LifeWatch

e-Science European Infrastructure for Biodiversity and Ecosystem Research



Ricerca di microalghe tossiche con approcci di DNA metabarcoding

Biomolecular Thematic Centre (BTC)



Maria Paola Tomasino

CENTRI TEMATICI & PIANO DELLE ATTIVITA' 2016-2017 CNR | Aula Bisogno Sede Centrale 15-17 febbraio 2016 ROMA



The terms HARMAFUL ALGAL BLOOMS (HABs) cover a heterogeneous set of events. HABs result from noxious and/or toxic algae that cause direct or indirect negative impact on aquatic ecosystems, costal resources and human health (M. Anderson, 2009)



Kudela et al. 2015 A Scientific Summary for Policy Makers. IOC/UNESCO, R.M.

4 categories of deleterious effects related to **marine microalgae**:

- Risks for human health
- Impact on living marine resources
- Impact on tourism and recreational activities
- Damage to the marine ecosystem (Zingone &. Enevolsden 2000)





About 110 over more than 5000 known microalgae produce toxins



These toxins are accumulated by marine animals and may cause several human syndromes

Paralytic shellfish poisoning (PSP) Diarrhetic shellfish poisoning (DSP) Neurotoxic shellfish poisoning (NSP) Amnesic shellfish poisoning (ASP) Ciguatera fish poisoning (CFP) Azaspiracid shellfish poisoning (AZP)

Respiratory problems and dermatitis Hepatotoxicity Clupeotoxism or cause fish kills and other damages to marine fauna



HABs are a worldwide phenomenon, whose socio-economic impact is increasing in severity, frequency and biogeographical range

cost of HABs

CONSERVATIVE ANNUAL COST

Marine HABs

| USA | ± | US\$ | 95 million |
|--------|---|------|-------------|
| Europe | > | US\$ | 850 million |
| Asia | > | US\$ | 1 billion |

Freshwater HABs

| USA | ± US\$ 4,6 billion |
|--------------|-------------------------------------|
| China | ± US\$ 6,5 billion (1998, Lake Tai) |
| Australia | ± US\$ 150 million |
| UK | ± US\$ 150 million |
| South Africa | ± US\$ 250 million |

Kudela et al. 2015 A Scientific Summary for Policy Makers. IOC/UNESCO

monitoring, and mitigation should be considered, because these activities can diminish the economic losses



http://www.geohab.info/

economic damages concern fisheries, aquaculture, tourism, desalination plants, drinking water supply, health care

- ✓ Support of HABs monitoring and management
- ✓ Prevention of HABs
- ✓ Modelling and prediction
- International coordination
- ✓ Research new technologies

HAB monitoring



The Global HAB Status Report, by the IOC UNESCO, IAEA, PICES and ICES, is based on data from various sources including OBIS and IODE/HAEDAT and will complement broader environment assessments.

New Tools and new Technologies to detect Harmful Microalgae (HMa)



To test the feasibility and the effectiveness of a High-Throughput Sequencing (HTS)-DNA metabarcoding approach to track geographic ranges of Harmful Microalgae (HMa)



What is a eDNA metabarcoding approach?

combines three concepts:





eDNA metabarcoding is a rapidly olving method for assessing biodiversity

PREPARATION OF THE Reference Database 18S-V4- Harmful Microalgae

CBOL Protist Working Group: Barcoding Eukaryotic Richness beyond the Animal, Plant, and Fungal Kingdoms

Jan Pawlowski
, Stéphane Audic, Sina Adl, David Bass, Lassaâd Belbahri, Cédric Berney, Samuel S. Bowser, Ivan Cepicka, Johan Decelle, Micah Dunthorn, Anna Maria Fiore-Donno, Gillian H. Gile, Maria Holzmann, [...], Colomban de Vargas
[view all]



Published: November 6, 2012 • DOI: 10.1371/journal.pbio.1001419

Barcode in Protist the V4 region of 18S rDNA

STEP 1:

BUILD UP THE REFERENCE DATABASE (18S-V4-HMa db)

HMa information retrieved from the IOC-UNESCO Taxonomic Reference List of Harmful Microalgae

HMa names and harmfulness data checked and updated, leading to a final list of 113 HMa belonging to 34 genera

18S sequences of HMa and their non-toxic congeneric downloaded from NCBI and PR₂

Screening and filtering of 18S sequences V4 hypervariable region extraction

Analysis of V4 discrimination power at three similarity levels

100% - 99.5% - 99%





http://www.marinespecies.org/hab

It provides a catalogue of the world's most harmful micro algal species

Eliminated

- Alexandrium balechii
- Chattonella japonica
- Prorocentrum micans

Added

• *Azadinium dexteroporum* (Percopo & Zingone, 2013)

New name for

• Alexandrium tamarense Species Complex (revisioned by John et al. 2014)

OUR Modifications!

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100% - 99.5% - 99%



18S-V4-HMa db DATABASE



Quality-checked 18S sequences available for 89 out of 113 HARMFUL MICROALGAE

no molecular 18S data available

V4 discrimination power

good- not shared V4 region

shared V4 region between toxic and non-toxic congeneric shared V4 region between two toxic congeneric

* shared V4 region among different genera

| | | 100% | 99.5% | 99% |
|----|--------------------------|------|--------------|-----|
| \$ | Alexandrium acatenella | | | |
| \$ | Alexandrium andersonii | | | |
| 0 | Alexandrium australiense | | | |

Discrimination of V4 at different similarity thresholds 63 species (of 89) at 100% 41 at 99.5% 33 at 99%



Take home messages

- Careful evaluation of similarity threshold in HTS studies on HMa distribution
- Need for 18S reference sequences for more HMa

18S-V4 HMa custom database



Harmful Microalgae across the WORLD

OCEAN SAMPLING DAY 18S-V4-dataset







L'Ocean Sampling Day (OSD)

June 21st -Summer solstice



••• • Map of OSD Participant sites



Ocean Sampling Day





Ocean Sampling Day @LTER-MC Stazione Zoologica Anton Dohrn (OSD4 station)







185 sampling sites



LTER-MC OSD 4

In the wet lab –Standardized protocols-







Labelling









Centralized DNA extraction at MPIMM Bremen, Germany and sequencing

STEP 2:

map the spatial distribution of Harmful Microalgae across OSD sites BLASTING THE REFERENCE DATA OBTAINED IN STEP 1 (18S-V4-HMa db) AGAINST





Ocean Sampling Day LGC 18S-V4-amplicon worldwide dataset 155 sites (5.04 M sequences)

IBBB

LifeWatch



Exploring spatial and temporal distribution of harmful microalgae with HTS-based DNA-metabarcoding data

M.P. Tomasino^{1,2}, R. Piredda², P. Sánchez³, B. Duarte⁴, B. Fosso¹, I. Caçador⁴ L. Amaral-Zettler⁵, G. Pesole¹, A. Zingone²

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ugu

48 different HMa species across 155 OSD sites

top 10 OSD sites in terms of the number of different HMa species detected

ASD DSD DSD other

| Top 10 richest OSD sites | ASP | PSP |) | DSP | | othe | r # | t of HMa |
|-------------------------------------|-----|-----|---|-----|---|------|-----|----------|
| OSD15_NW_MediterraneanSea | | 0 | 3 | | 1 | 1 | 1 | 15 |
| OSD51_Caribbean Sea | | 2 | 0 | | 1 | .0 | Э 🤇 | 12 |
| OSD78_Adriatic Sea | | 0 | 3 | | 1 | .0 | 3 | 12 |
| OSD124_Japan Sea | | 3 | 2 | | 1 | .0 | 5 | 12 |
| OSD154_Bay of Biscay | | 1 | 1 | | 1 | .0 | 3 🤇 | 11 |
| OSD1_English Channel | | 2 | 3 | | 1 | | 1 | 10 |
| OSD2_English Channel | | 3 | 1 | | 1 | | 5 | 10 |
| OSD22_MediterraneanSea_WesternBasin | | 1 | 1 | | 0 | .0 | 3 | 10 |
| OSD43_North Pacific Ocean | | 2 | 0 | | 0 | .0 | 3 | 10 |
| OSD54_North Atlantic Ocean | | 1 | 2 | | 2 | | 5 | 10 |
| OSD95_Singapore Strait | | 0 | 2 | | 1 | .0 | 7 | 10 |



top 10 sites in terms of abundance of harmful species

| Top 10 OSD toxic sites | #seqs | Most abundant Hma specie | % |
|--|-------|---|-------|
| OSD61_Vineyard Sound_North_Atlantic_Ocean_MA,US | 5088 | Alexandrium pseudogonyaulax /A. hiranoi | 98,92 |
| OSD157-1m-depth_Skagerrak_North Sea | 3129 | Alexandrium pseudogonyaulax /A. hiranoi | 98,82 |
| OSD152-5m-depth_Bedford Basin_North_Atlantic_Ocean | 2073 | Dinophysis spp. | 82,34 |
| OSD41_Sequim Bay Park site, WA, US | 1596 | Heterosigma akashiwo | 98,68 |
| OSD126_Eyafjordur_1_Greenland_Sea | 1365 | Alexandrium fundyense-Groupl | 54,95 |
| OSD130_Eyafjordur_5_Greenland_Sea | 1092 | Alexandrium fundyense-Groupl | 91,76 |
| OSD30_Tvarminne_Gulf_of_Finland | 1066 | Dinophysis spp. | 97,94 |
| OSD146_Fram Strait_Greenland_Sea | 1056 | Phaeocystis pouchetii | 97,54 |
| OSD159_Brest_Celtic_Sea | 875 | Alexandrium minutum /A. insuetum | 97,03 |
| OSD2_Roscoff_English_Channel | 796 | Phaeocystis globosa | 88,19 |

the main species for each site and their percentage over the total HMa sequences at that site

Distribution of the main harmful species across OSD sites grouped by type of harmful effect.



- High latitude sites (60° N) showed the highest HMa sequence abundance, while at lower latitudes the highest diversity of HMa was found
- Ichthyotoxic species were the most abundant and widespread followed by PSP- and DSPcausing species in the OSD dataset

Conclusions

- HTS-DNA metabarcoding proved to be a powerful tool for the assessment of HMa spatial patterns provided information on their distribution along with the detection of blooms at some sites and possible hot-spot sites for HMa diversity.
- These results open the way to a more widespread application of this approach in monitoring practices as well as in occasional surveys in areas not covered by such monitoring.

Deeper HTS at a selected OSD sites (LifeWatch dataset) are
 also explored



NumOfsegs

 Deeper HTS at a selected OSD sites (LifeWatch dataset) are also explored



LGC LW

 BioMaS pipeline (Bioinformatic analysis of Metagenomic AmpliconS) with HMa 18S-V4 db



- A custom V9-HMa database will be used to compare V4 and V9 detection and discrimination capability.
- Multimarker approach for discrimination: more variable markers, i.e. LSU-rDNA and ITS-rDNA, should be tested

HTS-DNA metabarcoding of HMa across temporal scale
 LTER-MareChiara, Gulf of Naples





Citizen Science initiative : MY OSD 2015 in the GULF of NAPLES

Thanks to the MyOSD initiative this year citizens were involved in the OSD worldwide scientific campaign.



MyOSD HUB: Stazione Zoologica of Anton Dohrn of Naples_OSD4 LTER-MC



The Stazione Zoologica Anton Dohrn of Naples, as HUB-MyOSD, has distributed Sampling Kits to citizens that have contributed, together with scientists, to a deeper understanding of the oceans.























MY OSD_2015 Naples











https://www.microb3.eu/events/workshops/osd-2016



Home

OSD & MyOSD 2016

to be developed



Date: Tuesday, June 21, 2016 Location: worldwide Topic: Ocean Sampling Day [] inv [0] Tweet Gel [0]

THANK YOU



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