

Database of Metagenomes of Sediments from Estuarine Aquaculture Farms in Portugal—AquaRAM Project Collection

Teresa Nogueira ^{1,2,*}, Daniel G. Silva ^{1,2}, Susana Lopes ³ and Ana Botelho ¹

¹ INIAV—National Institute for Agrarian and Veterinary Research, IP, 2780-157 Oeiras, Portugal

² cE3c—Center for Ecology, Evolution and Environmental Changes & CHANGE—Global Change and Sustainability Institute, 1749-016 Lisbon, Portugal

³ CIBIO—Centro de Investigação em Biodiversidade e Recursos Genéticos, 4485-661 Vairão, Portugal

* Correspondence: teresa.nogueira@iniav.pt

Abstract: Aquaculture farms and estuarine environments close to human activities play a critical role in the interaction between aquatic and terrestrial surroundings and animal and human health. The AquaRAM project aimed to study estuarine aquaculture farms in Portugal as a reservoir of antibiotic resistance genes and the potential of its spread due to mobile genetic elements. We have assembled a collection of metagenomic data from 30 sediment samples from oysters, mussels, and gilt-head sea bream aquaculture farms. This collection includes samples of the estuarine environment of three rivers and one lagoon located from the north to the south of Portugal, namely, the Lima River in Viana do Castelo, Aveiro Lagoon in Aveiro, Tagus River in Alcochete, and Sado River in Setúbal. Statistical data from the raw metagenome files, as well as the file sizes of the assembled nucleotide and protein sequences, are also presented. The link to the statistics and the download page for all the metagenomes is also listed below.

Citation: Nogueira, T.; Silva, D.G.; Lopes, S.; Botelho, A. Database of Metagenomes of Sediments from Estuarine Aquaculture Farms in Portugal—AquaRAM Project Collection. *Data* **2022**, *7*, 167. <https://doi.org/10.3390/data7110167>

Dataset: <https://www.mg-rast.org/mgmain.html?mgpage=search&search=AquaRAM>.

Dataset License: CC-BY-NC

Keywords: estuary river; aquaculture farm; metagenome; oyster aquaculture; sea bream aquaculture; sediment

Academic Editor: Pufeng Du

Received: 4 October 2022

Accepted: 27 October 2022

Published: 20 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Summary

In Portugal, more than 80 aquaculture projects are underway through the March 2020 operational program (<https://www.mar2020.pt/>, accessed on 31 October 2022). These projects aim to increase the productivity and diversification of seafood products, namely sea bass, sea bream, oysters, clams, and mussels, and to mitigate their diseases. Furthermore, the AquaRAM (Antimicrobial Resistance Determinants in Aquaculture Environments) project (<https://projects.inia.pt/aquaram/>, accessed on 31 October 2022) is an exploratory study with different funding and purposes, which aims to identify reservoirs of antimicrobial resistance by characterizing the antimicrobial resistance profile (resistome) and the mobilome (the set of mobile genetic elements) of aquaculture environments. Antibiotics are often used to mitigate diseases in aquaculture but can also increase antimicrobial resistance in existing bacteria, which can be transmitted to the environment and to humans through food consumption. Thus, the AquaRAM project provides information on the consequences of the overuse of antibiotics in aquaculture to control diseases that are increasing in food production, complementing the March 2020 projects. To our knowledge, AquaRAM is the first study in Portugal on this subject, while in China and Scandinavian countries, many researchers have addressed this issue.

This project focused on the extensive and semi-intensive aquaculture of shellfish (oysters and mussels) and gilt-head sea bream located in several Portuguese river estuaries to assess the possible risk to public health [1]. Since aquaculture environments are at the hinge between animal and human health, these data are crucial to implementing new strategies to control multi-resistant bacteria in aquaculture environments.

During the development of this project, a collection of metagenomes was generated whose data we grouped in this publication. It has been used to study the role of estuarine aquacultures in the spread of antibiotic resistance in animal production [1]. It could also be used to perform comparative metagenomics studies, through phylogenetic, functional, and metabolic diversity analysis, with other metagenomes from aquatic environments.

2. Data Description

2.1. Metagenomes Metadata

Figure 1 shows the geographical location of the sediment samples that were used in this work. These were collected from Portuguese aquaculture farms located in the estuaries of the Lima River in Viana do Castelo, the Aveiro Lagoon in Aveiro, the Tagus River in Alcochete, and the Sado River in Setúbal.

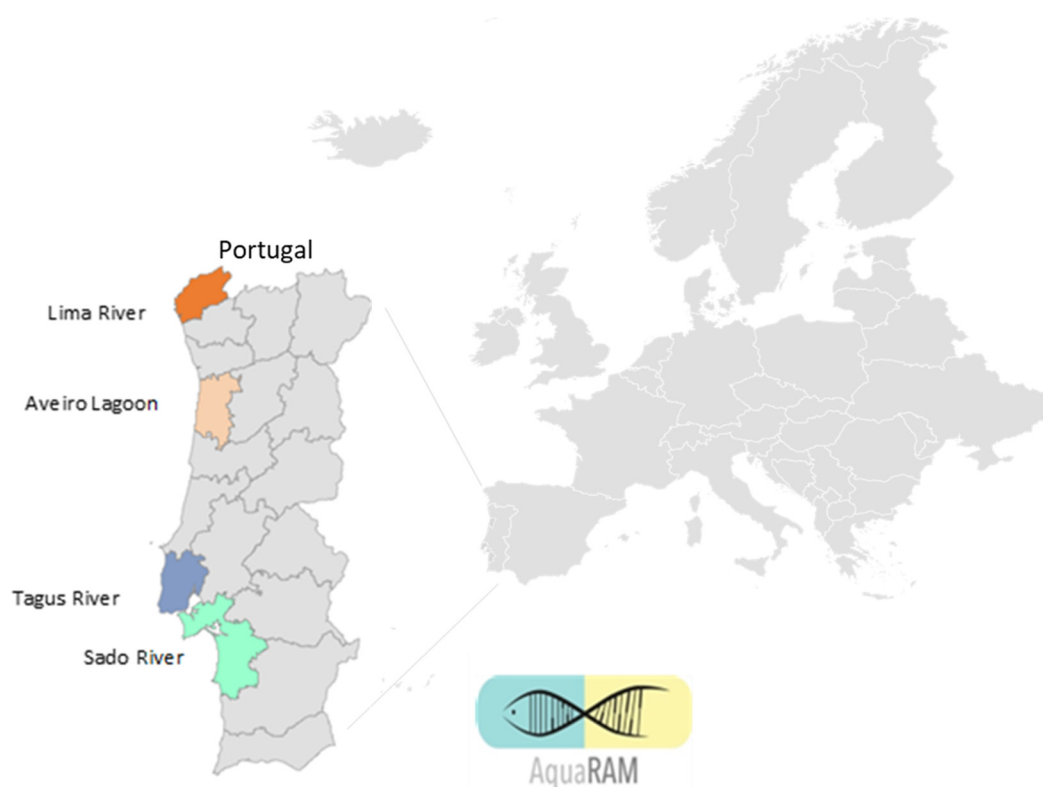


Figure 1. Map of mainland Portugal with the geographical regions where the samples of sediments from the aquaculture farms were collected. Regions where the estuaries of Lima River at Viana do Castelo (orange), Aveiro lagoon at Aveiro (salmon), Tagus River at Alcochete (dark blue), and Sado River (turquoise) are represented.

Table 1 summarizes the information on the collection of samples.

Table 1. Descriptors of the metagenomes samples.

Metagenome ID	Location	Region	Aquaculture Farm
mgm4949363.3	Aveiro Lagoon	Aveiro	Oyster
mgm4949369.3	Aveiro Lagoon	Aveiro	Oyster
mgm4949371.3	Aveiro Lagoon	Aveiro	Oyster
mgm4949372.3	Aveiro Lagoon	Aveiro	Oyster
mgm4949374.3	Lima River	Viana do Castelo	Oyster
mgm4949362.3	Lima River	Viana do Castelo	Oyster
mgm4949373.3	Lima River	Viana do Castelo	Oyster
mgm4949367.3	Tagus River	Alcochete	Mussel
mgm4949365.3	Tagus River	Alcochete	Mussel
mgm4949364.3	Tagus River	Alcochete	Mussel
mgm4949366.3	Tagus River	Alcochete	Mussel
mgm4949368.3	Tagus River	Alcochete	Mussel
mgm4949370.3	Tagus River	Alcochete	Mussel
mgm4954830.3	Sado River	Setúbal	Oyster
mgm4954821.3	Sado River	Setúbal	Oyster
mgm4954835.3	Sado River	Setúbal	Gilt-head bream
mgm4954834.3	Sado River	Setúbal	Gilt-head bream
mgm4954833.3	Sado River	Setúbal	Gilt-head bream
mgm4954831.3	Sado River	Setúbal	Gilt-head bream
mgm4954819.3	Sado River	Setúbal	Oyster
mgm4954820.3	Sado River	Setúbal	Oyster
mgm4954822.3	Sado River	Setúbal	Oyster
mgm4954818.3	Sado River	Setúbal	Oyster
mgm4954814.3	Sado River	Setúbal	Oyster
mgm4954832.3	Sado River	Setúbal	Oyster
mgm4954828.3	Sado River	Setúbal	Oyster
mgm4954836.3	Sado River	Setúbal	Oyster
mgm4954815.3	Sado River	Setúbal	Oyster
mgm4965131.3	Sado River	Setúbal	Oyster
mgm4954827.3	Sado River	Setúbal	Oyster

2.2. Metagenomse Statistics

All samples described in this work were sequenced by Illumina whole genome sequencing, and the metagenomes were processed and assembled on the MG-RAST (metagenomic rapid annotations using subsystems technology) server that performs an automatic phylogenetic and functional analysis of metagenomes. All generated files were stored in the MG-RAST server [1]. Table 2 summarizes the statistics of each metagenome, along with the link to the download page for each one.

Table 2. Statistics of the metagenomes.

Metagenome ID	Alpha Diversity Shannon	Average G/C Ratio Raw	Sequence Count Raw	Drisee Score Raw	Bp Count Raw	Bp Count Assembled	Protein Count 90%ID	Link
mgm4949363.3	591.63705 3453836	0.842	20,654,382	6.365	2,144,206,815	2,049,334,118	17,823,673	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949363.3
mgm4949369.3	591.63054 7118449	0.884	24,795,851	6.43	2,566,827,365	2,457,224,832	213,23,413	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949369.3
mgm4949371.3	597.26045 0471211	0.871	23,062,780	2.621	2,385,579,765	2,285,976,860	19,964,192	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949371.3
mgm4949372.3	600.49336 0405826	0.86	22,511,192	6.078	2,326,919,432	2,238,729,670	19,578,879	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949372.3
mgm4949374.3	466.27483 4842257	0.947	22,797,392	6.103	2,357,486,075	2,266,541,844	19,867,700	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949374.3
mgm4949362.3	528.76349 2952932	0.899	20,585,054	6.202	2,130,185,448	2,053,011,088	17,914,481	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949362.3
mgm4949373.3	545.35085 3121357	0.735	21,090,611	3.757	2,180,678,728	2,101,110,980	18,453,781	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949373.3
mgm4949367.3	667.56807 3322797	0.843	23,247,947	5.696	2,403,320,280	2,287,296,662	19,189,502	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949367.3
mgm4949365.3	632.41916 1801153	0.938	21,152,725	4.55	2,176,060,316	2,103,266,398	17,774,253	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949365.3
mgm4949364.3	645.83703 1370394	0.729	22,097,588	6.286	2,292,471,984	2,203,904,347	19,219,435	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949364.3
mgm4949366.3	655.79212 7270225	0.819	19,886,839	8.953	2,052,842,601	1,971,021,097	16,966,633	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949366.3
mgm4949368.3	607.27455 3767903	0.864	20,387,608	6.723	2,116,474,291	2,017,580,191	16,620,530	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949368.3
mgm4949370.3	640.51003 6790802	0.818	22,019,976	2.336	2,273,521,861	2,164,355,645	18,576,898	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4949370.3

mgm4954830.3	641.67086 2524688	0.861	33,669,439	8.871	3,440,035,053	3,204,168,236	28,274,537	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954830.3
mgm4954821.3	614.87486 7071491	0.835	23,650,874	8.182	2,410,762,118	2,242,709,031	19,160,418	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954821.3
mgm4954835.3	646.58651 569641	0.845	30,980,617	13.876	3,158,499,524	2,966,342,765	26,293,556	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954835.3
mgm4954834.3	510.78878 0077535	0.94	36,071,738	7.196	3,681,751,495	3,412,996,636	29,747,922	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954834.3
mgm4954833.3	406.13298 0410524	0.987	25,704,969	6.346	2,623,296,493	2,423,677,881	21,035,945	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954833.3
mgm4954831.3	486.01025 6625849	0.925	54,252,270	5.723	5,529,853,067	5,048,830,547	43,944,373	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954831.3
mgm4954819.3	592.29928 986737	0.79	33,489,968	2.503	3,410,510,642	3,221,617,003	28,358,490	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954819.3
mgm4954820.3	555.90834 6951917	0.804	39,087,412	9.767	3,979,101,606	3,722,807,329	32,696,139	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954820.3
mgm4954822.3	643.16462 9017245	0.806	52,405,104	7.122	5,326,248,790	4,958,596,344	43,897,599	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954822.3
mgm4954818.3	547.81610 6966442	0.891	34,206,159	2.935	3,483,067,311	3,117,389,965	25,059,315	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954818.3
mgm4954814.3	581.62934 7916998	0.847	46,313,426	2.513	4,708,594,822	4,350,188,356	25,985,847	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954814.3
mgm4954832.3	519.86726 1265164	0.792	42,577,822	6.879	4,328,343,205	4,033,164,629	35,873,696	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954832.3
mgm4954828.3	555.88482 5824692	0.811	51,868,875	2.268	5,275,370,523	4,884,381,025	43,110,558	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954828.3
mgm4954836.3	542.08169 1624614	0.797	49,865,118	7.48	5,071,677,080	4,681,279,515	41,401,104	https://www.mg-rast.org/mgmain.html?mgpage=overview&meta-genome=mgm4954836.3

mgm4954815.3	520.60499 3771978	0.764	46,046,824	2.593	4,683,855,947	4,363,590,883	38,763,159	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4954815.3
mgm4965131.3	556.11346 7628642	0.84	57,186,044	6.78	5,819,613,801	5,048,830,547	43,944,373	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4965131.3
mgm4954827.3	507.06715 9172064	0.788	40,965,911	3.355	4,160,446,023	3,881,700,575	34,366,229	https://www.mg-rast.org/mgmain.html?mgpage=overview&metagenome=mgm4954827.3

The Shannon alpha diversity index summarizes the phylogenetic abundances in each metagenome in terms of their richness (the number of species/clades) and evenness (inequalities between species abundances).

The average G/C ratio, base pairs count, and sequence counts of the raw sequence reads are also presented. The duplicate read inferred sequencing error estimation (DRI-SEE) score of the raw sequence gives an estimation of the sequencing error [2].

The columns “Bp count assembled” and “protein count 90%id” present two other measures regarding the size of the assembled metagenomes, namely, the number of base pairs of the assembled metagenomes and the number of proteins clustered with a 90% identity.

2.3. Metagenomes Database

Table 3 brings together the information on all the identifiers of each metagenome, as well as the hyperlink to the page with the link to all the downloadable MG-RAST pipeline output files.

Table 3. Identifiers and links to the metagenomes’ files.

Metagenome ID	Library ID	Library Name	Sample ID	Link
mgm4949363.3	mgl860512	mgshot_S4432Nr1	mgs860510	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949363.3
mgm4949369.3	mgl860515	mgshot_S4432Nr2	mgs860513	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949369.3
mgm4949371.3	mgl860518	mgshot_S4432Nr3	mgs860516	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949371.3
mgm4949372.3	mgl860521	mgshot_S4432Nr4	mgs860519	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949372.3
mgm4949374.3	mgl860524	mgshot_S4432Nr5	mgs860522	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949374.3
mgm4949362.3	mgl860527	mgshot_S4432Nr6	mgs860525	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949362.3
mgm4949373.3	mgl860530	mgshot_S4432Nr7	mgs860528	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949373.3

mgm4949367.3	mgl860533	mgshot_S4432Nr8	mgs860531	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949367.3
mgm4949365.3	mgl860536	mgshot_S4432Nr9	mgs860534	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949365.3
mgm4949364.3	mgl860539	mgshot_S4432Nr10	mgs860537	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949364.3
mgm4949366.3	mgl860542	mgshot_S4432Nr11	mgs860540	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949366.3
mgm4949368.3	mgl860545	mgshot_S4432Nr12	mgs860543	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949368.3
mgm4949370.3	mgl860548	mgshot_S4432Nr13	mgs860546	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4949370.3
mgm4954830.3	mgl866557	mgshot_S4659Nr1	mgs866555	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954830.3
mgm4954821.3	mgl866560	mgshot_S4659Nr2	mgs866558	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954821.3
mgm4954835.3	mgl866563	mgshot_S4659Nr3	mgs866561	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954835.3
mgm4954834.3	mgl866566	mgshot_S4659Nr4	mgs866564	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954834.3
mgm4954833.3	mgl866569	mgshot_S4659Nr5	mgs866567	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954833.3
mgm4954831.3	mgl866572	mgshot_S4659Nr6	mgs866570	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954831.3
mgm4954819.3	mgl866575	mgshot_S4659Nr7	mgs866573	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954819.3
mgm4954820.3	mgl866578	mgshot_S4659Nr8	mgs866576	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954820.3
mgm4954822.3	mgl866590	mgshot_S4659Nr12	mgs866588	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954822.3
mgm4954818.3	mgl866593	mgshot_S4659Nr13	mgs866591	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954818.3

mgm4954814.3	mgl866596	mgshot_S4659Nr14	mgs866594	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954814.3
mgm4954832.3	mgl866599	mgshot_S4659Nr15	mgs866597	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954832.3
mgm4954828.3	mgl866602	mgshot_S4659Nr16	mgs866600	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954828.3
mgm4954836.3	mgl866608	mgshot_S4659Nr18	mgs866606	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954836.3
mgm4954815.3	mgl866614	mgshot_S4659Nr20	mgs866612	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954815.3
mgm4965131.3	mgl876698	mgshot_S4659Nr21	mgs866615	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4965131.3
mgm4954827.3	mgl866623	mgshot_S4659Nr23	mgs866621	https://www.mg-rast.org/mgmain.html?mgpage=download&metagenome=mgm4954827.3

3. Methods

At each site, samples were taken from the top 5 cm layer, stored in sterile polypropylene centrifuge tubes (10 ml), and immediately transported refrigerated to the laboratory where they were kept at -20°C until analysis. The total DNA was extracted from the frozen sediment samples using the DNeasy PowerSoil Pro Kit (qiagen.com). DNA was sent to CeGaT (<https://www.cebga.com/>) in Germany in frozen isothermal boxes. Libraries for shotgun metagenome sequencing were prepared using an Illumina DNA (M) Tagmentation Library Prep kit from Illumina, followed by NovaSeq 6000, 2× 100 bp sequencing. The demultiplexing of the sequencing reads was performed with Illumina bcl2fastq (2.20), and adapters were trimmed with Skewer (version 0.2.2) [3]. The quality of the FASTQ files was analyzed with FastQC (version 0.11.5-cegat) [4]. The Illumina raw sequence data file pairs in the FASTQ format were assembled on the MG-RAST metagenomic analysis server [5]. The pipeline options chosen were the removal of artificial replicate sequences, any host-specific *Homo sapiens* NCBI v36 specie sequence, as well as low-quality sequences. The lowest Phred scores to count as a high-quality base were set to 15 and trimmed to, at most, 5 low Phred score bases. All the resulting files can be assessed at <https://www.mg-rast.org/mgmain.html?mgpage=search&search=mgp95904>.

Programmatic access to the files containing the assembled DNA contigs and protein sequences and the computation of the size of each metagenome was performed through bash scripting in a Linux environment.

4. User Notes

The MG-RAST server enables programmatical access to the collection AquaRAM using the following paths: <https://api.mg-rast.org/download/<Metagenome id>?file=299.1> (FASTA format files containing the assembled DNA contigs); and <https://api.mg-rast.org/download/<Metagenome id>?file=550.2> (FASTA format files containing the protein sequences), clustered at a 90% identity, where the string <Metagenome id> is listed in the first column of Table 2.

Author Contributions: Conceptualization, T.N.; methodology, T.N.; investigation, T.N., D.G.S., and S.L.; resources, A.B.; data curation, D.G.S. and T.N.; writing—original draft preparation, T.N.; writing—review and editing, D.G.S.; supervision, T.N.; project administration, T.N. and A.B.; funding acquisition, A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research and the APC were funded by Fundação para a Ciência e a Tecnologia, grant number ALG-01-0145-FEDER-028824 and PTDC/BIA-MIC/28824/2017.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The dataset generated in this study is publicly archived in the MG-RAST repository: <https://www.mg-rast.org/mgmain.html?mgpage=search&search=mgp95904>.

Acknowledgments: The authors would like to thank the team members of the AquaRAM project at INIAV (Cristina Ferreira, Sandra Cavaco, Leonor Orge, and Andreia Freitas), and at IPMA (Florabela Soares, Ana Maulvault, and Patrícia Anacleto) for providing, collecting, and preparing the sediment samples used in this work.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Silva, D.G.; Domingues, C.P.F.; João F. Figueiredo; Francisco Dionisio; Ana Botelho; Teresme. a Nogueira Estuarine Aquacultures at the Crossroads of Animal Production and Antibacterial Resistance: A Metagenomic Approach to the Resist. *Biology* **2022**, *11*.
2. Keegan, K.P.; Trimble, W.L.; Wilkening, J.; Wilke, A.; Harrison, T.; D'Souza, M.; Meyer, F. A Platform-Independent Method for Detecting Errors in Metagenomic Sequencing Data: DRISSE. *PLOS Comput. Biol.* **2012**, *8*, e1002541. <https://doi.org/10.1371/journal.pcbi.1002541>.
3. Jiang, H.; Lei, R.; Ding, S.-W.; Zhu, S. Skewer: A Fast and Accurate Adapter Trimmer for next-Generation Sequencing Paired-End Reads. *BMC Bioinform.* **2014**, *15*, 182. <https://doi.org/10.1186/1471-2105-15-182>.
4. Andrews, S. *FastQC: A Quality Control Tool for High Throughput Sequence Data*; Babraham Bioinformatics, Babraham Institute: Cambridge, UK, 2010.
5. Meyer, F.; Paarmann, D.; D'Souza, M.; Olson, R.; Glass, E.; Kubal, M.; Paczian, T.; Rodriguez, A.; Stevens, R.; Wilke, A.; et al. The Metagenomics RAST Server – a Public Resource for the Automatic Phylogenetic and Functional Analysis of Metagenomes. *BMC Bioinform.* **2008**, *9*, 386. <https://doi.org/10.1186/1471-2105-9-386>.