A molecular probe finds evidence of NIX pathogen in Pacific razor clams (*Siliqua patula*) in Oregon

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ABSTRACT

The Pacific razor clam, Siliqua patula, is an important recreational fishery species that lives in the intertidal zone of sandy beaches from Alaska to central California. Populations have had periodic, but significant, declines over the past 30-40 years. These declines have correlated with an increase in the presences of an unidentified, intranuclear bacterial parasite known as Nuclear Inclusion X (NIX). NIX, which was first identified in 1986, has generally been screened using a histological approach. We developed a PCR-based screen to reduce both the time and cost of identifying infected clams. Use of this screen resulted in amplified sequences with a 97% match to the previously published 16S rDNA sequence for NIX. The sequence data supports placement of NIX into the gamma-proteobacteria, and suggests that it is related to isolates from diseased corals. Clams collected from the northern coast of Oregon showed ~50% infection rate using the PCR screen. This is the first report of NIX present in clams from Oregon, as all previous work had been in the state of Washington. Future work will identify the incidence rate and geographical spread of the NIX parasite tt Oregon and Washington. hroughou

METHODS AND MATERIALS

Sample Protocols

- At Kalaloch Beach in Olympic National Park, clams were sampled along four transects using a pumped method.
- At beaches in Oregon, clams were dug haphazardly using a clam gun.

Figure 4. Method of sampling for razor clams and other infaunal organisms used at Kalaloch.



Probe Design

- Genomic DNA was extracted from clam gill tissue
- DNA was first amplified using the 27F/ 1492R primer set for the 16S rRNA gene
- This PCR product was used with a primer set (NIX F/NIX R) designed by R. Elston at Battelle Marine Lab to screen for NIX in clams

Sequencing and Phylogeny

- PCR products were enzymatically cleaned and sequenced (GeneWiz Inc., South Plainfield, NJ)
- Sequences were matched to the published (Kerk et al. 1992) sequence of the 16S gene of NIX
- A BLAST search was conducted, and a phylogeny was built using similar sequences in MEGA6.06



Figure 5. Sampling for clams in Oregon

BACKGROUND

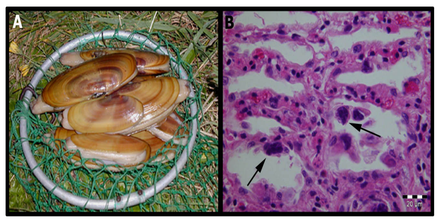
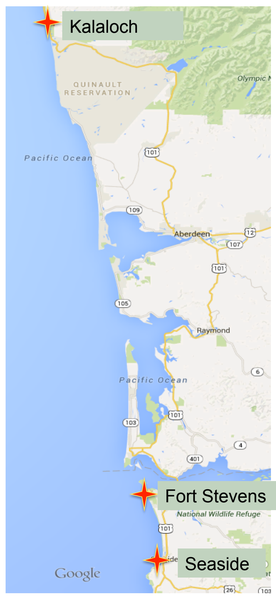


Figure 1: A) Razor clams harvested from Kalaloch beach, Olympic National Park, Washington. B) Stained razor clam gill tissue section with arrows illustrating NIX infections.



- Razor Clams (*Siliqua patula*; Fig 1A) are an important recreational fishery in Oregon and Washington
- Razor clam populations have declined significantly over the past 15 years (Fig. 3), possibly due to disease
- An unidentified bacteria called Nuclear Inclusion X (NIX) has been found in many clams and is thought to be the causative agent of a disease (Fig. 1B)

Figure 2. Map of Pacific Northwest coast. Study sites are indicated by a red star.

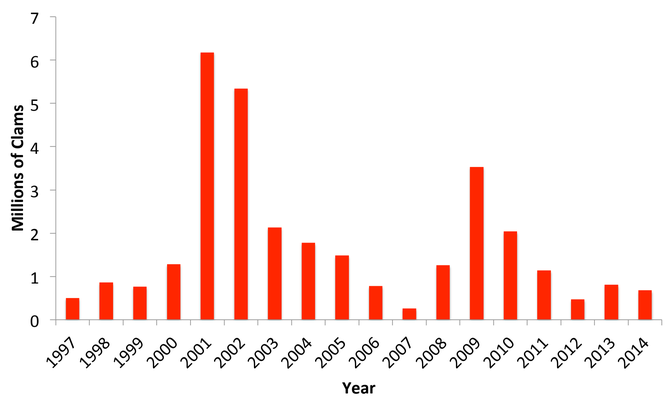


Figure 3: Annual adult razor clam population assessments at Kalaloch Beach (Olympic National Park). Populations have been struck by periodic declines over the last two decades.

RESULTS

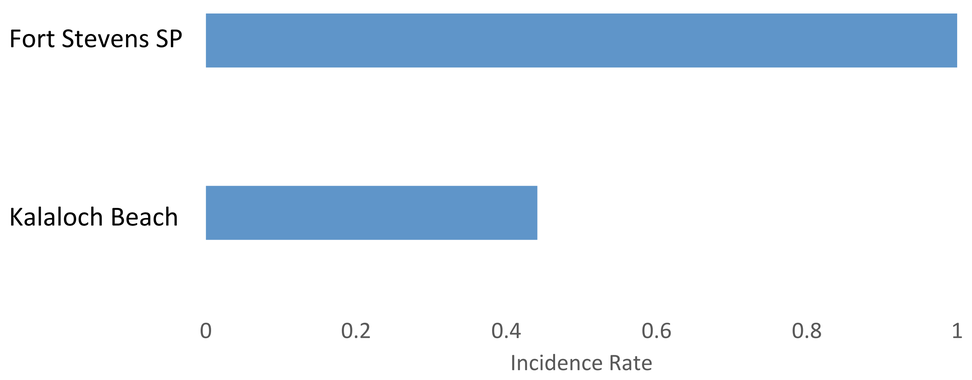


Figure 6: Incidence rate of razor clams testing positive for NIX bacteria from Klaloch Beach in Washington and Fort Stevens in Oregon. Incidence rates from Fort Stevens may not be indicative of overall incidence because only a small number of samples have been screened so far. However there is suggestion that

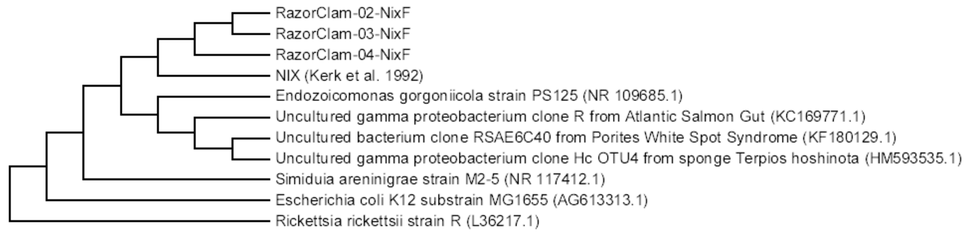


Figure 7: Maximum likelihood phylogeny of NIX sequences and related bacteria. This is a bootstrap consensus tree based on 500 replicates. The NIX sequence from Kerk et al. (1992) is the only published sequence of the NIX 16S gene. All other sequences are listed with their GenBank accession number.

DISCUSSION

- A successful screening method for the detection of NIX bacteria in razor clams was developed and tested.
- We have the first reports of NIX infected clams from Oregon, indicating a wider extent of the potential pathogen than previously suggested.
- In the coming summer various sites along the Oregon coast will be sampled and tested to determine the rate of incidence in Oregon.

ACKNOWLEDGEMENTS

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