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Article

Contribution of environmental factors to genetic variation in the Pacific white-sided dolphin

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SUMMARY

Environmental changes driven by pollution and climate change have affected many species globally. The Pacific white-sided dolphin, or *Lagenorhynchus obliquidens* (*L. obliquidens*), is threatened by water pollution, which is the product of human actions and can impact the genetic structure of species in affected areas. While researchers have studied genetic differences between populations of *L. obliquidens* in Japan, they failed to explore the potential environmental drivers of these differences. Our research aimed to identify the major environmental factors impacting the genetic variation of *L. obliquidens*, which will increase our understanding of how this species responds to changes in the environment. We studied several different variables, including salinity, ocean currents, temperature, and microplastic levels. Due to the significant presence and variation of microplastics in the ocean surrounding Japan, we hypothesized that this would have the most notable impact on the genetic variation of *L. obliquidens*. We began our research by gathering genetic sequencing data from a preexisting study focusing on populations along the Japanese coast. We collected environmental data and analyzed it alongside a genetic distance matrix, leveraging data analysis and machine learning tools in R. We found that the most impactful variable was current stability, which did not match our original prediction of microplastic levels having the most significant impact. We then investigated why ocean currents may have had such a strong impact on *L. obliquidens* and proposed that it has a connection to nutrient and prey distribution, which can be majorly impacted by changes in ocean currents.

INTRODUCTION

The environment of an organism is key to determining many of its characteristics. The genetic structure of several organisms has previously been shown to have some correlation with environmental variables, which is the product of certain genetic adaptions being better suited to different environments. A variety of environmental factors, such as temperature, salinity, pollution, and more, have been linked to genetic structure, particularly the variation or mutations that are prevalent within a given population (1-4). The environment is also rapidly changing in many areas due to climate change, making it even more relevant to studying modern species.

Lagenorhynchus obliquidens (*L. obliquidens*), commonly known as the Pacific white-sided dolphin, is threatened by the rate of global warming and struggling to adapt (5). This species typically resides in the colder regions of the Pacific Ocean in areas where the water is deep (6). While there used to be large populations of *L. obliquidens* in the Gulf of California, warming ocean waters have impacted the animals, reducing their population size by one order of magnitude per decade from 1980 through 2009 (7). The negative impact of warmer oceans is further shown by the northward shift of *L. obliquidens*. As populations in the Gulf of California have decreased, populations of *L. obliquidens* further north, along the west coast of Canada, have increased due to migration (7). These temperature changes can alter habitat and nutrient availability (8). Warming waters can also lead to acute thermal stress in organisms such as macroalgae, reducing the population (8). However, the study of shifting populations of *L. obliquidens* did not pinpoint the reason why they prefer more temperate waters; it simply indicated that the organisms have a general preference for more temperate waters and adjusted their range following that (7).

In addition to temperature having an impact on *L. obliquidens*, environmental variables like microplastic levels, salinity, and current can also affect populations (2-4). Microplastic levels can vary depending on population density in a certain area, as larger cities will produce more plastic and more isolated areas will have less waste. There are multiple examples of microplastics found along the coast of Japan, including polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) (9). PBDEs are common flame retardants that can be found in the air, water, and soil, meaning that organisms can be exposed through their environment alone (10). PCBs are most often used as insulating electrical fluids but can also be used in other hydraulic or lubricating fluids (11). According to a study of PBDE levels in Japan, the results were inconsistent with the urbanity of nearby areas and did not have a pattern, unlike PCBs. PCBs varied between urban and isolated areas, with significantly higher concentrations of PCBs in urban areas (9). Increased microplastic levels in the ocean lead to more organisms ingesting greater amounts of this potentially harmful substance, which impacts overall populations in addition to individual organisms in affected areas (12). In dolphins, microplastics can pose a health risk and cause conditions such as hyperthyroidism, in which an organism's thyroid releases high levels of hormones (13).

Salinity is an essential part of the environment for ocean-dwelling organisms, so any change in salinity levels can have major detrimental impacts on an ecosystem (14). Salinity can impact water circulation, as increased salinity leads to increased water density, which causes a more noticeable response in water circulation to precipitation and evaporation. In addition, increasing salinity decreases the freezing point of water, making it more difficult to form sea ice. This directly impacts ocean currents, and it contributes to global warming due to the reduced amounts of sea ice (15). Previously, studies have shown that salinity influences the genetic variation of ocean species, affecting genes linked to sodium-activated ion transport, kidney development, adipogenesis, and thermogenesis (16).

Ocean currents impact heat and nutrient distribution as well as the prey available to *L. obliquidens*, which typically feeds on small fish and squid (6, 17). The alteration in prey availability occurs because ocean currents affect migration patterns, changing the species diversity in a given area (17). Changes in ocean currents can result from climate change and volatile weather. For example, melting glaciers can lead to altered heat distribution, and inconsistent winds cause changing current strength (17).

Past research has shown a correlation between various environmental factors and genetic differences among populations. One study conducted in the Great Barrier Reef examined the impact of environmental factors on corals, specifically *Acropora millepora* and *Pocillopora damicornis* (1). The study found varying allele frequencies between two unique environments. It specifically studied water clarity and temperature within the reef and identified a connection between those two factors and the varied allele frequency (1). Given the intense environmental pressures experienced by *L. obliquidens*, we may be able to identify a similar connection between *L. obliquidens's* genetic structure and their environment.

Suzuki *et al.* surveyed the genetic structure of *L. obliquidens* off the coast of Japan (18). To do this, they sampled 123 organisms from twelve distinct regions across Japan from 1978 to 2021 and sequenced their genomes. The study identified two genetically distinct clusters of *L. obliquidens*, which were separated by location (18). While the study divided the samples by genetic cluster, it did not look at the potential environmental causes of those differences (18). Our research aims to fill that gap by determining the impact of various environmental variables on the genetic structure of *L. obliquidens* using that same genetic data.

To investigate the relationship between the environment and the genetic structure of the Pacific white-sided dolphin, we analyzed the impact of temperature, microplastic levels, and salinity on the observed genetic variation. Data on these variables was collected from multiple sources, namely the Japan Oceanographic Center, World Sea Temperatures, and a previous study of microplastic levels in Japan (9, 19-21). Each of these variables has previously been shown to impact the environment or genetics of some organisms (1-4). There was visible variation in microplastic levels depending on location, and a previous study found some correlation between microplastic levels and population density (9). Due to variations in microplastic levels depending on human population density, we hypothesized that microplastic levels would have the most significant genetic impact. Using the environmental and genetic data, we performed two types of analyses, one investigating the correlation between environmental variables and genetic distance and another to determine how well the variables can predict genetic clusters using machine learning methods. We found that ocean currents both had the highest correlation and were the best predictor of genetic cluster, and we concluded that this could be caused by the variations in nutrient distribution resulting from current deviations. Of course, further research into this correlation and its causes is necessary to determine the validity of this relationship. This research will increase our understanding of how *L. obliquidens* responds to environmental changes that may occur due to human actions.

RESULTS

To determine what environmental variables were correlated with the genetic structure of *L. obliquidens*, we performed several different analyses. A genetic distance matrix was analyzed alongside environmental data gathered from the locations of each population from the study. Using the locations given for each organism, we collected environmental data for salinity (PPT), surface temperature (°C), microplastic levels (ng/g dry weight), and current (knots) to analyze along with the genetic data (19-21, 9). We first performed clustering analysis to identify structures within our samples **(Figure 1)**. We identified some clusters in the upper left and center with more similarity.

We found a clear distinction in environmental variables between genetic clusters **(Figure 2)**. We looked at the data distribution for the most important variables from our previous analysis: current stability, salinity, and PBDEs **(Figure 2)**. Genetic cluster two exhibited significantly higher values for salinity than genetic cluster one ($t = -3.88$, $p < 0.05$). Genetic cluster one contained a narrower range of higher values for PBDE levels than genetic cluster two ($t = -3.01$, $p < 0.05$). Genetic cluster two was refined to a smaller range on the lower end of the spectrum for current stability, which is defined as the vector average current speed/scalar average speed, than genetic cluster one ($t = 6.12$, $p < 0.05$, 1).

In addition to looking at variability in the environmental data, we looked at how the different variables may be correlated. With some further analysis in R, we found that three ocean current variables, current stability, current speed, and current direction, were closely correlated with each other. None of the other variables showed a statistically significant correlation **(Table 1)**.

We confirmed the distinction between these clusters visually using PCoA **(Figures 3 and 4)**. Coloring by different variables indicated genetic structure might be driven by current stability. To more formally test the relationship between environmental and genetic data, we conducted a distance-based redundancy analysis (dbRDA), comparing the environmental data we collected to genetic distance **(Figure 5)**. This graph showed the strength of the correlation between various environmental variables and genetics compared to the genetic cluster of each dolphin identified in the previous study. The dbRDA did not produce statistically significant results, but the most notable variable was PCBs ($p < 0.1$, **Figure 5**). Among the remaining variables, there were varying amounts of correlation, with surface temperature having one of the weakest correlations.

Lastly, we created a random forest machine-learning model to predict the genetic cluster of an organism using environmental data, which allowed us to look at the importance of different variables. We first trained the model on the set of environmental and genetic data. The trained model then used input environmental data to predict the genetic cluster

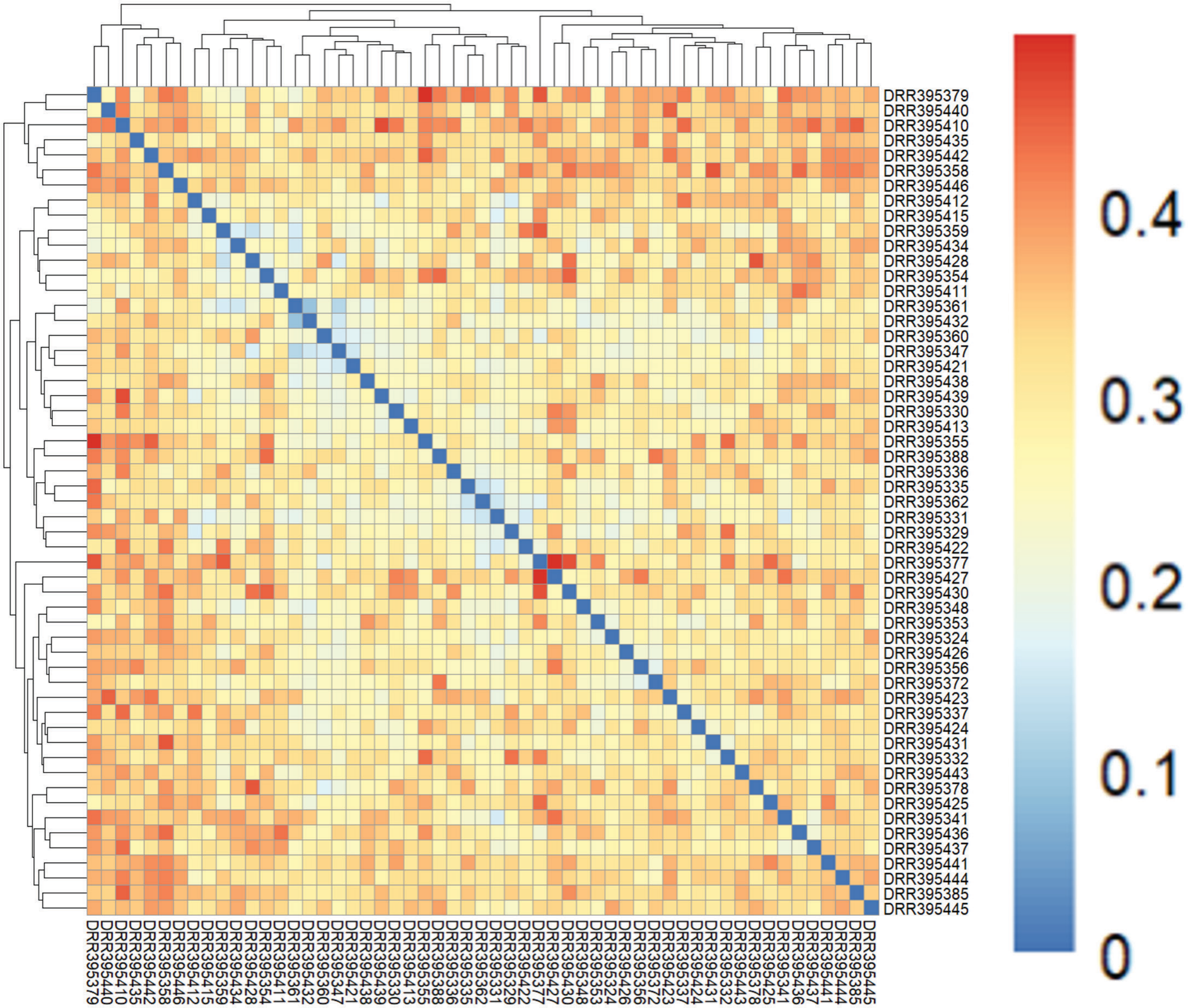

Figure 1: Pacific dolphins have two distinct genetic clusters. Heatmap visualizing genetic distance, a measure of the amount of genetic divergence between organisms, on a gradient where darker blue indicates lesser genetic distance while dark red indicates greater genetic distance. This is determined by comparing the different organisms in the dataset to determine the genetic distance based on single nucleotide variants. The figure shows there is more similarity between the organisms in the upper left, where individuals appear to cluster together.

of a given organism. The model predicted the data well and had a relatively high accuracy score of 0.786, calculated from the confusion matrix **(Table 2)**. Current stability was the most important variable in determining an individual's genetic cluster (mean decrease GINI = 1.24, **Figure 6**). Salinity also had a large impact (mean decrease GINI = 1.17, **Figure 6**). The remaining mean decrease GINI values were current direction, 0.833; PBDEs, 0.775; surface temperature, 0.770; PCBs, 0.756; and current speed, 0.670. We also noted that PBDEs were more influential in the model than PCBs.

DISCUSSION

In our study, we investigated the correlation between environmental variables and genetic structure in *L. obliquidens*. We initially hypothesized that microplastic levels would have the most significant correlation with genetic distance. To examine this claim, we performed several different types of analysis on genetic data compared with environmental data for various variables, including microplastics, ocean temperature, ocean currents, and salinity. We did find a small link between microplastic levels and genetics, but the strongest correlation was that of ocean current stability and genetics.

While PBDE and PCB were not the best predictors of genetic distance, they were correlated with genetic distance. However, despite both being microplastics, they affected genetic structure independently of each other, which was initially very surprising (as indicated by the perpendicular arrows in **Figure 5**). This may be a result of the different processes that produce each microplastic, causing them to act independently on genetic structure. Unlike PCBs, which varied between urban and isolated areas, PBDEs did not have a recognizable pattern (9). Despite this, PBDEs seemed to have a stronger correlation with genetics than PCBs

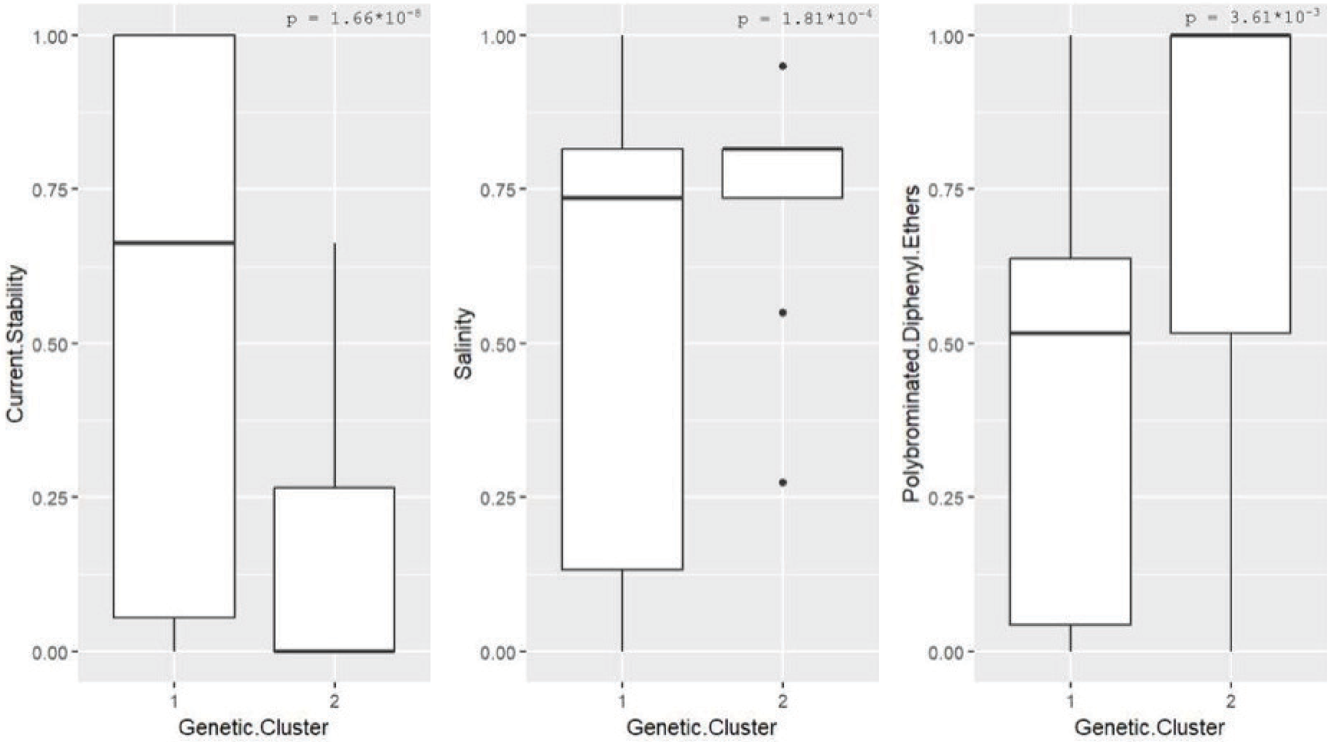

Figure 2: There is a significant difference in variable distribution between genetic clusters. The three box plots shown demonstrate the distribution of current stability, salinity, and PBDE values by genetic cluster. There is a significant difference between genetic clusters for all environmental variables. The y-axis represents the range of values for the given variable with lines at the quartile and median marks. As the data has been standardized by the maximum value for each variable, the y-axis is now unitless. Current stability is measured as a percentage, Salinity is measured in PPT, and microplastic levels are ng/g dry weight.

(Figure 6).

Though the dbRDA did not produce statistically significant results, ocean current stability was the most significant environmental variable based on the random forest analysis. Current stability had a major impact on determining the genetic cluster of an organism **(Figure 6)**. However, all three current variables, current stability, current direction, and current speed, are correlated with each other, which prevents us from identifying the independent impact of each individual variable **(Table 1)**.

While current does impact heat distribution, temperature did not have a noticeable impact on genetic variation, indicating that heat distribution is not the reason for the extreme impact of ocean currents. *L. obliquidens* are reportedly sensitive to heat, as populations in the Gulf of California have been reduced due to the warming climate in that area (6). However, there is a possibility that populations in different regions respond to changes in temperature differently. Instead of heat, the significance of currents may be caused by the large impact that they have on nutrient and prey distribution, meaning that varying currents can result in different diets and nutrients for separate populations (17). To confirm this hypothesis, additional research would need to be done into the varying diets of different populations.

One thing to note about the results of the dbRDA and random forest model is that they measure different relationships. The dbRDA uses a genetic distance matrix to identify the correlation between genetics and environmental variables, while the random forest model exclusively predicts the genetic cluster without looking at any exact genetic data. However, both analyses still support a correlation between current factors and genetics. Ocean currents are among the most prominent variables in predicting genetic clusters in the random forest. The dbRDA provides more nuanced correlation information, as genetic distance is a gradient value as opposed to the binary genetic cluster. Both analyses, however, measure the impact of environmental factors on some aspect of genetics.

Previous studies, including the source that provided the original data, have used similar methods of analysis as those performed here. The study of genetic clusters in the Pacific white-sided dolphin visualized the separation of the two clusters using a PCoA (18). Our study also utilized a PCoA but worked with a gradient of values instead of a binary category, allowing more room to look at variations in the data. Another study used a dbRDA to look at the relationship between environmental variables and genetics. The final dbRDA included fewer variables than ours and less information about variables that turned out to be less significant (22). Finally, the random forest method has previously been used to evaluate the relationship between the environment and genetics. However, this study focused more on specific genes and only used the random forest analysis, while we used this method in coordination with other analyses and provided a more general assessment of the relationship (23).

The lack of correlation between genetics and geographic

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Variable 1	**Variable 2**	**Correlation**	***p*-value**
Current Stability	Current Direction	-0.668	$1.76*10^{-2}$
Current Stability	Current Speed	0.786	$2.46 *10^{-3}$
Current Stability	PBDE	-0.333	$2.90*10^{-1}$
Current Stability	PCB	-0.209	$5.15*10^{-1}$
Current Stability	Temperature	-0.143	$6.57*10^{-1}$
Current Stability	Salinity	-0.470	$1.23*10^{-1}$
Current Direction	Current Speed	-0.655	$2.09*10^{-2}$
Current Direction	PBDEs	0.335	$2.87*10^{-1}$
Current Direction	PCBs	-0.133	$6.81*10^{-1}$
Current Direction	Temp	-0.412	$1.84*10^{-1}$
Current Direction	Salinity	0.452	$1.41*10^{-1}$
Current Speed	PBDEs	-0.150	$6.42*10^{-1}$
Current Speed	PCBs	0.097	$7.65*10^{-1}$
Current Speed	Temp	0.274	$3.89*10^{-1}$
Current Speed	Salinity	-0.436	$1.57*10^{-1}$
PBDE	PCBs	-0.160	$6.20*10^{-1}$
PBDE	Temp	0.181	$5.73*10^{-1}$
PBDE	Salinity	0.122	$7.05*10^{-1}$
PCB	Temp	0.487	$1.09*10^{-1}$
PCB	Salinity	-0.133	$6.80*10^{-1}$
Temp	Salinity	0.005	$9.87*10^{-1}$

Table 1: Current variables are correlated with each other. Looking at the correlation between different variables, different aspects of ocean currents had the strongest connection. None of the remaining variables showed statistically significant correlations.

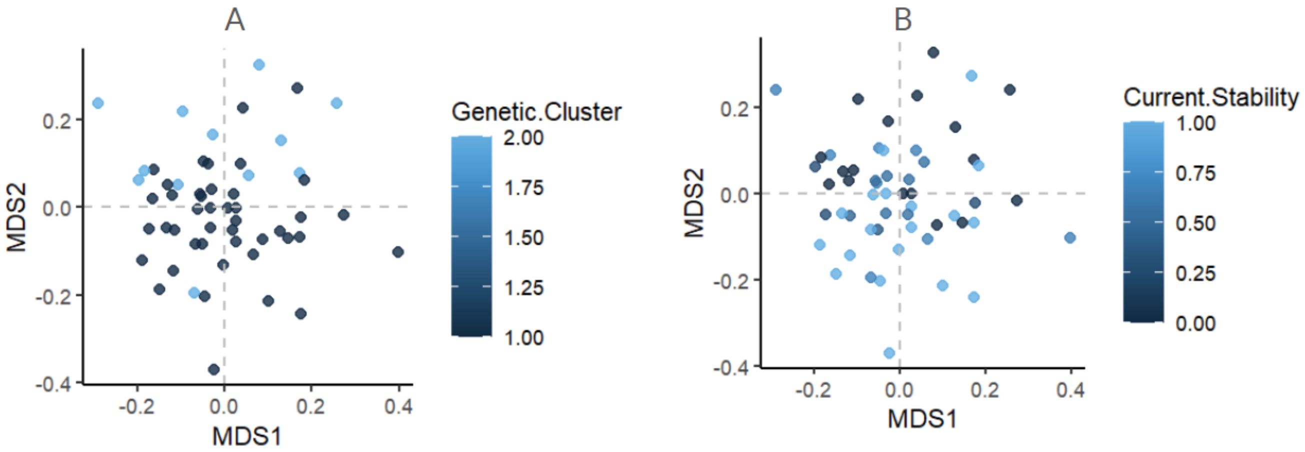

Figure 3: Graphs indicate a potential correlation between variables and genetic distance. Graph A is a principal coordinate analysis (PCoA) visualizing the genetic separation between genetic clusters 1 and 2. There is a clear division between genetic cluster 2 in the upper two quadrants and cluster 1 in the lower quadrants. PCoA B shows a gradient pattern of current stability values. Darker points with lower current stability values are shown more in the upper quadrants of the graph, while lighter points with higher current stability values are clustered in the bottom quadrants. Each point's positioning on the MDS1 and MDS2 axes relative to each other reflects the dissimilarity between the two organisms. The two axes are unitless as they measure the variance in these variables.

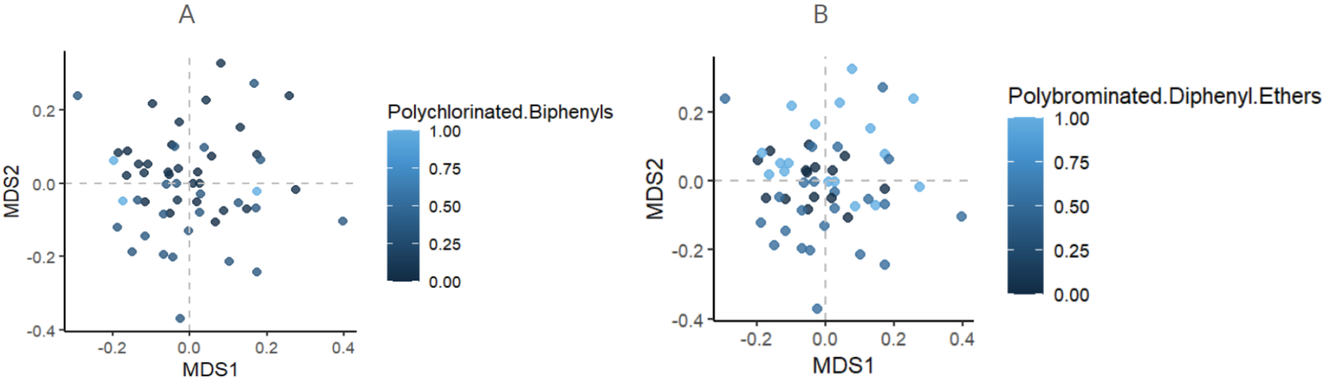

Figure 4: Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) have no visible correlation with genetic distance. Graphs A and B show no consistent patterns or groupings of microplastic levels, indicating a lack of strong correlation. Each point's positioning on the MDS1 and MDS2 axes relative to each other reflects the dissimilarity between the two organisms. The two axes are unitless as they measure the variance in these variables.

distance may be due to how we calculated distance. By using longitude and latitude as predictors, we ignored the geographic separation caused by the island of Japan. For example, the narrowness of the island means that locations on opposite sides are close spatially but distant as measured in miles of coastline. Because Japan is an island and divides the ocean into sections, each side of the island could have a very different current composition despite being physically close to each other. It's possible the relationship we found with the current variable may actually describe the separation of two populations formed by the island of Japan. Some evidence for this theory is the division of genetic clusters shown by the original study. Individuals in one group were almost entirely on one side of the island while the other group was split between the two sides (18). Salinity was not significantly correlated with genetic distance **(Figure 5)**. However, salinity was the second most consequential variable in the random forest model **(Figure 6)**. The two analyses may be measuring different types of relationships between environmental factors and genetics, but they are still both examining genetics, so the reason for this distinction is unclear. Salinity often has some influence on current, but it did not have a statistically significant correlation with current in this study (15). However, salinity does have some impact on water circulation and density, which may cause a connection with genetic structure (15).

Another area to consider is the potential mismatch between environmental and genetic data. Some organisms were sampled in 2010, while the environmental data is from 2022. This could mean that some of the environmental data is not accurate to what an organism actually experienced. For this reason, we filtered the data to only contain organisms sampled in 2010 onwards, but we did not have enough recent data to only focus on the past few years. Our data was also taken from several different sources as we were not able to perform the sampling ourselves. Because of this,

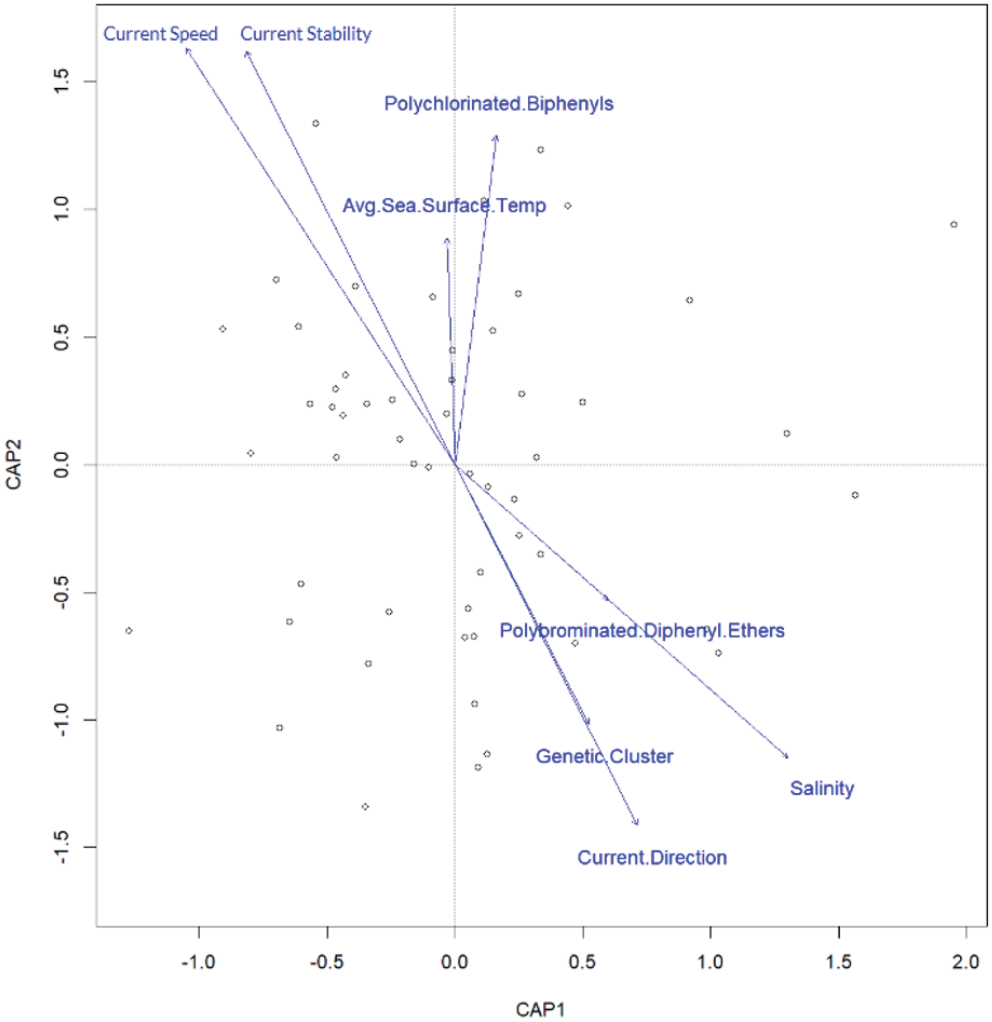

Figure 5: Current is strongly correlated with genetic distance. A distance-based redundancy analysis comparing environmental data with genetic distance to identify a correlation. CAP1 and CAP2 are unitless constrained ordination axes used to show the most possible variation explained by the given variables. The length of the arrows indicates how strong the correlation is, and the direction indicates where the greater values are. Notably, polychlorinated biphenyls did have the most significant correlation ($p < 0.1$), but it was not sufficient to prove a correlation.

	Predicted Genetic Cluster 1	Predicted Genetic Cluster 2
Expected Genetic Cluster 1	9	2
Expected Genetic Cluster 2	2	1

Table 2: The random forest model predicted genetic cluster. The model achieved a relatively high accuracy score of 0.714. The confusion matrix compares the predicted versus actual genetic clusters with only four out of fourteen predictions incorrect. The variation between the number of samples in each category is due to the random sample nature of the testing dataset, resulting in a skewed prediction distribution.

there was not always an exact match between organism location and environmental data location, but instead a close approximation. The filtered data resulted in a dataset that was skewed towards genetic cluster 1, which may have had an impact on the results of the random forest.

While the results of this study point towards ocean currents having a correlation with the genetics of *L. obliquidens*, the study was focused on a narrow area. We also note the possibility that current variables may be correlated with other environmental factors that were not tested in this study. To see if these results can be applied generally, a larger scale study including more diverse environmental variables and locations across the globe would be necessary. In addition to expanding the range of the study, it would be useful to

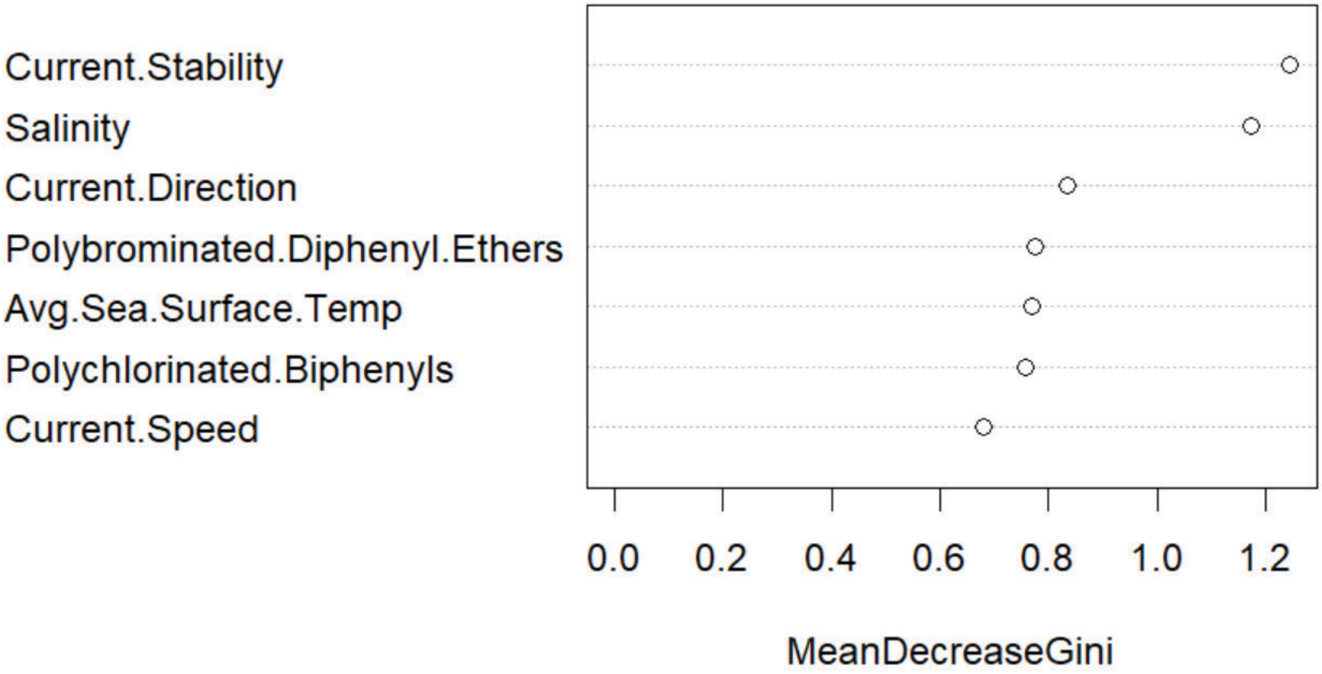

Figure 6: Current stability is the most important factor in the random forest model. A visualization of the importance of various variables for determining the outcome of the random forest. This shows that current stability has a very strong impact, followed by salinity and current direction. Mean decrease GINI measures how much the inclusion of a specific variable improves the model classification.

examine the impact of currents on other species to see if ocean currents have a significant impact on other organisms as well as *L. obliquidens*.

Another area for exploration is the physiological and genetic impact of each of these environmental variables on different organisms. Each aspect of the environment can affect organisms in different ways, so studying these differences is important to understanding the significance of our results. In addition, expanding the range of variables beyond those included in this study could provide insight into the most impactful variables. It would also be useful to look at the difference in these effects between distinct species, as variables could have varying impacts on dissimilar species.

Further research should also include the identification of specific genes that may be correlated with various environmental factors. In this study, we looked at general genome and genetic cluster correlation, but once significant environmental factors are identified, it would be interesting to explore specific genes that may be correlated with the environment. The causes behind these correlations would also provide more insight into how the environment is impacting these organisms. While this research clearly indicates a correlation between ocean currents and genetics, further research is necessary to understand the cause behind this correlation and how else the environment may relate to genetics in the Pacific white-sided dolphin and other organisms.

MATERIALS AND METHODS

Data Collection

We utilized existing sequencing data for the Pacific white-sided dolphin from Japan (18). We downloaded the raw whole genome sequencing data through NCBI BioProject (BioProject Number PRJDB13929). The data originally included 111 individuals from 12 different locations along Japan's coast and 12 individuals in captivity. We filtered this data to 55 individuals sampled in 2010 or later. These individuals spanned 9 different locations in the wild with an average of approximately 6 animals per location. Using this sequencing data, we created a genetic distance matrix by using Analysis of Next Generation Sequencing Data (ANGSD v = 0.938) (24). This data was composed primarily of the most recent yearly averages from these sources. To avoid bias, all environmental data were standardized using the 'decostand' command in vegan (25).

Principal Coordinate Analysis and Distance-Based Redundancy Analysis

Using the R packages pheatmap, vegan, and tidyverse, we generated a heatmap, several PCoAs, and a dbRDA to visualize the relationship between different environmental variables and the genetic distance between organisms (25-27). The PCoAs were used to identify the variables showing the strongest correlation with genetic distance, which we then processed with the dbRDA. We examined the relative variable importance from the dbRDA by studying the varying arrow lengths.

Random Forest

In parallel, we used a random forest to try to predict genetic clusters from environmental data. We confirmed the outcome using a random forest which predicted genetic clusters based on environmental factors instead of determining the correlation between genetic and environmental factors. Using

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the randomForest and caTools packages in R, we divided our data into training and testing sets, with 75 % (41 organisms) of the data in the training set and the remaining 25 % (14 organisms) in the testing set (28, 29). The training set was used to train the model to predict the genetic cluster of an organism based on given environmental data. These genetic clusters were identified by the original paper authors. The testing set was then used to test the model by having it predict known genetic clusters based on environmental data. Once we completed training and testing the model, we extracted the variable importance to determine which environmental variable had the most notable impact on the predictions made.

Box Plots and *t*-Tests

Finally, we looked at the distribution of different environmental variables by creating box plots divided by genetic cluster. To see how variables identified as important differed between groups, we created boxplots of the most important variables. These box plots visualized the data distribution for notable variables to help understand their significance. To determine significance, we conducted a *t*-test on important variables that distinguished genetic clusters. We corrected for multiple hypotheses by performing a Bonferroni correction. In addition to this, we checked for correlations between our environmental variables to avoid spurious results using Pearson's product-moment correlation test.

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REFERENCES

1. Lundgren, Petra, *et al.* "Genotype – Environment Correlations in Corals from the Great Barrier Reef." *Springer Link*, Feb. 2013, https://doi.org/10.1186/1471-2156-14-9.
2. Limonta, Giacomo, *et al.* "Microplastics Induce Transcriptional Changes, Immune Response and Behavioral Alterations in Adult Zebrafish." *Scientific Reports,* vol. 9, no. 1, Oct. 2019, p. 15775, https://doi.org/10.1038/s41598-019-52292-5.
3. Lal, Monal M., *et al.* "Swept Away: Ocean Currents and Seascape Features Influence Genetic Structure across the 18,000 Km Indo-Pacific Distribution of a Marine Invertebrate, the Black-Lip Pearl Oyster Pinctada Margaritifera." *BMC Genomics,* vol. 18, no. 1, Jan. 2017, p. 66, https://doi.org/10.1186/s12864-016-3410-y.
4. Kozela, Christopher, and Mark O. Johnston. "Effect of Salt Stress on Mutation and Genetic Architecture for Fitness Components in Saccharomyces Cerevisiae." *G3 Genes|Genomes|Genetics,* vol. 10, no. 10, Oct. 2020, pp. 3831–42, https://doi.org/10.1534/g3.120.401593.
5. Forman, S., *et al.* "Climate Change Impacts and Risks for Animal Health in Asia." *Revue Scientifique et Technique (International Office of Epizootics),* vol. 27, no. 2, 2008.
6. Layton, Taylor. "Lagenorhynchus Obliquidens (Pacific White-Sided Dolphin)." *Animal Diversity Web,* animaldiversity.org/accounts/Lagenorhynchus_obliquidens/. Accessed 23 Feb. 2023.
7. Salvadeo, Christian, *et al.* "Climate Change and a Poleward Shift in the Distribution of the Pacific White-Sided Dolphin in the Northeastern Pacific." *Endangered Species Research,* vol. 11, Mar. 2010, pp. 13–19, https://doi.org/10.3354/esr00252.
8. Smale, Dan A. "Impacts of Ocean Warming on Kelp Forest Ecosystems." *New Phytologist,* vol. 225, no. 4, Feb. 2020, pp. 1447–54, https://doi.org/10.1111/nph.16107.
9. Yeo, Bee Geok, *et al.* "PCBs and PBDEs in Microplastic Particles and Zooplankton in Open Water in the Pacific Ocean and around the Coast of Japan." *Marine Pollution Bulletin,* vol. 151, 2020, p. 110806, https://doi.org/10.1016/j.marpolbul.2019.110806.
10. Costa, L. G., and G. Giordano. "Polybrominated Diphenyl Ethers." *Encyclopedia of Toxicology (Third Edition),* edited by Philip Wexler, Academic Press, 2014, pp. 1032–34, https://doi.org/10.1016/B978-0-12-386454-3.00422-X.
11. Erickson, Mitchell D., and Robert G. Kaley. "Applications of Polychlorinated Biphenyls." *Environmental Science and Pollution Research,* vol. 18, no. 2, Feb. 2011, pp. 135–51, https://doi.org/10.1007/s11356-010-0392-1.
12. Wright, Stephanie L., *et al.* "The Physical Impacts of Microplastics on Marine Organisms: A Review." *Environmental Pollution,* vol. 178, July 2013, pp. 483–92, https://doi.org/10.1016/j.envpol.2013.02.031.
13. Aznar-Alemany, Òscar, *et al.* "Temporal Trends of Halogenated and Organophosphate Contaminants in Striped Dolphins from the Mediterranean Sea." *Science of The Total Environment,* vol. 753, Jan. 2021, p. 142205, https://doi.org/10.1016/j.scitotenv.2020.142205.
14. Roibás-Rozas, Alba, *et al.* "Modeling the Impact of Salinity Variations on Aquatic Environments: Including Negative and Positive Effects in Life Cycle Assessment." *Environmental Science & Technology,* vol. 56, no. 2, Jan. 2022, pp. 874–84, https://doi.org/10.1021/acs.est.1c04656.
15. Cullum, Jodie, *et al.* "Importance of Ocean Salinity for Climate and Habitability." *Proceedings of the National Academy of Sciences of the United States of America,* vol. 113, no. 16, 2016, pp. 4278–83. https://doi.org/10.1073/pnas.1522034113.
16. Pratt, Eleanor A. L., *et al.* "Seascape Genomics of Coastal Bottlenose Dolphins along Strong Gradients of Temperature and Salinity." Molecular Ecology, vol. 31, no. 8, Apr. 2022, pp. 2223–41, https://doi.org/10.1111/mec.16389.
17. Hays, Graeme C. "Ocean Currents and Marine Life." *Current Biology,* vol. 27, no. 11, 2017, pp. R470–73, https://doi.org/10.1016/j.cub.2017.01.044.
18. Suzuki, Miwa, *et al.* "Genomics Reveals a Genetically Isolated Population of the Pacific White-Sided Dolphin (Lagenorhynchus Obliquidens) Distributed in the Sea of Japan." *Wiley Online Library,* vol. 32, no. 4, Nov. 2022, pp. 881–91, https://doi.org/10.1111/mec.16797.
19. *1 Degree Gridded Current Statistics.* Japan Oceanographic Data Center, jdoss1.jodc.go.jp/vpage/ocs.html.
20. *1 Degree Gridded Salinity Statistics.* Japan Oceanographic Data Center, jdoss1.jodc.go.jp/vpage/bss.html.
21. *Japan Sea Water Temperatures.* World Sea Temperatures, www.seatemperature.org/asia/japan/.
22. Black, Kristina, *et al. Environmental Drivers of Genetic Adaptation in Florida Corals.*

23. Kijewski, Tomasz, *et al.* “Random Forest Assessment of Correlation between Environmental Factors and Genetic Differentiation of Populations: Case of Marine Mussels Mytilus.” *Oceanologia,* vol. 61, no. 1, Jan. 2019, pp. 131–42, https://doi.org/10.1016/j.oceano.2018.08.002.
24. Korneliussen, Thorfinn Sand, *et al.* “ANGSD: Analysis of Next Generation Sequencing Data.” *MC Bioinformatics,* vol. 15, Nov. 2014, https://doi.org/doi:10.1186/s12859-014-0356-4.
25. Oksanen, Jari, *et al. Vegan: Community Ecology Package.* 2017, CRAN.R-project.org/package=vegan.
26. Kolde, Raivo. *Pheatmap: Pretty Heatmaps.* 2019, CRAN.R-project.org/package=pheatmap.
27. Wickham, Hadley, *et al.* “Welcome to the Tidyverse.” *Journal of Open Source Software,* vol. 4, Nov. 2019, p. 1686, https://doi.org/10.21105/joss.01686.
28. Liaw, Andy, and Matthew Wiener. “Classification and Regression by RandomForest.” *R News,* vol. 2, Dec. 2002, pp. 18–22.
29. Tuszynski, Jarek. *CaTools: Tools: Moving Window Statistics,* GIF, Base64, ROC AUC, Etc. 2021, CRAN.R-project.org/package=caTools.