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Biomonitoring of Non-Native Species Through eDNA Metabarcoding Method and Risk Screening for Ballast Water in Northwest Türkiye

Yusuf Koray Küçük[a,b], Akasya Topçu[b], Esra Mine Ünal[c,d], Emre Keskin[b,c,d,g], Ali Serhan Tarkan[e,f], İlknur Meriç Turgut[b*]

[a] *Department of State Information Coordination Center, General Directorate of Security Affairs, Presidency of the Republic of Türkiye, Ankara, TÜRKIYE*
[b] *Department of Fisheries and Aquaculture Engineering, Faculty of Agriculture, Ankara University, Ankara, TÜRKIYE*
[c] *Ankara University Agricultural Faculty Department of Fisheries and Aquaculture Evolutionary Genetics Laboratory (eGL), Ankara, TÜRKIYE*
[d] *AgriGenomics Hub Animal and Plant Genomics Research Innovation Centre, Ankara, TÜRKIYE*
[e] *Department of Ecology and Vertebrate Zoology, Faculty of Biology and Environmental Protection, University of Lodz, Lodz, POLAND*
[f] *Department of Basic Sciences, Faculty of Fisheries, Muğla Sıtkı Koçman University, Menteşe, Muğla, TÜRKIYE*
[g] *Ankara University Aquaculture Research and Application Center (ASAUM), Ankara, TÜRKIYE*

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Corresponding Author: İlknur Meriç Turgut, E-mail: meric@agri.ankara.edu.tr
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ABSTRACT
The exponential development of maritime transport has made ballast water a primary vector for the spread of invasive organisms across the aquatic realm. This research aims to present a comprehensive overview of methodological and bioinformatic considerations for eDNA metabarcoding applied to ballast water from ships in İzmit Gulf, northwest Türkiye, with an emphasis on non-native species. The data related to DNA sequences for *COI*, *18Sv8*, *18Sv4*, *16S*, and *12S* presented a broad diverse taxonomic group for both microbial and macroscopic species, even for rare ones, with numbers of 93, 191, 241, 19, and 44, respectively. Additionally, the research unveiled the presence of highly invasive species such as *Rhopilema nomadica* and identified their invasiveness risk for İzmit Gulf, primarily due to elevated water temperatures in relation to climate change. The outlined results indicate that metabarcoding offers a potential tool for early detection of non-indigenous species and implementing management plans in view of current global warming interactions.

Keywords: İzmit Gulf, Monitoring, Biodiversity, Quantitative metabarcoding, Risk identification

1. Introduction

The remarkable evolution of ships in terms of technology, engineering, and social classification over the last century has significantly contributed to the advancement of maritime transport. As ships modernized, maritime trade experienced a surge, becoming a driving force for globalization (Ojaveer et al. 2018; Rey 2019). However, this rapid evolution, particularly the extensive displacement of ballast water has led to increased dominance of non-native species in the biodiversity of aquatic ecosystems. Recognizing that ballast water and sediment, representing 30-35% of the ship's carrying capacity, are a significant vector for both the transportation and spread of benthic and planktonic organisms, toxic dinoflagellates, and fish eggs, larvae and itselves, with some of these presumed to be non-native species (Verling et al. 2005; Gibb et al. 2013; Bradie 2016), there is a need to address the socio-economic, environmental, and human welfare impacts (Williams 2013).

In accordance with "The Control and Management of Ships' Ballast Water and Sediment Convention (BWMC)" framework in 2004 introduced by International Maritime Organization (IMO), the BWMC guidelines should be a main axis in port-based research for identifying areas and detecting target species. The identification of highly risky invasive species relies on comparing environmental identity and species composition in target ports as outlined by IMO guidelines from 2004. This "species-specific risk assessment" approach, which focuses on the biogeographic region in question when adopted in 2007 and upated in 2017, would be the most suitable method. To enhance this aspect, specific protocols tailored to exemptions could be developed by the Black Sea Commission operating under the Bucharest Convention and this would contribute to ensure the protection from potential threats posed by invasive species. The application of ballast water purification processes necessitates the use of advanced treatment technologies, particularly through mechanical and physico-chemical methods. In accordance with IMO standards, ballast water treatment systems are engineered to fulfill either the D-1 standard, which dictates the specific location for ballast water discharge, or the D-2 standard, which establishes permissible limits on viable organisms present in discharged

water. To ensure regulatory compliance, these onboard systems are equipped with monitoring tools that verify treatment efficacy and data logging mechanisms designed to meet IMO reporting requirements. In recent years, there has been a notable shift towards employing accurate and effective methods for determining the invasive potential of non-native species. Molecular metabarcoding methods, in particular, are increasingly outpacing morphological-based taxonomy (Shaw et al. 2017; Jeunen et al. 2019). These methods prove advantageous in monitoring and evaluating biodiversity effectively (Ghabooli et al. 2016; Blackman et al. 2017) via allowing numerous samples to be scanned in a short time period (Pochon et al. 2013; Zhan et al. 2013; Deiner et al. 2017) and exhibit heightened sensitivity for detecting rare, elusive, and cryptic species (Wee et al. 2023), particularly noteworthy within certain taxa (Fonseca et al. 2023). Moreover, the depending on the taxonomic knowledge abundance, species identification varies among groups due to the differences in primer selection and the completeness of the reference database (Pascher et al. 2022).

The acceleration of metabarcode-based studies, such as eDNA metabarcoding, enables comprehensive monitoring of marine biodiversity (Lacoursière-Roussel et al. 2018; Giroux et al. 2022). This approach is particularly useful for assessing ballast water and port areas for invasive species (Comtet et al. 2015; Xiong et al. 2016), detecting subtle population changes (Wright et al. 2019) and conducting ecological status assessments (Aylagas et al. 2018; Antich et al. 2021). Moreover, it allows for the simultaneous characterization of the spatio-temporal distribution of multiple taxa (Oka et al. 2021; Polanco-Fernández et al. 2021; Wee et al. 2023). The rapid response of applied molecular methods highlights their economic benefits in affirming the impact of port and shipping activities.

The accuracy and efficiency of the eDNA metabarcoding method depend significantly on the choice of primers used for PCR amplification (Alberdi et al. 2018; Gold et al. 2021). The selection of primers has a substantial impact on the taxonomic coverage and resolution of the metabarcoding studies, given that different primers target distinct genomic areas. Recognizing this, employing multiple primers (Ammon et al. 2018; Grey et al. 2018) can scan the employing of multiple primers (Ammon et al. 2018; Grey et al. 2018) has an ability to scan a diversified amount of species present in ballast water, reliably and capture a comprehensive spectrum. Employing several primers not only overcomes the constraints posed by primer biases (Alberdi et al. 2018; Chambert et al. 2018; Doi et al. 2019; Gold et al. 2021) but also provides a deeper understanding of the varied assemblages of microbes, algae, and aquatic organisms in ballast water. Using multiple sets of primers targeting various marker genes or regions (Borrell et al. 2017), researchers can enhance sensitivity in spotting rare species and gain a more thorough comprehension of the ecological dynamics and potential risks associated with the transoceanic convection of ballast water. So, it is essential to carefully choose and use a variety of primers for eDNA metabarcoding studies in ballast water to ensure the accuracy and comprehensiveness of the results. This approach ultimately contributes to informing management and mitigation strategies aimed at reducing the spread of invasive species through ballast water exchange. While a wide variety of metabarcoding primers have been developed for fish, revealing significant differences in taxonomic richness and discriminant power within species (Zhang et al. 2020), a comprehensive and comparative evaluation for aquatic species based on amplification or taxonomic classification is not yet available in the literature.

In order to document spatio-temporal changes in biodiversity, especially within İzmit Gulf, as a large-scale marine habitat, pose challenges and substantial costs (Gold et al. 2021; Pascher et al. 2022), a pooled eDNA metabarcoding approach was chosen considering the challenging nature of marine habitats and this method proves advantageous, making it time-consuming and costly to undertake individual assessments. This sample pooling strategy enabled the improvement of sensitivity, statistical power, and efficiency of the methodology while working with numerous samples moreover, this has a contribution like identifying low-abundance species, which are important signs of impending invasions, by integrating various samples into a single pool. Like restructuring the lab processes, minimizing batch impacts and preserving constant quality control throughout the evaluation were the other benefits of this type of sampling. A current study (He et al. 2023) also indicated that, working with greater water volume ensues an incline of eDNA-based species richness. This approach provides light on the ecological effects of ballast water exchange and the efficacy of current biosecurity measures in avoiding the introduction of invasive species; also offers an integrative/alternative one in comparison to traditional surveys (Stat et al. 2019; He et al. 2023).

To prevent against ecological disruptions (Elton 1942) and safeguard natural ecosystems and native species, it is imperative to proactively implement strategies and allocate resources (Tarkan et al. 2022). One effective approach involves the identification of suitable habitats for non-native species and an assessment of their potential threats. Screening techniques for assessing risks, such as Aquatic Species Invasiveness Screening Kit-AS-ISK (Copp et al. 2016), offer a valuable tool for appraising the potential risks posed by non-native species within a specific region (Tarkan et al. 2017). This, in turn, empowers us to prioritize and implement appropriate preventative measures.

This study centers on the critical environmental challenges posed by the extensive port facilities and marine transport in the northwest region of Türkiye, specifically the İzmit Gulf. Utilizing eDNA metabarcoding, our goal is to identify non-native species and gauge their invasive potential through an innovative risk screening tool. Thus wise, the importance of predicting the invasiveness of identified species and formulating effective management strategies for the area could be enabled.

2. Material and Methods

2.1. Study area

The study site encompasses the Marmara Sea, specifically within the Kocaeli province, focusing on Dilovası (11 port facilities), Yarımca (6 port facilities), and Hereke (3 port facilities) (Figure 1). This selection was made based on the high-density impact of voyages in Türkiye, making it a region of significant interest for the study.

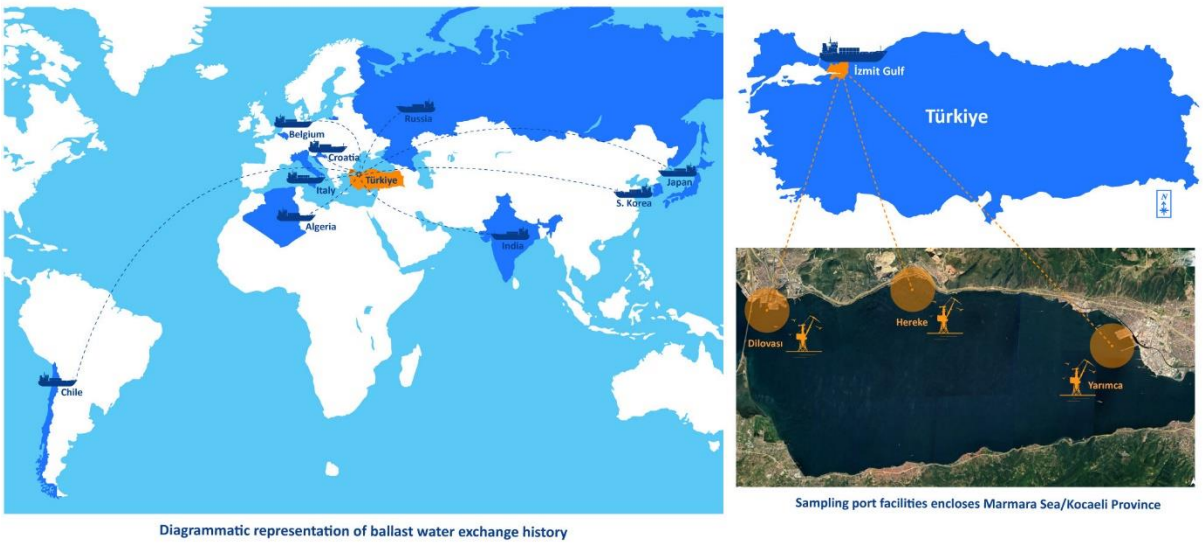

Figure 1- The documented last ballast water exchanges for ships and the selected sites (Dilovası, Yarımca, Hereke) showing port facilities in the Marmara Sea as a diagrammatic representation

2.2. Procedures of ballast water sampling

A total of 20 sampling ships was included in the investigation, from which ballast water samples were taken from 3 port facilities in İzmit Gulf. The sampling technique was carried out in triplicate over two separate seasons, specifically February and May. The Ministry of Transportation and Infrastructure utilized the Marine Traffic Programme to evaluate the present position and appropriateness of the ships for sampling. The ballast water exchange history of the vessels was investigated, and the most recent exchanges were recorded in the territorial seas of Russia, Italy, Croatia, Belgium, India, Algeria, South Korea, Japan, and Chile (Figure 1), as documented in both the Ballast Record Book and Port of Call List.

The sample collection was conducted in triplicate utilizing two particular methods, namely manhole and overflowing. Each replication consisted of 2 L of sample. The on-site filtration process involved passing each 2 L sample through Sterivex filters consisting of polyethersulfone (PES) membrane with a pore size of 0.22 µm. These filters are renowned for their ability to facilitate high flow rates and minimize protein adsorption. Subsequently, these filters were transferred to the laboratory while maintaining a controlled temperature environment.

Stringent methods were established throughout the trial to reduce contamination during sample collection, transport, and laboratory processing. Negative field controls, comprising deionized water samples subjected to the same treatment as the environmental samples, were incorporated at each site during collection to identify any possible contamination from the field. The gathered samples were conveyed under regulated, sterile settings to prevent any extraneous DNA contamination. In the laboratory, negative transport controls and equipment controls were utilized, wherein deionized water was filtered and processed concurrently with environmental samples to detect contamination introduced during DNA extraction or PCR. Moreover, laboratory technicians employed rigorous aseptic protocols, utilizing sterile gloves, pipette tips, and designated workspaces for each phase of the research. PCR configurations incorporated negative controls to guarantee the absence of contamination during amplification. These procedures jointly preserved the integrity of the eDNA samples and reduced the likelihood of false-positive results stemming from contamination.

Upon reaching the laboratory, the filtered samples from each set of three (6 L) were combined into a single composite sample. For every sampling instance, three sets of three 2 L samples were combined to form a total volume of 18 L. The purpose of this

was to enhance sensitivity, save costs and time, raise statistical power, and provide quality control in the composition of eDNA. Aggregating samples prior to filtration aids in the identification of species with low abundance and facilitates the efficiency of extensive monitoring initiatives (e.g., Deiner et al. 2017; Aylagas et al., 2018). Pooling enhances the identification of low-abundance species, but it can also lead to the omission of data regarding the diversity of individual samples.

Three types of controls were used during the entire water sampling and transport process according to Goldberg et al. (2013). These were negative field controls, negative transport controls and negative equipment controls, containing deionized water samples. All controls were treated the same as the site ones.

2.3. eDNA metabarcoding

Once the water samples were combined into a single batch measuring 18 L, the pooled sample underwent filtration using 36 Sterivex filters with a pore size of 0.22 μm. To ensure long-term preservation, the Sterivex filters were treated with Longmire solution. In addition, we collected three types of quality-control samples: a field control sample, a transit control sample, and a test control sample, using the approach outlined by Goldberg et al. (2013). The aforementioned samples were obtained from vessels that engage in the practice of exchanging ballast in the seas of Russia, Italy, Croatia, Belgium, Algeria, India, South Korea, Japan, and Chile.

The DNeasy® Blood and Tissue Kit (QIAGEN, Stockach, Germany) was used to isolate samples from Sterivex capsule filters, following the method described by Spens et al. (2017). The DNA isolates were assessed for quality and quantity using gel electrophoresis and the Qubit™ 3.0 Fluorometer, respectively. Firstly, the buffer solution and filter isolates from the same filter sample were merged after combining three isolates from each sample.

PCR analyses were performed using specific primer pairs recommended for each group of organisms. The primer pairs used were MiFish_U_F&R for fish (Bradley et al. 2016), mlCOIintF&jgHCO2198 for invertebrates (Leray et al. 2013), Vert-16S-eDNAF1&R for vertebrates (Miya et al. 2015), and V4F&R and V8F&R for microorganisms and eukaryotes (Vences et al. 2016). The DNA library was created using the two-step PCR technique described by Miya et al. (2015) and following the guidelines of Bourlat et al. (2016) for the Illumina TruSeq Nano DNA Library Preparation Protocol. The quality and quantity of the PCR products were assessed using the Qubit™ 3.0 Fluorometer and Bioanalyzer 2100 equipment, while the primer was employed to verify that the amplified product had the intended size. The Illumina MiSeq platform was employed for paired-end sequencing with 2 × 250 bp base pairs following library preparation.

The DNA library was generated using a two-step PCR approach, commonly referred to as the dual-indexing method, following the protocol described by Miya et al. (2015), with some changes to incorporate the Illumina TruSeq Nano DNA Library Preparation Kit. This approach guarantees the precise amplification and indexing of specific DNA sequences for sequencing.

Initial Polymerase Chain Reaction (PCR) for the purpose of generating amplicons:

- Distinct primer pairs were employed for different groups of organisms: MiFish_U_F&R for fish (Miya et al. 2015), mlCOIintF&jgHCO2198 for invertebrates (Leray et al. 2013), Vert-16S-eDNAF1&R for vertebrates (Miya et al. 2015), and V4F&R and V8F&R for microbes and eukaryotes (Vences et al. 2016).

- The PCR reactions were conducted in a 25 μL solution, consisting of 12.5 μL of 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), 0.2 μM of each primer, and 5 μL of DNA template.

- The thermocycling protocol consisted of an initial denaturation step at 95 °C for 3 minutes, followed by 35 cycles of 98 °C for 20 seconds, 55 °C for 15 seconds, and 72 °C for 30 seconds. The process concluded with a final extension step at 72 °C for 5 minutes.

- PCR amplification for the second time with indexing:

- The amplicons obtained from the initial PCR were utilized as templates in the subsequent PCR to incorporate Illumina Nextera XT dual indices and sequencing adapters.

- The PCR reactions were conducted in a 50 μL solution, consisting of 25 μL of 2X KAPA HiFi HotStart ReadyMix, 5 μL of each Nextera XT Index Primer, and 5 μL of the first PCR result.

- The thermocycling protocol consisted of an initial denaturation step at 95 °C for 3 minutes, followed by 8 cycles of denaturation at 98 °C for 20 seconds, annealing at 55 °C for 15 seconds, and extension at 72 °C for 30 seconds. The final extension step was performed at 72 °C for 5 minutes.
- Quantification and quality control of library samples:

- The PCR products that were marked with an index were cleansed using AMPure XP beads (Beckman Coulter, Brea, CA, USA) and measured using the Qubit™ 3.0 Fluorometer.

- The Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) was used to evaluate the size distribution and quality of the libraries.

- Sequencing:

- The libraries were combined in equal concentrations and subjected to sequencing on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle) for paired-end sequencing (2 × 300 bp).

2.4. Bioinformatic analysis

The OBITOOLS software package (Boyer et al. 2016) was employed for the bioinformatics workflow. The MiSeq device's fastq sequences' quality as well as the key statistics regarding these sequences were examined using the FastQC program (Andrews 2010). Illumina pairedend code, which considers coupling quality (phred score 30), was used to align and merge forward and backward reads pertaining to the same sample. Following the merging of the forward and reverse sequences, samples with different tags were demultiplexed within the same fastq file using the ngsfilter and obisplit commands. Sequence data was then prepared for processing separately and filtered based on count (10) and sequence length (minimum of 100 bp). Depending on the maximum read count in the negative controls, which was 9, we determined the cut-off number for the read count. As a result, we set the read number cut-off for each sample at less than 10. The relevant literature such as Yamamoto et al. (2017) and Gehri et al. (2021) also frequently uses this strategy. Raw data were examined for species exclusions linked to insertions and deletions that were not found in our study prior to filtering. More broad filters were used and tested down to 100 base pairs. To make taxonomic designations, all sequences were uploaded to NCBI GenBank as a batch megablast file, during which 98% of species level identifications based on similarity were omitted from the dataset.

2.5. Risk screening

The Aquatic Species Invasiveness Screening Kit (AS-ISK) decision-support tool was applied to assess the invasiveness risk of *Rhopilema nomadica* in the İzmit Gulf, referred to as the Risk Assessment (RA) area, based on detectable species identified through eDNA metabarcoding. The AS-ISK adheres completely to the "minimum standards" (Roy et al. 2018) for evaluating non-native species as outlined in the European Commission Regulation on the prevention and management of invasive non-native species. It has proven successful in accurately screening potentially invasive non-indigenous aquatic organisms in various RA areas globally (Vilizzi et al. 2021).

DNA metabarcoding data gathered in this investigation directly influenced the utilization of AS-ISK. *Rhopilema nomadica* was identified by conducting a thorough examination of DNA sequences obtained from samples of ballast water and it was the only non-native species that had the essential biological and ecological data required to address the 55 inquiries of the AS-ISK screening questionnaire.

The AS-ISK screening protocol consists of 55 questions (Copp et al. 2016). The initial 49 questions focus on the Basic Risk Assessment (BRA), examining species' biogeographical and biological aspects. The remaining six questions pertain to the Climate Change Assessment (CCA), requiring the assessor to evaluate how future climate conditions might influence the risks associated with the species' introduction, establishment, dispersal, and impact. Valid screening necessitates providing a response, a level of confidence in the response, and a justification for each question. Upon completing the screening, the species was assigned a BRA score and a BRA+CCA (composite) score, ranging from -20 to 70 and -32 to 82, respectively. Scores below 1 indicated a low risk of invasiveness, while higher scores classified the species as posing either a medium or high risk. The distinction between medium and high-risk levels was determined by a predetermined "threshold" value, which in this study was based on the calibrated BRA score of 6.5 for non-native jellyfishes in the Mediterranean Sea (Killi et al. 2020). The confidence levels associated with each question-related response in the AS-ISK were ranked as follows: 1= low, 2= medium, 3= high, and 4= very high. These confidence rankings aligned with those recommended by the Intergovernmental Programme on Climate Change (IPCC 2005). The overall confidence levels (CL_{Total}), as well as CL_{BRA} and CL_{CCA}, were calculated based on the allocated confidence level for each response across all 55 questions.

3. Results

3.1. eDNA metabarcoding

The data presented were generated from operational taxonomic unit groups (OTUs) consisted of 5 different primers as *12S*, *16S*, COI, *18Sv4* and *18Sv8* and the number of OTUs assigned to species using each primer sets were presented in Figure 2 and Figure 3. The first 20 readings were dominated by terrestrial species, mainly humans and cattle (Figure 2). Apart from these, arthropods,

microalgae, protists, ciliate parasites, rotifers, algae, annelids and bacteria were also present. As for fish species, only one species, *Chelon labrosus*, was identified among the top 20 species (Figure 2 and 3).

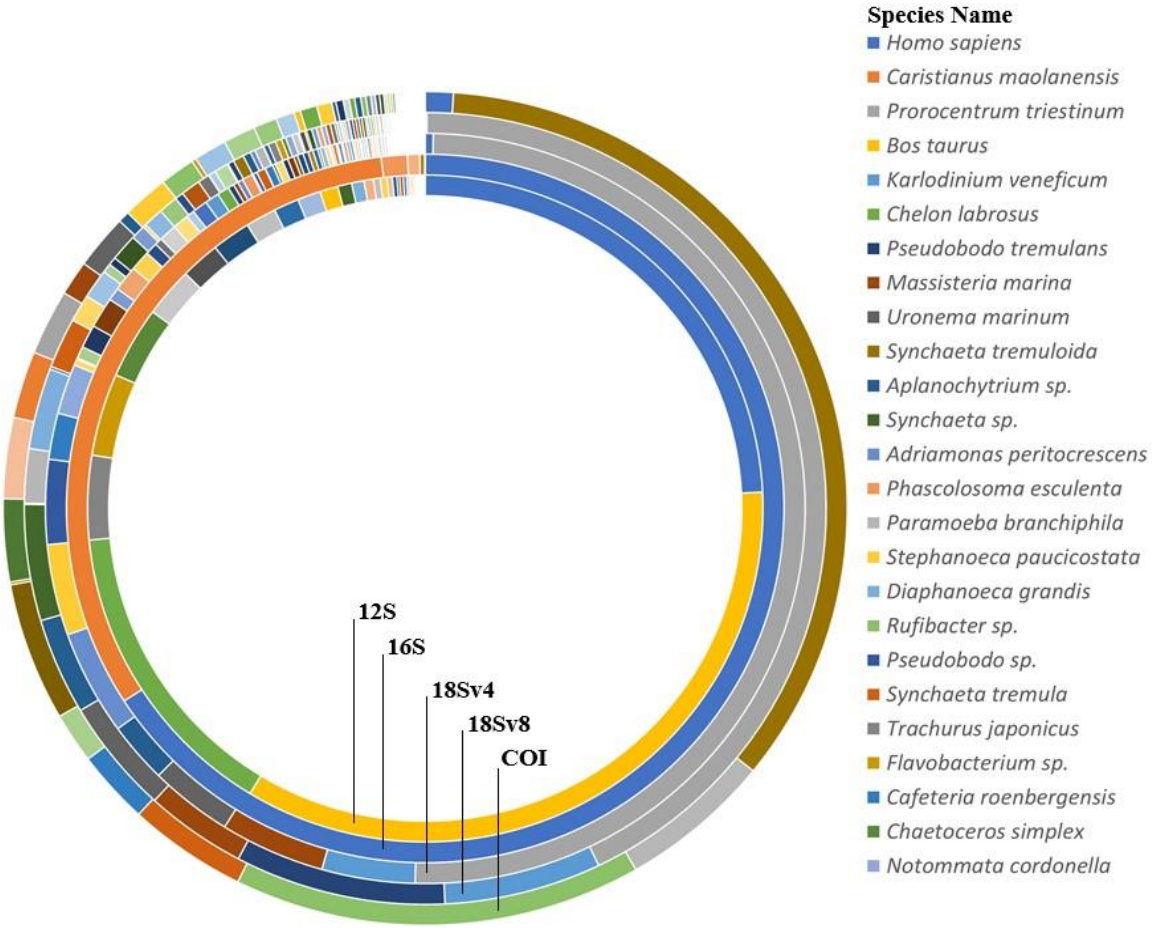

Figure 2- Results of OTUs assigned to species using all the primer sets in this study are presented. The complete list of samples is available in Supplementary Material 1. Circles belong to *12S*, *16S*, *18Sv4*, *18Sv8*, and *COI* from the inside out, respectively."

In the realm of biodiversity, the analysis revealed the identification of 93 species with *COI*, 191 species with *18Sv8*, 241 species with *18Sv4*, 19 species with *16S*, and 44 species with *12S* primers. Results from the *12S* primers indicated that 86% of the data pertained to the targeted group (fish/vertebrates), with 14% identified as those with fewer than 10 reads and species exhibiting less than 98% matching, flagged as suspicious reads. The *16S* primers demonstrated that only 11% of the results belonged to the target group (vertebrates), while the remaining 89% belonged to groups that were incorrectly marked regard to number of reads and percentage of matches. For other primers targeting specific groups, the matching success was calculated as 15% for *COI* targeting invertebrates, 23% for *18Sv4* primers targeting eukaryotic microorganisms, and 27% for *18Sv8*.

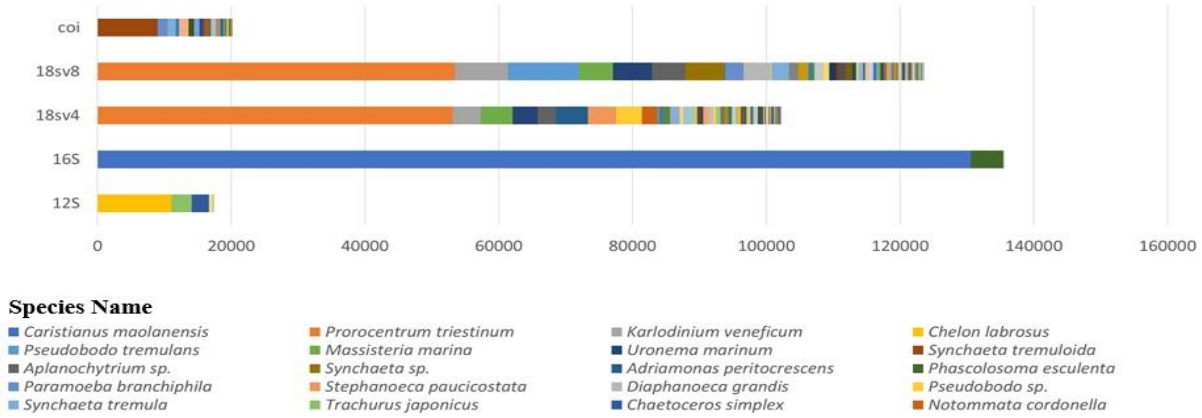

Figure 3- Results of OTUs assigned to species (only the first 20 species with the highest number of reads are presented) for each primer set separately. The complete list of samples is available in Supplementary Material 1.

This work showed us that non-indigenous species as *Nostoc* sp., *Prorocentrum micans*, *R. nomadica*, *Alexandrium minutum* and *Prorocentrum mexicanum*, even *Penaeus vannamei*, most cultured crustacean, could be detected (Supplementary Material 1), but the reads of these species were found suspicious. A pathogenic parasite and an amoeba known as *Uronema marinum* and *Paramoeba branchipila*, also were seen according to OTUs, respectively (Figure 3).

The identification of *Rhopilema nomadica*, as well as other non-native species including *Nostoc sp.*, *Prorocentrum micans*, *Alexandrium minutum*, and *Prorocentrum mexicanum*, demonstrated the efficacy of the multi-primer method.

3.2. Risk screening

According to the calibrated threshold values, BRA scores for *Rhopilema nomadica* indicated a high-risk category for the İzmit Gulf, with a score of 22.5 (Table 1). Considering the potential impact of climate change, it increased to 26.5, signifying an even higher risk for the species to become invasive in this RA area under predicted climate change conditions. Several factors and traits contributed to the increase in the BRA score, with most being biological and ecological features, followed by biogeographical and historical attributes. The history of invasiveness elsewhere is by far the most important factor increasing the overall score in biological and ecological attributes, whereas undesirable threats, reproduction, and dispersal mechanisms where the most score-increasing factors. However, factors like domestication/cultivation in biological and ecological attributes and limited resource exploitation and lack of tolerance attributes by the species in the RA in biological and ecological features lowered the overall score. The mean CL associated with responses to the BRA, CCA and BRA+CCA questions were as follows: CL_{BRA} = 2.41±0.08, CL_{CCA} = 2 and CL_{TOTAL} = 2.36±0.07. These values indicate medium-to-high confidence in all cases (Supplementary material 2).

Table 1- Scoring output from the AS-ISK for nomad jellyfish *Rhopilema nomadica* in the İzmit Gulf

Section/category	***Score***
Biogeography/Historical	13.5
Domestication/Cultivation	0.0
Climate, distribution and introduction risk	3.0
Invasive elsewhere	10.5
Biology/Ecology	9.0
Undesirable (or persistence) traits	5.0
Resource exploitation	0.0
Reproduction	2.0
Dispersal mechanisms	2.0
Tolerance attributes	0.0

4. Discussion

Here, in the present study, according to the recent occurrence of extensive marine mucilage, mainly in İzmit Gulf, has highlighted the deficiencies in wastewater treatment facilities for anthropogenic waste and the lack of effective monitoring of existing facilities. Given the high pollution pressure on this region, introducing of invasive species could have a detrimental impact on the Gulf biodiversity, and the prepotent monitoring could not be carried out in this context due to the limited number of inspections conducted by port state control officers under the BWMC. Our observations for this study, also, stress out mandatory regulation set by the IMO, in compliance with the Ballast Water Performance Standard which many companies have facilitated through ballast water treatment until September 2024.

To identify potentially invasive species, we used various sets of primers targeting distinct genomic areas to amplify and analyze eDNA from a wide range of taxa in order to thoroughly examine the biodiversity and possible dangers associated with ballast water exchange. We were able to overcome the possible biases and restrictions brought with single primer techniques by using different primer sets, leading to a more thorough and precise evaluation of the microbiological and macroscopic diversity found in ballast water. Our results using this multi-primer approach shed light on the complexity and variability of species compositions in ballast water, facilitating a deeper comprehension of the ecological implications and assisting in the development of efficient biosecurity measures to mitigate the introduction and spread of invasive species through ballast water discharge.

The eDNA metabarcoding data for this study provides valuable insights into the species biodiversity (Bautista et al. 2023) within complex environmental samples, particularly ballast waters (Antich et al. 2021; Dugal et al. 2023). This method allows for both species- and taxon-specific identification by aligning genetic sequences (barcodes) with reference sequences in a database, applying universal primers (Pascher et al. 2022). The application of various primers, including *COI*, *18Sv8*, *18Sv4*, *16S*, and *12S*, revealed a wide range of species diversity in ballast water samples. The study showcased the adaptability and utility of eDNA metabarcoding, capturing diverse taxonomic groups and providing a comprehensive understanding of microbial and macroscopic organisms transported through ballast water, with 93, 191, 241, 19, and 44 species identified using each respective primer set as reported by Dugal et al. (2023).

The importance of primer selection in eDNA metabarcoding cannot be overstated, as highlighted in recent studies by van Driessche et al. (2023) and Bautista et al. (2023). These studies emphasize the significant impact of primer choice on the precision and reliability of results. Variations in species detection among different primer sets, such as the superior matching success of *COI* primers targeting invertebrates compared to *18Sv4* and *18Sv8* primers, underscore the critical nature of selecting primers tailored to the taxonomic groups of interest (Keskin & Atar 2013). Additionally, the analysis of *12S* primers yielded results flagged as questionable reads, highlighting the need for cautious interpretation and consideration of primer biases. This raises the possibility of false positives, although Zhang et al. (2020) presented conflicting results and suggested a richer taxonomic composition with the use of *12S* primers over *16S* primers based on sequence references.

The discrepancies in species detection across various primer sets highlight the necessity for meticulous primer selection to improve the precision and taxonomic breadth of eDNA metabarcoding research. Contamination by human DNA is a recognized issue in environmental DNA research, and *Homo sapiens* DNA was identified in our samples. This phenomenon can be ascribed to multiple factors, such as human activities proximate to the sampling locations, airborne pollutants during sample acquisition, or laboratory manipulation. To mitigate these risks, we instituted stringent contamination control measures, including the application of negative field, transport, and equipment controls treated identically to the actual samples, and the enforcement of rigorous laboratory practices such as the utilization of sterile equipment and designated workspaces. Notwithstanding these efforts, the ubiquitous presence of human DNA renders total eradication of contamination challenging. Nonetheless, its existence functions as a crucial procedural safeguard, confirming that contamination control methods were implemented and successful. The identification of human DNA is unlikely to influence our main goal of evaluating the biodiversity of non-native and invasive species, given the studies are concentrated on recognizing species of ecological significance. This study highlights the necessity of continuous efforts to improve contamination control and augment the dependability of eDNA metabarcoding in biodiversity research.

The occurrence of *Homo sapiens* (human) DNA in ballast water eDNA metabarcoding studies poses a technical challenge, primarily due to contamination factors (Rishan et al. 2023; Wee et al. 2023). Contamination can arise from human activities near sampling sites, introducing human genetic material during sample collection and handling (Furlan and Gleeson, 2016; Valdivia-Carrillo et al. 2021). Laboratory procedures, including DNA extraction, PCR amplification, and sequencing, may also introduce human DNA from researchers or surfaces, leading to potential contamination (Goldberg et al. 2016, Huerlimannet al. 2020, Valdivia-Carrillo et al. 2021). Sequencing errors and the use of overlapping taxonomic primers can contribute to false-positive results. Additionally, human DNA from home sewage systems may be present in ballast water discharged from treated wastewater. The persistence of eDNA further complicates the assessment, potentially indicating the presence of species outside their natural habitats (Giroux et al. 2022). To mitigate this issue, researchers should use primers designed to minimize human DNA amplification, implement rigorous quality control measures such as negative controls and blank samples, and maintain sterile analytical procedures to manage potential sources of contamination (Carraro et al. 2020; McClenaghan et al. 2020; Rishan et al. 2023).

Several factors contribute to the presence of non-target species in the current eDNA metabarcoding process. Firstly, the choice of primers significantly influences the specificity of amplification. If the selected primers share partial similarity with off-target species, they may unintentionally amplify undesired sequences. Another reason is primer bias, a characteristic of various primer sets that can lead to unequal amplification of DNA from different taxa, potentially overrepresenting some species and underrepresenting others. Additionally, environmental samples may undergo DNA degradation, which can vary among organisms (Sanchez et al. 2022). This degradation results in shorter fragments that might only partially match the primer sequences, leading to the amplification of degraded DNA from unintended species (Thomsen et al. 2012; Sassoubre et al. 2016). Another significant concern is contamination from various sources, including laboratory chemicals, tools, and human handling. These impurities may contain DNA from non-target species, leading to the inadvertent amplification of off-target species during PCR. Cross-reactivity is another issue, where certain primer sets mistakenly amplify DNA from organisms or species that share genetic sequences. Additionally, during amplification, PCR artifacts such as chimeras, in which non-target DNA sequences mix with target sequences, can occur, potentially resulting in false-positive detections.

Researchers should carefully construct and assess primer sets for specificity and coverage to reduce the amplification of non-target organisms. Their potential can be found using in silico techniques, which can include screening against reference databases and testing on known DNA samples. While minimizing biases, a multi-primer technique mixing various primer sets might enhance the detection of various taxonomic groups. Incorporating negative controls and blank samples into laboratory protocols aids in monitoring and spotting potential contamination. The reliability and accuracy of eDNA metabarcoding analyses can be improved by using strict quality control procedures as optimizing calibration and validation at every single stage of procedures (Rishan et al. 2023) and careful result interpretation to separate real detections from false positives. By solving these problems, eDNA metabarcoding might produce more precise and instructive information on species biodiversity from environmental samples like ballast waters and as well as, some data proposed that the contribution to eDNA method with taxonomic based species identification could be adopted (Jeunen et al. 2019; Rey 2019) for the elimination of environmental factors leads to DNA degradation in eDNA researches.

The results of this work emphasize the need for eDNA metabarcoding studies to use a multi-primer method, as each primer set has advantages and disadvantages in terms of taxonomic coverage and specificity. Achieving a more comprehensive assessment of biodiversity in ballast waters involves carefully selecting a combination of primers targeting various marker genes (Xiong et al. 2022; Bautista et al. 2023). This method ensures a broader taxonomic representation, enhancing the ability to detect rare species—vital indicators of potential invasive species incursions (Freeland 2017; Pawluczyk et al. 2015; Lacoursière-Roussel et al. 2018).

Considering the invasive potential amongst the species for this work, *Uronema marinum*, a pathogen, can pose a risk to fish population already limited this highly polluted area, İzmit Gulf with systemic tissue damagement and high mortality (Li et al. 2018; Huang et al. 2021) coherent with the very first findings of Türe (2021). Nonetheless, among the species within the assessment scope, only *R. nomadica* stands out as it is known for forming blooms along the Eastern Mediterranean coasts of Türkiye. In these regions, it can account for up to 60% of the total catch in trawls, purse seines, and gillnets (Turan et al. 2011). This species has established populations causing substantial swarms in the Levantine Basin of the Mediterranean Sea (Galil et al. 1990; Kıdeys & Gücü 1995). It is suggested that *R. nomadica* entered the RA area via currents and ship ballast waters, using the Suez Canal as a conduit (Killi et al. 2020). Occurrences of *R. nomadica* blooms have been documented across various mediterranean coastal regions in Türkiye, often leading to net clogging in fishing activities (Öztürk & İşinibilir 2010; Turan et al. 2011). Furthermore, its traumatogenic effects have resulted in instances of hospitalization (Gülşahin 2017). Given its preference for warmer waters, the positive climate change score (+4) suggests that *R. nomadica* could potentially benefit from global warming conditions. While increasing temperatures might impact multiple species similarly, the potential for this species to expand beyond its native range to more northerly territories could be augmented due to elevated water temperatures (Walther et al. 2002). While our study predominantly confirms ballast water transportation for this species, it is worth noting that global warming might also facilitate its establishment in new regions.

In addition to *R. nomadica*, other invasive species detected in the current study could also experience shifts in distribution and ecological impact as a result of climate change. For instance, *U. marinum*, already posing a risk in the heavily polluted İzmit Gulf, could see its pathogenic effects exacerbated under warmer water conditions, as elevated temperatures may increase host susceptibility and accelerate pathogen life cycles (Li et al. 2018). Climate-induced stress on native fish populations could make them more vulnerable to pathogens like *U. marinum*, potentially causing more significant ecosystem imbalances and impacting local fisheries. Furthermore, rising water temperatures, combined with changing salinity and oxygen levels, could alter habitat suitability for multiple detected species, potentially facilitating their spread beyond current ranges (Walther et al. 2002). Such shifts could increase competitive pressures on native species, disrupt trophic relationships, and lead to unexpected ecological impacts (Hellmann et al. 2008). For example, invasive species adapted to warmer, low-oxygen environments may outcompete native species as climate change alters baseline ecosystem conditions, promoting their establishment and success (Rahel & Olden 2008).

4.1. Linking metabarcoding data to the AS-ISK screening protocol

The combination of eDNA metabarcoding with the AS-ISK screening process showcases the practical implementation of molecular approaches in ecological risk assessment. The extensive data obtained through metabarcoding offered a strong foundation for evaluating the invasive capacity of identified species, as is the case for *R. nomadica* in the present study. By employing numerous primers, a wide range of taxonomic groups were included, hence improving the identification of infrequent and hard-to-find species that could otherwise go unnoticed.

This paper contributes to the expanding body of research on the application of eDNA metabarcoding for the surveillance of invasive species in ballast water. Prior research has shown that metabarcoding is useful for identifying non-native species and assessing their spread (Comtet et al. 2015; Xiong et al. 2016). This study provides a comprehensive method for monitoring the risks of invasive species in maritime ecosystems by integrating metabarcoding data with current risk assessment frameworks, such as AS-ISK.

Combining samples prior to filtering has been demonstrated to enhance the identification of species that are present in low quantities and simplify extensive monitoring initiatives. The pooling technique employed in this work was selected to augment the sensitivity and efficiency of species detection, particularly in a vast and intricate sample environment like ballast water. Pooling samples has benefits, including enhanced statistical power and the capacity to identify rare species by amalgamating DNA from several sources; nevertheless, it also presents certain constraints. A major concern is the possible loss of information on the geographical and temporal diversity of species distributions, as aggregation obscures differences that may occur across individual samples. This may impact the capacity to precisely evaluate species abundance and diversity, resulting in the over- or underestimating of certain taxa. The statistical efficacy of the pooling method is contingent upon the sample size and the quantity of water treated, which subsequently affect the detection probability of uncommon or low-abundance species. While pooling is useful for comprehensive biodiversity assessments, it entails error margins due to the unequal distribution and shedding rates of eDNA among species. Future research could enhance its findings by employing a hybrid approach that integrates pooled and individual sampling to attain a more thorough picture of species diversity. This approach offers advantages such as heightened sensitivity, cost-effectiveness, and time efficiency (Deiner et al. 2017; Aylagas et al. 2018). This method improves the capacity

to obtain a complete and detailed overview of the variety of life forms present in the studied environment. However, it is important to carefully assess any possible drawbacks, such as the potential reduction in the variability of individual samples.

5. Conclusions

DNA-based tools emerge as a promising alternative to traditional taxonomic surveys, particularly marine habitats facing pollution from ballast water discharge to enhance a prepotent ecological monitoring. In this regard, eDNA metabarcoding proves to be a crucial tool offering extensive taxonomic coverage, even for cryptic, rare and elusive species, with the simultaneous benefits of high identification sensitivity, cost-efficiency, and rapid scanning of entire ecosystems. Metabarcoding can play a pivotal role in supporting management initiatives aimed at reducing the risk of established species and focusing efforts on preventing introductions and spreading. Therefore, a rigorous tracking of invasive species is imperative for long-term and sustainable biomonitoring of aquatic environments. The data from this study also underscores the importance of using a variety of primers to mitigate biases and enhance the precision of species identification. Utilizing novel eDNA metabarcoding with species-selective primers, this combined approach of risk identification and eDNA metabarcoding contributes to a better understanding of early detection, management strategies, and policymaking concerning invasive species, especially for the conservation of marine and freshwater systems.

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Appendix 1- List of *12S, 16S, 18Sv4, 18Sv8* and *COI* primer results and related figures

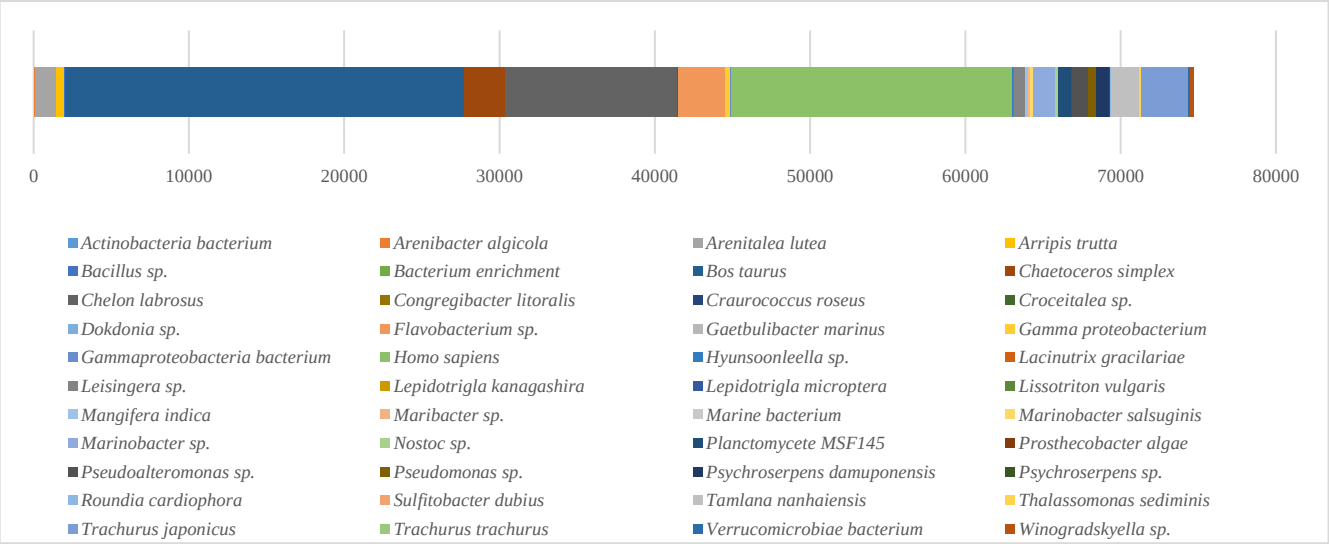

Figure S1. *12S* (Fish/Vertebrate) *Red indicates those either with less than 10 read counts and/or less than 97% identification rate

Table S1. *12S* (Fish/Vertebrate) Primer Results

Species	Read Count	Classification
Actinobacteria bacterium	49	Bacterium
Arenibacter algicola	29	Bacterium
Arenitalea lutea	1399	Flavo Bacterium
*Arripis trutta**	500	Fish
Bacillus sp.	28	Bacterium
Bacterium enrichment	15	Bacterium
Bos taurus	25751	Cow
Chaetoceros simplex	2598	Diatom
Chelon labrosus	11054	Fish
Congregibacter litoralis	19	Bacterium
Craurococcus roseus	14	Bacterium
Croceitalea sp.	51	Bacterium
Dokdonia sp.	23	Flavo Bacterium
Flavobacterium sp.	2985	Flavo Bacterium
Gaetbulibacter marinus	17	Bacterium
Gamma proteobacterium	336	Bacterium
Gammaproteobacteria bacterium	93	Bacterium
Homo sapiens	18092	Human
Hyunsoonleella sp.	14	Flavo Bacterium
Lacinutrix gracilariae	115	Bacterium
Leisingera sp.	647	Bacterium
Lepidotrigla kanagashira	2	Fish
Lepidotrigla microptera	67	Fish
Lissotriton vulgaris	1	Newt
Mangifera indica	135	Plant
Maribacter sp.	95	Flavo Bacterium
Marine bacterium	47	Bacterium
Marinobacter salsuginis	194	Bacterium
Marinobacter sp.	1385	Bacterium
Nostoc sp.	241	Cyanobacteria
Planctomycete MSF145	827	Bacterium
Prosthecobacter algae	14	Bacterium
Pseudoalteromonas sp.	1102	Bacterium
Pseudomonas sp.	446	Bacterium
Psychroserpens damuponensis	903	Bacterium
Psychroserpens sp.	74	Flavo Bacterium
Roundia cardiophora	23	Diatom
Sulfitobacter dubius	14	Bacterium
Tamlana nanhaiensis	1794	Bacterium
Thalassomonas sediminis	158	Bacterium
Trachurus japonicus	3045	Fish
Trachurus trachurus	3	Fish
Verrucomicrobiae bacterium	65	Bacterium
Winogradskyella sp.	239	Flavo Bacterium

Appendix 1-(continued)

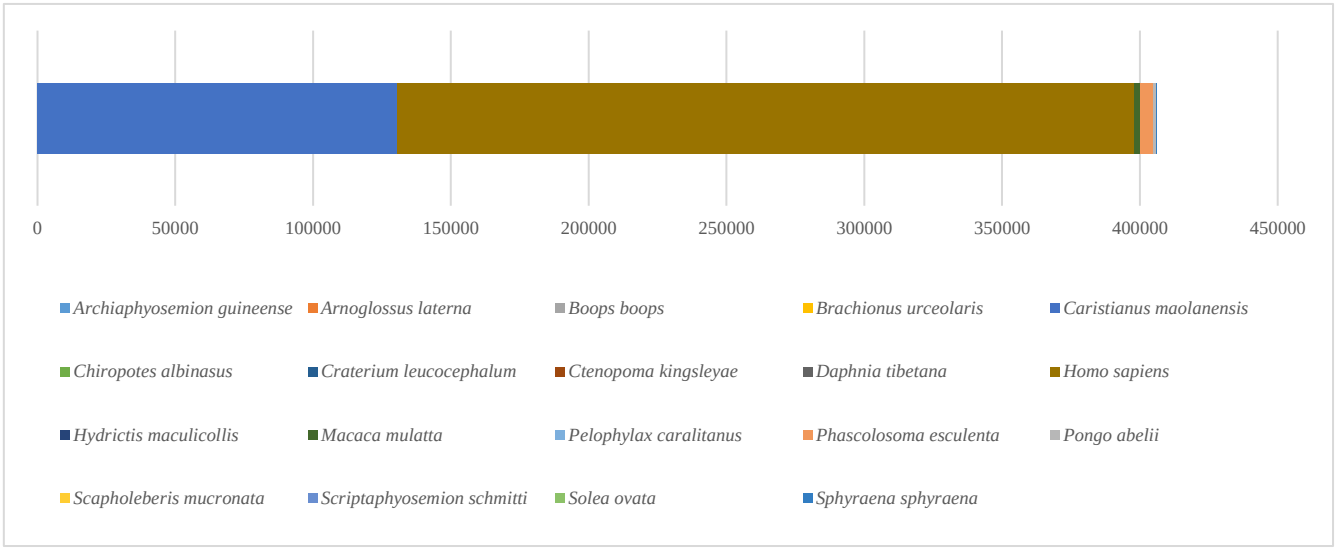

Figure S2. ***16S*** **(Vertebrate) privot** *Red indicates those either with less than 10 read counts and/or less than 97% identification rate

Table S2. ***12S*** **(Fish/Vertebrate) Primer Results**

Species	**Read Count**	**Classification**
*Archiaphyosemion guineense**	3	Fish
Arnoglossus laterna	1	Fish
Boops boops	1	Fish
Brachionus urceolaris	2	Rotifer
Caristianus maolanensis	130608	Insect
Chiropotes albinasus	4	Monkey
Craterium leucocephalum	7	Fungi
Ctenopoma kingsleyae	1	Fish
Daphnia tibetana	1	Crustacean
Homo sapiens	267354	Human
Hydrictis maculicollis	55	Sea otter
Macaca mulatta	2233	Macacus rhesus
Pelophylax caralitanus	1	Frog
Phascolosoma esculenta	4793	Seaworm
Pongo abelii	882	Sumatran orangutan
Scapholeberis mucronata	11	Crustacean
Scriptaphyosemion schmitti	1	Fish
Solea ovata	1	Fish
Sphyraena sphyraena	1	Fish

Appendix 1-(continued)

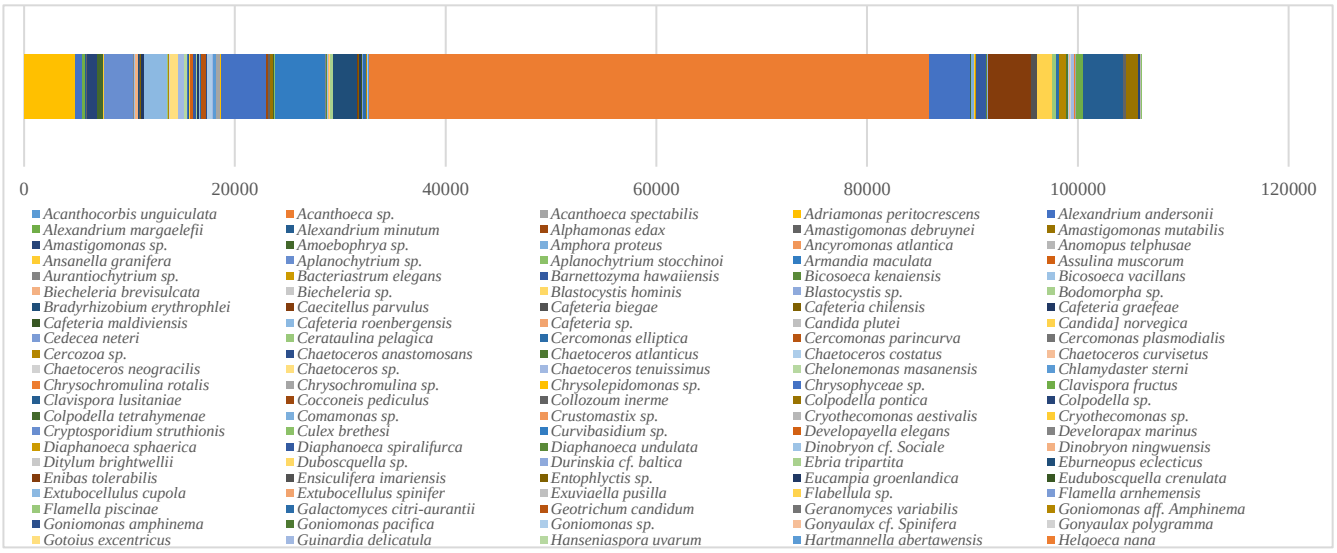

Figure S3. ***18Sv4*** **Eukaryote (Plankton/Algae/Diatom) privot** *Red indicates those either with less than 10 read counts and/or less than 97% identification rate

Table S3. ***18Sv4*** **Eukaryote (Plankton/Algae/Diatom) Primer Results**

Species	Read Count	Classification
*Acanthocorbis unguiculata**	1	Choanocyte
Acanthoeca sp.	4	Choanocyte
Acanthoeca spectabilis	52	Choanocyte
Adriamonas peritocrescens	4793	Flagellate
Alexandrium andersonii	641	Dinoflagellate
Alexandrium margaelefii	328	Dinoflagellate
Alexandrium minutum	49	Dinoflagellate
Alphamonas edax	4	Flagellat
Amastigomonas debruynei	105	Invertebrate
Amastigomonas mutabilis	15	Invertebrate
Amastigomonas sp.	916	Invertebrate
Amoebophrya sp.	588	Dinoflagellate
Amphora proteus	1	Diatome
Ancyromonas atlantica	16	Protist
Anomopus telphusae	10	Rotifer
Ansanella granifera	85	Dinoflagellate
Aplanochytrium sp.	2778	Parasite
Aplanochytrium stocchinoi	3	Parasite
Armandia maculata	1	Sea worm
Assulina muscorum	1	Algae
Aurantiochytrium sp.	64	Protist
Bacteriastrum elegans	3	Diatom
Barnettozyma hawaiiensis	3	Yeast
Bicosoeca kenaiensis	1	Flagellate
Bicosoeca vacillans	80	Flagellate
Biecheleria brevisulcata	264	Dinoflagellate
Biecheleria sp.	15	Dinoflagellate
Blastocystis hominis	3	Algae
Blastocystis sp.	1	Algae
Bodomorpha sp.	58	Algae
Bradyrhizobium erythrophlei	4	Bacterium
Caecitellus parvulus	84	Flagellate
Cafeteria biegae	25	Nanoflagellate
Cafeteria chilensis	103	Nanoflagellate
Cafeteria graefeae	336	Nanoflagellate
Cafeteria maldiviensis	3	Nanoflagellate
Cafeteria roenbergensis	2108	Flagellate
Cafeteria sp.	11	Nanoflagellate
Candida plutei	10	Yeast
Candida norvegica	3	Fungi

Cedecea neteri	2	Bacterium
Cerataulina pelagica	93	Diatome
Cercomonas elliptica	1	Algae
Cercomonas parincurva	4	Algae
Cercomonas plasmodialis	2	Algae
Cercozoa sp.	112	Algae
Chaetoceros anastomosans	1	Diatome
Chaetoceros atlanticus	2	Diatome
Chaetoceros costatus	9	Diatome
Chaetoceros curvisetus	20	Diatome
Chaetoceros neogracilis	11	Diatome
Chaetoceros sp.	806	Diatome
Chaetoceros tenuissimus	551	Diatome
Chelonemonas masanensis	286	Eukaryote (whip)
Chlamydaster sterni	6	Eukaryote
Chrysochromulina rotalis	14	Kelp
Chrysochromulina sp.	23	Kelp
Chrysolepidomonas sp.	27	Algae
Chrysophyceae sp.	17	Algae
Clavispora fructus	2	Yeast
Clavispora lusitaniae	79	Yeast
Cocconeis pediculus	4	Diatome
Collozoum inerme	3	Algae
Colpodella pontica	2	Flagellate (carnivore)
Colpodella sp.	3	Flagellate (carnivore)
Colpodella tetrahymenae	6	Flagellate (carnivore)
Comamonas sp.	44	Bacterium
Crustomastix sp.	4	Algae
Cryothecomonas aestivalis	3	Algae
Cryothecomonas sp.	61	Algae
Cryptosporidium struthionis	1	Parasite
Culex brethesi	6	Mosquito
Curvibasidium sp.	37	Fungi
Developayella elegans	246	Flagellate
Develorapax marinus	1	Algae
Diaphanoeca sphaerica	47	Choanocyte
Diaphanoeca spiralifurca	225	Choanocyte
Diaphanoeca undulata	31	Choanocyte
Dinobryon sociale	2	Algae
Dinobryon ningwuensis	5	Algae
Ditylum brightwellii	1	Diatome
Duboscquella sp.	5	Parasite
Durinskia baltica	6	Dinoflagellate
Ebria tripartita	35	Algae
Eburneopus eclecticus	1	Arthropod
Enibas tolerabilis	9	Choanocyte
Ensiculifera imariensis	14	Dinoflagellate
Entophlyctis sp.	12	Fungi
Eucampia groenlandica	183	Diatome
Eduboscquella crenulata	6	Dinoflagellate
Extubocellulus cupola	28	Diatome
Extubocellulus spinifer	7	Diatome
Exuviaella pusilla	45	Dinoflagellate
Flabellula sp.	6	Amoeba
Flamella arnhemensis	2	Amoeba
Flamella piscinae	4	Amoeba
Galactomyces citri-aurantii	32	Yeast
Geotrichum candidum	547	Yeast
Geranomyces variabilis	15	Fungi
Goniomonas amphinema	15	Nanoflagellate
Goniomonas pacifica	1	Nanoflagellate
Goniomonas sp.	548	Nanoflagellate
Gonyaulax cf. Spinifera	1	Dinoflagellate
Gonyaulax polygramma	15	Dinoflagellate
Gotoius excentricus	3	Dinoflagellate
Guinardia delicatula	4	Diatome
Hanseniaspora uvarum	7	Yeast
Hartmannella abertawensis	246	Amoeba
Helgoeca nana	84	Eukaryote (whip)

Homo sapiens	357	Human
Incisomonas marina	144	Flagellate
Karlodinium veneficum	4204	Dinoflagellate
Katablepharis japonica	13	Algae
Kazachstania africana	24	Fungi
Kluyveromyces marxianus	179	Yeast
Kluyveromyces sp.	215	Yeast
Labyrinthulochytrium haliotidis	220	Fungi
Lepidoglyphus destructor	5	Mite
Leptocylindrus convexus	80	Diatome
Leptocylindrus danicus	1	Diatome
Lingulamoeba sp.	8	Amoeba
Lithodesmioides polymorpha	6	Diatome
Malacoceros fuliginosus	2	Worm (annelid)
Malassezia globosa	2	Fungi
Mantamonas plastica	135	Flagellate
Massisteria marina	4737	Algae
Massisteria sp.	22	Algae
Massisteria voersi	30	Algae
Metromonas simplex	31	Amoeba
Microcaecilia unicolor	1	Amphibian
Micrometopion nutans	83	Amphibian
Minorisa minuta	69	Plankton
Monorhizochytrium globosum	170	Algae
Monosiga brevicollis	1	Eukaryote (whip)
Navicula trivialis	12	Diatome
Neocercomonas sp.	2	Algae
Nolandella sp.	363	Amoeba
Notommata cordonella	2289	Rotifer
Ovulinata parva	20	Algae
Parabirojimia similis	19	Algae
Paraphysomonas butcheri	153	Algae
Paraphysomonas mikadiforma	113	Algae
Paraphysomonas sp.	105	Algae
Parvicardium exiguum	5	Bivalvia
Paulinella micropora	1	Algae
Pectinaria koreni	61	Trumpet worm
Perideraion elongatum	2	Diatome
Philodina sp.	14	Rotifer
Pichia fermentans	71	Yeast
Pichia kudriavzevii	217	Yeast
Picomonas judraskeda	42	Plankton
Pierrecomperia catenuloides	22	Diatome
Pirsonia guinardiae	58	Parasite
Plagiopyliella pacifica	27	Ciliate
Planomonas brevis	2	Flagellate
Planomonas elongata	62	Flagellate
Planomonas micra	17	Flagellate
Platyophrya bromelicola	10	Algae
Polarella glacialis	2	Dinoflagellate
Polykrikos kofoidii	1	Dinoflagellate
Polyoeca dichotoma	1	Eukaryote (whip)
Prorocentrum mexicanum	62	Dinoflagellate
Prorocentrum triestinum	53124	Dinoflagellate
Protaspis sp.	11	Algae
Protostelium nocturnum	19	Amoeba
Pseudobodo sp.	3859	Zooflagellate
Pseudochilodonopsis mutabilis	6	Algae
Pseudochlorella pringsheimii	8	Algae (green)
Pseudocohnilembus persalinus	5	Algae
Pseudophyllomitus vesiculosus	26	Flagellate
Pseudostaurosira madagascariensis	15	Diatome
Pyramimonas sp.	1	Algae (green)
Pyxinia crystalligera	15	Algae
Reckertia gemma	354	Algae
Rhizophlyctis rosea	1	Fungi
Rhoicosphenia abbreviata	9	Diatome
Rhopilema nomadica	71	Jelly fish
Roubikia sp.	1	Insect

Saccamoeba sp.	4	Bacterium
Salpingoeca macrocollata	5	Eukaryote (whip)
Salpingoeca urceolata	13	Eukaryote (whip)
Savillea micropora	5	Eukaryote (whip)
Schizochytrium minutum	11	Algae
Scrippsiella sp.	1054	Dinoflagellate
Sicyoidochytrium sp.	5	Protist
Sourniaea diacantha	4	Dinoflagellate
Spizellomyces pseudodichotomus	14	Fungi
Spondylosium pulchellum	1	Plant
Spumella sp.	31	Algae
Stellarchytrium dubum	47	Algae
Stephanoeca diplocostata	1	Eukaryote (whip)
Stephanoeca norrisii	1	Eukaryote (whip)
Stephanoeca paucicostata	2489	Eukaryote (whip)
Stephanoeca paucicostata	1678	Eukaryote (whip)
Stephanopyxis turris	479	Eukaryote (whip)
Strombidium sp.	5	Ciliate (planktonic)
Symbiodinium sp.	5	Dinoflagellate
Syncystis mirabilis	1	Parasite
Syracosphaera pulchra	6	Algae
Teleaulax amphioxeia	2	Algae
Teleaulax gracilis	11	Algae
Telonema subtilis	1402	Protist
Tetraselmis cordiformis	17	Algae (green)
Tetraselmis rubens	409	Algae (green)
Tetraselmis sp.	233	Algae (green)
Thalassiosira gessneri	6	Diatome
Thalassiosira gravida	1	Diatome
Thalassiosira profunda	690	Diatome
Thalassiosira sp.	22	Diatome
Thaumatomastigidae sp.	187	Algae
Thaumatomastix sp.	108	Algae
Thecamoeba sp.	53	Amoeba
Thraustochytriidae sp.	87	Algae (brown)
Thraustochytrium multirudimentale	38	Protist
Thraustochytrium sp.	271	Fungi
Tokophrya quadripartita	1	Fungi
Trachyrhizium urniformis	11	Amoeba
Triparma pacifica	131	Algae
Tunicothrix wilberti	1	Algae
Ulkenia aff. visurgensis	3	Fungi
Umbraulva japonica	1	Algae (green)
Uncinata gigantea	729	Ciliate
Uronema marinum	3753	Parasite
Vannella samoroda	1	Amoeba
Ventrifissura artocarpoidea	283	Algae
Ventrifissura sp.	1155	Algae
Vexillifera abyssalis	176	Amoeba
Vexillifera bacillipedes	1	Amoeba
Vexillifera sp.	50	Amoeba
Wangodinium sinense	1	Dinoflagellate
Wolffia angusta	19	Plant
Yarrowia deformans	1	Yeast
Yarrowia lipolytica	75	Fungi
Yarrowia sp.	1	Yeast

Appendix 1-(continued)

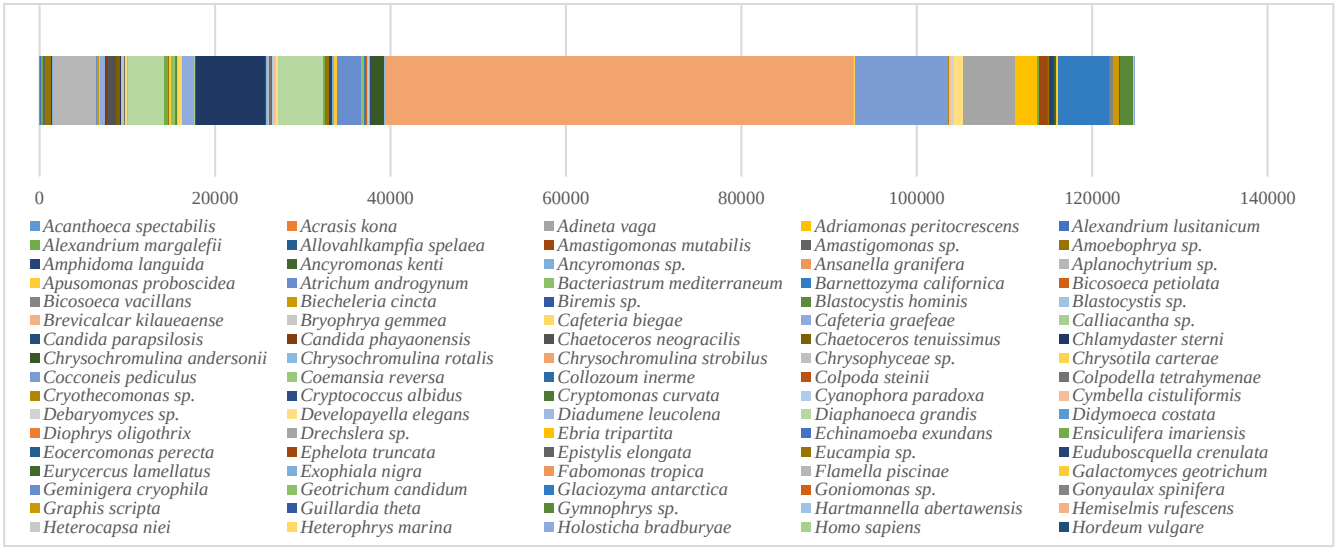

Figure S4. ***18Sv8*** **Eukaryote (Plankton/Algae/Diatom) privot** *Red indicates those either with less than 10 read counts and/or less than 97% identification rate

Table S4. ***18Sv8*** **Eukaryote (Plankton/Algae/Diatom) Primer Results**

Species	**Read Count**	**Classification**
Acanthoeca spectabili	44	Protozoa
*Acrasis kona**	2	Protozoa
Adineta vaga	4	Rotifer
Adriamonas peritocrescens	48	Flagellate
Alexandrium lusitanicum	57	Dinoflagellate
Alexandrium margalefii	242	Dinoflagellate
Allovahlkampfia spelaea	10	Amoeba
Amastigomonas mutabilis	26	Protozoa
Amastigomonas sp.	163	Apusozoa
Amoebophrya sp.	736	Dinoflagellate
Amphidoma languida	71	Dinoflagellate
Ancyromonas kenti	6	Protozoa
Ancyromonas sp.	64	Eukaryote
Ansanella granifera	99	Dinoflagellate
Aplanochytrium sp.	4923	Eukaryote
Apusomonas proboscidea	4	Flagellate
Atrichum androgynum	48	Plant
Bacteriastrum mediterraneum	6	Bacteria
Barnettozyma californica	42	Fungi
Bicosoeca petiolata	2	Bicosoecida
Bicosoeca vacillans	34	Bicosoecida
Biecheleria cincta	188	Dinoflagellate
Biremis sp.	2	Diatome
Blastocystis hominis	6	Parasite
Blastocystis sp.	18	Parasite
Brevicalcar kilaueaense	26	Fungi
Bryophrya gemmea	4	Plant
Cafeteria biegae	30	Eukaryote
Cafeteria graefeae	596	Eukaryote
Calliacantha sp.	16	Eukaryote
Candida parapsilosis	6	Fungi
Candida phayaonensis	242	Fungi
Chaetoceros neogracilis	980	Diatome
Chaetoceros tenuissimus	429	Diatome
Chlamydaster sterni	88	Algae
Chrysochromulina andersonii	42	Seaweed
Chrysochromulina rotalis	11	Seaweed
Chrysochromulina strobilus	22	Seaweed
Chrysophyceae sp.	315	Algae

Chrysotila carterae	8	Algae
Cocconeis pediculus	16	Algae
Coemansia reversa	2	Arthropod
Collozoum inerme	4	Eukaryote
Colpoda steinii	18	Eukaryote
Colpodella tetrahymenae	6	Eukaryote
Cryothecomonas sp.	22	Kelp
Cryptococcus albidus	18	Yeast
Cryptomonas curvata	36	Flagellate
Cyanophora paradoxa	24	Flagellate
Cymbella cistuliformis	2	Diatome
Debaryomyces sp.	17	Yeast
Developayella elegans	244	Plankton
Diadumene leucolena	24	Anemone
Diaphanoeca grandis	4120	Eukaryote (whip)
Didymoeca costata	12	Eukaryote (whip)
Diophrys oligothrix	8	Kelp
Drechslera sp.	12	Fungi
Ebria tripartita	28	Algae
Echinamoeba exundans	2	Eukaryote
Ensiculifera imariensis	388	Dinoflagellate
Eocercomonas perecta	12	Kelp
Ephelota truncata	2	Protozoa
Epistylis elongata	2	Kelp
Eucampia sp.	69	Diatome
Eudubosquella crenulata	18	Dinoflagellate
Eurycercus lamellatus	4	Arthropod
Exophiala nigra	2	Fungi
Fabomonas tropica	10	Eukaryote
Flamella piscinae	6	Amoeba
Galactomyces geotrichum	237	Yeast
Geminigera cryophila	4	Algae
Geotrichum candidum	470	Fungi
Glaciozyma antarctica	4	Yeast
Goniomonas sp.	50	Algae
Gonyaulax spinifera	2	Dinoflagellate
Graphis scripta	6	Fungi
Guillardia theta	2	Algae
Gymnophrys sp.	86	Eukaryote
Hartmannella abertawensis	68	Eukaryote
Hemiselmis rufescens	2	Algae
Heterocapsa niei	66	Dinoflagellate
Heterophrys marina	462	Eukaryote
Holosticha bradburyae	1374	Ciliate
Homo sapiens	89	Human
Hordeum vulgare	4	Plant
Hyphochytrium catenoides	12	Eukaryote
Ichthyophonus irregularis	114	Parasite
Ipomoea trifida	42	Plant
Karlodinium veneficum	7908	Dinoflagellate
Katablepharis japonica	16	Algae
Kluyveromyces marxianus	241	Yeast
Korotnevella pelagolacustris	2	Protozoa
Labyrinthuloides minuta	103	Kelp
Laetisaria fuciformis	23	Fungi
Leptocylindrus convexus	18	Diatome
Leptocylindrus danicus	2	Diatome
Leptomyxa reticulata	22	Amoeba
Leptosphaeria biglobosa	50	Fungi
Leucosporidium sp.	165	Fungi
Leucosporidium yakuticum	25	Fungi
Lingulamoeba leei	32	Amoeba
Malassezia globosa	29	Fungi
Mallomonas akrokomos	232	Algae
Mallomonas tonsurata	82	Algae
Mamiella gilva	136	Plankton
Mantamonas plastica	214	Flagellate
Marchantia quadrata	2	Plant
Massisteria marina	5093	Kelp

Massisteria sp.	104	Kelp
Massisteria voersi	32	Kelp
Melosira varians	2	Diatome
Micromonas pusilla	4	Algae
Monosiga brevicollis	8	Eukaryote (whip)
Myrothecium sp.	158	Fungi
Nausithoe rubra	8	Jelly fish
Neohodgsonia mirabilis	2	Plant
Neoparamoeba branchiphila	33	Parasite
Neoparamoeba sp.	394	Parasite
Nolandella sp.	344	Amoeba
Nurscia albofasciata	10	Arthropod
Nusuttodinium poecilochroum	264	Dinoflagellate
Ovulinata parva	16	Kelp
Paraflabellula hoguae	4	Amoeba
Paramoeba aestuarina	276	Kelp
Paramoeba branchiphila	2773	Amoeba
Paramoeba perurans	324	Parasite
Paraphysomonas imperforata	178	Algae
Parauronema virginianum	160	Protozoa
Paulinella chromatophora	4	Amoeba
Pectinaria koreni	46	Sea worm
Penaeus duorarum	4	Shrimp
Pentapharsodinium sp.	11	Dinoflagellate
Peridinium sociale	2	Dinoflagellate
Phoma herbarum	70	Fungi
Pichia fermentans	114	Yeast
Pichia sp.	32	Fungi
Picomonas judraskeda	40	Plankton
Pinus taeda	2	Plant (pine tree)
Pirsonia guinardiae	99	Flagellate
Plagiopyliella pacifica	8	Kelp
Planomonas brevis	54	Flagellate
Planomonas elongata	12	Flagellate
Platyamoeba contorta	2	Amoeba
Prorocentrum mexicanum	1484	Dinoflagellate
Prorocentrum micans	10	Dinoflagellate
Prorocentrum triestinum	53452	Dinoflagellate
Pseliodinium pirum	26	Dinoflagellate
Pseudobodo sp.	139	Algae
Pseudobodo tremulans	10618	Algae
Pseudo-nitzschia delicatissima	41	Diatome
Pseudoparamoeba pagei	2	Amoeba
Pyramimonas tetrarhynchus	10	Algae (green)
Rhizoclosmatium sp.	6	Fungi
Rhodotorula mucilaginosa	29	Fungi
Rhogostoma schuessleri	2	Kelp
Rhopilema nomadica	41	Jelly fish
Salpingoeca urceolata	22	Choanocyte
Savillea micropora	148	Choanocyte
Scrippsiella sp.	405	Dinoflagellate
Scrippsiella trochoidea	1004	Dinoflagellate
Slooffia sp.	10	Fungi
Soletellina diphos	2	Bivalvia
Strombidium sp.	2	Ciliate
Strombidium stylifer	2	Ciliate
Synchaeta sp.	5918	Rotifer
Synchaeta tremula	2560	Rotifer
Syncystis mirabilis	2	Parasite
Synura sp.	162	Algae
Taphrina vestergrenii	2	Fungi
Tetraselmis marina	976	Algae (green)
Thalassiosira minima	14	Algae
Thaumatomastix sp.	110	Kelp
Thaumatomonas seravini	546	Kelp
Thraustochytrium sp.	304	Algae (brown)
Tintinnidium mucicola	2	Kelp
Toxorhynchites amboinensis	6	Mosquito
Trichia sordida	6	Protozoa

Trichodina meretricis	232	Kelp
Tripos tenuis	2	Dinoflagellate
Ulkenia profunda	2	Fungi (Marine)
Uronema marinum	5849	Parasite
Vacuolaria virescens	20	Algae (green)
Vannella calycinucleolus	340	Amoeba
Vannella samoroda	702	Amoeba
Vannella sp.	158	Amoeba
Ventrifissura artocarpoidea	1402	Kelp
Vexillifera abyssalis	98	Amoeba
Vexillifera armata	64	Amoeba
Vexillifera sp.	21	Amoeba
Yarrowia deformans	4	Fungi
Yarrowia lipolytica	28	Fungi

Appendix 1-(continued)

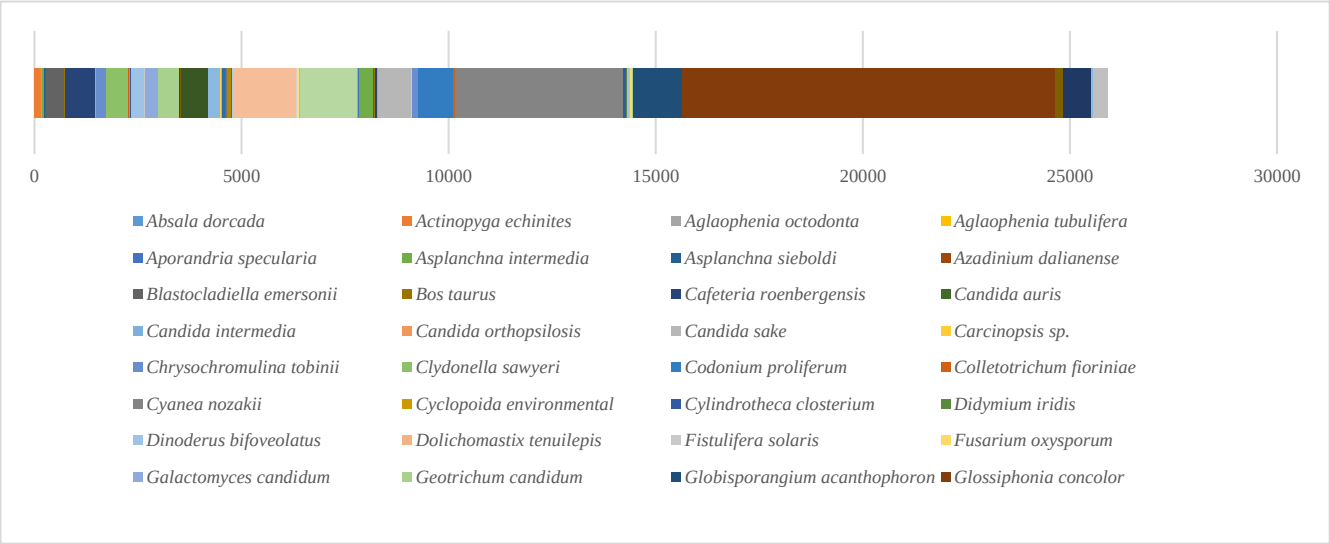

Figure S5. *COI* (Invertebrate) privot *Red indicates those either with less than 10 read counts and/or less than 97% identification rate

Table S5. *COI* (Invertebrate) Primer Results

Species	Read Count	Classification
*Absala dorcada**	1	Heterocera
Actinopyga echinites	169	Tripang
Aglaophenia octodonta	11	Hydrozoa
Aglaophenia tubulifera	1	Hydrozoa
Aporandria specularia	5	Heterocera
Asplanchna intermedia	60	Rotifer
Asplanchna sieboldi	2	Rotifer
Azadinium dalianense	26	Dinoflagellate
Blastocladiella emersonii	460	Fungi
Bos taurus	1	Cow
Cafeteria roenbergensis	739	Flagellate
Candida auris	1	Yeast
Candida intermedia	9	Yeast
Candida orthopsilosis	1	Yeast
Candida sake	1	Yeast
Carcinopsis sp.	3	Insect
Chrysochromulina tobinii	234	Kelp
Clydonella sawyeri	547	Ameoba
Codonium proliferum	1	Hydrozoa
Colletotrichum fioriniae	3	Fungi
Cyanea nozakii	1	Jellyfish
Cyclopoida environmental	44	Copepod
Cylindrotheca closterium	7	Diatome
Didymium iridis	9	Mold
Dinoderus bifoveolatus	321	Insect
Dolichomastix tenuilepis	2	Algae (green)

Fistulifera solaris	10	Diatome
Fusarium oxysporum	1	Fungi
Galactomyces candidum	327	Yeast
Geotrichum candidum	507	Yeast
Globisporangium acanthophoron	13	Fungi
Glossiphonia concolor	4	Worm
Gonium pectorale	4	Algae (green)
Gorilla beringei	13	Gorilla
Haliplus fasciatus	4	Insect
Heterocapsa circularisquama	660	Dinoflagellat
Homo sapiens	277	Human
Hordeum vulgare	9	Barley
Hypochilus bonneti	10	Spider
Lichtheimia ramosa	8	Fungi
Lizzia blondina	10	Hydrozoa
Lytocarpia myriophyllum	1	Hydrozoa
Malassezia globosa	73	Fungi
Maribacter sp.	41	Bacterium
Minutocellus polymorphus	14	Diatom
Neoparamoeba sp.	115	Ameba
Nitzschia frustulum	1	Diatom
Nitzschia palea	5	Diatom
Pan troglodytes	29	Chimpanzee
Paramoeba branchiphila	1515	Parasite
Paramoeba perurans	44	Ameoba
Paravannella minima	42	Ameoba
Penaeus vannamei	5	Shrimp
Penilia avirostris	1392	Crustacean
Phaeocystis pouchetii	11	Algae
Phoma sp.	1	Fungi
Phytophthora boehmeriae	6	Fungi
Phytophthora cajani	15	Fungi
Phytophthora moyootj	16	Fungi
Pichia kudriavzevii	349	Yeast
Plecotus auritus	2	Bat
Plecotus ognevi	2	Bat
Pneumocystis jirovecii	4	Fungi
Pongo abelii	28	Sumatra orangutan
Prorocentrum micans	54	Dinoflagellate
Pseudoceratina purpurea	2	Sponge
Pseudogymnoascus pannorum	9	Fungi
Pseudopedobacter saltans	7	Bacterium
Pyropia haitanensis	813	Algae (red)
Pythium biforme	5	Fungi
Pythium emineosum	150	Fungi
Rhagoletis zephyria	11	Fruit Mosquito
Rhodotorula mucilaginosa	831	Fungi
Rhopilema nomadica	62	Jellyfish
Rufibacter sp.	4055	Bacterium
Saccharomyces cerevisiae	1	Fungi
Scapholeberis mucronata	60	Crustacean
Schizophyllum commune	26	Fungi
Scrippsiella precaria	41	Dinoflagellate
Selenops sp.	30	Arthropod
Shiraia bambusicola	1	Fungi
Squamamoeba japonica	51	Ameoba
Symbiodinium sp.	1	Microalgae
Synchaeta oblonga	15	Rotifer
Synchaeta tremula	1181	Rotifer
Synchaeta tremuloida	9012	Rotifer
Taphrina wiesneri	10	Plant pathogen
Thecamonas trahens	185	Bacterium
Tremella fuciformis	673	Fungi
Trichoderma hamatum	1	Fungi
Tristramella simonis	49	Fish
Vanderwaltozyma polyspora	8	Fungi
Vexillifera sp.	350	Ameoba

Appendix 2- *Rhopilema nomadica* AS-ISK analysis

AS-ISK v2

Taxon and Assessor details	
Category	Invertebrates (marine)
Taxon name	*Rhopilema nomadica*
Common name	Nomad jellyfish
Assessor	Ali Serhan Tarkan
Risk screening context	
Reason and socio-economic benefits	
Risk assessment area	Izmit Gulf
Taxonomy	
Native range	
Introduced range	
URL	

			Response	Justification (references and/or other information)	Confidence
A. Biogeography/Historical					
1. Domestication/Cultivation					
1	1,01	Has the taxon been the subject of domestication (or cultivation) for at least 20 generations?	No	No report is available for domestication or cultivation of this species	Medium
2	1,02	Is the taxon harvested in the wild and likely to be sold or used in its live form?	No	No report found on this	Medium
3	1,03	Does the taxon have invasive races, varieties, sub-taxa or congeners?	Yes	Yu, H., Li, C., Li, R., Xing, R., Liu, S., Li, P., 2007. Factors influencing hemolytic activity of venom from the jellyfish Rhopilema esculentum Kishinouye. Food and Chemical Toxicology, 45(7), 1173-1178.	High
2. Climate, distribution and introduction risk					
4	2,01	How similar are the climatic conditions of the Risk Assessment (RA) area and the taxon's native range?	Medium	According to Köppen-Geiger classification scheme	Medium
5	2,02	What is the quality of the climate matching data?	Medium	According to Köppen-Geiger classification scheme	Medium
6	2,03	Is the taxon already present outside of captivity in the RA area?	No	There is no report or documentation that the species present outside of captivity in the RA area	Medium
7	2,04	How many potential vectors could the taxon use to enter in the RA area?	>1	There is a report that it was detected in ballast water of the ships (Koray, 2022) and it could transported by natural ways (currents) from Mediterranean Sea. Koray, K. 2022. Gemi Balast Suları ile Taşınan Yabancı Türlerin eDNA Metabarkodlama Yöntemiyle Tespiti ve Risk Analizleri: İzmit Körfezi. Institute of Science. Ankara Universtiy	Medium
8	2,05	Is the taxon currently found in close proximity to, and likely to enter into, the RA area in the near future (e.g. unintentional and intentional introductions)?	Yes	This species have been recorded (established) (Gulsahin & Tarkan 2011) from neighbouring sea basin (Aegean Sea) so given no pyhisical barries between seas and high ship traffic it is likely to enter into RA. Gülşahin, N., Tarkan, A. N., 2011. The first confirmed record of the alien jellyfish Rhopilema nomadica Galil, 1990 from the southern Aegean coast of Turkey. Aquatic Invasions, 6 (Suppl 1), S95-S97.	High
3. Invasive elsewhere					
9	3,01	Has the taxon become naturalised (established viable populations) outside its native range?	Yes	Cinar, ME, Bilecenoğlu, M, Yokeş M.B, Ozturk B, Taşkin E, Bakir K, et al.(2021). Current status (as of end of 2020) of marine alien species in Turkey. PLoSONE16(5): e0251086.	Very high
10	3,02	In the taxon's introduced range, are there known adverse impacts to wild stocks or commercial taxa?	Yes	Galil, B. S., 1993. Lessepsian migration: new findings on the foremost anthropogenic change in the Levant basin fauna. Ist. Sci. Ambientali Mar., Santa Margherita Ligure (Italy), 307-318.	High
11	3,03	In the taxon's introduced range, are there known adverse impacts to aquaculture?	No	No evidence	Medium
12	3,04	In the taxon's introduced range, are there known adverse impacts to ecosystem services?	Yes	Turan, C., Gürlek, M., Özbalcılar, B., Yağlıoğlu, D., Ergüden, D. et al., 2011. Jellyfish bycatch data by puse seine, trawl and net fisheries during March-April 2011 in the Mediterranean coasts of Turkey, p.1- First National Workshop on Jellyfish and Other Gelatinous Species in Turkish Marine Waters,Bodrum, 20–21 May 2011. Turkish Marine Research Foundation,(In: Turan, C., Öztürk, B. eds.) Istanbul, Turkey.	High
13	3,05	In the taxon's introduced range, are there known adverse socio-economic impacts?	Yes	ÖZTÜRK, B. & ISINIBILIR, M., 2010. An alien jellyfish Rhopilema nomadica and its impacts to the Eastern Mediterranean part of Turkey. Journal of the Black Sea/Mediterranean Environment, 16 (2): 149-156.	High

Appendix 2-(continued)

B. Biology/Ecology					
4. Undesirable (or persistence) traits					
14	4,01	Is it likely that the taxon will be poisonous or pose other risks to human health?	Yes	Gusmani, L., Avian, M., Galil, B., Patriarca, P., Rottini, G., 1997. Biologically active polypeptides in the venom of the jellyfish Rhopilema nomadica. Toxicon, 35(5), 637-648.	High
15	4,02	Is it likely that the taxon will smother one or more native taxa (that are not threatened or protected)?	No	No report/evidence	Medium
16	4,03	Are there any threatened or protected taxa that the non-native taxon would parasitise in the RA area?	No	No evidence	Medium
17	4,04	Is the taxon adaptable in terms of climatic and other environmental conditions, thus enhancing its potential persistence if it has invaded or could invade the RA area?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
18	4,05	Is the taxon likely to disrupt food-web structure/function in aquatic ecosystems if it has invaded or is likely to invade the RA area?	No	The species has not been found to occur and establish in RA area so it is higly unlikely that it could disrupt the ecosystem as such	Medium
19	4,06	Is the taxon likely to exert adverse impacts on ecosystem services in the RA area?	Yes	It is likely that it affects fishing activities by clogging the nets and disrupts gears	Medium
20	4,07	Is it likely that the taxon will host, and/or act as a vector for, recognised pests and infectious agents that are endemic in the RA area?	No	No evidence	Medium
21	4,08	Is it likely that the taxon will host, and/or act as a vector for, recognised pests and infectious agents that are absent from (novel to) the RA area?	No	No evidence	Medium
22	4,09	Is it likely that the taxon will achieve a body size that will make it more likely to be released from captivity?	Not applicable	This species is not kept at captivity	Medium
23	4,10	Is the taxon capable of sustaining itself in a range of water velocity conditions (e.g. versatile in habitat use)?	Yes	ÖZTÜRK, B. & ISINIBILIR, M., 2010. An alien jellyfish Rhopilema nomadica and its impacts to the Eastern Mediterranean part of Turkey. Journal of the Black Sea/Mediterranean Environment, 16 (2): 149-156.	High
24	4,11	Is it likely that the taxon's mode of existence (e.g. excretion of by-products) or behaviours (e.g. feeding) will reduce habitat quality for native taxa?	No	No evidence	Medium
25	4,12	Is the taxon likely to maintain a viable population even when present in low densities (or persisting in adverse conditions by way of a dormant form)?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
5. Resource exploitation					
26	5,01	Is the taxon likely to consume threatened or protected native taxa in the RA area?	No	No evidence	Medium
27	5,02	Is the taxon likely to sequester food resources (including nutrients) to the detriment of native taxa in the RA area?	No	No evidence	Medium
6. Reproduction					
28	6,01	Is the taxon likely to exhibit parental care and/or to reduce age-at-maturity in response to environmental conditions?	No	The species does not have such features	Medium
29	6,02	Is the taxon likely to produce viable gametes or propagules (in the RA area)?	No	No report for maturation nor reproduction is avalable in RA area	Medium
30	6,03	Is the taxon likely to hybridise naturally with native taxa?	Not applicable	This species has no such reproduction system allowing hybridization	High
31	6,04	Is the taxon likely to be hermaphroditic or to display asexual reproduction?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
32	6,05	Is the taxon dependent on the presence of another taxon (or specific habitat features) to complete its life cycle?	No	No evidence	Medium
33	6,06	Is the taxon known (or likely) to produce a large number of propagules or offspring within a short time span (e.g. < 1 year)?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
34	6,07	How many time units (days, months, years) does the taxon require to reach the age-at-first-reproduction?	6	months - N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High

Appendix 2-(continued)

7. Dispersal mechanisms					
35	7,01	How many potential internal vectors/pathways could the taxon use to disperse within the RA area (with suitable habitats nearby)?	One	ballast waters: Koray, K. (2022). Gemi Balast Suları ile Taşınan Yabancı Türlerin eDNA Metabarkodlama Yöntemiyle Tespiti ve Risk Analizleri: İzmit Körfezi. Institute of Science. Ankara Universtiy	Medium
36	7,02	Will any of these vectors/pathways bring the taxon in close proximity to one or more protected areas (e.g. MCZ, MPA, SSSI)?	No	No relevant information is available	Medium
37	7,03	Does the taxon have a means of actively attaching itself to hard substrata (e.g. ship hulls, pilings, buoys) such that it enhances the likelihood of dispersal?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
38	7,04	Is natural dispersal of the taxon likely to occur as eggs (for animals) or as propagules (for plants: seeds, spores) in the RA area?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
39	7,05	Is natural dispersal of the taxon likely to occur as larvae/juveniles (for animals) or as fragments/seedlings (for plants) in the RA area?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
40	7,06	Are older life stages of the taxon likely to migrate in the RA area for reproduction?	No	No evidence	Medium
41	7,07	Are propagules or eggs of the taxon likely to be dispersed in the RA area by other animals?	No	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	Medium
42	7,08	Is dispersal of the taxon along any of the vectors/pathways mentioned in the previous seven questions (35–41; i.e. either unintentional or intentional) likely to be rapid?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	High
43	7,09	Is dispersal of the taxon density dependent?	No	No evidence	Medium
8. Tolerance attributes					
44	8,01	Is the taxon able to withstand being out of water for extended periods (e.g. minimum of one or more hours) at some stage of its life cycle?	No	No evidence	Medium
45	8,02	Is the taxon tolerant of a wide range of water quality conditions relevant to that taxon? [In the Justification field, indicate the relevant water quality variable(s) being considered.]	No	No evidence	Medium
46	8,03	Can the taxon be controlled or eradicated in the wild with chemical, biological, or other agents/means?	No	No report in regard	Medium
47	8,04	Is the taxon likely to tolerate or benefit from environmental/human disturbance?	Yes	Purcell, J. E., Uye, S. I., Lo, W. T., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. Marine Ecology Progress Series, 350, 153-174.	High
48	8,05	Is the taxon able to tolerate salinity levels that are higher or lower than those found in its usual environment?	No	No evidence	Medium
49	8,06	Are there effective natural enemies (predators) of the taxon present in the RA area?	Yes	N. Killi, A.S. Tarkan, S. Kozic, G.H. Copp, P.I. Davison, L. Vilizzi Risk screening of the potential invasiveness of non native jellyfishes in the Mediterranean Sea Mar. Pollut. Bull., 150 (2020) 110728,	Medium
C. Climate change					
9. Climate change					
50	9,01	Under the predicted future climatic conditions, are the risks of entry into the RA area posed by the taxon likely to increase, decrease or not change?	Increase	Based on climate change projections (mainly on global warming) and the species warm-water character	Medium
51	9,02	Under the predicted future climatic conditions, are the risks of establishment posed by the taxon likely to increase, decrease or not change?	No change	As there is no report on establishment of the species in RA area - that's not likely	Medium
52	9,03	Under the predicted future climatic conditions, are the risks of dispersal within the RA area posed by the taxon likely to increase, decrease or not change?	Increase	Based on climate change projections (mainly on global warming) and the species warm-water character	Medium
53	9,04	Under the predicted future climatic conditions, what is the likely magnitude of future potential impacts on biodiversity and/or ecological integrity/status?	No change	As there is no report on establishment of the species in RA area - that's not likely	Medium
54	9,05	Under the predicted future climatic conditions, what is the likely magnitude of future potential impacts on ecosystem structure and/or function?	No change	As there is no report on establishment of the species in RA area - that's not likely	Medium
55	9,06	Under the predicted future climatic conditions, what is the likely magnitude of future potential impacts on ecosystem services/socio-economic factors?	No change	As there is no report on establishment of the species in RA area - that's not likely	Medium

Appendix 2-(continued)

Statistics	
Scores	
BRA	**22,5**
BRA Outcome	**-**
BRA+CCA	**26,5**
BRA+CCA Outcome	**-**
Score partition	
A. Biogeography/Historical	**13,5**
1. Domestication/Cultivation	*0,0*
2. Climate, distribution and introduction risk	*3,0*
3. Invasive elsewhere	*10,5*
B. Biology/Ecology	**9,0**
4. Undesirable (or persistence) traits	*5,0*
5. Resource exploitation	*0,0*
6. Reproduction	*2,0*
7. Dispersal mechanisms	*2,0*
8. Tolerance attributes	*0,0*
C. Climate change	**4,0**
9. Climate change	*4,0*
Answered Questions	
Total	***55***
A. Biogeography/Historical	**13**
1. Domestication/Cultivation	*3*
2. Climate, distribution and introduction risk	*5*
3. Invasive elsewhere	*5*
B. Biology/Ecology	**36**
4. Undesirable (or persistence) traits	*12*
5. Resource exploitation	*2*
6. Reproduction	*7*
7. Dispersal mechanisms	*9*
8. Tolerance attributes	*6*
C. Climate change	**6**
9. Climate change	*6*
Sectors affected	
Commercial	**9**
Environmental	**4**
Species or population nuisance traits	**18**

Thresholds	
BRA	**-**
BRA+CCA	**-**
Confidence	
BRA+CCA	**0,59**
BRA	**0,60**
CCA	**0,50**