, a pencil, a marker, gum tapes, and optional items including a smartphone, a PET bottle of water, and a hat for themselves to use during the program.

2.3 | Evaluation of the program

2.3.1 | Participants and sampling sites

Participants collected eDNA samples in pairs to ensure safety at sampling sites. To evaluate eDNA collection by participants, we excluded two pairs of participants who were accompanied by the scientists to collect eDNA in the results in this article. After the exclusion, a cumulative total of 84 pairs participated in the program. The number of participants by sex, age group, and the number of times that they had participated in other surveys in Earthwatch Japan is summarized as basic information on the participants (Table 1). Permission to publish basic information was obtained from Earthwatch Japan. The net number of participants who could collect eDNA was 67 pairs; 15 pairs overlapped in 2020 and 2021, and three pairs (including one pair that overlapped in 2020 and 2021) were canceled in 2021 because of bad weather or damage to the sampling kit during eDNA collection.

Based on the latitude and longitude data in the data sheet, the sampling sites decided by the participants were plotted on the map. The cumulative total of sampling sites reached 81 including eight overlapped sites because one participant selected a sampling site in 2021 that was the same sampling site selected by a different participant in 2020, and seven participants selected sampling sites in 2021 that were the same or close to their sampling sites in 2020.

2.3.2 | eDNA collected by participants

We evaluated the efficiency of eDNA collection by participants using the total number of fish taxonomic groups and the year-on-year reproducibility of eDNA collection by participants. We reported the total number of taxonomic groups because most eDNA sequences have been identified at the species or genus level, while the others were identified at the larger taxonomic group level.

To calculate year-on-year reproducibility, we selected the seven overlapped sites in 2020 and 2021. Among participants who collected eDNA in both 2020 and 2021, seven participants selected sampling sites in 2021 that were the same or close to their sampling sites in 2020. The sampling dates of seven overlapped sites are shown in Table D2 in Appendix S4. Two eDNA samples collected by the same participant at the same site in 2020 and 2021 were compared and the percentage of the common taxonomic groups detected in 2020 and 2021 was calculated to evaluate the year-on-year reproducibility of eDNA collection by the participants. The percentage was calculated without considering the hierarchical taxonomic structure.

The metabarcoding results include noise in the processes from extraction to PCR, library preparation processes, and index-hopping of artificial base sequences to identify derived samples due to reactions within the sequencer. There is a risk that a small amount of high-concentration DNA in one sample may be mixed into another in a laboratory. The methods of denoising have been proposed in several previous studies (Davis et al., 2018; Esling et al., 2015). However, in this study, the number of fish taxonomic groups was counted without denoising because the total number of fish taxonomic groups does not change, with or without denoising. The total number of sites where a taxonomic group was detected and the percentage of the common taxonomic groups detected in 2020 and 2021 might be slightly different from that with denoising but was meaningful as a feature of the eDNA survey.

2.3.3 | Effectiveness of environmental education

We evaluated the effectiveness of environmental education for participants using a questionnaire survey of the participants (Appendix S3). The effectiveness of environmental education was evaluated by participants as a form of self-reporting ranking from "Level 1" to "Level 5." The questionnaire was created using Google Forms, and a questionnaire survey was conducted in March 2022. The questionnaire consisted of a page for obtaining informed consent, a page for basic information, and 15 pages with 19 questions, including motivations for participation, evaluation of the eDNA collection method, achievement of the program's objectives, and effectiveness of the program's environmental education. The answer options for the questions about motivations for participation (Q1 and Q2) were created based on Rotman et al. (2012) and West and Pateman (2016) (Table C3 in Appendix S3). The Results section in this article shows the effectiveness of environmental education and the costs perceived by participants for collecting eDNA. Please see Appendix S3 for the results of the basic information (Table C1), the motivations for participation (Table C2), the evaluation of the eDNA collection method (Figure C1), the achievement of the program's objectives (Figure C2), and the overall program (Figure C3). The cumulative total number of participants in the program was 84 pairs. However, the net number of participants who could collect eDNA was 67 pairs due to the overlap between 2020 and 2021 and the cancellation of eDNA collection in 2021. Of the 67 pairs, 44 voluntarily cooperated in the questionnaire survey, and 36 of 44 pairs returned valid questionnaire responses. Informed consent was obtained from all the respondents.

2.3.4 | Costs perceived by participants for eDNA collection

The time and physical costs perceived by participants for collecting eDNA and the issues of the program were extracted from the

questionnaire survey and participants' personal reports. Personal reports were mainly collected from comments in the remarks column of the data sheet (Appendix S3) to record field data and comments about eDNA collection. All participants submitted the data sheet, and about two-thirds wrote comments because the remarks column was optional.

3 | RESULTS

3.1 | Participants and sampling sites

Basic information on sex, age group, and the number of times that they had participated in other surveys with Earthwatch Japan is shown in Table 1. Participants of all ages from teens to 80s participated in the program (Table 1). For most participants, it was the first time they participated in community science programs with Earthwatch Japan (Table 1).

The latitude of the sampling sites ranged from 26.2 to 43.3 °N, and the longitude of the sampling sites ranged from 127.8 to 145.5 °E (Figure 3). Although eDNA was frequently collected near Tokyo (Figure 3b), it was collected from all over Japan.

3.2 | eDNA collected by participants

A total of 572 taxonomic groups of fish were detected from eDNA collected at the sampling sites. The mean number of taxonomic groups per site was 37.7. Of the 572 taxonomic groups, 441 were identified at the species level and 95 were identified at the genus level. The others were identified at the subfamily, family, or larger taxonomic group levels. Several families, such as Sparidae, Blenniidae, Mugilidae, Gobiidae, and Tetraodontidae, were commonly detected at most sampling sites (Figure 4). In contrast, others such as Polynemidae were only detected at a few sampling sites (Figure 4). Even at the same sampling site (blue diamonds in Figure 3), the mean percentage of common taxonomic groups identified from eDNA collected by the same participant between 2020 and 2021 was 37.2% of the taxonomic groups detected at the sampling site (Table 2).

3.3 | Effectiveness of environmental education

In the questionnaire survey, we asked participants to evaluate the effectiveness of environmental education in the program as a form of self-reporting ranking from "Level 1" to "Level 5." The five-level evaluation (Level 5, Improve – Level 1, Not improve) of respondents' understanding of biodiversity, marine environments, and eDNA indicated the high efficiency of the environmental education in the program. 77.8%, 83.3%, and 91.7% respondents answered > level 3 in the five-level evaluation about the understanding of biodiversity, marine environments, and eDNA, respectively (Figure 5a–c). The means of the five-level evaluation of the understanding of biodiversity, marine environments, and eDNA were 4.33, 4.28, and 4.5, respectively. Comments of respondents indicated that the understanding of biodiversity, marine environments, and eDNA was improved by the eDNA collection work and the webinar after eDNA collection.

The five-level evaluation (Level 5, Meaningful – Level 1, Not meaningful) of eDNA collection work and the obtained fish name data from eDNA indicated that eDNA collection work itself was meaningful to participants in the program. In the five-level evaluation of eDNA collection work, 94.4% respondents answered > level 3 (Figure 5d). The mean of the five-level evaluation of the eDNA collection work was 4.65. In the five-level evaluation of fish name data obtained from eDNA, 88.8% respondents answered > level 3

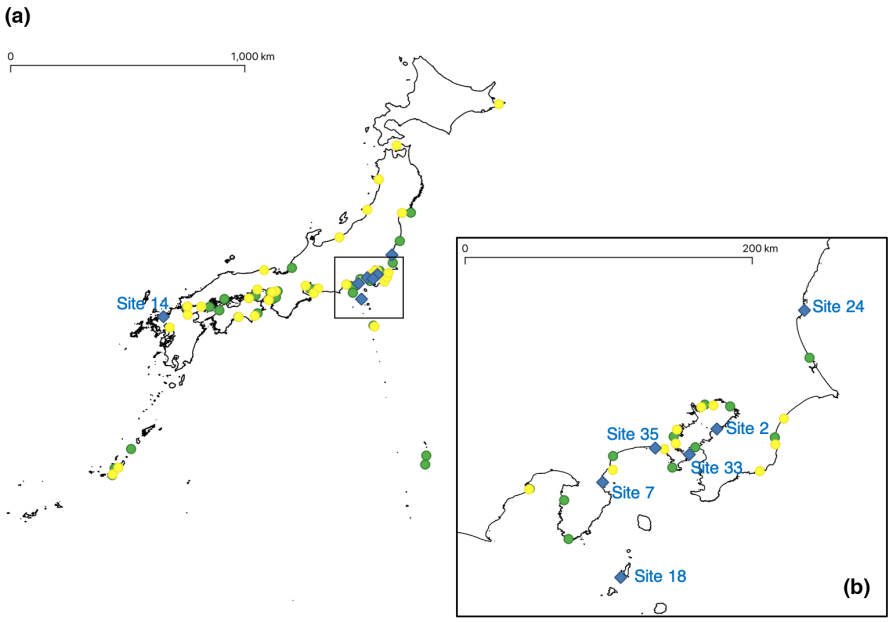

FIGURE 3 Sampling sites of environmental DNA in "Fish survey project using environmental DNA." Green circles represent the sampling sites in 2020 whereas yellow circles represent the sampling sites in 2021. Blue diamonds represent the seven overlapped sampling sites selected by participants in both 2020 and 2021. (a) Map of the whole of Japan, (b) Map of the coastal line near Tokyo where sampling sites were concentrated.

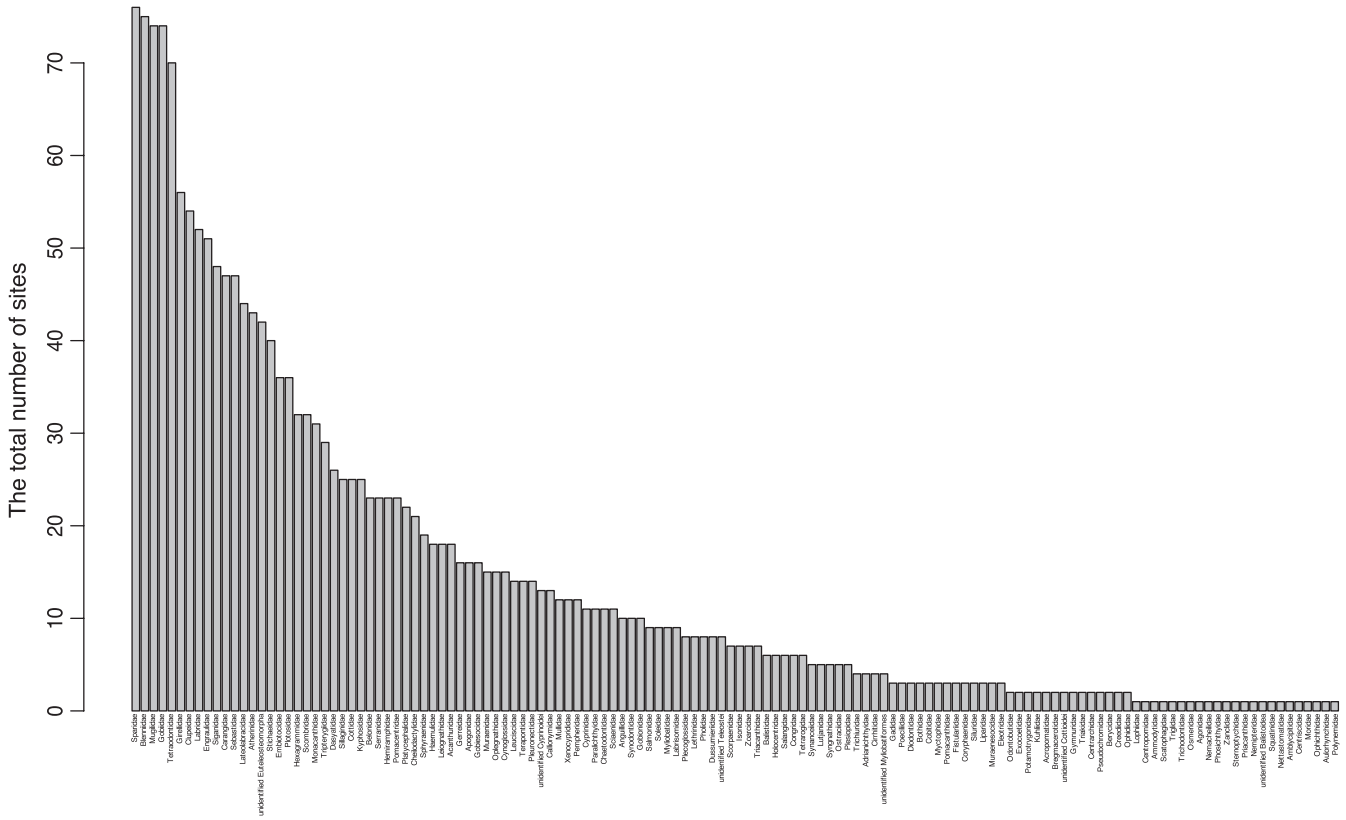

FIGURE 4 Histogram of the total number of sampling sites where the environmental DNA (eDNA) of the fish family was detected in 2020 and 2021. The x-axis represents fish families, whereas the y-axis represents the total number of sampling sites where the eDNA of the fish family was detected. Although most eDNA were identified at the species or genus level, the x-axis represents only the family level to keep the visibility of the figure. Sparidae, Blenniidae, Mugilidae, Gobiidae, and Tetraodontidae are shown in the left and Polynemidae in the rightmost in Figure 4. The raw data of the histogram are described in Appendix S4.

TABLE 2 The common taxonomic groups identified from environmental DNA (eDNA) collected by the same participant at the same sampling site in 2020 and 2021. The number of taxonomic groups identified from eDNA in 2020 and 2021, and the number and percentage of common taxonomic groups between 2020 and 2021 are shown.

	No. of taxonomic groups in 2020	2021	Common	Percentage
Site 2	26	23	13	36.1%
Site 7	48	50	25	34.2%
Site 14	39	55	27	40.3%
Site 18	70	66	37	37.4%
Site 24	30	42	19	35.8%
Site 33	61	36	23	31.1%
Site 35	56	66	38	45.2%
Average				37.2%

(Figure 5e). The mean of the five-level evaluation of the obtained fish name data from eDNA (mean: 4.51) was also high (Figure 5e), but slightly lower than that of eDNA collection work (mean: 4.65) (Figure 5d). In the free text boxes, there were comments that fish species actually observed at the sampling sites were detected, or fish species had changed from those observed before. On the other hand, some respondents would like scientists to comment more on their fish name data and the environments of their sampling sites in the webinar because it was difficult to find important information (e.g., rare fish or indicator fish species for a good environment) just by looking at their fish name data. Those who were familiar with fish and those who knew information about the fish at the sampling sites in advance were satisfied with fish name data, but those who did not seem to were not and wanted to obtain more information about the fish from experts.

3.4 | Costs perceived by participants for collecting eDNA

Six respondents indicated the costs perceived by them for collecting eDNA in free text boxes in the questionnaire survey. The cost was mainly associated with the time and effort of selecting sampling sites. Some of the participants lived far from their candidate sampling sites and could not visit the sites in advance. In a few candidate sites, it was difficult to draw seawater with a bucket because of obstacles such as fences, or no parking lot. The participants had to find another place near the candidate sampling site.

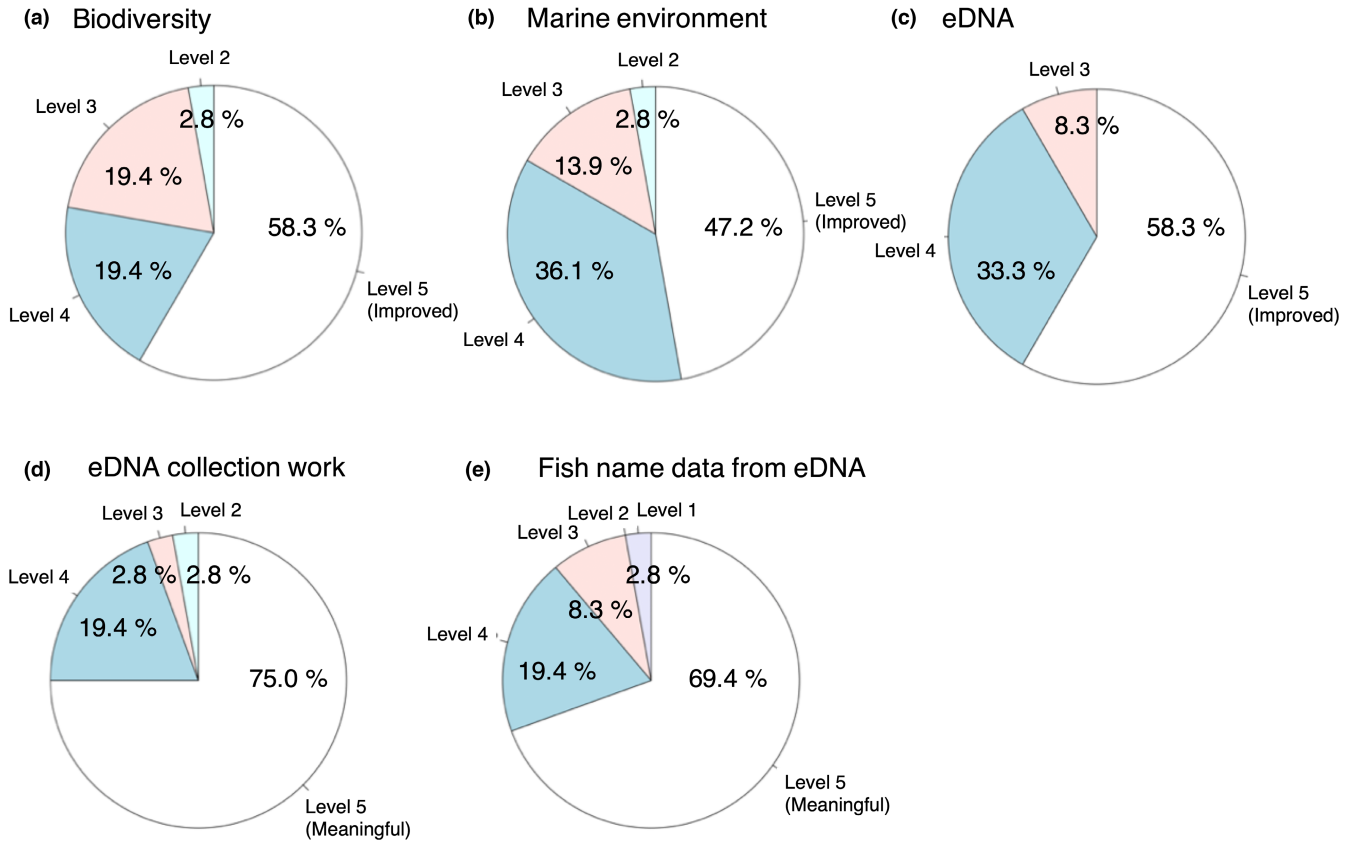

FIGURE 5 Percentage of five-level evaluations about the environmental education of the project in the questionnaire survey. Upper graphs are the pie chart of answers to the question of whether the project improves the understanding of "biodiversity"/"marine environment"/"environmental DNA (eDNA)." Level 5 is "Improved," whereas level 1 is "Not improved." Lower graphs are the pie chart of answers to the question whether "eDNA collection work"/"Fish name data from eDNA" is meaningful to the respondent. Level 5 is "Meaningful," whereas level 1 is "Not meaningful." In Figure 5e 2.8% of Level 1 corresponds to a respondent who did not visit a web page that displays fish name data from eDNA.

As with the questionnaire survey, several participants wrote the time and effort of selecting the sampling site to collect eDNA in the data sheet.

Through personal reports from participants, several participants indicated that they were troubled by the weather. Since the sampling points are on the coast, it was necessary to pay attention to the weather, especially typhoons. There were nine cases in which eDNA collection work was not completed within the predetermined survey period in 2021. eDNA collection work was postponed in six of these nine cases and completed the following week.

4 | DISCUSSION

In this study, we evaluated the community science eDNA survey program, "Fish survey project using eDNA," conducted in 2020 and 2021. This study revealed that the community science program using eDNA is effective for data collection and environmental education. Even if the participants did not see the actual organisms, the effectiveness of the environmental education was high. The effectiveness can be further enhanced by improving the methods of communicating more information about fish name data and selecting sampling sites.

4.1 | eDNA collected by participants

A total of 572 taxonomic groups of fish were detected from the samples of eDNA collected at a cumulative total of 81 coastal sites from all over Japan (Figures 3 and 4). Of the 572 taxonomic groups, 441 were identified at the species level, and 95 were identified at the genus level. Most fish species identified from eDNA were only detected at a few sampling sites (e.g., Polynemidae in the rightmost column in Figure 4). Even at the same sampling site, the mean percentage of common taxonomic groups identified from eDNA collected by the same participant between 2020 and 2021 was 37.2% of the taxonomic groups detected at the sampling site (Table 2). This does not mean that the eDNA data collected by participants are inaccurate. The species composition identified from eDNA was affected not only by regionality but also by microhabitat, the date and time of sampling, weather (e.g., winds and rains) on the sampling day, the stochastic effect of collecting

a certain species' eDNA, and population dynamics, including fish immigration, migration, and oviposition. Therefore, it is desirable to continue the survey and conduct multiple surveys throughout the year. By continuing to conduct multiple surveys throughout the year or annual surveys over multiple years, we will be able to see how often we should sample to identify species that are stably living there. The "Fish survey project using eDNA" is scheduled to continue until 2023, and maybe beyond 2023. The multi-year eDNA data in the program will provide more useful information on fish diversity.

4.2 | Effectiveness of environmental education

The respondents of the questionnaire survey highly evaluated the program from the perspective of environmental education. According to the results of the questionnaire survey, more than 75% of respondents considered that the program improved their understanding of biodiversity, marine environments, and eDNA (Figure 5a–c). 86.1% of respondents answered that they participated in this program to acquire new knowledge (Table C2), and more than 90% of respondents answered that eDNA collection work was meaningful to them (Figure 5d). Therefore, the project of eDNA collection can provide environmental education to participants.

The questionnaire survey also revealed that the evaluation of the fish name data obtained from the samples of eDNA that participants collected was slightly lower than that of the eDNA collection work (Figure 5d,e). In the free text boxes of the questionnaire survey, some respondents commented on the request of providing more information about the fish name data by scientists on the webinar after eDNA collection. At the webinar, the scientists presented on the detectability of fish eDNA, changes in fish distribution, and the effect of global warming on marine environments. More presentations on each fish name data, interspecific interactions, and ecosystems in the webinar will improve the understanding of fish name data. It will also improve the understanding of biodiversity (Figure 5a). In addition, the frequent use of web pages, Facebook, and Twitter will enhance a conversation about fish name data between scientists and participants or between participants. We consider that it is effective to create a fish encyclopedia and to provide information on culturally or economically important species and invasive species including domestic invasive species, on web pages and SNSs such as Facebook and Twitter. Information on rare species is also important, but it has a risk that their habitats will be disclosed. We should be very careful or avoid talking about rare species on public SNSs.

In this study, we evaluated the effectiveness of environmental education based on the form of self-reporting. Although self-reporting is a less-stressful method for participants, it is more objective to quantitatively measure the effectiveness of environmental education through quizzes or tests. For example, we might obtain clear evidence by giving quizzes before and after eDNA collection in secondary school classrooms or college programs and measure the effectiveness quantitatively. This is an issue that should be investigated in the future.

4.3 | Costs perceived by participants for collecting eDNA

Six respondents reported the time and effort of selecting the sampling site to collect eDNA in the questionnaire survey. Proposing suitable sampling sites from scientists will reduce the time and effort of selecting the sampling site. Before the questionnaire survey, we expected many participants to report problems related to the time and physical costs of eDNA collection work because it requires participants to concentrate on not contaminating samples or equipment for about two hours to collect eDNA and physical labor such as drawing seawater. However, in the questionnaire survey, the time and effort of selecting the sampling site were reported more than the time and physical cost of eDNA collection work itself. A respondent commented that it was difficult to collect eDNA samples but good to know how difficult it was. This may be because most of the participants had the purpose of experiencing the eDNA collection work itself.

To reduce the time and physical cost perceived by participants to select sampling sites, scientists could present several candidate sampling sites in advance for participants who do not have familiar coastal sites. Coastal sites where the other participants collected eDNA before in the program may become such candidate sampling sites. In the future, we should test whether the cost perceived by participants to select sampling sites is reduced by having scientists present some candidate sampling sites in advance.

Several previous studies reported community science programs using eDNA (Biggs et al., 2015; Larson et al., 2017; Meyer et al., 2021; Miya et al., 2022; Tøttrup et al., 2021). Biggs et al. (2015) investigated the distribution of the great crested newt *Triturus cristatus* in ponds in the UK. A total of 86 volunteers, including professional workers, collected eDNA samples from 239 ponds. Larson et al. (2017) investigated the presence of invasive crayfishes *Faxonius rusticus*, which was reclassified by changing the genus of *Orconectes* to *Faxonius* (Crandall & De Grave, 2017), and *Pacifastacus leniusculus* in large lakes in North America. Ninety-four volunteers, including organization staff, employees, students, or teachers, collected eDNA samples at 9 sites. Sampling eDNA from freshwater in ponds, lakes, and rivers may be less costly and risky than that from seawater at coastal sites. We must carefully consider the time and physical costs perceived by participants for collecting eDNA because the costs change depending on the environments of the sampling sites. The program conducted by Miya et al. (2022) is the closest to the "Fish survey project using eDNA" among the previous studies, but it was a pilot study involving only six samples of eDNA by participants and did not evaluate the costs of collecting eDNA and the effects of environmental education. In the "Fish survey project using eDNA," eDNA collection was conducted by a cumulative total of 84 pairs of participants of various ages (Table 1). This is similar to the situation

of the online community science programs using photographs and is suitable for evaluating community science monitoring using eDNA. This study will contribute to the development and improvement of community science monitoring using eDNA.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest.

DATA AVAILABILITY STATEMENT

The obtained data of eDNA collected by participants is available in an open database ANEMONE DB (https://db.anemone.bio/).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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