Finding the Practical Value of Environmental DNA Data: A Case Study with Invasive European Green Crab

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A thesis

Submitted in partial fulfillment of the

requirements for the degree of

Master of Marine Affairs

University of Washington

2021

Committee:

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Program Authorized to Offer Degree:

School of Marine and Environmental Affairs

University of Washington

Abstract

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Advances in environmental DNA (eDNA) applications hold promise for revolutionizing environmental monitoring and management, providing increased detection sensitivity at reduced cost and survey effort. However, implementation of eDNA methods in decision-making contexts lags significantly behind technical progress in research, mainly due to uncertainty in data interpretation and a lack of standard operating procedures for how to best integrate eDNA approaches into existing management efforts. I address these key challenges by developing a Bayesian model that uses heterogeneity in molecular detection probability to interpret patterns of eDNA signals, integrating information from both traditional and eDNA monitoring methods to jointly estimate local species density. Critically, the joint model offers a framework for quantifying the marginal benefit of eDNA data, specifically how the addition of eDNA data

improves the precision of species density estimates. I illustrate the approach using environmental DNA information from a marine invasive species, *Carcinus maenas* (European green crab), across Washington State, USA. Importantly, I document green crab eDNA beyond the previously known invasion front and find exponential increases in the value of environmental DNA data at these locations. In addition, the joint model quantifies uncertainty in estimates of species density and provides the necessary interface for combining molecular and traditional data streams. These results provide managers the analytical framework needed to successfully integrate environmental DNA information into management decisions and suggest that eDNA approaches are best suited for mapping invasion fronts and locations with low species density.

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Introduction

Applications of environmental DNA (eDNA) represent one of the most critical advances in aquatic conservation in the last decade (Cristescu & Hebert, 2018; Sutherland et al., 2015). Aquatic eDNA refers to the intra- and extra- cellular forms of DNA released by an organism into the water (Pawlowski et al., 2020). Since the first documented use of eDNA methods for the detection of invasive American bullfrogs (*Lithobates catesbeianus*) (Ficetola et al., 2008), the fields of conservation and ecology have seen a wave of eDNA studies, with wide ranging applications across a myriad of ecosystems and target taxa (Bohmann et al., 2014; Deiner et al., 2017; Thomsen & Willerslev, 2015). Rapid adoption of eDNA-based techniques is also facilitated by the increasing availability and affordability of techniques like quantitative polymerase chain reaction (qPCR), digital droplet PCR (ddPCR), and high throughput sequencing (HTS) that can detect trace amount of DNA in environmental samples (Jerde, 2019). These molecular techniques allow for collection of high-resolution biological information where traditional monitoring may be infeasible, labor-intensive, or reliant upon diminishing taxonomic expertise (Kelly et al., 2014). Furthermore, some eDNA assays have been shown to be more sensitive than traditional sampling methods in detecting rare individuals (Goldberg et al., 2013; Jerde et al., 2011). Together, the advances and success of eDNA sampling are promising for the detection of rare, cryptic, or elusive aquatic species, specifically invasive species.

Invasive species pose a global threat to biodiversity, transforming habitats by displacing native species, increasing genetic homogeneity, changing community structure, and altering fundamental ecological and physical processes (Molnar et al., 2008). Establishment of non-native populations can have dramatic economic effects by diminishing fisheries, fouling ships' hulls, and clogging intake pipes, and some species can directly impact human health by

facilitating disease spread (Ruiz et al., 1997). Early detection and monitoring are key components of successful invasive species management strategies (Lodge et al., 2006). During the invasion process, the lag time between arrival and rapid population growth provides an opportunity for detection and eradication, and detection at early stages of establishment has previously facilitated successful eradications of nascent invasions (Anderson, 2005; Wimbush et al., 2009). However, the effort required to detect a species is inversely proportional to its population size (Hayes et al., 2005). Historically, cost-effective management strategies have had to balance high survey costs for small populations and high eradication costs if the survey fails to detect an incipient population in the initial stages of invasion (Lodge et al., 2006). Genetic approaches can have improved detection of rare individuals, thereby increasing the efficacy of surveys for small populations (Dougherty et al., 2016; Harper et al., 2018; Hunter et al., 2015; Schütz et al., 2020; Tucker et al., 2016; Wilcox et al., 2016).

Despite substantial technical advances in eDNA research, few examples exist of eDNA methods incorporated into routine monitoring and management practices. Notable exceptions include the United Kingdom's acceptance of eDNA qPCR results as evidence for the presence of the protected great crested newt, *Triturus cristatus*; there, developers can be prohibited from developing wetlands where there have been positive eDNA detections (Biggs et al., 2015; Natural England, 2017). Protocols for eDNA sampling from streams and eDNA monitoring of bighead and silver carps (*Hypophthalmichthys* spp.) were developed by the U.S. Fish and Wildlife Service and U.S. Department of Agriculture to guide field and laboratory eDNA methods, as well as outline recommendations for sampling plans and schedules to be implemented by regional sampling agencies (Carim et al., 2016; Woldt et al., 2020). Specifically, eDNA methods have also been adopted as a key element of the Asian carp (*Hypophthalmichthys*

spp.) monitoring program in the economically important Laurentian Great Lake ecosystem. Here, the initial molecular detections in the Great Lakes sparked highly politicized debate, given that the detections were found beyond the known invasion front and past the electric barrier intended to prevent Asian carp dispersal into Lake Michigan, USA (Jerde et al., 2011, 2013). Uncertainty regarding whether the species had actually invaded the Great Lakes led to multiple federal court cases regarding closing the Chicago Area Water System locks and dams, which brought eDNA into the public eye (Cristescu & Hebert, 2018; Jerde, 2019). Importantly, eight months after eDNA evidence indicated Asian carp presence north of the barriers, a commercial fisherman caught an adult bighead carp within 4 km upstream of the nearest positive eDNA detection.

Typically, however, methodological development outpaces thoughtful consideration and a systematic experimental approach to determine how to use DNA evidence to support management decisions. Consequently, managers have been slow to adopt eDNA-based approaches in decision making frameworks, (Bohmann et al., 2014; Darling & Mahon, 2011) due to gaps in understanding of the dynamics of eDNA in space and time, as well as eDNA methods' susceptibility to false negative detections and false positive detections (Goldberg et al., 2016; O'Donnell et al., 2017). Although all sampling methods have potential errors, there are many mechanisms for eDNA methods to indicate a false presence, and the fear of a false positive detection is cited as the primary obstacle to adopting eDNA-based methods in species monitoring (Jerde, 2019). Understanding and managing uncertainty is also not exclusively a scientific issue (Darling & Mahon, 2011). When the relationship between the presence of target DNA and presence of viable individuals is unknown, data is not reliable enough to satisfy legal standards and justify public confidence (Kelly et al., 2014).

Previous reviews highlight the "potential" of eDNA methods to dramatically improve biodiversity assessments and targeted detection of species of concern, as well as the "potential" for unreliability and augmenting of existing uncertainty in environmental management and assessment (Beng & Corlett, 2020; Bohmann et al., 2014; Darling & Mahon, 2011; Yoccoz, 2012). Moving from evaluating the potential value of eDNA data to the practical value of eDNA data will depend on addressing uncertainties in data interpretation (Cristescu & Hebert, 2018; Lacoursière-Roussel & Deiner, 2021). Novel and appropriate statistical methods therefore must be employed to fully realize the potential of eDNA techniques, improve and quantify confidence in eDNA detections, and achieve efficient and strategic use of management and monitoring resources (Yoccoz, 2012).

Recent work advances eDNA data interpretation by extending site occupancy modeling methods to estimate species presence and absence using eDNA data (Lahoz-Monfort et al., 2016). Such models account for imperfect detection when inferring species occupancy and can overcome bias introduced by false negative and false positive detections (Hunter et al., 2015; Schmelzle & Kinziger, 2016; Schmidt et al., 2013). Occupancy models suggest that there are two classes of sites, those that are occupied and those that are not, and these models assume no unmodelled heterogeneity among sites in the probability of detecting a species at a site where it occurs (Royle and Nichols 2003; Altwegg & Nichols, 2019). In reality, variability in local abundance of the species between sites is one important factor that can induce heterogeneity in detection probability with genetic methods, resulting in low estimates of occupancy probability at sites where a species is present but rare. A low molecular detection rate can therefore reflect low abundance, rather than low probability of occupancy. Particularly for species with relatively low eDNA shedding rates, and subsequently relatively low molecular detection probability

(Andruszkiewicz Allan et al., 2021; Crane et al., 2021), using eDNA data to its fullest potential is hamstrung by the inadequacy of the models currently used for data interpretation.

Royle and Nichols (2003) aimed to overcome this limitation by describing a modeling approach that links heterogeneity in abundance to heterogeneity in detection probability, allowing for estimation of abundance from repeated observations of a species. This heterogeneous detection probability model provides a framework for estimating species density based on abundance-induced variation in detection probability with eDNA methods (Royle & Nichols, 2003). Building on this framework, I present a model formulated using Bayesian inference that jointly models information from both traditional and eDNA monitoring methods to estimate local species density. The joint model aids management decisions by informing interpretation of molecular detections, the most appropriate use of eDNA sampling efforts, and the relative sensitivities of molecular and traditional sampling methods.

To determine the value of eDNA information for detecting an invasive species, here I apply the joint model to eDNA detection data of European green crab, *Carcinus maenas*, in Washington State. Green crab is one of the International Union for Conservation of Nature's (IUCN) world's 100 worst alien invasive species (Lowe et al., 2000). The first green crab was detected in Washington waters in 1998, after warm El Nino-Southern Oscillation (ENSO) currents spread larvae of California populations up to British Columbia, Canada (Behrens Yamada & Hunt, 2000). Green crab is now classified as a deleterious species in Washington State because of perceived risks to coastal resources, and this action mandated monitoring and control in State waters (Grason et al., 2018). To address this invasion, Washington Sea Grant launched a community science network, Crab Team, in 2015, that conducts early detection monitoring for invasive green crab across the Washington portion of the Salish Sea (Grason et

al., 2016). Since green crabs were first detected along these shorelines in 2016, Washington Department of Fish and Wildlife (WDFW), federal and state agencies, and several sovereign tribal nations have joined the effort to increase the statewide capacity to surveil and manage the nearly 3,000 km of Washington's inland shoreline.

Having estimated the density of green crab across the study sites using the joint model, I then quantify the value of eDNA information in the context of green crab detection. This value can be interpreted as eDNA's marginal benefit, or the changes in the precision of green crab density estimates upon inclusion of eDNA data in a joint model of species density. These changes in precision highlight the circumstances under which adding eDNA sampling is likely to reduce the uncertainty around estimates of species density, thereby increasing confidence that management has been scaled appropriately. By providing a framework for inferring confidence in patterns of eDNA detections and quantifying uncertainty around estimates of species density, I provide a channel for data interpretation between eDNA results and management, as well as a tool for integrating uncertainty into decision-making using genetic surveys.

Methods

I first illustrate the specification of the joint model estimating species density using both traditional and environmental DNA sampling. I then describe the joint model's application in the context of green crab, beginning with eDNA sample collection and processing and moving to model application and evaluation of eDNA's value through both data and simulations.

i. Joint model description

I present a joint model that uses two streams of information—traditional trap data and eDNA qPCR detections—, linked through a shared species density. This density is estimated through repeated observations by both traditional monitoring methods and eDNA methods. Traditional monitoring methods, like trapping or trawling, can relate repeated capture rates to an underlying species density. Here I model species counts as a negative binomial process; the negative binomial is a mixture of Poisson distributions with means that vary according to a gamma distribution (White & Bennetts, 1996). The negative binomial is frequently used in wildlife modeling in instances where the density of a species varies spatially, allowing for more variation in abundance than can be described by a constant mean Poisson model (Lindén & Mäntyniemi, 2011). The observed count, Y, of a species on occasion *j* at site *i* can be considered a draw from a negative binomial distribution with a mean species density, μ, at site *i* and an overdispersion parameter, σ, that controls overdispersion relative to the square of the mean (Eq. 1.1).

$$Y_{i,j} \sim \text{NegBinomial}(\mu_{i,} \sigma) \qquad \textit{Eq. 1.1}$$

Guided by the principle that the probability of detection with eDNA methods increases as the underlying species density increases, I describe the probability of a true molecular detection, p11, at site *i* as a log-linear function of mean species density, μ, scaled with coefficient β (Eq. 1.2). The logit link converts the linear function to the scale of a probability, between 0 and 1. Recognizing the susceptibility of eDNA methods to false positive errors (Roussel et al., 2015; Sepulveda et al., 2020), I incorporate a false positive probability, p10, that represents two sources of false positive detections: (1) presence of target DNA in the sample but absence of

target organism at the associated site, arising from processes like laboratory contamination or transportation of target cells from far away locations, and (2) absence of target DNA in the sample but a positive molecular detection, arising from processes like non-specific amplification. The false positive probability, p10, contributes to the overall molecular detection probability, p, at site *i*, which is estimated through repeated molecular observations of the site by employing a species-specific quantitative PCR (qPCR) assay. To avoid computational challenges associated with estimating a parameter near zero, the false positive probability is estimated as e^{p10} (Annis et al., 2017). The number of positive qPCR detections, N, out of the number of trials, K, on occasion *j* at site *i* can be considered a draw from a binomial distribution, with a probability of success on a single trial, p (Eq. 1.4).

$$\text{logit}(p11_i) = \log(\mu_i) * \beta \qquad \textit{Eq. 1.2}$$

$$p_i = \exp(p10) + p11_i \qquad \textit{Eq. 1.3}$$

$$N_{i,j} \sim \text{Binomial}(K_{i,j}, p_i) \qquad \textit{Eq. 1.4}$$

I formulate this joint model under a Bayesian statistical framework, which treats unobserved quantities as random variables, and available data are used to make statements about the probability distributions of the unobserved quantities, which can provide the basis for mathematically coherent decision analysis (Williams & Hooten, 2016). These statements about the unobserved quantities address uncertainty in the ecological process, as well as the sampling and observation of the process. Assessing uncertainty is fundamental to science and important in guiding decision making, and Bayesian methods offer an opportunity to make scientific

statements tempered by quantified uncertainty (Hobbs & Hooten, 2015). Additionally, Bayesian methods allow model augmentation with additional sources of information through explicit assignment of prior probabilities of the unobserved quantities (Ellison, 1996). These prior distributions reflect knowledge about the joint model's estimated parameters and can be critical in overcoming parameter identifiability issues (Griffin et al., 2019).

Bayesian inference is based on a joint likelihood that is a product of the likelihoods from the independent observations or datasets, as well as the prior probability distributions of the estimated parameters (Figure 1).

$$[\boldsymbol{\mu}_i, \sigma, \beta, p10 \mid \mathbf{Y}_{i,k}, \mathbf{N}_{i,j}, \mathbf{K}_{i,j}] \propto \prod_{i=1}^{n} \prod_{j=1}^{m} \prod_{k=1}^{p} \text{NegBinomial}(\mathbf{Y}_{i,k} \mid \boldsymbol{\mu}_i, \sigma) \times \text{Binomial}(\mathbf{N}_{i,j}, \mathbf{K}_{i,j} \mid p10, \boldsymbol{\mu}_i, \beta) \times \text{gamma}(\boldsymbol{\mu}_i \mid \alpha, \beta) \times \text{gamma}(\sigma \mid \alpha, \beta) \times \text{normal}(p10 \mid \mu, \sigma^2) \times \text{gamma}(\beta_1 \mid \alpha, \beta)$$

Figure 1: Model joint posterior distribution expressed as the product of the two independent likelihoods and the prior distributions for the estimated parameters for sites 1…*n*, eDNA sampling occasions 1…*m*, and trap sampling occasions 1…*p*. Bolded symbols are vectors. Gray symbols indicate fixed quantities, while black symbols indicate random variables.

The Bayesian statistical model was specified within Stan, a probabilistic programming language written in C++ that implements full Bayesian statistical inference using Markov chain Monte Carlo. The Stan language was accessed through R version 4.0.4 as an interface, using the package 'rstan' to allow for integration with the R software environment and to access the output, including posterior inferences and generated quantities, like the log likelihood (Guo et al., 2020; R Development Core Team, 2021).

ii. Green crab eDNA data collection

eDNA field sampling

Twenty sites with varying known presence and abundance of green crab were chosen for eDNA sampling (Figure 2, Supplemental Figure 1), and all sites were distinct with relation to green crab movement on the time scale relevant for sampling effort. All eDNA field sampling equipment was decontaminated by soaking in 10% bleach between each use. Five water samples (1 L) were collected 1-5 meters apart at each site by submerging Nalgene bottles attached to a 1.7-meter pole about 0.5 meters into the water. Water samples were placed on ice and vacuum filtered within four hours of collection, except for samples from the KVI site, where samples were stored at 4°C and filtered 24 hours after collection due to vacuum equipment malfunction. I filtered 500-600 mL of each water sample for eDNA with a cellulose acetate filter (47 mm diameter, 0.45 µm pore size) using a vacuum pump and side arm flask, and the filter was preserved in 900 µL of Longmire buffer (Renshaw et al., 2015). Filters were stored at -80°C for 1-3 weeks before DNA extraction.

eDNA sample processing

To extract DNA from filters, I used a phenol:chloroform:isoamyl alcohol protocol (modified from Renshaw et al., 2015). About 500 mL of acid washed glass beads (425-600 µM, Sigma Aldrich) were added to each filter and vortexed for 60 seconds to dislodge cells from the filter. Each sample was incubated at 56°C with 20 µL of proteinase K (10 mg/mL). After incubation, 900 µL of phenol:chloroform:isoamyl alcohol (25:24:1) was added to each sample, and tubes were shaken vigorously for 60 seconds and centrifuged at 14,000 rpm for 5 minutes. Each sample then underwent two consecutive chloroform washes by transferring the aqueous layer to 700 µL of chloroform:isoamyl alcohol (24:1), shaking vigorously for 60 seconds, and centrifuging at 14,000 rpm for 5 minutes. The aqueous layer (about 500 µL) was then transferred

to a tube containing 20 µL 5 M NaCl and 500 µL 100% isopropanol, and the samples precipitated overnight (about 15 hours) at -20°C. After the overnight incubation, the samples were centrifuged at 14,000 rpm for 10 min, liquid was poured off slowly, and samples were vacuum centrifuged at 45°C for 15 min. Each eluate was resuspended in 200 µL of UV sterilized, molecular grade water. One negative control (900 µL of Longmire buffer) was extracted during each set of DNA extractions, for a total of three extraction negative controls. DNA purity of each sample was quantified on a spectrophotometer (Nanodrop, Thermo Scientific), and DNA concentration was quantified on a fluorometer (Qubit, Invitrogen).

Each eDNA extract was amplified by quantitative polymerase chain reaction (qPCR) using a *C. maenas*-specific assay developed by Roux et al. (2020). The assay targets a 100 bp fragment of the cytochrome c oxidase 1 (CO1) region and uses forward primer 5′-ATGAACAGTCTATCCTCCTTTAG-3′, reverse primer 5′-GAAAGAACGCATATTGATAATAGTTG-3′, and TaqMan™ MGB probe (Applied Biosystems) 6FAM-AGTTGATTTAGGGATTTTC- MGB (Roux et al., 2020). Three qPCR replicates were run for each eDNA extract in the following 25 µL reaction: 5.75 µL of nuclease-free water, 1.25 µL forward primer (10 µM), 1.25 µL reverse primer (10 µM), 0.25 µL probe (10 µM), 12.5 µL TaqPath™ ProAmp™ Master Mix (Applied Biosystems), 2 µL Bovine Serum Albumin (500 ng/µL), and 2 µL of DNA template. Cycling conditions were as follows: 15 min at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Three negative PCR controls containing 2 µL of molecular grade water were included in each reaction, and each extraction negative control was run in triplicate. All qPCR reactions were performed on Applied Biosystems StepOnePlus Real-Time PCR System an analyzed with StepOne Software v2.3. Any DNA template passing the fluorescence threshold in fewer than 38 cycles was considered a

positive amplification, since 38 Ct is the average Ct value corresponding to the assay's limit of detection with 50% chance of detection (Roux et al., 2020). The identity of 13 qPCR products from four sites were confirmed through unidirectional Sanger sequencing with the forward primer.

Inhibition Testing

To ensure negative qPCR detections were not systematically due to PCR inhibition, I measured potential inhibition occurrence by analyzing the quantification threshold (Ct) deviation of a spiked internal positive control. A synthetic (gBlock) positive control was spiked into samples with no positive amplifications (Integrated DNA Technologies). The double-stranded 125 bp gBlock fragment contains green crab specific primer and probe sequences and was otherwise comprised of random nucleotides. For sites where all eDNA replicates previously tested negative for green crab, one eDNA sample per site was tested for inhibition. For sites where some eDNA replicates tested negative for green crab, each previously negative eDNA sample was tested for inhibition. Each qPCR reaction contained all previously mentioned reaction components, with 1 µL of DNA template and 1 µL of the gBlock positive control at a final concentration of 0.195 copies/µL. Three qPCR replicates containing 1 µL of the gBlock positive control at a final concentration of 0.195 copies/µL was also included in the reaction. Inhibition occurrence was measured as the difference in Ct, ΔCt, between the Ct value of the spiked eDNA sample and the mean of the three positive gBlock controls ($Ct_{sample} - Ct_{control}$) (Volkmann et al., 2007). Historically, a ΔCt greater than three cycles in spiked samples is considered evidence of inhibition (Hinlo et al., 2017), and here I consider evidence of inhibition as ΔCt greater than 2, considering that three cycles is almost one order of magnitude difference

in concentration in an efficient reaction and that many of the samples in this study were within 2 cycles of the assay's limit of detection. Each DNA sample underwent 1-3 passes through a OneStep PCR Inhibitor Removal spin column (Zymo Research Corp.) until inhibition occurrence was not detected (Supplemental Table 1).

iii. Joint model application: green crab density estimates

The robust trapping effort for green crab along the Pacific Coast and inland shores of Washington provided a unique opportunity to investigate the reliability and information contribution of eDNA data, and information from trapping efforts conducted concurrently with eDNA sampling were used in the joint model. Baited traps are the primary traditional detection tool used for green crab, and, though implemented by a collective of separate management entities for different purposes, trapping efforts in Washington employ sufficiently similar gear and techniques to permit comparison of detection rates across sites. Traps were set for an overnight soak, and traps were collected within 24 hours of placement. The latitude and longitude of the traps are recorded, and any trapped green crab individuals are counted and subsequently removed from the system. Trap types included in the dataset were limited to those demonstrating similar catchability for green crab and mechanisms of trapping. Specifically, two types of trap were included: the Gee-brand galvanized steel minnow trap (5.08 cm opening, 0.635 cm mesh), and the square Fukui fish trap (1.27 cm mesh).

Twenty of these monitored sites were chosen for eDNA field sampling to represent a range of known presence and abundance of green crab, as well as a range of trapping effort (Supplemental Figure 1). Trapping effort at these sites ranged from three to 420 traps set over the selected trapping period, and water samples were collected two weeks before or after trap

collection, with the exception of the Stackpole site (STA) (Supplemental Figure 2). At STA, only three traps were set during the sampling period, and no green crabs were recovered. To reflect the relatively high density of green crab determined through previous, greater trapping efforts, trapping data at STA collected eight weeks before eDNA sampling were included in the dataset (Supplemental Figure 2). Each trap set was treated as an independent draw from the negative binomial distribution in the model (eq. 1.1). Although the removal nature of the trapping effort (i.e., sampling without replacement of the trap) violates the assumption of independence among samples typical for wildlife models, I assumed the removal efforts likely did not substantially change the relative densities of green crab at the sampled sites over the sampling period (Supplemental Figure 2).

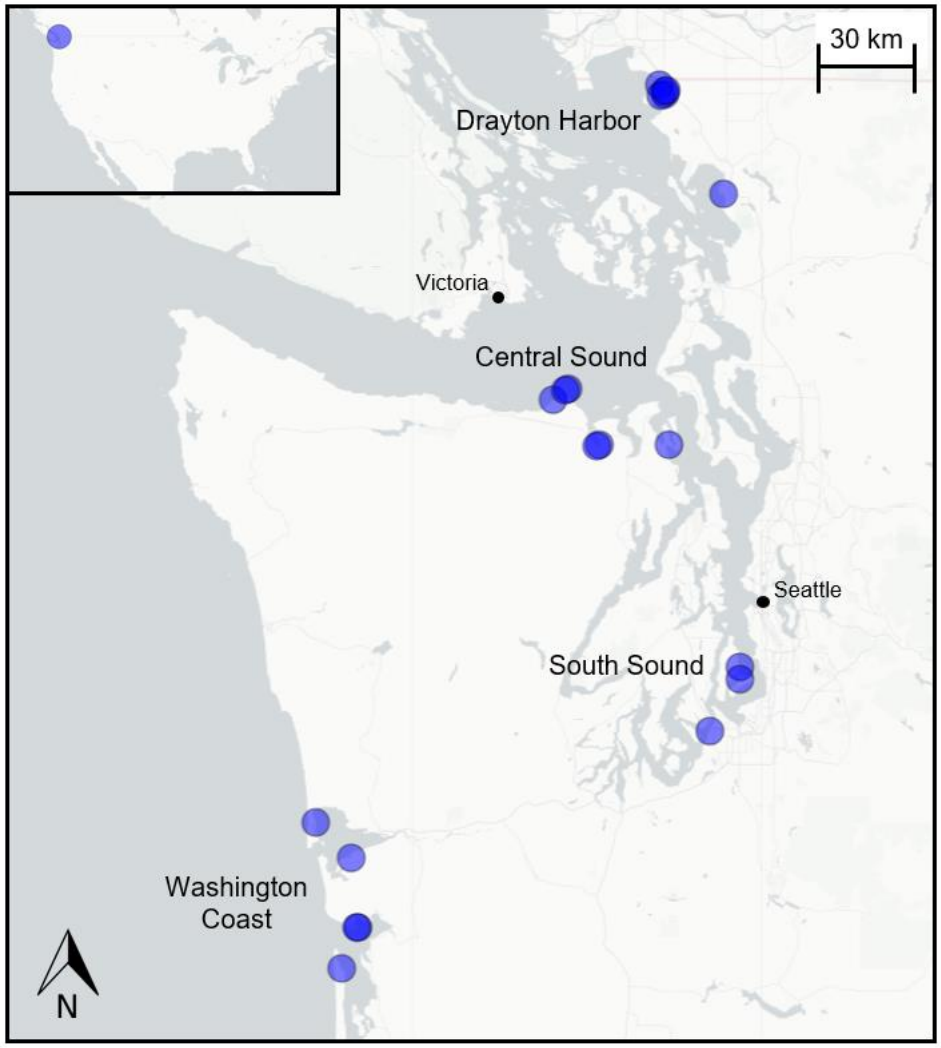


Figure 2: Map of study area and sites of eDNA sampling combined with traditional trapping surveys. Five replicate water samples were collected at 20 sites along the Washington Coast and the Washington portion of the Salish Sea (blue dots). Inset map indicates study location in the context of the United States.

An informative prior distribution was included for the estimated parameter, p10, using negative control data related to misspecification and contamination. After qPCR assay development, Roux *et al*. conducted specificity testing, where the assay was tested with DNA extracts from closely related species. These tests included DNA extracts from eight species within the same order as *C. maenas*, Decapoda, from British Columbia, Canada, which neighbors the areas where environmental samples were collected in this study (Roux et al., 2020). Given

this specificity testing and the 45 contamination-related negative controls (no-template controls, field blanks, DNA extraction blanks), a conservative prior distribution of beta(1,8) was used for the p10 parameter to reflect the knowledge that the false positive detection probability is likely less than 0.125, given the specificity and negative control information. These beta distribution parameters were converted to lognormal distribution parameters through moment matching and were used in a normal prior distribution for the p10 parameter, since the parameter is specified as exp(p10) in the joint model (Eq. 1.3) (Hobbs & Hooten, 2015). Vague prior distributions were used for the other model parameters, where the β prior distribution was gamma(2,1), the mu prior distribution was gamma(0.25, 0.25), and the phi prior distribution was gamma(0.25, 0.25). Gamma distributions were used for the mu and phi prior distributions because the gamma distribution is the conjugate prior distribution for the negative binomial likelihood (Hobbs & Hooten, 2015). The gamma distribution was used for the β prior distribution to reflect the belief that the probability of a true molecular detection is directly proportional to the underlying green crab density, and therefore β is non-negative.

The joint model was run with both the eDNA and trapping data from the 20 sampled sites, with a step size of 0.5 and 4 chains with 5,000 warm-up and 5,000 sampling iterations per chain. Model convergence was determined by analyzing the resulting trace plots. The 95% credibility interval for all estimated parameters was calculated as the highest density interval (HDI), where all points within the interval have a higher probability density than points outside the interval and does not assume equal tails. Additionally, a trap-only model was run using only trapping data from the 20 sampled sites (Eq. 1.1), with a step size of 0.5 and 4 chains with 5,000 warm-up and 5,000 sampling iterations per chain. Model convergence was determined by analyzing the resulting trace plots.

iv. Evaluation of eDNA data's marginal benefit

As information increases, uncertainty decreases. I therefore considered a reduction in uncertainty around green crab density estimates as a measure of the marginal value of eDNA data, relative to the baseline information contained in trap data. Precision in the estimates of green crab density, μ_i, at site *i*, was quantified using a coefficient of variation, which measures the variability in a series of numbers scaled by the mean and therefore facilitates comparisons of variability across green crab densities of differing orders of magnitude (Abdi, 2010). The coefficient of variation was calculated as the standard deviation of the posterior distributed divided by its mean for the parameter μ_i, and was calculated for both the joint model, incorporating both eDNA and trapping information, and the trap-only model, using only trapping information. To determine the changes in precision in the parameter estimates when eDNA information is included in the model, the difference between the coefficient of variation associated with μ_i from the trap-only model and the coefficient of variation associated with μ_i from the joint model was calculated (ΔCV, $CV_{trap} - CV_{joint}$). To investigate whether the marginal benefit varied depending on the level of traditional sampling effort, I explored the relationship between ΔCV and trapping effort.

v. Simulation study

I conducted simulation studies to evaluate how both precision and accuracy of green crab density estimates were influenced by sampling strategy, given a range of variability in green crab trapping effort level and species densities. Both eDNA qPCR data and green crab trap count data were simulated for five green crab densities (0, 0.1, 0.25, 1, 3 crabs/trap) and six trapping efforts

(3, 5, 10, 15, 30, 60 traps), for a total of 30 scenarios, and these scenarios were introduced to the model as 30 simulated sites. These scenarios represented the range of green crab densities and trapping efforts observed in this study. Using the model's estimated parameters relating the underlying green crab density to the probability of molecular detection, I simulated eDNA data representing five water samples with triplicate qPCR reactions for each simulated site. Using the model's estimated parameters relating trap counts to green crab density, I simulated trap counts of green crab for each simulated site. Each set of 30 sites were simulated 50 times to reduce stochasticity. The simulated datasets were used in both the joint model and trap-only model to estimate the underlying green crab density at each simulated site, μ_{sim}, and both models were run with a step size of 0.5 and 4 chains with 1,000 warm-up and 1,000 sampling iterations per chain. The coefficient of variation was calculated as the standard deviation of the posterior distributed divided by its mean for the parameter, μ_i, and was determined for both the joint model and trap-only for all 30 simulated sites. The ΔCV ($CV_{sim,\ trap}$ $CV_{sim,\ joint}$) was averaged across all simulations representing each of the 30 replicated scenarios, and the 95% confidence intervals for each scenario were determined.

Also, the accuracy of the green crab density estimates was determined for all simulated scenarios. The proportion of simulation replicates that yielded a 95% credibility interval containing the true density, μ_{sim}, was calculated for each scenario. The 95% credibility interval was calculated as the highest density interval (HDI). Also, the difference between the estimated $\hat{\mu}_{sim}$ and the true μ_{sim} for all scenarios and replicates was calculated. This difference was identified for both the joint and trap-only model by finding the mean difference between $\hat{\mu}_{sim}$ and μ_{sim} and the 95% confidence interval of the difference for all simulation replicates.

To evaluate the relative sensitivities of the two sampling methods, eDNA sampling and trap sampling, the joint model's parameter estimates were used to determine the sampling efforts necessary to "detect" a green crab with 95% confidence. A detection refers to either capturing at least one green crab in a trap or producing at least one positive qPCR amplification. For the trapping effort, the probability of capturing one or more green crabs in one trap, given a range of underlying crab densities, μ, (0-3 crabs/trap) was first calculated. Then the minimum number of traps necessary to catch one crab with 95% confidence, given the calculated probability of capturing, was subsequently determined. For the eDNA sampling effort, effort was defined as the number of water bottles with triplicate qPCR. The probability of molecular detection, as the sum of the true positive molecular detection (p11) and false positive detection (p10) probabilities, was first calculated given a range of underlying crab densities, μ, (0-3 crabs/trap) for three binomial trials. Then the minimum number of water samples necessary to yield one molecular detection with 95% confidence, given the calculated probability of detection, was subsequently determined.

Results

i. Green crab genetic and traditional monitoring data collection

A total of 128 eDNA water samples were collected and 384 qPCR reactions were performed from samples at 20 sites along the Washington coast and Salish Sea. At least one positive amplification occurred at 13 sites, ranging from one amplification to 15 amplifications out of the total 15 qPCR replicates per site (five biological replicates, three technical replicates per site) (Supplemental Table 1). The average number of positive amplifications per water bottle was 0.40, out of 3 replicates. Among the 20 sampled sites, there were 1274 trap observations,

ranging from three to 420 traps set over the sampling period (Supplemental Figure 2). The mean number of crabs/trap at each site ranged from 0 to 6.04 crabs/trap, and the variance in the mean number of crabs/trap at each site ranged from 0 to 23.00 crabs/trap. Green crabs were trapped at nine of the 20 sampled sites over the sampling period, and eDNA samples yielded at least one positive detection at all nine of these sites. Four sites yielded at least one positive eDNA detection where no green crabs were trapped over the sampling period (Figure 3).

Sites exhibited a range of initial qPCR inhibition, and eDNA samples underwent multiple passes through a PCR inhibitor removal column to mitigate the effects of inhibiting compounds. All samples underwent one to two passes through inhibitor removal columns, except for samples from the Stackpole site (STA), which exhibited the greatest amount of inhibition and underwent three passes through an inhibitor removal column. All samples exhibited a Ct shift from the average of three gBlock positive control replicates fewer than 2 Ct, with the Stackpole site still exhibiting the greatest evidence of inhibition (Supplemental Table 1).

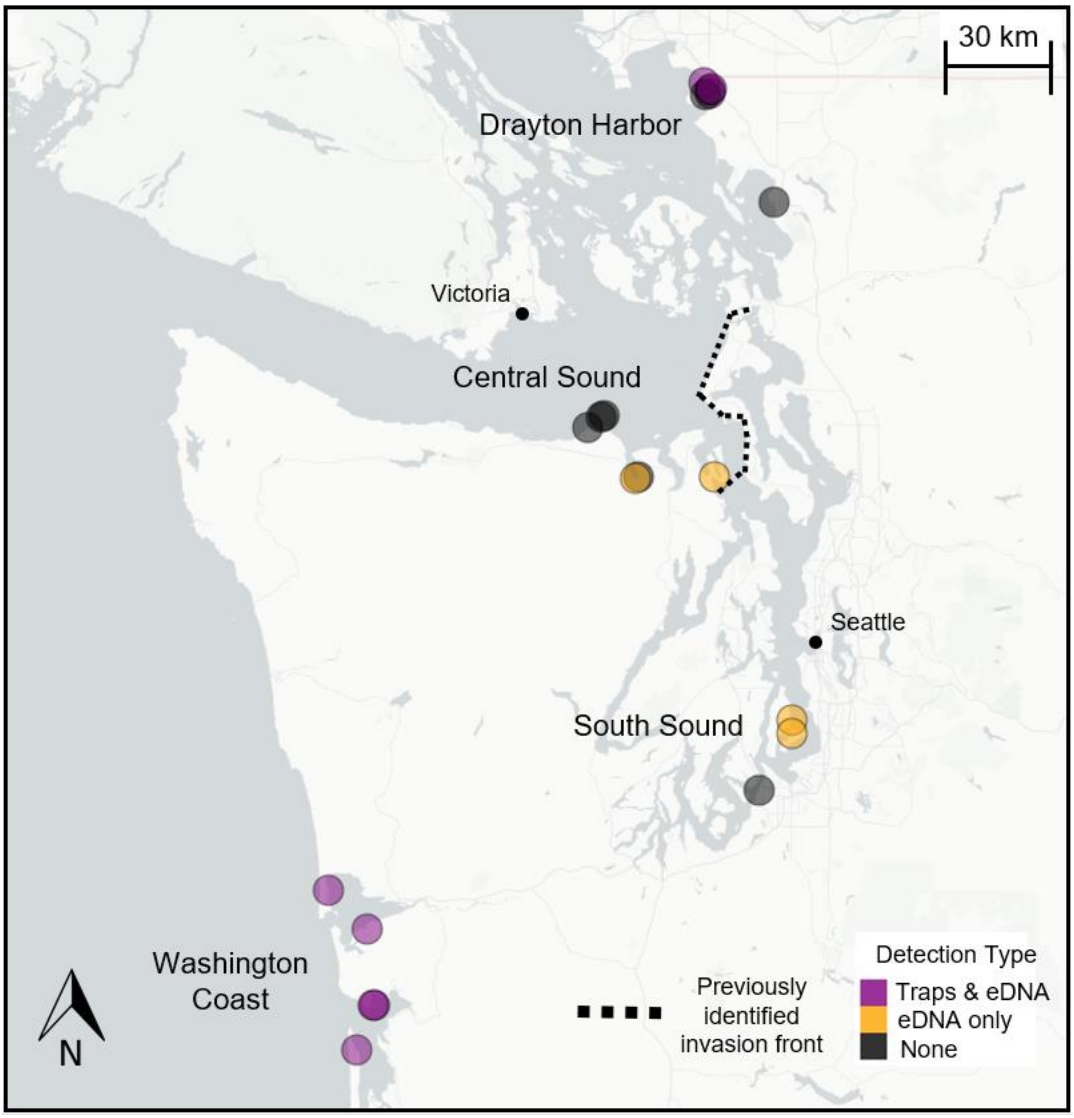


Figure 3: Environmental DNA and trapping detections of green crab over the sampling period. Purple dots indicate sites where green crabs were trapped and eDNA samples yielded at least one positive detection. Yellow dots indicate sites where no green crabs were trapped and eDNA samples yielded at least one positive detection. Black dots indicate sites where no green crabs were trapped and eDNA samples yielded no positive detections. The black dotted line designates the previously identified invasion front, where green crabs have never been observed further south into the Puget Sound.

ii. Detection of green crab eDNA beyond known invasion front

Both the joint and trap-only models converged with no divergent transitions, and the trace plots showed random scatter around a mean value, suggesting that the chains mixed well. The

joint model estimated relatively high green crab densities in locations beyond the previously known invasion front (Figure 4). Most notable was the estimation of relatively high green crab densities on Vashon Island, a location more than 60 km south into the Puget Sound than green crabs have been visually observed. Based on the posterior distribution of the green crab density estimate at the Rabb's Lagoon site on Vashon Island, the probability that the true green crab density was greater than 0.01 crabs/trap is 0.964, and the probability that the green crab density was greater than 0.1 crabs/trap is 0.730 (Table 1). This relatively high density of green crab is similar to density estimates at sites at Drayton Harbor, where historically green crabs have been recovered in traps under high trapping efforts. The joint model estimated green crab densities of 0.090, 0.561, 0.102, and 0.068 crabs/trap at California Creek, Dakota Creek, Pillars, and Willsei's, respectively. Additionally, the eDNA samples from KVI Beach, also on Vashon Island, yielded one positive eDNA detection, and the mean estimate of green crab density at KVI Beach is 0.046 crabs/trap. Together, these molecular detections and estimated green crab densities support a low probability that green crabs are absent at Vashon Island (Table 1). Also, the joint model estimated a false positive detection probability of 0.018 (Table 2).

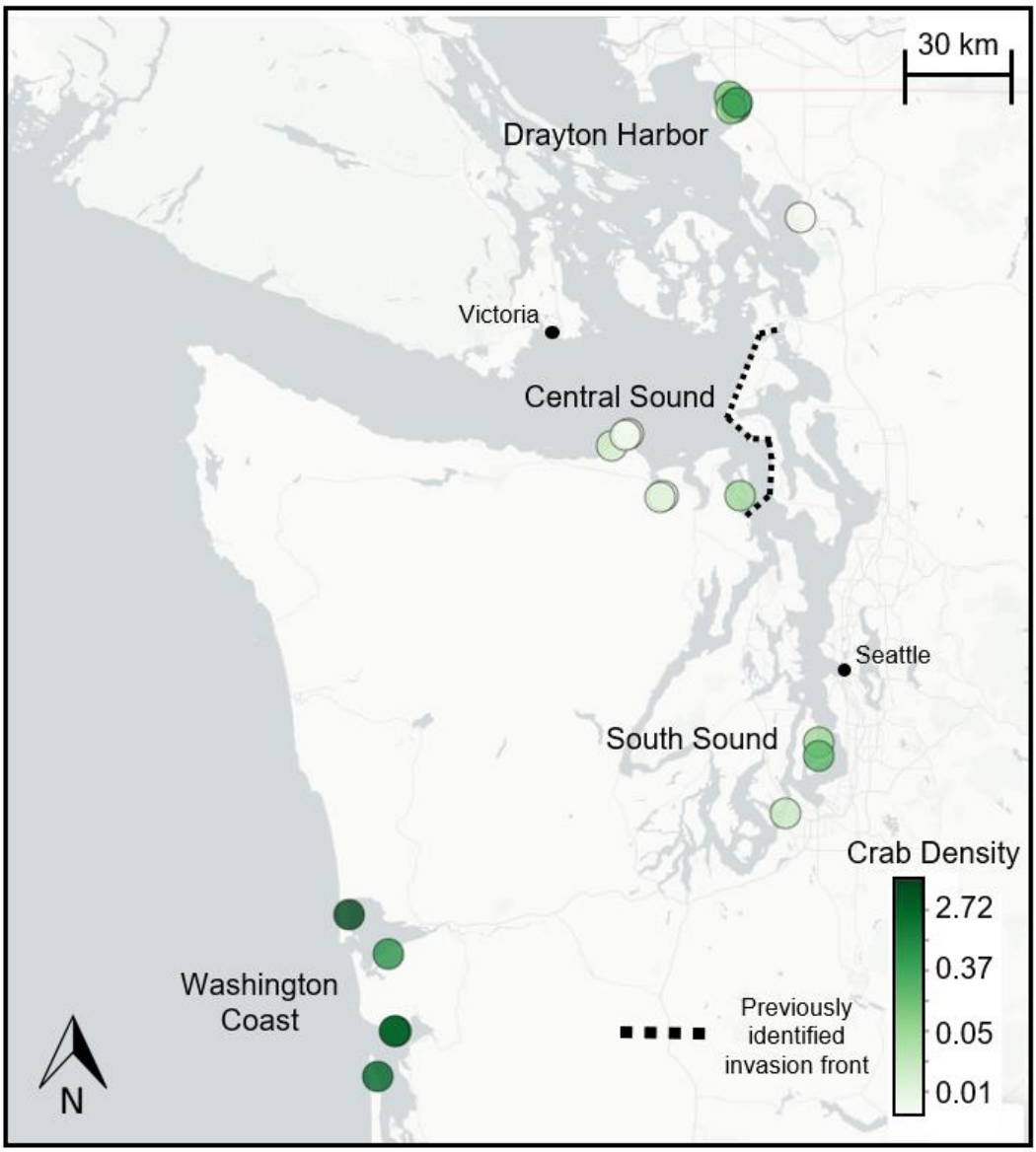


Figure 4: Mean of the joint model's posterior distributions of estimated green crab density at the 20 sampled sites. Colors indicate the green crab density (crabs/trap) estimated by the joint model, and the colors are presented on a log scale. The black dotted line designates the previously identified invasion front, where green crabs have never been visually observed further south into the Puget Sound.

Table 1: Estimated green crab density, $\hat{\mu}$**,** at sampled sites. Table includes the mean and 95% credibility interval of the parameter's posterior distribution.

Site (ID)	Region	mean $\hat{\mu}$	95% Credibility Interval
Chuckanut Creek	Bellingham Bay	0.003	4.47e-22, 0.016

California Creek	Drayton Harbor	0.090	0.048, 0.135
Dakota Creek	Drayton Harbor	0.561	0.405, 0.724
Noname Creek	Drayton Harbor	0.004	3.59e-22, 0.020
Pillars	Drayton Harbor	0.102	0.072, 0.135
Willsei's	Drayton Harbor	0.068	0.003, 0.152
Chicken Coop Creek	Central Sound	0.006	1.14e-19, 0.028
Dungeness Base Lagoon	Central Sound	0.013	3.60e-21, 0.061
Dungeness East Lagoon	Central Sound	0.013	1.39e-18, 0.065
Graveyard Spit Channel	Central Sound	0.003	4.41e-18, 0.012
Indian Island	Central Sound	0.045	8.56e-19, 0.179
Jimmycomelately Creek	Central Sound	0.008	9.69e-17, 0.036
KVI Beach	South Sound	0.046	5.68e-15, 0.184
Rabb's Lagoon	South Sound	0.219	3.21e-9, 0.489
Titlow	South Sound	0.016	2.06e-21, 0.077
John's River	Washington Coast	0.736	0.460, 1.055
Ocean Shores	Washington Coast	5.913	4.338, 7.570
Stackpole	Washington Coast	2.816	2.034, 3.698
Tokeland East	Washington Coast	1.894	1.005, 2.945
Tokeland West	Washington Coast	3.081	2.171, 4.072

Table 2: Parameters estimated by the joint model, with the mean and 95% credibility intervals (highest density interval calculation) of the 20,000 sampling iterations. σ is the overdispersion parameter in the negative binomial distribution of species counts (eq. 1.1), β is the coefficient relating species density to true positive molecular detection probability (eq. 1.2), and p10 is the false positive molecular detection probability (eq. 1.3).

Parameter	Mean Estimate	95% Credibility Interval
σ	0.94	0.70, 1.20
β	1.07	0.72, 1.42
p10	-4.02	-4.86, -3.22
exp(p10)	0.018	0.008, 0.040

One sampled site, Jimmycomelately creek (JIM), in the Central Sound produced one positive qPCR detection, yet the 43 traps set over the sampling period recovered 0 green crab individuals. During 2020, no green crabs were recovered in traps, although historically green crabs have been trapped in Sequim Bay, which is the water body at the mouth of

Jimmycomelately creek. The estimated mean green crab density at this site was 0.008 crabs/trap. At Graveyard Spit Channel, the eDNA samples yielded no positive molecular detections, and no green crabs were trapped out of the 86 traps set during the sampling period. The estimated mean green crab density at this site was 0.003 crabs/trap. However, in 2020, 1369 traps were set, and three green crabs were recovered (0.002 crabs/trap), and in April 2021, three more crabs were recovered at this site, indicating that it is nearly certain that crabs were present in the channel during the time of sampling but not detected by eDNA sampling. Additionally, the Stackpole site (STA) had the largest evidence of PCR inhibition among the sites. Likely due to this inhibition, this site was an outlier in that the estimated green crab density was relatively high, with a mean density estimate of 2.829 crabs/trap, yet only 2 of 15 qPCR replicates produced a positive amplification (Table 1, Supplemental Table 1).

iii. Quantifying uncertainty to find the value of eDNA information

The Bayesian modeling framework allows quantification of uncertainty around the estimated parameters, which is useful in determining the information benefit provided by eDNA data. At sites with a lower trapping effort, the joint model's posterior estimates of green crab density included narrower credibility intervals, compared to a model estimating green crab density using only trapping information (Figure 5). Additionally, the joint model estimated low green crab densities at sites where a low trapping effort was insufficient in detecting green crab presence (Figure 5).

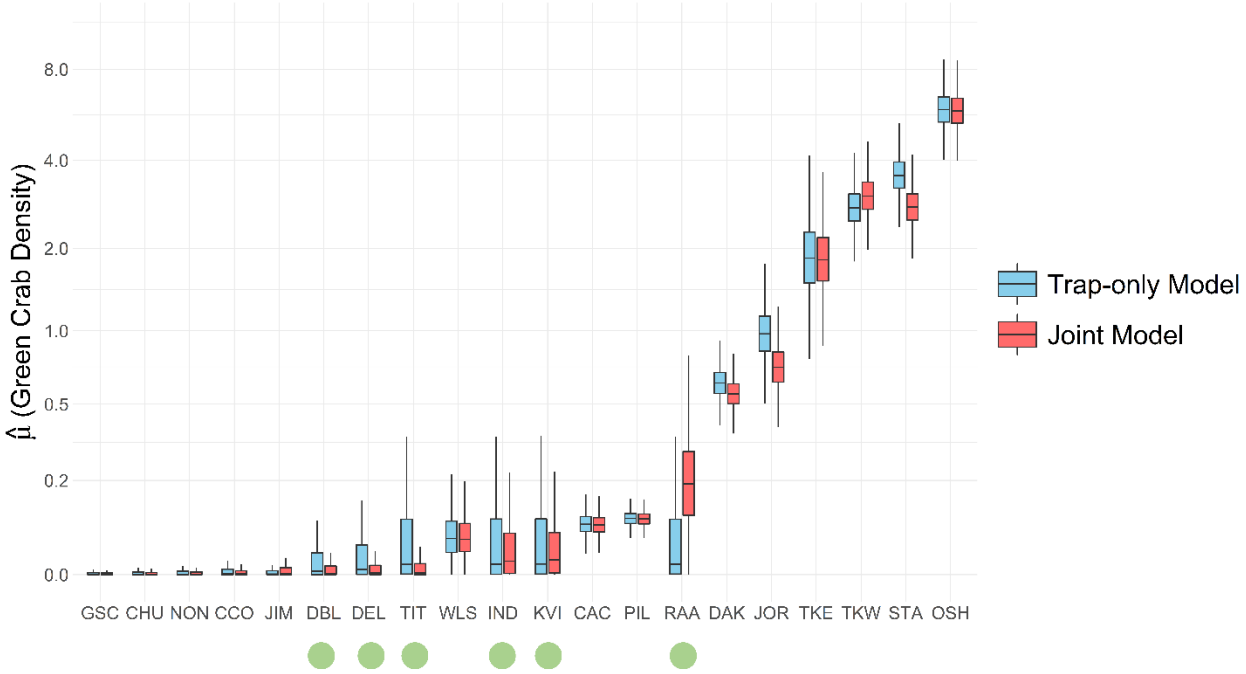


Figure 5: Boxplots indicating the posterior distributions of estimated green crab density at each of the twenty sampled sites (not including the unambiguous absence sites). Red boxplots are the estimated densities using the joint model, incorporating both trapping and eDNA information, and blue boxplots are the estimated densities using the trap-only model, using only trapping information. The lower and upper hinges correspond to the first and third quartiles, and the upper and lower whiskers represent the highest and lowest values within 1.5*IQR (inter-quartile range). Outliers have been removed. Green dots indicate sites where fewer than eight traps were set during the sampling period.

The coefficient of variation was used to quantify the precision in estimates of green crab density independent of the order of magnitude of the density. As the trapping effort decreased, I found exponential increases in the marginal benefit (ΔCV) of eDNA data (Figure 6). The sites with the greatest ΔCV were at sites along the invasion front in the Central and South Puget Sound (Figure 6). Therefore, the addition of eDNA information dramatically increased the precision of green crab density estimates at sites along the invasion front and at sites characterized by low trapping efforts.

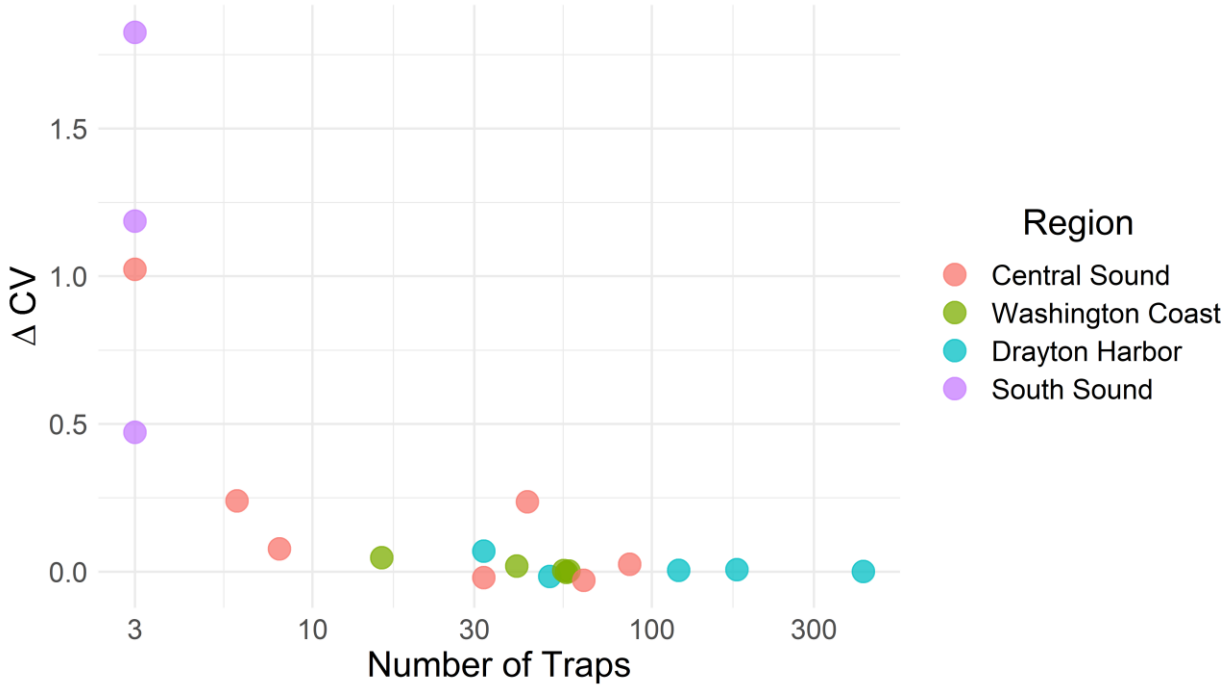


Figure 6: The difference in the coefficient of variation (ΔCV) in the posterior distributions of the estimated green crab densities between a model using only trapping information (trap-only model) and a model using both trapping and eDNA information (joint model).

iv. eDNA's greatest marginal benefit at low species densities and trapping effort

Simulations suggested that the marginal benefit of eDNA data was not only greater at lower trapping efforts, but also at lower green crab densities. These simulations indicated that the marginal benefit of eDNA data increased as trapping effort decreased for all densities of green crab, but the marginal benefit increases were larger under lower green crab densities (Figure 7B). Additionally, decreases in green crab density corresponded to wider confidence intervals surrounding the changes in precision with the addition of eDNA information (Figure 7B).

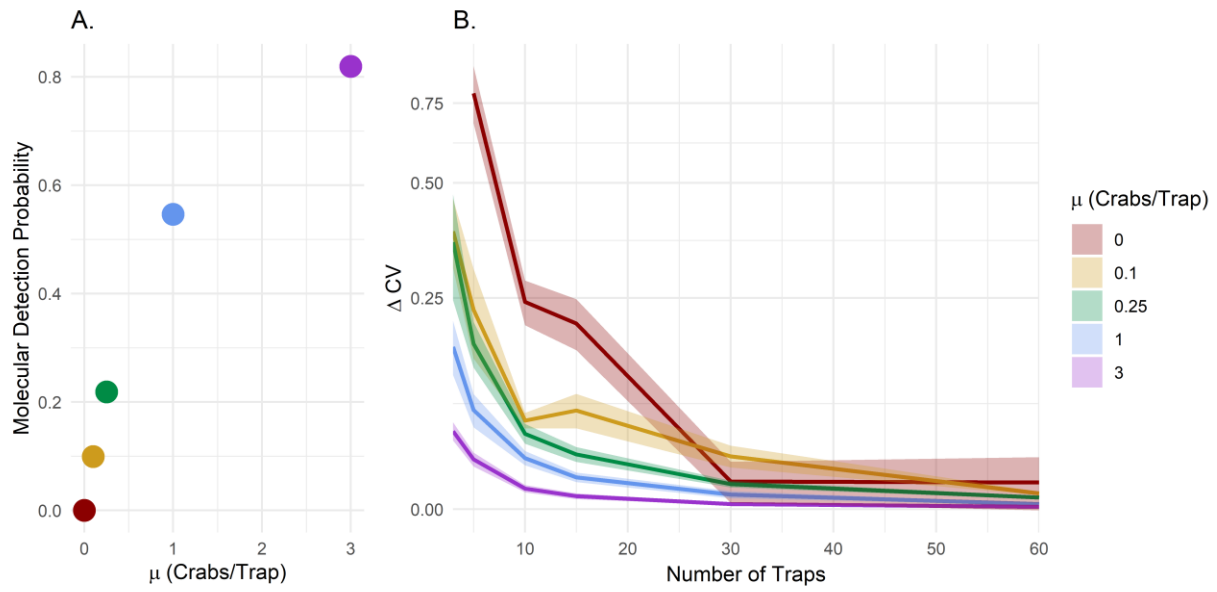


Figure 7: ***A.*** Relationship between true green crab density, μ, and molecular detection probability (p10+p11) as estimated by the joint model. Points and colors indicate the five crab densities and associated molecular detection probability included in simulations. ***B.*** The marginal benefit of eDNA data at each simulated crab density and trapping effort. The information benefit is represented by the difference in the coefficient of variation (ΔCV) in the posterior distributions of the estimated green crab densities between a model using only trapping information (trap-only model) and a model using both trapping and eDNA information (joint model). Lines represent the mean ΔCV for all simulation scenario replicates, and the shaded areas represent the ΔCV 95% confidence interval for all simulation scenario replicates.

When investigating changes in the accuracy of green crab density estimates in the joint and trap-only models, both models produced accurate estimates of green crab density. For both the joint and trap-only models, 100% of simulation replicates for all scenarios yielded 95% credibility intervals of density estimates that contained the true green crab density, μ_{sim}, except for $\mu_{sim} = 0$, since $\hat{\mu}_{sim}$ is constrained to never be zero due to the log-linear relationship between μ_{sim} and the probability of molecular detection (eq. 1.2).

To identify the relative sensitivities of the two sampling methods, eDNA sampling and trap sampling, the joint model's estimated parameters were used to determine the sampling effort necessary to "detect" a green crab with 95% confidence. The detection sensitivity, defined as the

probability of capturing at least one crab in one trap or the probability of one positive qPCR amplification among three trials, was higher for eDNA sampling than for trap sampling, suggesting that the information provided by water bottle is greater than the information provided by one trap (Figure 8). Therefore, when comparing the sampling effort necessary to detect one green crab, the effectiveness of one unit of eDNA sampling, defined as one water bottle or triplicate qPCR, was greater than one unit of trap sampling (Figure 8).

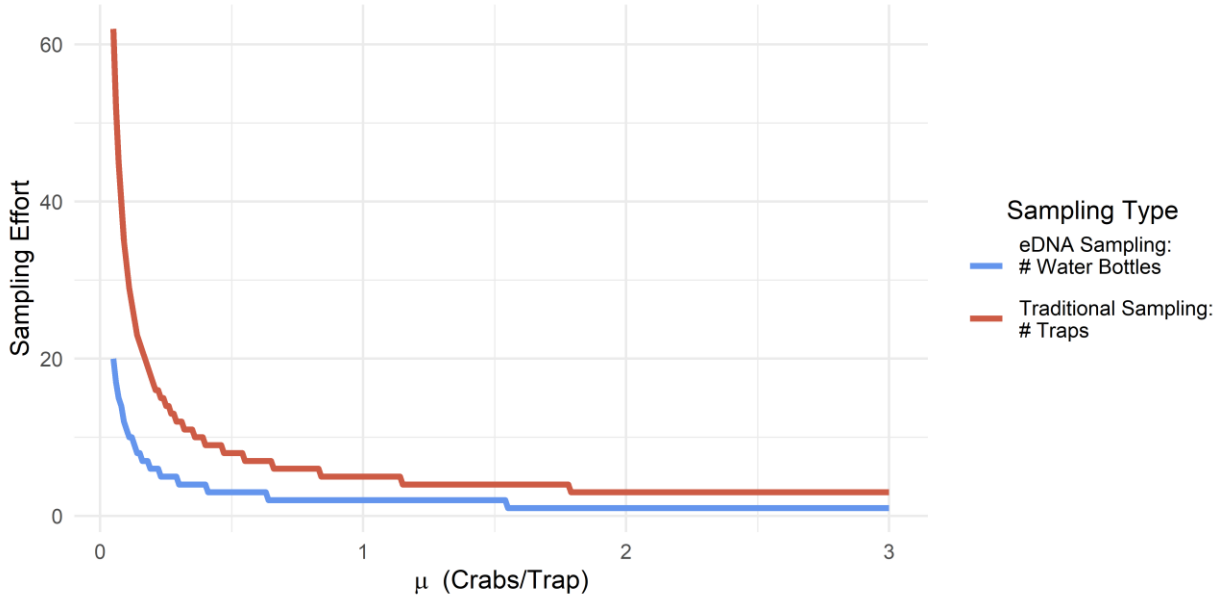
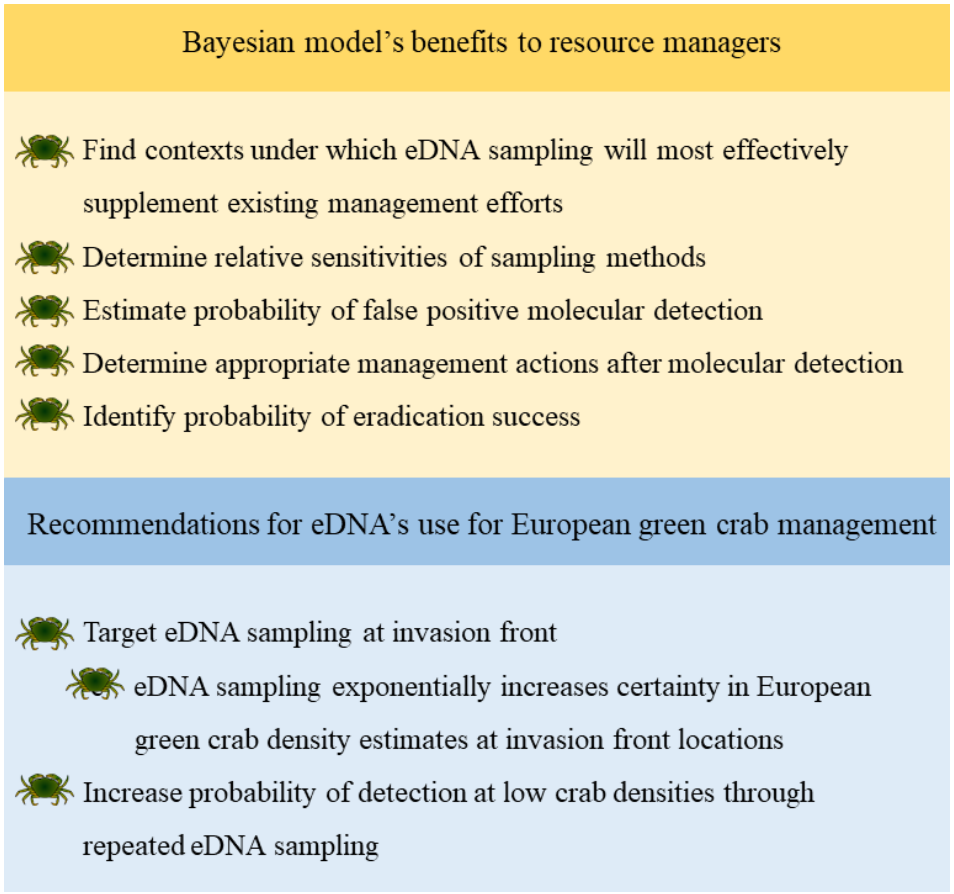


Figure 8: The sampling effort necessary to "detect" a green crab with 95% confidence. Lines designate the type of sampling effort (water bottles, traps).

Discussion



As interest grows in incorporating genetic surveys into management practices, environmental managers will need to make decisions regarding the appropriate use of eDNA-based monitoring methods, and these decisions will be informed by understanding the contexts under which eDNA information provides the greatest value. Given that many of these decisions have prominent economic and social consequences, informed management decisions need to be made with known levels of confidence in survey results. The ability to identify the reliability of eDNA surveys will be critical in assessing species presence and subsequent management actions. I present a framework for interpreting eDNA data using a Bayesian model that combines data from two detection methods to jointly estimate species density. The joint model allows for

quantification of the marginal benefit of eDNA data under varying contexts, which can identify the degree to which eDNA data changes the precision of species density estimates. The joint model explicitly estimates the probability of a false positive detection and can also inform the degree of certainty in estimates of species density, which can be critical when eDNA surveys suggest unexpected results.

Improving interpretation of eDNA data through Bayesian modeling

This joint model improves upon previous work adapting occupancy modeling approaches to facilitate eDNA data interpretation (Griffin et al., 2019; Lahoz-Monfort et al., 2016; Pilliod et al., 2013; Schmidt et al., 2013). These previous approaches suggest that there are two classes of sites—those that are occupied and those that are not—and crucially, that the probability of detecting a species is constant within a given ecological context. This assumption can be insufficient in the context of eDNA surveys, where local abundance can induce heterogeneity in detection probability (Altwegg & Nichols, 2019; Royle & Nichols, 2003). Therefore, under this occupancy framework, sites with the highest species abundance have the greatest occupancy probability, and the probability of occupancy for the less abundant sites are scaled relative to the high abundance sites. The joint model presented here uses the heterogeneity in molecular detection probability to estimate species density, rather than occupancy, and operates under the assumption that the probability of a true detection increases as species density increases.

The joint nature of the model uses the unique strengths of two sampling methods, whose observations separately arise from a shared underlying species density, and the two streams of information can mutually inform their limitations. The joint model assumes perfect species identification in traditional sampling methods and incorporates prior information regarding the

specificity of the molecular assay, both of which provide detection data necessary to estimate the false positive detection probability of the molecular survey. The combined likelihood borrows strength from the sites with greater trapping effort over the sampling period to infer detection biases across all locations and to inform species density at data-limited sites. Also, the joint model can inform the relative sensitivities of the two sampling methods, as well as the relative information contributions at varying sampling efforts.

The myriad interactions between extraorganismal genetic material and its environment challenge the interpretation of eDNA detections, which subsequently hamstrings eDNA's application in conservation. Environmental factors including flow rates, turbulence, temperature, water chemistry, and UV light can affect the dilution, persistence, and strength of an eDNA signal (Andruszkiewicz et al., 2017; Barnes & Turner, 2016; Deiner & Altermatt, 2014; Sansom & Sassoubre, 2017). This joint model, implemented under a Bayesian framework, aims to mitigate this challenge by capturing uncertainty in how eDNA detections arise from true species presence and density. The uncertainty around the point estimates of underlying species density can therefore be quantified, which can facilitate interpretation of eDNA data and management decision making (Ellison, 1996). However, capturing this full uncertainty relies upon sufficient inter-site environmental variability, which is unlikely in this study given its relatively small sample size of 20 sites. Also, these results are likely season-specific, since season can affect the retention and stability of DNA in water (Hunter et al., 2015), as well as the shedding rate of the organism (Goldberg et al., 2016).

Importantly, the joint model's results can aid appropriate management responses after a molecular detection. The most common management application of eDNA methods is their use as a trigger for non-molecular sampling, as exemplified in the eDNA surveillance program for

Asian carp in the Great Lakes region (Woldt et al., 2020). However, after a positive eDNA detection, managers are faced with the challenge of unknown subsequent management intensity. Given difficulties in confirming a species' absence with traditional methods (Morrison et al., 2007; Russell et al., 2017), managers will likely need to establish a tolerable level of risk regarding species density. The results of our joint model identify the detection sensitivities of both eDNA and traditional methods, which can inform the trapping effort necessary to obtain this tolerable species density with a known level of certainty (Figure 8).

Quantifying the practical value of eDNA information

The joint model can investigate the value of incorporating genetic surveys into existing management practices, therefore moving eDNA data's contribution to management practices from "potential" value to practical value. The marginal benefit of eDNA data, or the changes in the precision of species density estimates upon the addition of eDNA data, is highest at sites characterized by low trapping effort and low species density. This result is supported by both data from green crab eDNA detections in Washington State (Figure 6), as well as simulations based on the joint model's estimated parameters (Figure 7). With data from green crab detections, I find exponential increases in precision (ΔCV) in green crab density estimates as trapping effort decreases, suggesting that the addition of eDNA information provides much more precise estimates of green crab density at data-limited locations (Figure 6). Simulations indicate that eDNA's marginal information benefit includes more precise estimates of species density at all densities (0 – 3 crabs/trap) at lower trapping efforts, and eDNA data's marginal benefit increases as species density decreases (Figure 7).

Invasive species managers are faced with a fundamental tension regarding management resource allocation between prevention, detection, and eradication of invasive species (Mehta et al., 2007; Vander Zanden et al., 2010), and the introduction of genetic surveys into environmental monitoring adds another set of costs and benefits when considering optimal detection strategies. However, by quantifying the information gained by including eDNA data into detection surveys, managers can more effectively incorporate known benefits of genetic information. Targeting management efforts earlier and in more appropriate locations has previously been demonstrated to improve the cost effectiveness of invasive species management (Leung et al., 2002), and some existing eDNA monitoring programs already prioritize eDNA surveillance at locations on the putative leading edge of invasion (Jerde et al., 2011). This study is the first to quantify the value of eDNA data, showing that by targeting eDNA sampling at the edge of the species' known distribution where trapping efforts remain low, managers can gain greater confidence in the estimates of species density than without added eDNA information. Importantly, the joint model's ability to quantify precision around the density estimates provides a way for managers to integrate uncertainty into decisions regarding the location and intensity of future trapping efforts.

The joint model not only indicates where the marginal benefit of eDNA sampling is highest, but also where eDNA's marginal benefit is negligible, which is valuable information for developing a monitoring plan under limited resources. This study identifies a lower limit of molecular detection and indicates that eDNA sampling is unlikely to improve management at locations with high trapping effort or a high species density (Figure 6, Figure 7). For example, eDNA samples were collected in Dungeness National Wildlife Refuge, an area rich in marine life that contains one of the world's longest sand spits. The watershed in this area is also home of

the Jamestown S'Klallam Tribe, providing abundant resources from its tidelands and marine waters (Jamestown S'Klallam Tribe, 2007). U.S. Department of Fish and Wildlife implements an intense removal trapping procedure in the national refuge. In 2020 in Graveyard Spit Channel, 1369 traps were set, and three green crabs were recovered. In April 2021, three more crabs were recovered at this site, and their size indicated that they were almost certainly present at the channel during the time of eDNA sampling, even though there were no molecular or trap detections during the sampling period in this study. The joint model's estimated mean green crab density at this site during this study's sampling window is 0.003 crabs/trap, and throughout the entirety of 2020, the calculated density based on trapping is 0.002 crabs/trap. These results indicate a green crab density that yields a low probability of detection through molecular methods, as well as an existing management practice with high effort that is unlikely to be improved through the addition of eDNA sampling.

Increasing certainty at the green crab's invasion front

In the context of green crab, the most notable example of eDNA data's value at the invasion front is the estimation of relatively high green crab densities at sites well beyond green crab's previously known distribution (Table 1, Figure 4). By interpreting the pattern of eDNA signals, the joint model indicates green crab presence with high certainty on Vashon Island, suggesting that the local species density is perhaps low and previously undetectable using traditional monitoring methods implemented at a low effort. Based on the posterior distribution of the green crab density estimate at the Rabb's Lagoon site on Vashon Island, one of the sites beyond the previously known invasion front, the probability that the true green crab density is greater than 0.01 crabs/trap during the sampling period is 0.964, and the probability that the

green crab density is greater than 0.1 crabs/trap is 0.730 (Table 1, Figure 4). This finding is consistent with recent studies showing that sufficient eDNA sampling applied across large geographic areas can reveal unexpected patterns and new occurrences of species missed by traditional approaches (Mckelvey et al., 2016; Tucker et al., 2016), and the Bayesian modeling framework allows these statements of new occurrences to be tempered by quantified uncertainty (Hobbs & Hooten, 2015). It is important to note, however, that the model estimates species density, which is a coarse description of the presence of a species at a site when considering eDNA detections. Here, presence refers to the existence of the species, and this species presence potentially includes larval and dead individuals, both of which can shed eDNA into their environment but might not necessarily indicate a viable population or incipient invasion.

Gaining greater certainty in a species' true absence through the addition of eDNA information also represents a critical piece of management-relevant information (Carim et al., 2020). Simulations in this study suggest that the marginal benefit of eDNA information is highest when green crabs are truly absent, suggesting that incorporating multiple sampling types for species detection is a valuable strategy for increasing certainty that a species is absent (Figure 7). Environmental DNA information has previously been used in species eradication campaigns, given that the sensitive molecular monitoring technique is particularly useful where the veracity of negative results may be of equal importance as confirmation of positive detections (Carim et al., 2020; Davison et al., 2019; Larson et al., 2020). Monitoring eradication success often proves challenging, particularly since premature declarations of success and ceased management operations can result in continued ecological impacts and increased management costs, as well as loss of public confidence and social license for future work (Rout et al., 2009). Although eDNA sampling faces similar challenges to traditional sampling in confirming a species' absence, as

evidenced by the findings at Graveyard Spit Channel, the ability to quantify certainty in eradication success can allow managers to make informed decisions regarding future management efforts.

Joint model considerations and limitations

The fear of false-positive detections is often cited as the primary hurdle for adopting eDNA approaches for species monitoring (Jerde, 2019). However, addressing misunderstandings and clear communication regarding the term "false positive" can improve confidence in the utility of eDNA methods (Darling et al., 2021). Different mechanisms contribute to false positive errors, and previous work makes important distinctions between false positive samples (i.e. errant detection in an individual sample) and false positive sites (i.e. errant detection at an unoccupied site) (Chambert et al., 2015; Darling et al., 2021; Guillera-Arroita et al., 2017). The joint model explicitly includes a molecular false positive probability, which incorporates both the probability of a false positive sample and the probability of a false positive site through information included in the parameter's prior distribution and unambiguous presence sites with a high trapping intensity.

Notably, however, the false positive probability in this joint model does not include the probability that the molecular methods are detecting unviable organisms or larval individuals. Under the joint model, molecular detections can indicate the presence of the species at a site, but not that the site contains a viable, self-sustaining green crab population or an incipient invasion. This type of false positive is particularly concerning for green crab, since green crab larvae move up and down in the water column in sync with tides (Moksnes et al., 2014), which may be more easily detectable than their benthic adult and juvenile counterparts. Circulation models find that

hydrodynamic variables and seasonal variation predict the likelihood that green crab larvae can be retained by circulation and behavior long enough to reach maturity and resettle at the end of their development (Banas et al., 2009), suggesting that the molecular detection of larvae alone does not necessarily indicate a high probability of invasion. Future work should investigate sampling timing to optimize the detection of green crab during periods of molting and high activity, while minimizing the detection of green crab during their larval stages. However, if sampling is targeted in highly suitable green crab habitat, detecting larval green crab eDNA can offer valuable management information. Given that ineffective communication among diverse research partners can act as a barrier to the efficient use of molecular data for ecological inferences and conservation decision making (Mosher et al., 2020), careful and clear communication of the model's results and limitations can enable managers to harness the full utility of molecular methods.

False negative detections present another consideration when using molecular methods to detect species presence and density (Goldberg et al., 2016; Hunter et al., 2019). PCR inhibition has the potential to mask even high eDNA copy numbers, which profoundly affects molecular detection estimates (Jane et al., 2015). For example, DNA extracted from turbid water often contains humic acid and tannin compounds, created through non-enzymatic decay of the organic material, and these compounds can inactivate DNA polymerase and inhibit the PCR amplification process, reducing PCR efficiency or causing PCR failure (Albers et al., 2013; Goldberg et al., 2016). In this study of green crab, the Stackpole site along the Washington Coast is located near natural stream outflow, where the water is often visibly tannic. The eDNA samples from this site exhibited substantial inhibition, likely due to the presence of inhibiting organic compounds. Additionally, the multiple post-extraction DNA purification columns used

to mitigate inhibition at this site likely resulted in loss of DNA yield and subsequent dilution of the eDNA signal (McKee et al., 2015). Together, the inhibiting compounds and efforts to mitigate PCR inhibition likely resulted in eDNA samples from this site yielding fewer positive amplifications than would be expected, given the relatively high green crab density indicated by trapping efforts. Future work should consider robust data collection regarding PCR inhibition measurement and mitigation, incorporating inhibition data into the joint model's relationship between true species density and probability of true molecular detection.

The joint model assumes that both types of sampling occur concurrently and reflect a shared environmental status. However, the two types of sampling were not truly concurrent (Supplemental Figure 2), and the window of trapping data included in the model was chosen to include the narrowest possible time frame surrounding eDNA sampling that could reflect known presence or absence of green crab, while also resulting in model convergence. Additionally, sites were defined in terms of spatial units reflecting practicable management units, rather than a consistent geographic delimitation surrounding the location of eDNA sampling. Improvements to the joint model could include a probability of molecular detection as a function of distance and time from trap collection, which could offer valuable insight into the spatial and temporal footprint of eDNA sampling.

Finally, the model's specification itself should be evaluated through robust model checking. The reliability of inference from a fitted model depends on how well the model approximates reality, and this study primarily relies upon convergence diagnostics, which allows for evaluation of the algorithm fitting the model, rather than the model itself (Conn et al., 2018; Hobbs & Hooten, 2015). Given the log-linear relationship between true species density and the probability of a true molecular detection, the estimated species density can never be zero, which

does not optimally approximate reality, and other model specifications might offer better predictive accuracy than the existing specified model. In addition to improved model checking, a robustness analysis should be conducted, including prior sensitivity analyses or simulation analyses where model assumptions are violated (Conn et al., 2018). However, this study serves primarily as a proof-of-concept, offering a novel framework to investigate the practical value of eDNA information.

Model framework to inform and improve management in other contexts

The marginal benefit of eDNA information is likely species- and system-specific. The sensitivity of molecular detection methods likely differs across taxa due to varying life histories and eDNA shedding rates and mechanisms, which would affect the probability of detection relative to traditional methods. For example, previous work suggests that organisms with a hard exoskeleton, including green crab, have a lower rate of eDNA shed and lower detection sensitivity than other aquatic organisms (Andruszkiewicz Allan et al., 2021; Crane et al., 2021; Forsström & Vasemägi, 2016; Tréguier et al., 2014). Furthermore, occupancy models indicate a higher detection probability with traditional survey methods than eDNA methods for semi-aquatic snakes, suggesting that the marginal benefit of eDNA data could be negligible for some species (Rose et al., 2019). Varying eDNA persistence and transport across different aquatic systems will also affect the way in which eDNA detections arise from true species density, where the marginal benefit of eDNA information is likely lower in environments with greater flow rates and subsequent dilution of the eDNA signal (Lacoursière-Roussel et al., 2016; Pilliod et al., 2013; Robson et al., 2016; Shogren et al., 2017). However, the proposed joint model provides a

framework for use in other contexts to understand the probability of false positive detections and how the marginal benefit of eDNA data varies across species and systems.

Environmental DNA's information benefit in data-limited contexts potentially extends beyond the marine invasion front. Since more than 80% of global catch comes from data-poor/limited fisheries, most of the world's fisheries are managed under great uncertainty (Costello et al., 2012). This lack of information limits science-based management strategies necessary for a fishery's long-term sustainability. Formal assessments of the biological status of fisheries rely on detailed stock assessments with intensive data requirements and cost (FAO, 2010). As eDNA methods advance in their ability to overcome data collection obstacles, like accessing aquatic systems inaccessible to traditional gears (Tucker et al., 2016) or in remote environments (Ore et al., 2015), eDNA information can provide data inputs necessary in otherwise data-limited settings. Our results suggest that these information benefits would be most pronounced for fisheries lacking formal assessment, so especially on a global scale, managing fisheries without advanced detection technologies like eDNA represents an opportunity loss (Jerde, 2019).

Conclusion

Given the limited resources available to government agencies charged with controlling invasive species, there is significant value in identifying and implementing optimal invasive species management strategies. Applications of eDNA methods represent one of the most significant advances in invasive species surveillance in the recent decade, yet uncertainty inherent in eDNA sampling means managers are often hesitant to direct management actions based solely on molecular evidence. Although previous work identifies the potential for DNA-

based methods to amplify the uncertainty already associated with invasive species risk assessment (Benke et al., 2007; Darling & Mahon, 2011; Sikder et al., 2006), I demonstrate that incorporating environmental DNA information in invasive species surveillance increases certainty in estimates of species density, especially at low species densities and data-limited locations. I present a joint model that combines a pattern of eDNA signals with information from well-characterized sites through traditional monitoring methods to make more reliable inferences about data-limited sites. The joint model aids eDNA data interpretation and contributes to a growing body of analyses providing frameworks for inferring confidence in patterns of eDNA detections (Furlan et al., 2016; Guillera-Arroita et al., 2017; Lahoz-Monfort et al., 2016). While environmental DNA methods can improve detection of invasive species at low abundances, improved statistical methods to interpret patterns of environmental DNA detections can empower informed management responses.

Acknowledgements

I would first like to thank Dr. Ryan Kelly for inspiring me with his endless curiosity and for being a wonderful mentor in every possible sense of the word. Dr. Emily Grason and Dr. P. Sean McDonald from Washington Sea Grant (WSG) empowered me to make this project my own and provided valuable insight and perspectives that were critical in considering eDNA's practical value in the context of green crab. The trapping data used in this study was the product of a huge effort conducted by WSG's Crab Team, WA Department of Fish and Wildlife, U.S. Department of Fish and Wildlife, and several sovereign tribal nations. Alex Stote (WSG), Amy Linhart (WSG), and Bethany McKim (USFWS) provided eDNA sampling assistance, and Neil

Harrington (Jamestown S'Klallam Tribe) provided valuable insight into the history of green crab in Sequim Bay. Ana Ramón-Laca (NOAA) offered her time and expertise and answered my many qPCR-related questions. Dr. Eily Allan and Dr. Zachary Gold provided encouragement and helpful comments on thesis drafts. Most importantly, although our time physically together in the Marine Affairs building (particularly the “party carrel”) was short-lived, I would like to thank the SMEA Class of 2021 for being supportive friends and motivating me to live in the world inquisitively and compassionately.

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