Temperature sensitivity of the interspecific interaction strength of coastal marine fish communities

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Abstract

Interspecific interactions influence the dynamics and stability of a marine ecological community, and thus, are key to understanding marine ecosystem dynamics. The effects of temperature on interaction strengths are important for understanding and forecasting how global climate change impacts marine ecosystems; however, it currently remains unclear how temperature influences interaction strengths in marine communities. We herein performed quantitative fish environmental DNA (eDNA) metabarcoding on 550 seawater samples that were collected twice a month from 11 coastal sites in the Boso Peninsula, Japan, and analyzed eDNA monitoring data (a proxy of fish community dynamics) using nonlinear time-series analytical tools. We detected and quantified fish-fish interactions as information flow between eDNA time series, and reconstructed interaction networks for the top 50 frequently detected species from the 11 sites. Water temperature positively influenced fish-fish interaction strengths in general, supporting the view that warming temperature intensifies interspecific interactions. Furthermore, the impact of water temperature on interspecific interaction strengths varied among fish species, suggesting that fish species identity influences the effects of temperature on interactions. Therefore, an increase in water temperature induced by global climate change may intensify fish interactions, consequently influencing marine community dynamics and stability.

Keywords: *Community dynamics; Empirical dynamic modeling; Environmental DNA; Fish; Interaction network; Quantitative sequencing*

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Introduction

Interspecific interactions are key to understanding and predicting the dynamics of ecological communities (May 1972; Wootton & Emmerson 2005; Tang *et al.* 2014; Wootton & Stouffer 2015; Ushio *et al.* 2018a; Ratzke *et al.* 2020). Theoretical and empirical studies have shown that various properties of interspecific interactions, namely, the number of interactions, sign (positive or negative), strength, and pair-wise correlations of interactions (e.g., prey-predator interactions), influence the dynamics, stability, and diversity of ecological communities in terrestrial and aquatic ecosystems (Mougi & Kondoh 2012; Tang *et al.* 2014; Allesina *et al.* 2015; Ushio *et al.* 2018a; Ratzke *et al.* 2020; Ushio 2022). Interaction strength is a fundamental property, and previous studies examined the relationship between interaction strengths and ecological community properties (Wootton & Emmerson 2005; Wootton & Stouffer 2015; Ushio *et al.* 2018a; Ratzke *et al.* 2020). The dominance of weak interactions stabilizes the dynamics of a natural fish community (Ushio *et al.* 2018a). Interaction strengths have been reported to decrease with increases in the diversity of experimental microbial communities (Ratzke *et al.* 2020), and this holds true even for more diverse ecological communities (Ushio 2022).

Environmental variables exert significant effects on interspecific interaction strengths. The effects of temperature on interaction strengths are important for understanding and forecasting the impact of the ongoing global climate change on ecosystems. The relationship between temperature and interaction strengths has been investigated in terrestrial and aquatic ecosystems for decades (Coley & Aide 1991; Kishi *et al.* 2005; Adams & Zhang 2009; Kratina *et al.* 2012; Hein *et al.* 2014; Thakur *et al.* 2017). In terrestrial ecosystems, Coley & Aide (1991) proposed that interactions between plants and insect herbivores were generally stronger in warmer regions, which may result in stronger negative density dependence for tree species and contribute to higher plant diversity in a tropical region (Forrister *et al.* 2019). The influence of temperature on interaction strengths has also been investigated in other systems; freshwater fish-fish interactions (Hein *et al.* 2014), freshwater fish-prey interactions (Kishi *et al.* 2005), and terrestrial arthropods interactions (Rall *et al.* 2010). In addition, Wieczynski et al. (2021) showed that species-level functional traits (e.g., body size and shape) may underlie the relationships between temperature and interaction strengths. Therefore, a more detailed understanding of the complex interactions between temperature, interaction strengths, and community-level function (e.g., primary production) is critical for predicting ecosystem responses to climate change.

In marine ecosystems, interspecific interactions in ecological communities play a fundamental role in system dynamics, such as population dynamics, primary production, and nutrient cycling (Hannisdal *et al.* 2017; Ushio *et al.* 2018a; Penn *et al.* 2019), as well as ecosystem services, including food supply (Smith *et al.* 2019). However, it currently remains unclear how temperature influences the strengths of interspecific interactions in marine communities despite their importance. This may be due to the difficulties associated with detecting and measuring interaction strengths among multiple species in marine ecosystems; the identification of fish species and the quantification of their abundance under field conditions are challenging, and the quantification of their interactions is even more difficult. Overcoming these difficulties and understanding how temperature influences the strength of interspecific interactions of marine ecological communities will provide insights into how marine communities are assembled and how they may respond to the ongoing and future global climate change.

Environmental DNA (eDNA), defined here as extra-organismal DNA left behind by macro-organisms (Bohmann *et al.* 2014), has been attracting increasing attention as an indirect genetic marker for inferring the presence of species for biodiversity monitoring (Deiner *et al.* 2017; Cristescu & Hebert 2018). A simple protocol for collecting eDNA samples from aquatic environments facilitates continuous biodiversity monitoring at multiple sites (Deiner *et al.*

2017), and eDNA metabarcoding (the simultaneous detection of multiple species using universal primers and a high-throughput sequencer) provides useful information on the dynamics of ecological communities (Bálint *et al.* 2018; Miya *et al.* 2020; Miya 2022; Ushio 2022). Recent studies demonstrated that eDNA metabarcoding combined with frequent water sampling enabled the efficient monitoring of high-diversity ecological communities (Bista *et al.* 2017; Djurhuus *et al.* 2020; Ushio 2022). Furthermore, if eDNA metabarcoding is performed quantitatively (e.g., by including spike-in DNAs, Ushio *et al.* 2018b), these data may contain information on species abundance.

More importantly, recent studies have shown that information on interspecific interactions may be embedded in multispecies eDNA time series, particularly when the data is "quantitative" (Ushio 2022). Advances in nonlinear time series analyses have enabled the quantification of interspecific interactions only from time series data. For example, transfer entropy (TE) is a method based on the information theory that quantifies information flow between two variables (Schreiber 2000; Runge *et al.* 2012). Information flow may be an index of interspecific interactions when applied to ecological data. Convergent cross mapping (CCM) is a causality detection tool in empirical dynamic modeling (Sugihara *et al.* 2012), which is based on the dynamical theory. TE and CCM have more recently been understood under the information theory framework, and unified information-theoretic causality (UIC) may also quantify information flow between variables (rUIC package, Osada & Ushio 2021). These advanced statistical tools facilitate the detection and quantification of interspecific interactions from quantitative, multispecies eDNA time-series data.

In the present study, we collected eDNA samples twice a month from 11 coastal sites at the southern tip of the Boso Peninsula, central Japan (Fig. 1a) for two years (a total of 550 samples). This region is located on the Pacific side of Honshu Island around 35°N and is markedly affected by the warm Kuroshio Current, cold Oyashio Current, and inner-bay water from Tokyo Bay. These geographic and oceanographic characteristics form latitudinal temperature gradients, allowing us to investigate temperature effects on interspecific interactions. We obtained quantitative eDNA metabarcoding data by combining MiFish eDNA metabarcoding data (relative abundance data) and the eDNA concentration data obtained through quantitative PCR (qPCR) for one of the most common fish species, *Acanthopagrus schlegelii* (e.g., absolute abundance data of *A. schlegelii* eDNA was utilized as internal spike-in DNA; see Methods). Based on quantitative, multispecies eDNA time-series data, interspecific interaction strengths were quantified as information flow between species using a nonlinear time series analysis (Schreiber 2000; Osada & Ushio 2021). We hypothesized that (1) a positive relationship exists between temperature and interaction strengths at the community level, as found in terrestrial ecosystems, and (2) the relationship between the patterns of temperature and interaction strengths varies among fish species because of species-specific ecologies and the behavior of component species.

Mateirals and Methods

Study sites and environmental observations

We selected 11 sites (Stations [Sts.] 1–11) for seawater sampling along the shoreline of the southern tip of the Boso Peninsula (Fig. 1a). The 11 stations are located within or surrounded by rocky shores with intricate coastlines. They are arranged such that the five stations on the Pacific and Tokyo Bay sides are approximately at the same latitudinal intervals, with St. 6 in between. The northernmost stations on both sides (Sts. 1 and 11) are markedly affected by the cold Oyashio Current (Yang *et al.* 1993) and inner-bay water from Tokyo Bay (Fujiwara &

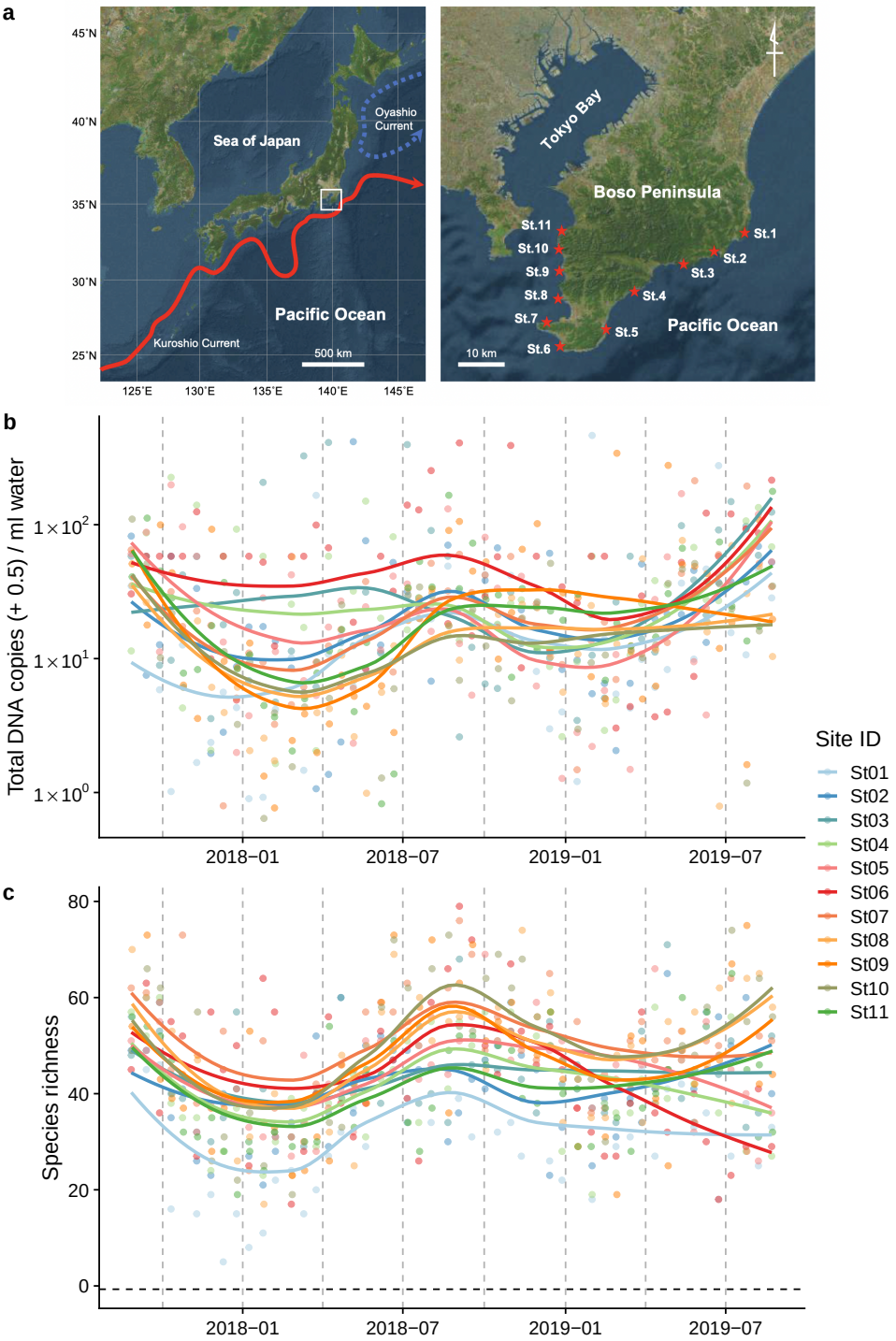

Figure 1 | Study sites and overall dynamics of environmental DNA (eDNA) concentrations and the number of fish species detected. (**a**) Study sites in the Boso Peninsula. The study sites are influenced by the Kuroshio current (red arrow; left panel) and distributed along the coastal line in the Boso Peninsula (right panel). (**b**) Total eDNA copy numbers estimated by quantitative eDNA metabarcoding (see Methods for detail). (**c**) Fish species richness detected by eDNA metabarcoding. Points and lines indicate raw values and LOESS smoothing lines, respectively. The line color indicates the sampling site. Warmer colors generally correspond to study sites with a higher mean water temperature.

Yamada 2002), respectively, while the remaining stations (Sts. 2–10) are markedly affected by the warm Kuroshio Current flowing northward along the Pacific coast as well as its branches (Soh 2003). Before seawater sampling at each station, we measured seawater temperature (°C) and salinity (‰) using a portable water-quality meter (WQC-30, Toa DKK, Tokyo, Japan). In addition, we recorded the sampling start time, latitude/longitude, weather and sea conditions, and turbidity.

Collection of eDNA samples

Detailed information on seawater sampling and on-site filtration is provided in Supporting Information. We collected seawater samples twice a month (i.e., around the first and second quarter moons) for two years between August 2017 and August 2019 by casting a bucket fastened to a rope at the 11 sites. We filtered the collected seawater using a combination of a filter cartridge and disposable syringe until the final filtration volume reached 1,000 mL for each replicate and obtained duplicate samples at each site. We added RNAlater (Thermo Fisher Scientific, DE, USA) into the cartridge to prevent eDNA degradation. We made a filtration blank (FB) by filtering 500 mL of purified water in the same manner at the end of each day of water sampling.

Laboratory protocol

Detailed information on the laboratory protocol is provided in Supporting Information. We thoroughly sterilized the workspace and equipment in the DNA extraction, pre-PCR, and post-PCR rooms before all experiments. We used filtered pipette tips and conducted all eDNA extraction, pre-PCR, and post-PCR manipulations in three different dedicated rooms that were separate from each other to safeguard against cross-contamination.

We extracted eDNA from filter cartridges using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the methods developed by Miya et al. (2016) with slight modifications (Miya & Sado 2019; Minamoto *et al.* 2021). After removing RNAlater from filter cartridges, they were subjected to lysis using proteinase K. We purified the collected DNA extracts using the above kit following the manufacturer's protocol and the final elution volume was set to 200 μL. An extraction blank (EB) was also created during this process.

We employed two-step PCR for paired-end library preparation using the MiSeq platform (Illumina, CA, USA). We generally followed the methods developed by Miya et al. (2015) and subsequently modified by Miya & Sado (2019). Detailed information on library preparation is provided in Supporting Information.

We performed the first-round PCR (1st PCR) using a mixture of the following six primers: MiFish-U-forward, MiFish-U-reverse, MiFish-E-forward-v2, MiFish-E-reverse-v2, MiFish-U2-forward, and MiFish-U2-reverse (Table 1). The 1st PCR amplified a hypervariable region of the mitochondrial 12S rRNA gene (*ca.* 172 bp; hereafter called the "MiFish sequence"). We also prepared a 1st PCR blank (1B) during this process, in addition to FB and EB. After the 1st PCR, PCR products were purified, quantified, diluted to 0.1 ng/μL, and used as templates for the second-round PCR (2nd PCR).

We performed the 2nd PCR to append dual-indexed sequences and flow cell-binding sites for the MiSeq platform. We prepared a 2nd PCR blank (2B) during this process in addition to FB, EB, and 1B. In total, we made 195 blanks (FB = 60, EB = 50, 1B = 50, 2B = 35) to monitor contamination during the on-site filtration, subsequent DNA extraction, and 1st and 2nd PCR of 550 samples.

We pooled each of the individual paired-end libraries in an equal volume, performed electrophoresis on an agarose gel (Invitrogen, CA, USA), and excised the target amplicons (*ca.* 370

bp). The concentrations of the size-selected libraries were measured, diluted to 10–12.0 pM, and sequenced on the MiSeq platform using the MiSeq v2 Reagent Kit for 2 × 150 bp PE (Illumina, CA, USA) following the manufacturer's protocol.

Table 1. Primer sequences used in the present study

Primer information	Primer sequence[1,2,3,4,5]
1st PCR primers	
MiFish-U-forward	*ACACTCTTTCCCTACACGACGCTCTTCCGATCT*
	NNNNNN GTCGGTAAAACTCGTGCCAGC
MiFish-U-reverse	*GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*
	NNNNNN CATAGTGGGGTATCTAATCCCAGTTTG
MiFish-E-forward-v2	*ACACTCTTTCCCTACACGACGCTCTTCCGATCT*
	NNNNNN RGTTGGTAAATCTCGTGCCAGC
MiFish-E-reverse-v2	*GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*
	NNNNNN GCATAGTGGGGTATCTAATCCTAGTTTG
MiFish-U2-forward	*ACACTCTTTCCCTACACGACGCTCTTCCGATCT*
	NNNNNN GCCGGTAAAACTCGTGCC
MiFish-U2-reverse	*GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*
	NNNNNN CATAGGAGGGTGTCTAATCCCCGTTTG
2nd PCR primers	
2nd-PCR-forward	AATGATACGGCGACCACCGAGATCTACAC
	XXXXXXXX *ACACTCTTTCCCTACACGACGCTCTTCCGATCT*
2nd-PCR-reverse	CAAGCAGAAGACGGCATACGAGAT
	XXXXXXXX *GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*

[1]Normal characters indicate target-specific universal primers (i.e., MiFish primers). [2]The six random bases (Ns) in the middle of the 1st PCR primers were appended to enhance cluster separation on flow cells. [3]Italic characters indicate Illumina sequencing primers. [4]**X** indicates index sequences to identify each sample. [5]Underlined characters indicate P5/P7 adapter sequences for Illumina sequencing.

Sequence analysis

We performed data preprocessing and analyses of raw MiSeq reads from the MiSeq run using PMiFish ver. 2.4 (https://github.com/rogotoh/PMiFish, Miya *et al.* 2020). Detailed information on sequence data processing is provided in Supporting Information. Forward (R1) and reverse (R2) reads were merged while discarding short reads (<100 bp) after tail trimming and paired reads with too many differences (>5 positions) in the aligned region (*ca.* 65 bp). Primer sequences were removed from merged reads and reads without the primer sequences underwent quality filtering to remove low quality reads. Preprocessed reads were dereplicated, and all singletons, doubletons, and tripletons were removed from subsequent analyses to avoid false positives (Edgar 2010). Dereplicated reads were denoised to generate amplicon sequence variants (ASVs) that removed all putatively chimeric and erroneous sequences (Edgar 2016; Callahan *et al.* 2017).

ASVs were subjected to taxon assignments to species names with a sequence identity of > 98.5% with the reference sequences (two nucleotide differences allowed) and a query coverage of ≥ 90%. ASVs with identical fish species names were clustered as molecular operational

taxonomic units (MOTUs) following the procedure described in Supporting Information. MiFish DB ver. 43 was used for taxon assignment, comprising 7,973 species distributed across 464 families and 2,675 genera. The ASV table was rarefied to the approximate minimum read number (*ca.* 20,000 reads). To refine the above taxon assignments, family-level phylogenies were reproduced from MiFish sequences from MOTUs and reference sequences (contained in MiFish DB ver. 43) belonging to these families. A total of 103 family-level trees were visually inspected and taxon assignments were revised. The final list of detected species is provided in Table S1.

Estimation of DNA copy numbers

The nonlinear time series analytical tools used in the present study require quantitative time-series data. To estimate fish eDNA concentrations, we initially measured the eDNA concentrations of the most common fish species in the region, Japanese Black Seabream (*A. schlegelii*), using qPCR, which was detected in 504 out of 550 seawater samples (91.6%). By dividing *A. schlegelii* sequence reads by the *A. schlegelii* eDNA quantity in each sample, we estimated the number of sequence reads generated per *A. schlegelii* eDNA copy for each sample. Note that sequence reads generated per eDNA copy may vary depending on factors such as the level of PCR inhibition and PCR amplification efficiency; however, in general, this "internal spike-in DNA" method has been shown to reasonably estimate the quantity of eDNA concentrations (Ushio *et al.* 2018b; Ushio 2022). More importantly, species-specific biases that may be introduced in the internal spike-in DNA method do not cause serious biases in the outcomes of our nonlinear time-series analysis because it standardizes time series to have a zero mean and a unit variance before analyses and also only utilizes the fluctuation patterns of time series. When we did not detect any *A. schlegelii* eDNA by qPCR, we replaced the "zero" value with the minimum eDNA copy numbers of *A. schlegelii* (0.346 copies/μl DNA). Similarly, when we did not detect any *A. schlegelii* eDNA sequence reads by metabarcoding, we replaced the "zero" value with the minimum eDNA sequence reads of *A. schlegelii* (12 reads/sample). These corrections estimated how many sequence reads were generated per eDNA copy for all samples. Details are provided in Supporting Information.

Nonlinear time series analysis to reconstruct fish interaction networks

We reconstructed fish interaction networks using a nonlinear time series analysis based on quantitative eDNA time-series data from multiple species and sites. Since the reliable detection of interspecific interactions requires sufficient information in the target time series, we arbitrarily selected the top 50 most frequently detected species and excluded other rarer fish species from the analysis. We quantified information flow between two fish species' eDNA time series by the "unified information-theoretic causality (UIC)" method implemented in the "rUIC" package (Osada & Ushio 2021) of R (R Core Team 2021). UIC tests the statistical clarity of information flow between variables in terms of TE (Schreiber 2000) computed by cross mapping (Sugihara *et al.* 2012). In contrast to the standard method used to measure TE, UIC quantifies information flow as follows:

$$TE = \log\left(\frac{p(y_{t+tp} \mid x_t, x_{t-\tau}, \ldots, x_{t-(E-1)\tau}, z_t)}{p(y_{t+tp} \mid x_{t-\tau}, x_{t-2\tau}, \ldots, x_{t-(E-1)\tau}, z_t)}\right) \quad , \ldots(1)$$

where x, y, and z represent a potential causal variable, effect variable, and conditional variable (if available), respectively. $p(A \mid B)$ represents conditional probability: the probability of A conditioned on B. t, tp, τ, and E represent the time index, time step, a unit of time lag, and the optimal embedding dimension, respectively. For example, if TE between y_{t+1} and

$x_{t-\tau}, x_{t-2\tau}, \ldots, x_{t-(E-1)\tau}$ is measured, it tests the causal relationship between y_{t+1} and x_t. Optimal E was selected by measuring TE as follows:

$$TE = \log\left(\frac{p(x_{t+tp} \mid y_t, x_t, x_{t-\tau}, \ldots, x_{t-(E-1)\tau})}{p(x_{t+tp} \mid y_t, x_t, x_{t-\tau}, \ldots, x_{t-(E_R-1)\tau})}\right) \quad , \ldots(2)$$

where E_R ($< E$) is the optimal embedding dimension of lower dimensional models. Eqn. [2] is a TE version of simplex projection (Sugihara & May 1990). TE measured according to Eqn. [1] gains the advantage of previous causality tests, i.e., standard TE methods (Schreiber 2000; Runge *et al.* 2012) and convergent cross mapping (CCM) (Sugihara *et al.* 2012), the algorithm of which is explained and implemented in https://github.com/yutakaos/rUIC.

By using UIC, we quantified TE between fish eDNA time series. As environmental variables, water temperature, salinity, wave height, and tide level were considered as conditional variables. This means that the effects of the environmental variables on the fish eDNA abundance were removed in the analysis when quantifying interspecific interaction strengths. We standardized the eDNA time series to have zero means and a unit of variance before the analysis. If TE between two fish eDNA time series was statistically clearly higher than zero, we interpreted it as a sign of an interspecific interaction. Furthermore, in the present study, the amount of information flow was used as an index of interaction strength. We quantified TE for all species pairs in each site, and site-specific fish interaction networks based on eDNA time series were reconstructed and visualized.

Quantification of the temperature sensitivity of fish species interactions

After reconstructing fish interaction networks, we analyzed the relationships among fish species interaction strengths and biotic and abiotic variables. We performed all analyses using R v4.1.2 (R Core Team 2021). Generalized additive mixed models (GAMMs) were performed using the "mgcv" package of R (Wood 2004). We visualized results with "ggplot2" (Wickham 2009) and "cowplot" (Wilke 2017). In the present study, we used the term "statistical clarity" instead of "statistical significance" to avoid misinterpretations, according to the recommendations by Dushoff et al. (2019).

In the first analysis, the relationships between fish-fish interaction strengths and the characteristics of the study sites were analyzed by GAMM. In the analysis, the median values of TE of each fish species (i.e., the median value of the strengths of interspecific interactions that a focal fish species gives and receives at each site) were an explained variable, and a site characteristic was an explaining variable. Site characteristics included water temperature, species richness, latitude, and total fish eDNA concentrations. A gamma distribution was assumed as the error distribution, the "log" function was used as a link function, and fish species was used as a random effect (i.e., in R, `gamm(TE ~ s(site characteristics), family = Gamma(link="log"), random = list(species = ~1))`, where `s()` indicates a smoothing term). The biological assumption behind this modeling is that site characteristics, such as water temperature, may linearly or nonlinearly influence fish species interactions. Moreover, the effects of site characteristics may randomly vary depending on the fish species. Nevertheless, in this analysis, we were primarily interested in detecting the effects of site characteristics on fish species interactions. The site effect was considered to be statistically clear at $P < 0.05$.

In the second analysis, we focused on the effects of water temperature on the interaction strengths of each fish species because water temperature was the most important variable that affected fish species interactions in the present study (see Results). In the analysis, to simplify the interpretations and visualizations of the temperature sensitivity of fish species interactions, general linear models (GLMs) were performed (note that qualitatively similar results were found

even if we applied GAM to each fish species; see Results). TE, an index of the interspecific interaction strength, was an explained variable and water temperature was an explaining variable. A gamma distribution was assumed as the error distribution and the "log" function was used as a link function (i.e., in R, `glm(TE ~ temperature, family = Gamma(link="log))` was applied). GLM was separately performed for each fish species. In the analysis, positive and negative slopes indicated that the strength of the interspecific interaction increased with increasing and decreasing water temperatures, respectively.

Results and Discussion

Taxonomic diversity and dynamics of fish eDNA

Our multiple MiSeq runs generated 71,892,685 reads in total and detected 1,130 molecular operational taxonomic units (MOTUs). We inspected their family-level phylogenies to increase the accuracy of taxonomic assignments, recognizing 856 MOTUs across 33 orders, 167 families, and 466 genera (Table S1). This taxonomic diversity was similar to that of the local fauna (948 species across 33 orders, 158 families, and 493 genera) compiled from a literature survey and museum collections (Table S2).

Sequence reads in the ASV table were converted to estimated eDNA concentrations by using the eDNA concentrations of a common fish species detected across most samples (Japanese Black Seabream, *A. schlegelii*) (i.e., an analog of the internal spike-in DNA method; see Methods and Fig. S1). The converted ASV table included 23,863 detections and we selected the top 50 most frequently detected species for our time-series analysis. The detection of these 50 species ranged between 148 and 532 with a mean of 296, and their total detection reached 14,793 (62.0%).

Fish eDNA concentrations and fish species richness showed a clear intra-annual pattern (i.e., seasonality; Fig. 1b, c), which were higher in warmer months (e.g., between July and October) than in colder months (e.g., between January and March) at all sites. Total eDNA concentrations and species richness positively correlated with water temperature (Fig. S2). Seasonal changes in eDNA concentrations were consistent with the patterns of seasonal occurrences in tropical and subtropical fish species in the Boso Peninsula, to which they are transported by the warm Kuroshio Current, settling on the coastal waters during the warmer months and subsequently disappearing during the colder months (Senou *et al.* 2006; Saito 2019).

Reconstruction of the fish species interaction network

We reconstructed fish species interaction networks based on the quantitative, multispecies fish eDNA time series. Regarding the 50 frequently detected fish species, we quantified pairwise information flow between fish species. Figure 2 shows reconstructed fish interaction networks for the 50 frequently detected fish species in the Boso Peninsula. At the regional scale, the correlation between the number of interactions and the number of species detected per sample was statistically clear ($P < 0.05$; Fig. S3). On the other hand, the correlation between the number of interactions and water temperature was statistically unclear (Fig. S3). At the study-site scale, fish species with higher eDNA concentrations (i.e., nodes with a larger size in Fig. 2b–i) had slightly more interactions, suggesting that the dominant fish species may have more opportunities to interact with other fish species. Fish species belonging to the same order or family were aligned close to each other in Figure 2; however, there was no clear phylogenetic effect on the number of interactions.

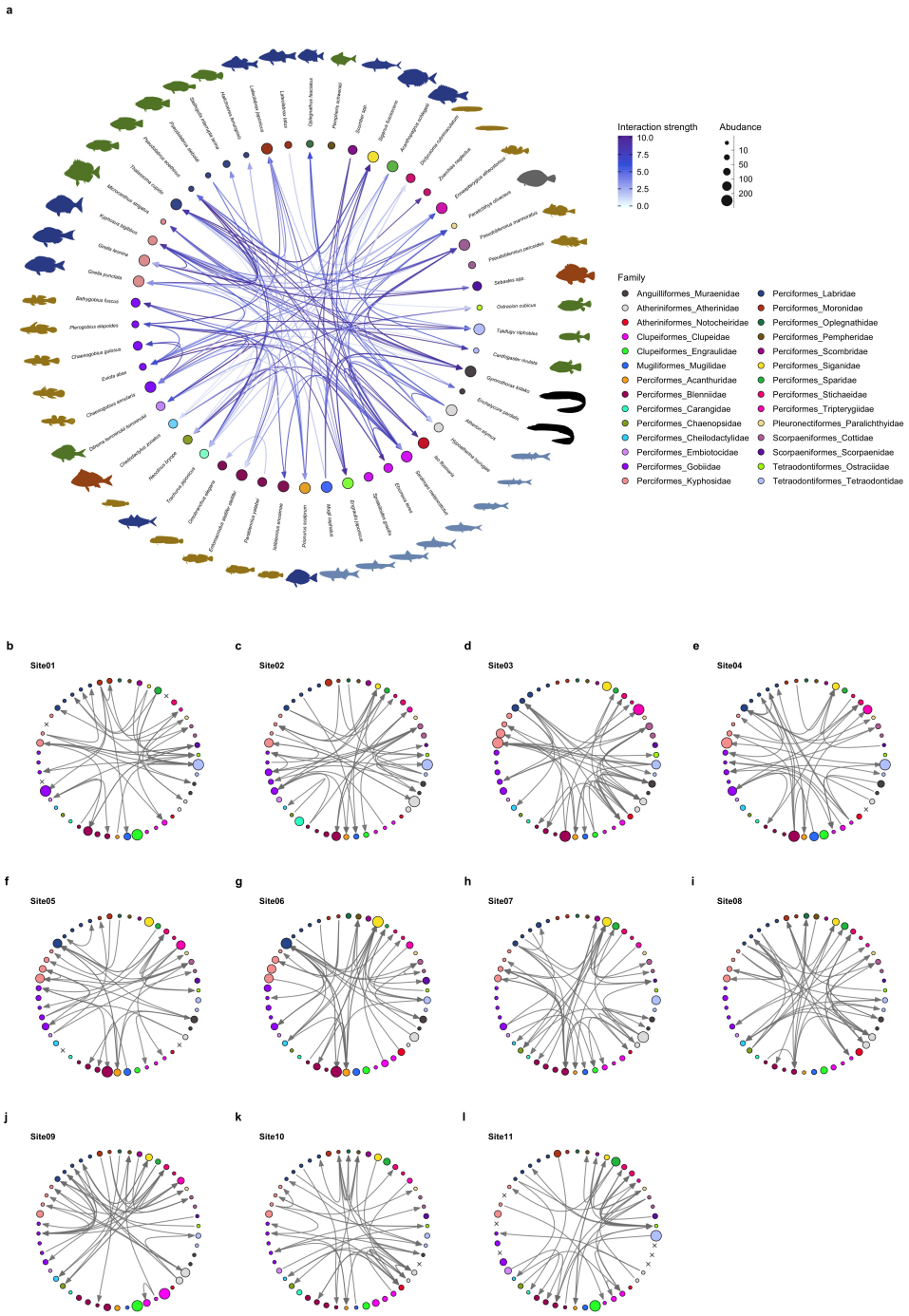

Figure 2 | Interaction networks of the fish community in the Boso Peninsula coastal region. (**a**) The "average" interaction network reconstructed by quantifying information transfer between eDNA time series. Transfer entropy (TE) was quantified by leveraging all eDNA time series from multiple study sites to draw this network. Only information flow larger than 80% quantiles was shown as interspecific interactions just for visualization. The edge color indicates relative interaction strengths, and fish illustration colors represent their ecology (e.g., habitat and feeding behavior). (**b**–**l**) Site-specific fish interaction networks. Each site-specific network was reconstructed by multispecies eDNA time series from a single site. Nodes represent fish species as in the averaged network in **a**. Only information flow larger than 80% quantiles was shown as interspecific interactions just for visualization. In **a**–**l**, node colors and node sizes indicate the fish family and fish abundance (total eDNA copy numbers of the fish species), respectively.

Most of the statistically clear information flow may be interpreted from the viewpoint of fish-fish interspecific interactions. The largest information flow was detected from *Pseudoblennius marmoratus* to *Pseudolabrus eoethinus* (Table S3). These fish species have overlapping habitats, and thus, they may interact. Bidirectional information flow was detected between *P. eoethinus* and *Gymnothorax kidako*. They are both carnivorous fish species and may conduct joint hunting. *Chaenogobius annularis* and *Takifugu niphobles* are often found together in the sand between reefs. *T. niphobles* frequently dive in the sand, and benthos and mysids that are dug up during the dive may be prey for *C. annularis*. Supplementary discussions about the detected information flow are described in Supporting Information (Supplementary Discussion and Table S3).

Interaction strengths and environmental variables

We investigated how interaction strengths changed with environmental variables (e.g., water temperature) and ecological properties (e.g., species richness). The interaction strengths of fish species were statistically clearly associated with the latitudes and median water temperatures of the study sites (GAMM, $P < 0.05$; Fig. 3a, b). Fish communities in warmer regions generally interacted more strongly with each other; however, a large variation in the interaction strength was noted. Species richness had statistically clear, but nonlinear effects on fish species interactions (Fig. 3c). On the other hand, total eDNA concentrations showed statistically unclear associations with interaction strengths (Fig. 3d). Water temperature may influence fish physiological activity (Kishi *et al.* 2005; Claireaux *et al.* 2006; Oyugi *et al.* 2012), and thus, the community-level influence of water temperature may also arise from the individual-level influence of water temperatures on fish. Based on the results showing that water temperature generally positively influenced interaction strengths, fish physiological activity may increase with water temperature, at least within the temperature range of our study systems.

We also investigated how temperature influenced the interaction strength of individual fish species. Figure 4a shows how interaction strengths among fish species changed with water temperature. The interaction strengths of many fish species increased at higher water temperatures (Fig. 4a; species with a red regression line), which was consistent with the community-level results showing that fish communities in warmer regions had stronger interaction strength (Fig. 3b). Among the 50 frequently detected species included in the networks, 29 fish species showed a positive relationship between the interaction strength and water temperature and this relationship was statistically clear for 20 out of the 29 fish species (Fig. 4b). Only five fish species showed a statistically clear negative relationship between the interaction strength and water temperature. The results obtained were qualitatively similar even if we assumed a nonlinear relationship between the interaction strength and water temperature (GAM; Fig. S4). Previous studies showed that fish physiological activities, such as feeding rates, growth rates, and swimming speed, were influenced by water temperature (Kishi *et al.* 2005; Claireaux *et al.* 2006; Oyugi *et al.* 2012). The direction (i.e., positive or negative) of the temperature effects on fish metabolic activities may be species-specific (Oyugi *et al.* 2012), and this species specificity may underlie the species-specific effects of temperature on the interaction strength.

Potential limitations of the present study

The present results showing that the strengths of fish-fish interactions depend on water temperature rely on several assumptions that were not fully investigated, and thus, careful interpretations and discussions are required. The extent to which fish eDNA concentrations accurately represent fish abundance is an open question. Previous studies demonstrated that the quantity of eDNA may be a proxy of fish abundance and/or biomass (i.e., a positive correlation between the quantity of eDNA and fish abundance/biomass) (Takahara *et al.* 2012; Thomsen *et al.* 2012; Yamamoto

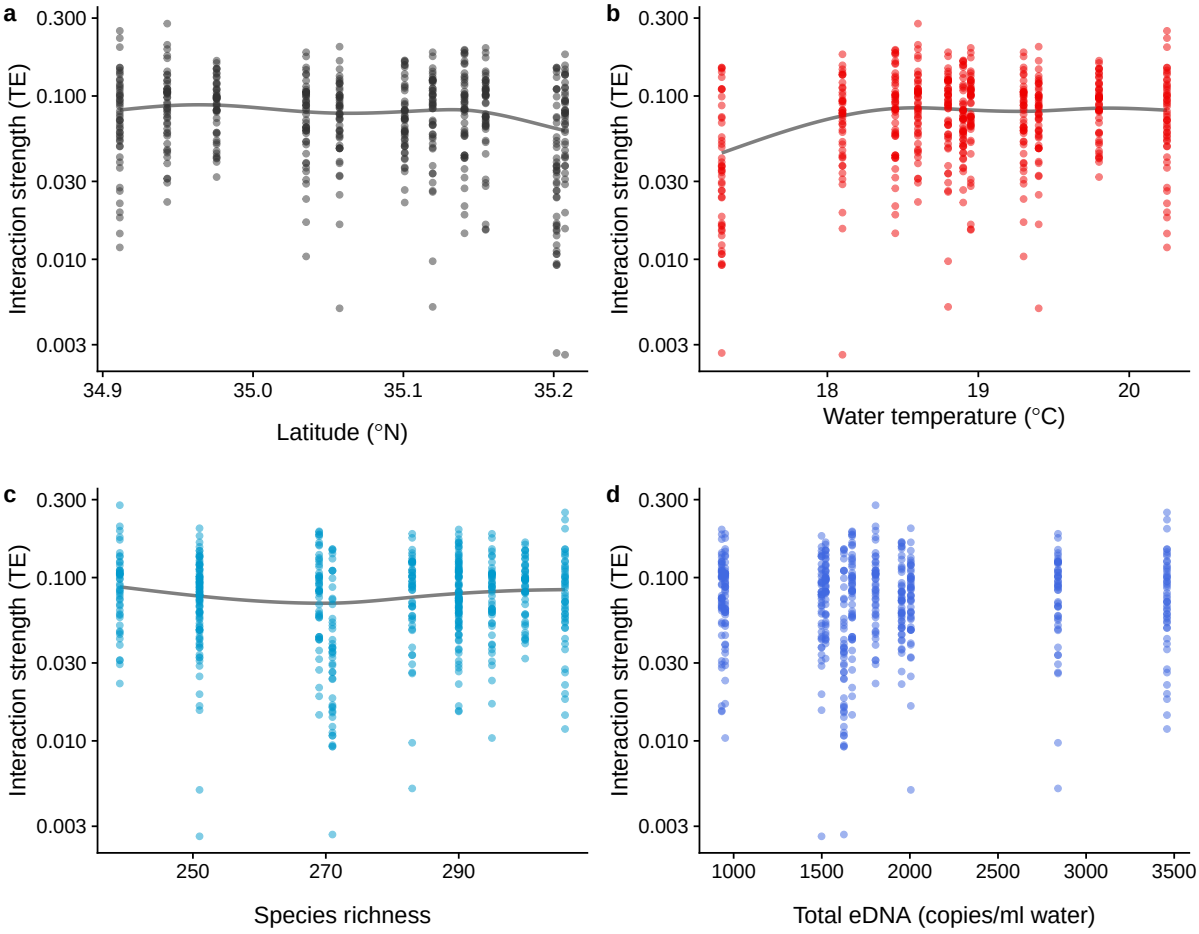

Figure 3 | Dependence of interaction strengths on biotic and abiotic variables. The panel shows the relationship between fish species interaction strengths (quantified as information flow; transfer entropy [TE]) and (**a**) latitude, (**b**) the median annual water temperature of each study site, (**c**) species richness, and (**d**) total eDNA copy numbers. The *y*-axis indicates TE as an index of the interaction strength between fish species. The line indicates the nonlinear regression line estimated by the general additive mixed model (GAMM).

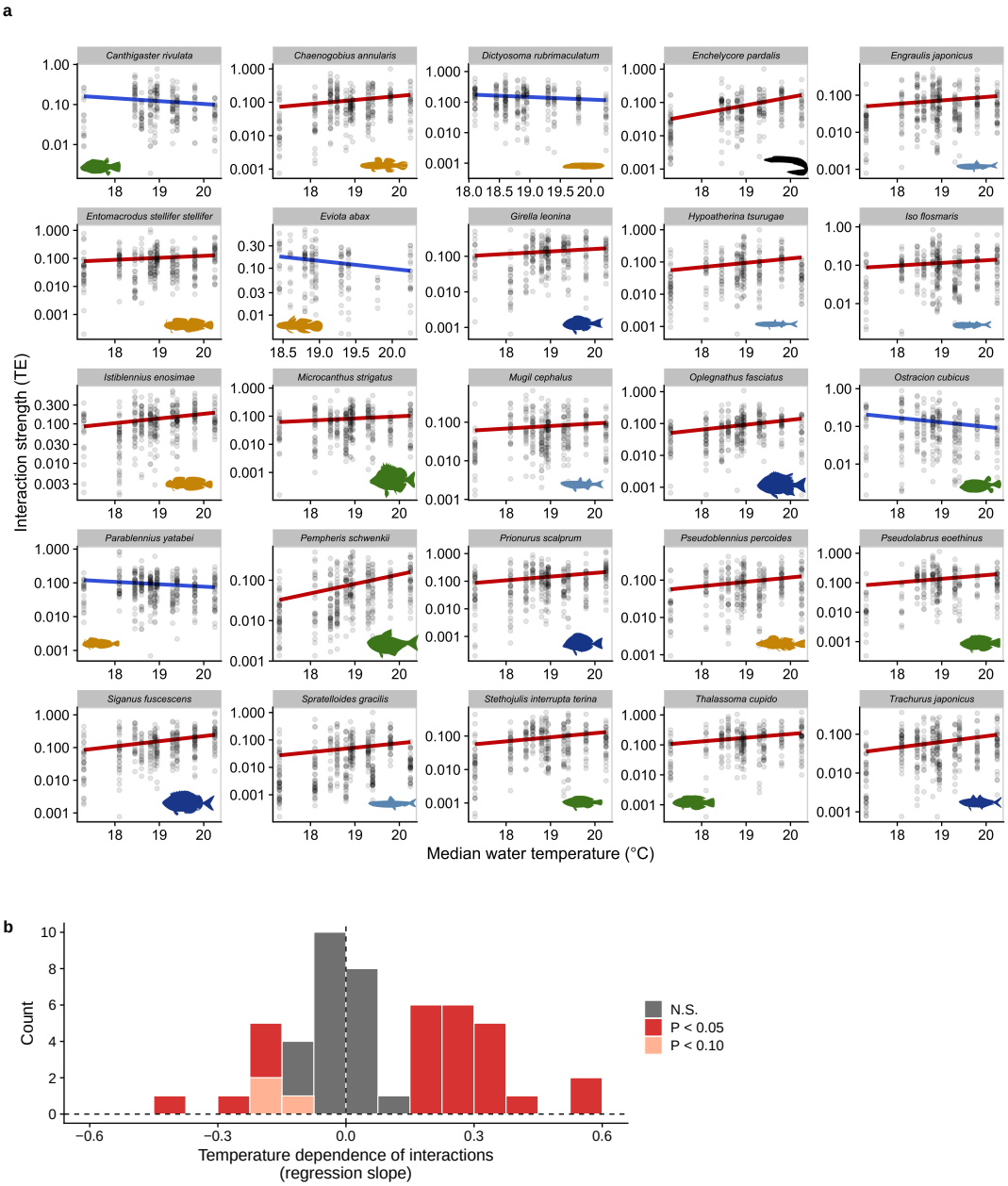

Figure 4 | Temperature dependence of fish species interactions. (**a**) Temperature effects on fish species interactions quantified as information flow (transfer entropy; TE). Points indicate the TE value of each interaction for each species at each site. Interactions include giving and receiving effects. Red and blue lines indicate statistically clear positive and negative relationships between temperature and interaction strengths estimated by the generalized linear model (GLM). (**b**) Distributions of temperature effects on interactions (i.e., slopes of the regression between interaction strengths and water temperatures estimated by GLM). Red, pink, and gray colors indicate statistically clear ($P < 0.05$; $P < 0.10$) and unclear (not statistically clear; N.S.) slopes between interaction strengths and water temperatures, respectively.

et al. 2016). Our nonlinear time series analysis used the information embedded in fluctuations in time series, and time series are always standardized before the analysis. Therefore, species-specific differences in the copy numbers of the genetic marker and eDNA release rates, which may bias the estimation of absolute abundance, do not cause serious biases in the reconstruction of the networks and estimations of interaction strengths. However, accurate estimations of the absolute abundance of fish will improve the accuracy of analyses, and the integration of eDNA concentration data, knowledge on eDNA dynamics (i.e., release and degradation rates), and the hydrodynamic modeling of ocean water flow will be a promising approach (e.g., as in Fukaya *et al.* 2021).

Another limitation is that the reconstructed interaction network was not fully validated in the present study. Although previous studies demonstrated that network reconstructions based on a time series analysis work reasonably well and show meaningful patterns (Sugihara *et al.* 2012; Ushio *et al.* 2018a; Runge *et al.* 2019; Ushio 2022), experiment/observation-based validations of the reconstructed network have not yet been performed. Potential causal interactions between fish species may be reasonably interpreted (Supplementary Discussion); however, developing a framework to efficiently validate "previously unknown" interactions detected by eDNA data and a nonlinear time series analysis is an interesting future direction.

Furthermore, although we showed that positive temperature-interaction strength relationships are common in the Boso peninsula coastal region, the range of water temperatures in the present analysis was relatively narrow and there is currently no approach to generalize these results. Nonetheless, the advantages of the eDNA method are the low cost, time, and labor needed for field sampling as well as the potential scalability of the library preparation process (Buchner *et al.* 2021; Ushio *et al.* 2022). Therefore, frequent (Ushio 2022) and large spatial-scale ecological monitoring is possible, which will provide a more detailed understanding of the temperature-interaction strength relationships of organisms on a larger spatial scale.

Conclusions

The present study demonstrated that the strengths of fish species interactions changed with water temperature. Intensified species interactions in warmer water may change the correlation and distribution of interaction strengths, which may consequently influence community dynamics and stability (Tang *et al.* 2014; Allesina *et al.* 2015; Ushio *et al.* 2018a). Therefore, a more detailed understanding of the effects of environmental conditions on species interactions will contribute to improvements in our capability to understand, forecast, and even manage ecological communities and dynamics, which is particularly important under ongoing global climate change. Developments and improvements in eDNA techniques and statistical analyses are still required; however, our framework integrating a eDNA analysis and advanced statistical analyses is promising for understanding and forecasting ecological dynamics.

Endnotes

Data and code availability

Sequence data were deposited in DDBJ Sequence Read Archives (DRA) (DRA accession number = DRA014111). Analysis scripts are available at Github (https://github.com/ong8181/eDNA-BosoFish-network).

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Conflict of Interest Statements

The authors declare no conflicts of interest.

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