Oyster Aquaculture - Case Studies

Using Standard BioTools Microfluidic Technology

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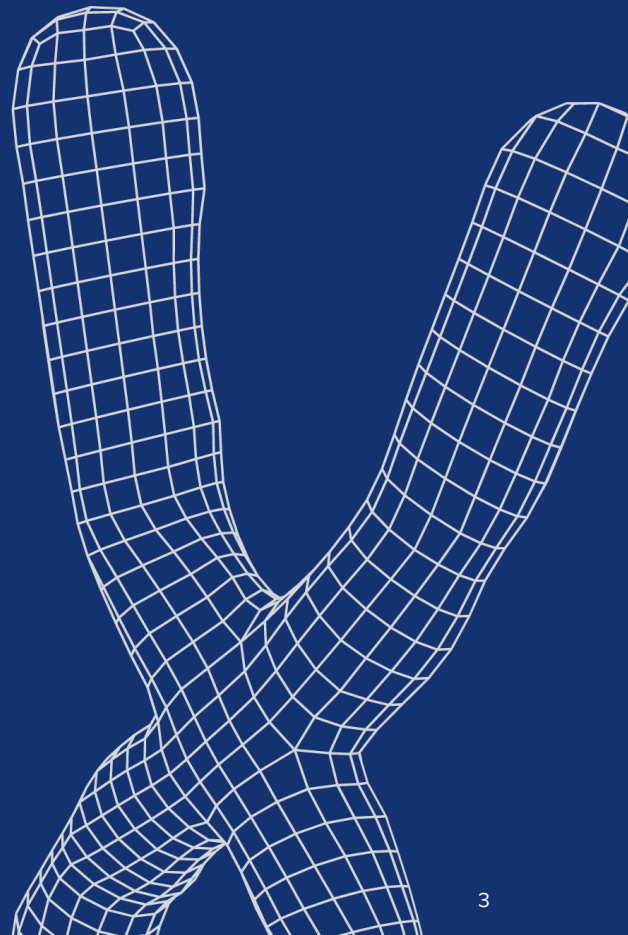

Genotyping Using EDNA In Oysters

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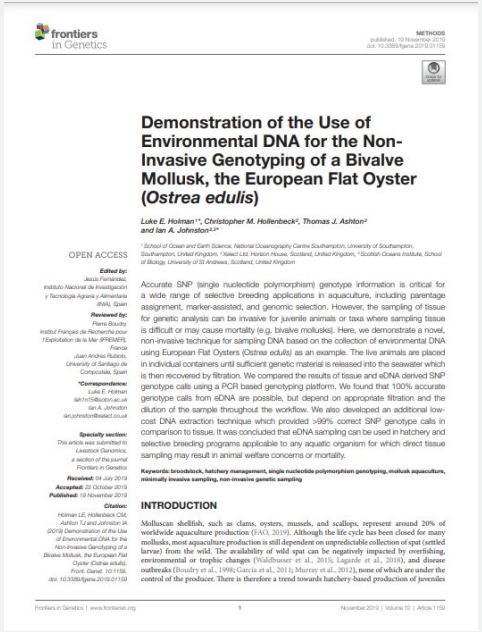

frontiers in Genetics

METHODS

published: 19 November 2019
doi: 10.3389/fgene.2019.01159

Demonstration of the Use of Environmental DNA for the Non-Invasive Genotyping of a Bivalve Mollusk, the European Flat Oyster (*Ostrea edulis*)

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Accurate SNP (single nucleotide polymorphism) genotype information is critical for a wide range of selective breeding applications in aquaculture, including parentage assignment, marker-assisted, and genomic selection. However, the sampling of tissue for genetic analysis can be invasive for juvenile animals or taxa where sampling tissue is difficult or may cause mortality (e.g. bivalve mollusks). Here, we demonstrate a novel, non-invasive technique for sampling DNA based on the collection of environmental DNA using European Flat Oysters (*Ostrea edulis*) as an example. The live animals are placed in individual containers until sufficient genetic material is released into the seawater which is then recovered by filtration. We compared the results of tissue and eDNA derived SNP genotype calls using a PCR based genotyping platform. We found that 100% accurate genotype calls from eDNA are possible, but depend on appropriate filtration and the dilution of the sample throughout the workflow. We also developed an additional low-cost DNA extraction technique which provided >99% correct SNP genotype calls in comparison to tissue. It was concluded that eDNA sampling can be used in hatchery and selective breeding programs applicable to any aquatic organism for which direct tissue sampling may result in animal welfare concerns or mortality.

Keywords: broodstock, hatchery management, single nucleotide polymorphism genotyping, mollusk aquaculture, minimally invasive sampling, non-invasive genetic sampling

INTRODUCTION

Molluscan shellfish, such as clams, oysters, mussels, and scallops, represent around 20% of worldwide aquaculture production (FAO, 2019). Although the life cycle has been closed for many mollusks, most aquaculture production is still dependent on unpredictable collection of spat (settled larvae) from the wild. The availability of wild spat can be negatively impacted by overfishing, environmental or trophic changes (Waldbusser et al., 2015; Lagarde et al., 2018), and disease outbreaks (Boudry et al., 1998; Garcia et al., 2011; Murray et al., 2012), none of which are under the control of the producer. There is therefore a trend towards hatchery-based production of juveniles

Objective: Accurate SNP (single nucleotide polymorphism) genotype information is critical for a wide range of selective breeding applications in aquaculture, including parentage assignment, marker-assisted, and genomic selection

Utilization of Standard BioTools products:

- Standard Biotools D3 assay design portal
- Standard Biotools EP1 platform
- Standard Biotools 96.96 Dynamic Array genotyping chip

Conclusions:

- Here we show that the collection of eDNA can be used to accurately genotype bivalve mollusks and potentially other aquatic organisms.
- The influence of DNA extract dilution on genotyping accuracy was assessed to produce a practical protocol for the European flat oyster that can be used by researchers and aquaculture professionals as a template to develop viable alternatives to invasive tissue sampling in similar species.
- We also demonstrated that eDNA extracted using this protocol is of sufficient quality and quantity for multi-locus genotyping, which is necessary for most applications in aquaculture breeding programs.

Holman, etl al. *"Demonstration of the Use of Environmental DNA for the Non-Invasive Genotyping of a Bivalve Mollusk, the European Flat Oyster (Ostrea edulis)"* Frontiers (2019)

Genotyping Using eDNA In Oysters

Any further question?

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Background

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- Molluscan shellfish, such as clams, oysters, mussels, and scallops, represent around 20% of worldwide aquaculture production.
- Wild spat can be negatively impacted by overfishing, environmental or trophic changes and disease outbreaks none of which are under the control of the producer. There is therefore a trend towards hatchery-based production of juveniles for on-growing in the sea.
- Hatchery-based production allows for genetic improvement of stock via selective breeding, which has the potential to improve economically important traits such as growth and disease resistance by 10%–15% per generation.
- One particular challenge of molluscan aquaculture is the availability of non-invasive DNA sampling techniques for parentage assignment and advanced marker-assisted or genomic selection strategies.
- Recent advances in the isolation of environmental DNA (eDNA) potentially offer a non-invasive alternative to tissue sampling
- Typing accuracy was assessed to produce a practical protocol for the European flat oyster that can be used by researchers and aquaculture professionals as a template to develop viable alternatives to invasive tissue sampling in similar species.
- We also demonstrated that eDNA extracted using this protocol is of sufficient quality and quantity for multi-locus genotyping, which is necessary for most applications in aquaculture breeding programs.

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Genotyping Using EDNA In Oysters

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Methods

- Oysters were acclimatized in a 50 L seawater aquarium at 16°C for 60 days, with 700 L/hour external filtration.
- They were derived from native stock from the Argyll area and were fed a maintenance diet of powdered algal biomass.
- Each oyster was externally rinsed with reverse osmosis (RO) filtered water and placed into a polypropylene vessel with 500 ml seawater made from artificial salt.
- After being introduced, duplicate water samples of 75 ml were taken from each vessel 72 h and a 5 mm2 section of mantle was dissected and stored in 100% ethanol.
- A 75 ml artificial seawater control sample was taken before filling the vessels and all 75 ml water samples were filtered using a vacuum filtration manifold and 47 mm 0.45 µm Cellulose Nitrate filters.

Genotyping proceeded with the Fluidigm EP1 platform using the manufacturer's protocols.

SNP genotypes were called using k-means clustering under the default settings in the Fluidigm Genotyping Analysis Software.

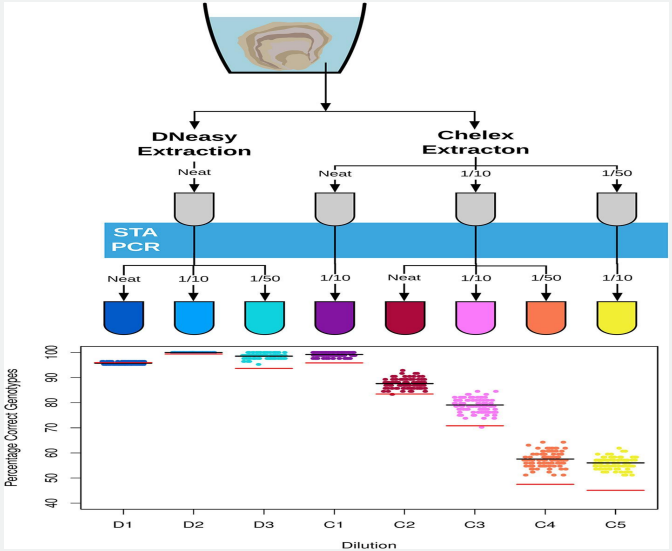

Figure 1: Demonstrate DNA extraction and the genotyping methodology.

Holman, etl al. "*Demonstration of the Use of Environmental DNA for the Non-Invasive Genotyping of a Bivalve Mollusk, the European Flat Oyster (Ostrea edulis)*" Frontiers (2019)

Genotyping Using EDNA In Oysters

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Results and conclusions

Results:

- The Fluidigm EP1 platform was used to synthesize 16 SNPs with the highest mean MAF for the discovery of target DNA in parentage assignment. To minimize the amount of potentially PCR inhibiting co-purified contaminants from DNA extractions, several different dilutions with RO water were trialed.
- Results showed that the overall SNP call rate was calculated as the proportion of allele calls across the 96 assays (16 SNPs, 6 replicates per SNP) for the eDNA sample that matched the tissue sample.
- Two of the 16 trialed assays failed to produce any identifiable clusters, indicating no polymorphism among the tested oysters or a non-functioning assay, and the tissue samples for the remaining 14 assays gave high quality clusters reliably identified using k-means clustering (SNP call data).

All three strategies provided the highest accuracy between eDNA and tissue DNA genotypes for the DNeasy and Chelex extractions.

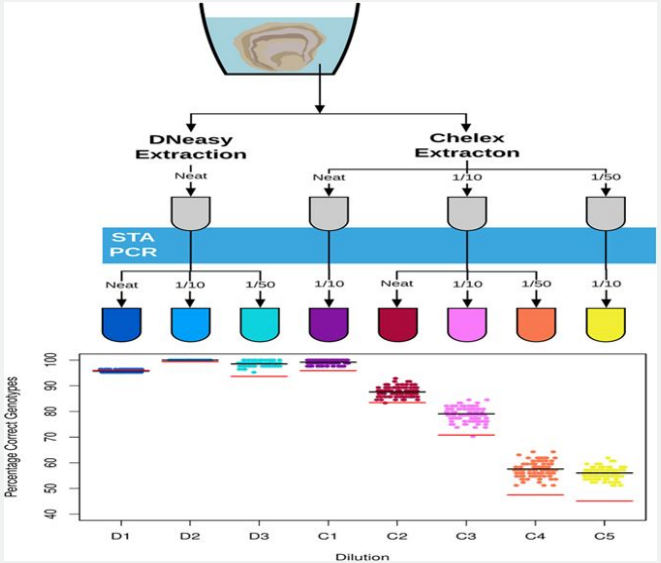


Figure 1: Demonstrating the results from extraction methodology, STA PCR (Fluidigm workflow),genotyping methodology and SNP call rates.

Holman, etl al. *"Demonstration of the Use of Environmental DNA for the Non-Invasive Genotyping of a Bivalve Mollusk, the European Flat Oyster (Ostrea edulis)"* Frontiers (2019)

Genotyping Using EDNA In Oysters

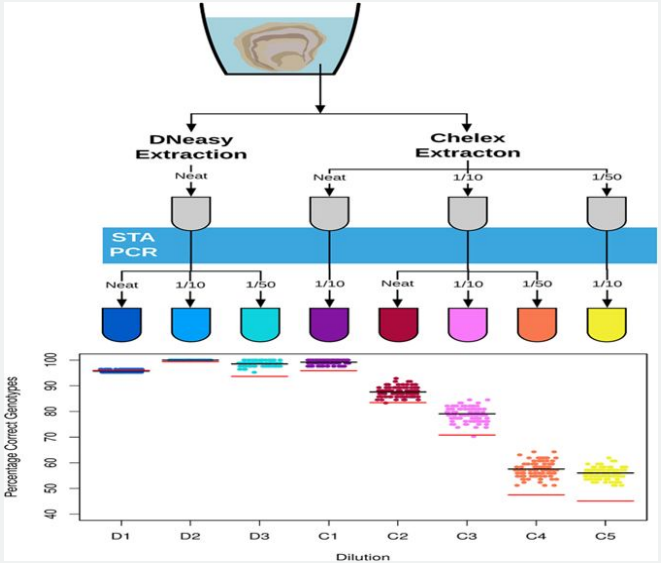
Any further question?

Talk to us

Results and conclusions

Conclusions:

- eDNA is used to accurately genotype bivalve mollusks and other aquatic organisms.
- A protocol for the European flat oyster was developed to develop viable alternatives to invasive tissue sampling.
- The protocol is of sufficient quality and quantity for multi-locus genotyping, which is necessary for most applications in aquaculture breeding programs.
- Invasive methods involving the removal of internal tissue or fluid are routinely reported in marine and freshwater mussels



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Genotyping For Population Genetic Analysis

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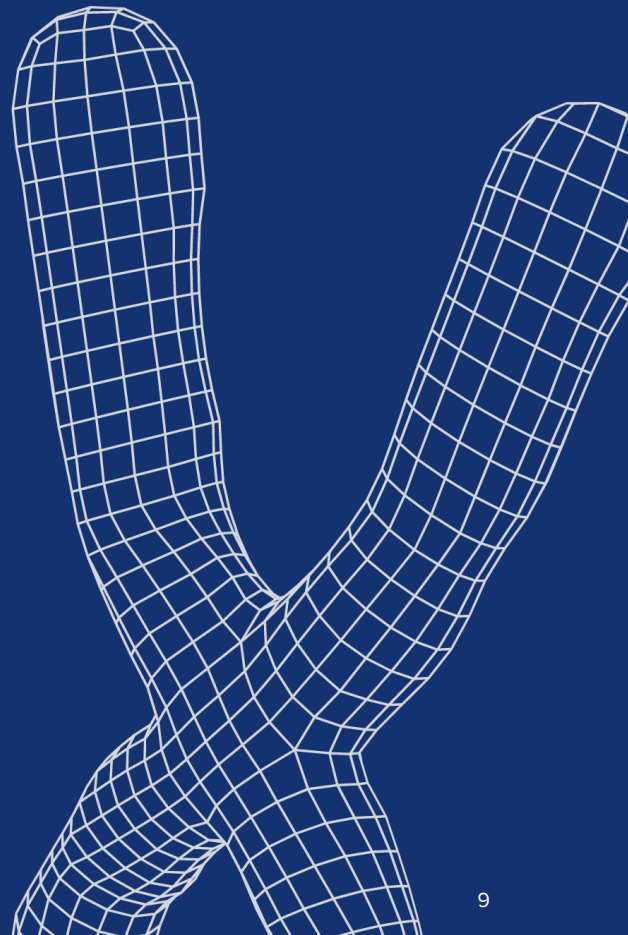

Genotyping For Population Genetic Analysis

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Conservation Genetics (2019) 20:1057–1071
https://doi.org/10.1007/s10592-019-01194-0

RESEARCH ARTICLE

Multiple drivers of interannual oyster settlement and recruitment in the lower Chesapeake Bay

Brendan Turley[1,2] · Kimberly Reece[1] · Jian Shen[1] · Jeong-Ho Lee[3] · Ximing Guo[3] · Jan McDowell[1]

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Abstract
Despite global investment in shellfish restoration activities, relatively little attention has been given to predicting optimal restoration sites and testing these expectations. We used a coupled biological-physical connectivity model as a guide to plant two distinct hatchery-spawned strains of the eastern oyster, *Crassostrea virginica*, in the Lafayette River, Virginia during the summer of 2013 at two locations corresponding to virtual spawning locations within the connectivity model. We utilized single nucleotide polymorphism markers to test the model predictions by genotyping oysters recruited after planting two hatchery-spawned strains and examining interannual recruitment variability for two successive years. Two spat were identified as hybrids of one of the planted strains and resident oyster genotypes. We also observed a genetic influence from an oyster strain used previously for restoration. Differences in environmental conditions between the two years of monitored recruitment likely affected larval dispersal and survival, contributing to observed interannual differences in the newly recruiting cohorts. Oyster spat from 2013 were genetically more similar to resident adults sampled in the Lafayette River, while the 2014 spat exhibited genotypic frequencies more similar to adults from surrounding rivers. The winds during the spawning seasons differed between years prevailing conditions for retention in 2013 and mixing of water masses in 2014. We recommend that the monitoring of restoration activities should consider relevant environmental conditions and observe multiple years of recruitment to assess the genetic impacts of restoration plantings and variable reproductive success.

Keywords Citizen science · Connectivity · *Crassostrea virginica* · Restoration · Seascape genetics · Single nucleotide polymorphism

Introduction

Traditionally, population genetic studies of larval and juvenile dispersal have focused primarily on the correlation between genetic and geographic distances to understand population connectivity. More recently, landscape genetics methods used in terrestrial environments have considered environmental data in addition to geographic distances to explain regional population structure; however, the marine environment presents unique challenges (Selkoe et al. 2016). For example, ocean surface currents forced by winds and tidal cycles are two important factors impacting the distribution and survival of estuarine organisms. As a result, the physical dynamics of estuaries are vital to understanding resident organisms, and hydrodynamic models that incorporate biological components are valuable for formulating population connectivity hypotheses. Restoration projects located in estuaries could benefit from the combination of physical modeling and population genetic analyses to better predict optimal restoration sites and track the reproductive influence of restoration introduced animals.

Molecular markers have been used for tracking the efficacy of restoration projects by monitoring the genetic contribution of planted animals to newly recruiting individuals with varying success. In 2002, the Virginia Marine

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10592-019-01194-0) contains supplementary material, which is available to authorized users.

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Objective: The main goal of this study was to see if restoration efforts had an effect on the annual recruitment of oysters to the point where genetic contributions could be seen.

- Utilization of Standard BioTools products:
- Fluidigm Biomark HD
- Fluidigm SNP Type Panel
- Fluidigm 48.48 Dynamic Array genotyping chip

Conclusions:

- This study examined whether restoration efforts affected yearly oyster recruitment to uncover genetic contributions.
- Our investigation found that one of the two experimental oyster strains recruited new oysters. Two of the NEH® hatchery-selected strain's young were found to be hybrids.
- The research showed that the populations from which new oysters come into the river change from year to year in the Lafayette River. It's possible that the flow of water in 2013 was just right for oysters that were born outside of the Lafayette to settle in the Lafayette, but HABs may have had a bigger effect on post-larval survival in the late summer. In 2014, on the other hand, HABs happened early and weren't as bad.

Turley, et. al. *"Multiple drivers of interannual oyster settlement and recruitment in the lower Chesapeake Bay"* Conservation Genetics (2019)

Genotyping For Population Genetic Analysis

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Background

- Maritime population connectivity is the study of how populations are related and connected.
- It is important for the management of species for economic reasons, management of reserve areas, and restoration activities.
- Population connectivity as a function of several processes for organisms with a larval planktonic life history strategy, including larval transport, larval dispersal, and post-larval survival.
- This study examined the connectivity of oyster spat and adult oyster populations, as well as the effects of larval lay, metamorphosis, and recruitment. The results of this project can be measured and tested with a variety of methods.

Turley, et. al. "*Multiple drivers of interannual oyster settlement and recruitment in the lower Chesapeake Bay*" Conservation Genetics (2019)

Genotyping For Population Genetic Analysis

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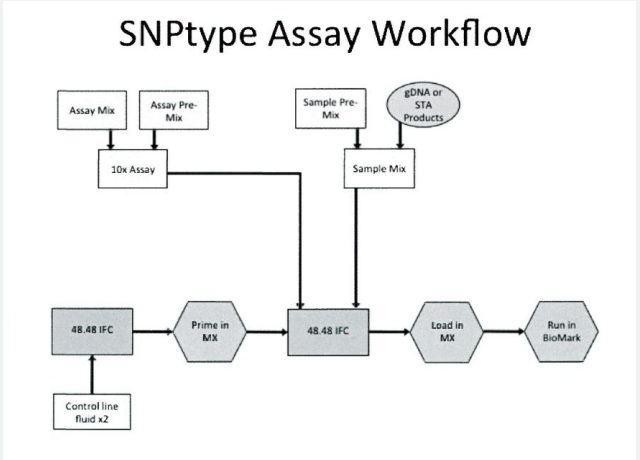
Methods

- DNA was extracted from sterile gill, mantle, and adductor-muscle tissue stored in 95% ethanol.
- 95 loci were converted to SNP Type assays using D3TM Assay Design software and tested for repeatability.
- Oysters were genotyped utilizing Fluidigm 48×48 ®'s Dynamic Array IFC technique. Before the SNP Type test process, pre-amplification PCR was done using specific target amplification (STA) primers for each SNP locus to standardize the starting DNA template for each sample.
- The diluted STA products as template on a 48×48 Dynamic Array IFC, which genotypes 47 individual samples plus one control and 48 SNP loci simultaneously.
- SNP Genotyping Analysis 4.1.2 used a k-means clustering approach to handle BioMark HD fluorescence signals.
- The genotype of each individual was stable among duplicate runs, however fluorescence levels varied.
- One of our SNP panel's 48 loci was duplicated to test repeatability.
- Before analysis, the duplicate locus was deleted. Reproducibility difficulties limited statistical studies to 41 loci.



Flow diagram for this study showing the SBI SNP Type Assay Workflow (via Genotyping).

Turley, et. al. "*Multiple drivers of interannual oyster settlement and recruitment in the lower Chesapeake Bay*" Conservation Genetics (2019)

Genotyping For Population Genetic Analysis

Any further question?

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Results and conclusions

Results:

- Gill, mantle, and adductor-muscle tissue was collected in a sterile way so that DNA could be taken from it.
- Patterns of spat recruitment in the Lafayette River were different from the patterns of where and when post-larval oysters settled, which were based on shell string surveys.
- All of the shell-string sites were settled at about the same time each year.
- Between 2013 and 2014, however, both the time and space patterns were different.
- In the 2013 shell-string survey, settlement of post larval oysters was first seen in mid-June and steadily rose until early August, when it reached its peak (Fig. 4).

Conclusions:

- Oyster spat from 2013 were genetically more similar to resident adults sampled in the Lafayette River, while the
- 2014 spat exhibited genotypic frequencies more similar to adults from surrounding rivers. The winds during the spawning seasons differed between years providing conditions for retention in 2013 and mixing of water masses in 2014.
- We recommend that the monitoring of restoration activities should consider relevant environmental conditions and observe multiple years of recruitment to assess the genetic impacts of restoration plantings and variable reproductive success

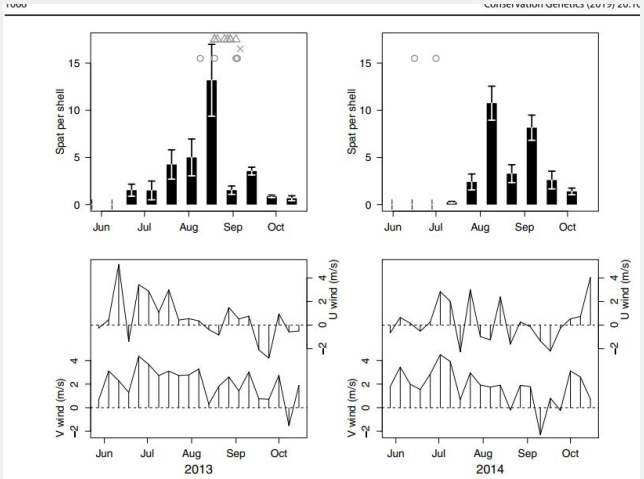


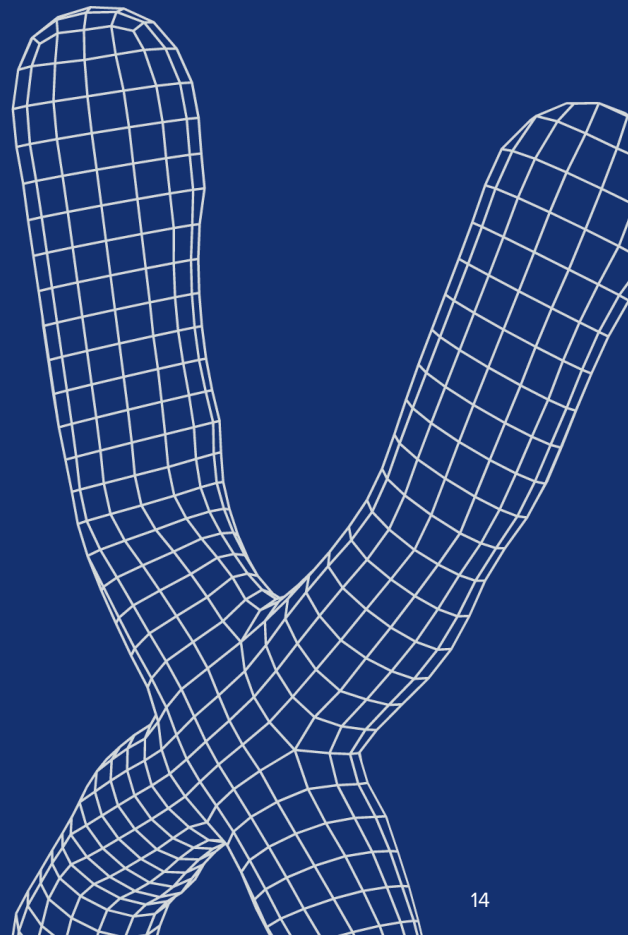
Figure 4. Demonstration of settlement of post larval oysters .

Turley, et. al. "*Multiple drivers of interannual oyster settlement and recruitment in the lower Chesapeake Bay*" Conservation Genetics (2019)

Gene Expression For Pathogen Detection

Any questions?

Talk to us



Gene Expression For Pathogen Detection

Any further question?

Talk to us

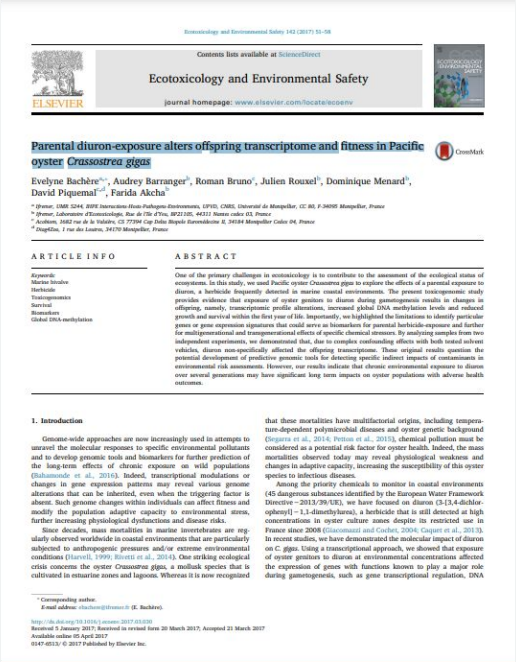
Objective: Utilize Pacific oysters to explore the effects of parental lineage to diuron (a herbicide) detected in marine coastal environments.

Utilization of Standard BioTools products:

- Fluidigm Biomark HD
- Fluidigm GE Type Panel
- Fluidigm 96.96.48 Dynamic Array genotyping chip

Conclusions:

- These original results question the potential development of predictive genomic tools for detecting specific indirect impacts of contaminants in environmental risk assessments.
- However, our results indicate that chronic environmental exposure to diuron over several generations may have significant long-term impacts on oyster populations with adverse health outcomes.



Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Parental diuron-exposure alters offspring transcriptome and fitness in Pacific oyster *Crassostrea gigas*

Evelyne Bachère[a,*], Audrey Barranger[b], Roman Bruno[c], Julien Rouxel[b], Dominique Menard[b], David Piquemal[c,d], Farida Akcha[b]

ARTICLE INFO

ABSTRACT

1. Introduction

Bachere, et al. "*Parental diuron-exposure alters offspring transcriptome and fitness in Pacific oyster Crassostrea gigas*" Ecotoxicol Environ Saf. (2017)

Gene Expression For Pathogen Detection

Any further question?

Talk to us

Background

- Genome-wide techniques are being employed to unravel cellular responses to specific environmental contaminants and build genomic tools and biomarkers to anticipate the long-term impacts of chronic exposure on wild populations
- Even without the triggering stimulus, transcriptional modulations or gene expression patterns can indicate genomic abnormalities that can be inherited.
- Individual genomic mutations can impact fitness and community adaptation to environmental stress, raising physiological dysfunctions and illness risks
- Oysters a major ecological catastrophe.
- Chemical pollution is a risk factor for oyster health, even if these mortalities are caused by temperature-dependent polymicrobial illnesses and oyster genetic background.
- The large mortalities observed today may indicate physiological frailty and adaptive capability changes, making this oyster species more susceptible to infectious illnesses.
- Diuron (a herbicide) affects oysters molecularly, as shown in recent investigations.
- Diuron is found in high concentrations in oyster culture zones.

Bachere, et al. "*Parental diuron-exposure alters offspring transcriptome and fitness in Pacific oyster Crassostrea gigas*" Ecotoxicol Environ Saf. (2017)

Gene Expression For Pathogen Detection

Any further question?

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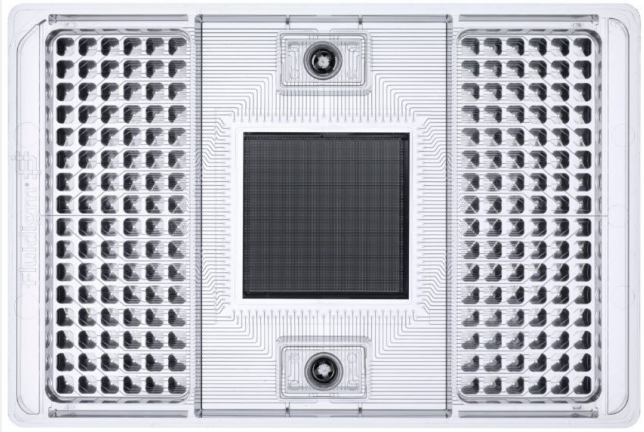
Methods

- cDNAs were generated from 300 ng of total individual RNA samples and pre-amplified using the TaqMan PreAmp kit for high-throughput qPCR.
- Biomark microfluidic qPCR was used to evaluate the relative gene expression of 96 genes in triplicate for 90 samples via 96.96 IFC.
- 14 cycles (95 °C/15 s and 60 °C/4 min) were used for pre-amplification. 35 amplification cycles (95 °C/15 s and 60 °C/60 s) were performed. qPCR fluorescence was collected between 60 and 95 °C in 0.5 °C increments for melting curve analysis.
- Biomark HD qPCR results were examined, and the relative expression was normalized.



Standard Biotools 96.96 IFC

Bachere, et al. "*Parental diuron-exposure alters offspring transcriptome and fitness in Pacific oyster Crassostrea gigas*" Ecotoxicol Environ Saf. (2017)

Gene Expression For Pathogen Detection

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Results and conclusions

Results:

- The present toxicogenomic study provides evidence that exposure of oyster genitors to diuron during gametogenesis results in changes in offspring, namely, transcriptomic profile alterations, increased global DNA methylation levels and reduced growth and survival within the first year of life.
- Importantly, we highlighted the limitations to identify particular genes or gene expression signatures that could serve as biomarkers for parental herbicide-exposure and further for multigenerational and transgenerational effects of specific chemical stressors.
- By analyzing samples from two independent experiments, we demonstrated that, due to complex confounding effects with both tested solvent vehicles, diuron non-specifically affected the offspring transcriptome

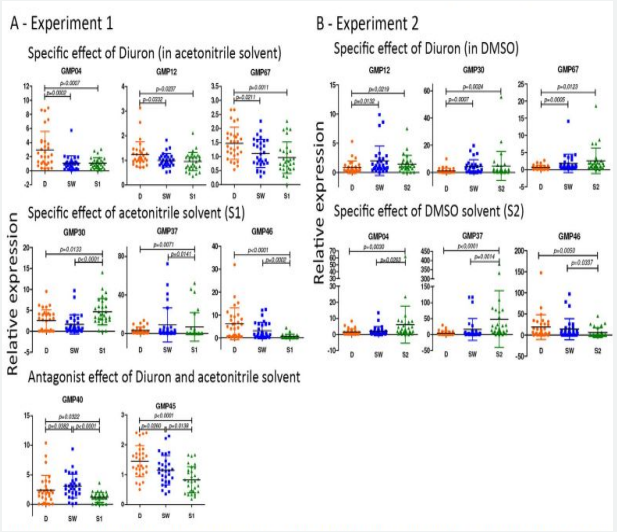

Fig. 2. Examples of various effects of parental exposure on gene expression in the spat from Exp1 (A) and Exp2 (B). Gene expression profiles characteristic of the specific effects of diuron and of the solvents, acetonitrile or DMSO, and characteristic of the antagonistic effects of diuron and its solvent.

Bachere, et al. *"Parental diuron-exposure alters offspring transcriptome and fitness in Pacific oyster Crassostrea gigas"* Ecotoxicol Environ Saf. (2017)

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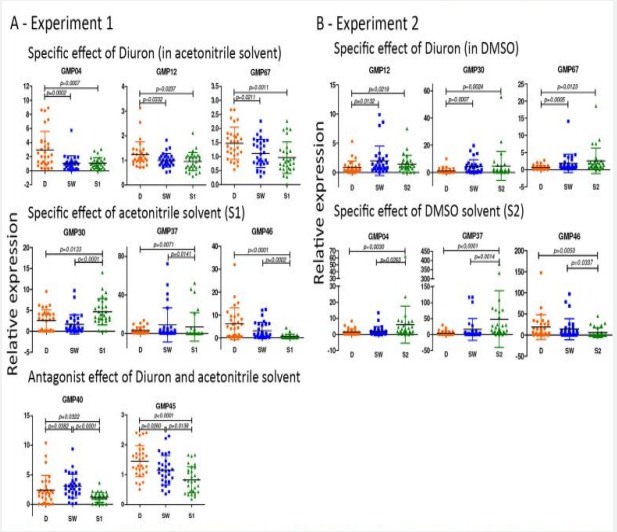

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Modern aquaculture is powered by X9

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