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ORIGINAL ARTICLE

Detecting endangered pinto abalone (*Haliotis kamtschatkana*) using environmental DNA: Comparison of ddPCR, qPCR, and conventional diver surveys

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Abstract

Abalone populations along the Pacific Coast of North America are threatened. In the Salish Sea (Washington, USA), pinto abalone (*Haliotis kamtschatkana*) have failed to recover from intensive harvest after over 25 years of fishery closure, prompting a growing restoration effort. As these efforts expand, a persistent challenge is simply locating this rare and highly cryptic species in the field, limiting the ability to identify critical habitat and locate wild adults to serve as restoration broodstock. Here, we tested the use of environmental DNA (eDNA) to detect pinto abalone. Using a quantitative PCR (qPCR) assay previously developed for larval pinto abalone, we first evaluated its sensitivity to abalone eDNA in aquaria settings, finding a positive relationship between abalone biomass and the concentration of abalone DNA. We then tested abalone eDNA detection in the field by collecting replicate water samples from abalone restoration sites, using an occupancy model to estimate detection probability in relation to abalone biomass estimated via diver surveys. Both eDNA concentration and detection probability were positively associated with diver-estimated abalone biomass. By modifying the assay for droplet digital PCR (ddPCR), detection probability increased by 32%–89% over qPCR. eDNA surveys using ddPCR had higher error (CV = 96.9%) than diver surveys (CV = 29.4%) but were more efficient, taking approximately 1/10th of the person-hours per site of a diver survey. For the final phase of the study, we collected water samples at 80 sites throughout the region, obtaining positive abalone eDNA detections at 11 sites with qPCR and 19 additional sites with ddPCR. Our results provide novel survey data on abalone populations within the Salish Sea and show that eDNA is a viable tool for cost-effective, efficient, and non-invasive abalone detection.

KEYWORDS
abalone, endangered species, environmental DNA, polymerase chain reaction, survey methods

1 | INTRODUCTION

Abalone are a family of large marine snails that have experienced widespread overexploitation leading to a nearly 70% reduction in worldwide fisheries harvests since the 1970s (Cook, 2019). Along the Pacific Coast of North America, all of the seven extant abalone species are listed by the IUCN Red List as either endangered or critically endangered (Peters & Rogers-Bennett, 2021). The widest ranging of these species, pinto or northern abalone (*Haliotis kamtschatkana* Jonas, 1845), have become rare in many parts of their range (Neuman et al., 2018). In the State of Washington, pinto abalone have undergone a 97% decline since the early 1990s despite a 1994 harvest ban (Carson et al., 2019; Carson & Ulrich, 2019). Pinto abalone are now considered threatened with local extinction, which led the State of Washington to list the species as a State Endangered Species in 2019. Low remaining populations of pinto abalone in Washington waters are thought to have resulted in an Allee effect, where densities of broadcast spawning adults are below critical thresholds for successful reproduction (Rothaus et al., 2008). This hypothesis is supported by demographic data showing an aging population with extremely low juvenile recruitment (Bouma et al., 2012; Carson & Ulrich, 2019).

In response to this decline, a restoration aquaculture program has outplanted over 40,000 hatchery-raised juvenile abalone at 21 sites in the San Juan Archipelago through 2021. Despite successes and plans for expansion, major challenges for the recovery program include finding sufficient mature wild adults for broodstock, as well as basic detection and monitoring of individuals in the field (Carson et al., 2019). Although available evidence suggests pinto abalone exhibit strong site fidelity and little movement within their habitat, they are highly cryptic and often hide among rock crevices, making it difficult to obtain an accurate census (Carson et al., 2019). The detection rate of abalone from diver surveys is low, ranging from 20% to 40%, suggesting limited efficacy of diver surveys for abalone detection (Carson et al., 2019). Diver surveys are also costly and time consuming, requiring teams of 4–6 scientific divers over multiple weeks of sampling per year. Yet, the need for abalone population monitoring is expected to increase with anticipated restoration program expansion. It is therefore desirable to have an additional means of surveying wild abalone for the broodstock program and to identify suitable habitat for restoration, as well as captive-bred abalone outplanted at restoration sites.

Over the past decade, environmental DNA (eDNA) techniques—in which DNA from tissues, excrement, gametes, or larvae shed from organisms is identified from environmental samples such as water and soil—have been increasingly used for ecosystem profiling and species detection (Rourke et al., 2022). Metabarcoding of eDNA has shown promise in characterizing marine communities (e.g., Jeunen et al., 2019; O'Donnell et al., 2017; Port et al., 2016), while single species eDNA surveys for both fish and invertebrates have seen success using quantitative and digital PCR (e.g., Bolte et al., 2021; Plough et al., 2018; Roux et al., 2020; Uthicke et al., 2018). These techniques are particularly suitable for marine environments due to their relative inaccessibility. Shallow subtidal communities, for example, are traditionally surveyed using either SCUBA or remotely operated submersibles, which is time consuming and inefficient, or trawling, which is efficient to sample soft-sediment benthic communities yet destructive. Environmental DNA sampling has strong potential in these environments because it is non-invasive, efficient, sensitive, and cost-effective (Bohmann et al., 2014; Pikitch, 2018). Several studies have proven the effectiveness of eDNA in marine environments despite potential decay and advection of DNA by currents (Collins et al., 2018; Jeunen et al., 2019; Kelly et al., 2018). Environmental DNA may be detected in the marine environment for up to 48 h (Collins et al., 2018), yet there is evidence that eDNA accurately reflects distinct marine communities at discrete spatial scales of as little as tens of meters (Jeunen et al., 2019; O'Donnell et al., 2017; Port et al., 2016). Environmental DNA techniques also detect taxa regardless of life history or degree of motility; for example, eDNA has been successful in detecting sessile invertebrates that brood their young in addition to highly mobile taxa such as fishes, and highly dispersive taxa such as barnacles and mussels (O'Donnell et al., 2017). Perhaps most importantly in the context of pinto abalone restoration and management, eDNA approaches appear to be ideal for the detection of rare and/or threatened species (Jerde et al., 2011; Pikitch, 2018; Thomsen et al., 2012). Rare species that were either undetected or detected with limited success using traditional methods have been discovered using eDNA (Pfleger et al., 2016; Pikitch, 2018; Thomsen et al., 2012). This suggests that eDNA may be a powerful tool for detection of endangered pinto abalone.

Here, we first validated the use of eDNA sampling in aquaria with known abalone densities. We then tested the detection of abalone eDNA in field settings, during and after annual dive surveys at restoration sites. A site occupancy model was developed based on repeated sampling at restoration sites with abalone density estimates from diver surveys. Finally, we use eDNA techniques to investigate abalone populations throughout the San Juan Archipelago and Strait of Juan de Fuca, with the goal of identifying potential abalone populations that may have been overlooked by previous conventional surveys. Quantitative PCR (qPCR) was the primary method used, but we also adapted the assay for droplet digital PCR (ddPCR) due to its increased sensitivity and precision, absolute quantification, and resistance to PCR inhibition.

2 | MATERIALS AND METHODS

2.1 | Aquaria sampling

Abalone restoration aquaculture operations occur at the Kenneth K. Chew Center for Shellfish Research and Restoration at NOAA's Manchester Research Station in Port Orchard, WA. In December 2020, a single 1 L seawater sample was taken from each of 10 different flow-through seawater aquaria (all tanks = 175 L volume) with a range of juvenile (1–2 yearr old) abalone densities. Sample bottles

were transported on ice to the laboratory, where they were filtered within 3 h of collection. Samples were vacuum filtered onto cellulose nitrate membrane filters (0.45 μm pore size, 47 mm diameter; Nalgene #1452045) and preserved in absolute ethanol. To obtain abalone biomass in each tank, a subset of 30 animals in each tank was weighed (wet weight of tissue and shell), and the mean weight was multiplied by total abalone numbers.

2.2 | Pilot testing at restoration sites

For field sampling, initial pilot testing was employed to inform the optimal sampling approach. These tests were done in conjunction with annual dive surveys at abalone restoration sites in the San Juan Islands during February and March 2021. At each of 12 different restoration sites with known abalone presence, divers obtained 1 L water samples at near-bottom, 2 m off-bottom, and the surface. Additional surface water control samples were taken during transit to field sites in open water far (>1–2 km) from shore, or at marinas at the conclusion of survey days. Samples were kept in a cooler on ice until they were vacuum filtered and preserved as described above within 6 h of collection. Sample bottles were thoroughly sterilized with 25% bleach and a deionized water rinse before each use. Bottles were filled with deionized water to prevent collapse underwater, and deionized water was exchanged with seawater when opened underwater. These initial tests revealed that abalone eDNA was detected at three of 12 abalone restoration sites. At one of these sites, eDNA was detected at both near bottom and 2 m off the bottom, while at the other two sites, only near-bottom samples had positive detections. Abalone eDNA was not detected in surface water samples or at the control sites (marinas, offshore locations). Based on these results, subsequent field surveys used near-bottom sampling with larger volumes (2 L) and larger PCR reaction volumes to increase detection probability (see below for PCR details).

2.3 | Field eDNA surveys

Field eDNA surveys occurred during June and July 2021. To select sites for field surveys, benthic habitat maps and aerial imagery were used to identify potential abalone habitat in the San Juan Archipelago. For the Strait of Juan de Fuca, only aerial imagery was used because habitat maps were not available for this region. We targeted areas with dense kelp canopies (primarily *Nereocystis luetkeana* with some *Macrocystis pyrifera* in the western Strait of Juan de Fuca) that tend to have rocky reef habitat suitable for abalone. Coordinates for sites of interest were saved as waypoints for field surveys. At each waypoint, the research vessel was navigated into shallow waters at a typical abalone habitat depth of 5–10 m, and 2 L seawater samples were taken via Niskin bottle. The Niskin bottle was rigged with a lead downrigger ball 1 m below the bottle that served as a bottom indicator; once the bottom was reached, a weighted messenger was deployed to trip the bottle closure mechanism. Thus, water samples were taken 1 m above the bottom. Just below the Niskin bottle, a CastAway CTD (SonTek) logged time, depth, temperature, salinity, and geographic coordinates of each site (Table S1). Samples were filtered and preserved as described above on the vessel en route to each successive site, and all sampling equipment including the Niskin bottle and downrigger ball were soaked in a 25% bleach solution, followed by a tap water rinse, in between sites.

2.4 | Occupancy modeling

To determine the probability of detecting *H. kamtschatkana* eDNA in the field, we applied an occupancy modeling technique that evaluated the probability of eDNA detection as a function of abalone biomass estimated by diver surveys at $N = 4$ different abalone restoration sites. These sites have received outplants of juvenile abalone for varying numbers of years and have had varying abalone survival rates, resulting in a range of biomass at each site. At each site, restoration plots ranged from 82 to 102 m^2 at depths of 5–10 m. Surveying techniques are described in detail by Carson et al. (2019). Briefly, at each site, divers established the perimeter of the restoration plot by running a transect tape to each of the four corners of the plot, marked by fixed steel pitons with floating yellow line. The plot was then divided into five 2 m-wide lanes with leaded lines and divers counted and measured the shell length of every abalone encountered; care was taken to look for hidden abalone, with small boulders carefully moved to look for abalone hiding in crevices. To account for potential emigration of abalone off-plot, divers also searched a 2 m perimeter outside the plot, effectively doubling the total search area to 172–200 m^2 per plot. Dive surveys were conducted in February and March 2021. In late June / early July 2021, eDNA above the plots was sampled on four different days via Niskin bottle as described above for field surveys; thus, each of the four sites was repeatedly sampled four times. Additional diver survey data from prior years (2011–2016) in which replicate surveys were conducted were used to compare error of diver surveys to eDNA surveys.

To calculate abalone biomass at each site, abalone mass was estimated from shell length data using the *H. kamtschatkana* length–weight model from Zhang et al. (2007). Summed abalone biomass for each site was then divided by the total area surveyed. These estimates were used as covariates in a Bayesian hierarchical occupancy model computed with the R package *eDNAoccupancy* (Dorazio & Erickson, 2018). Probability of eDNA occurrence at a site (ψ) and probability of eDNA occurrence in a PCR replicate (p) were assumed to be constant, while probability of eDNA occurrence in a sample (θ) was modeled as a function of diver-estimated abalone biomass at each site. The number of iterations for the Markov chain Monte Carlo algorithm was set at 11,000, with posterior summary statistics determined after a burn-in of 1000. Sample occupancy probabilities are reported as the median with 95% credible intervals.

2.5 | DNA extraction and qPCR

DNA was extracted from one-half of membrane filters using the Qiagen DNeasy kit with Qiashredder columns as described by Goldberg et al. (2011). DNA was eluted with 100 µl Qiagen AE buffer. A fragment of the cytochrome c oxidase subunit I (COI) mtDNA gene was targeted using the TaqMan qPCR protocol developed for larval *H. kamtschatkana* by Vadopalas et al. (2006). These authors tested the specificity of this assay against DNA from 29 species of marine gastropods, and while COI from other species in the genus *Haliotis* were amplified, *H. kamtschatkana* is the only haliotid likely to be located in Washington waters (Vadopalas et al., 2006). PCR reactions were set up in a laminar flow PCR cabinet treated with UV sterilization for 15 min before and after each use. A 500 bp gBlock synthetic gene fragment (Integrated DNA Technologies) of the *H. kamtschatkana* COI target was used as a control, as well as to develop a gene copy number standard curve using 10-fold serial dilutions. All PCR runs included gBlock standards and no template controls. Six replicates ($N = 6$) of each sample were run on an Applied Biosystems StepOnePlus Real Time PCR System. For tank and field samples, two of the six replicates included VIC-labeled TaqMan Endogenous Internal Positive Control (IPC; Applied Biosystems) reagents to assess PCR inhibition. qPCR conditions were as follows: 1× TaqMan Environmental Master Mix (Applied Biosystems), 320 nM forward primer (Integrated DNA Technologies), 320 nM reverse primer (Integrated DNA Technologies), and 160 nM FAM-labeled TaqMan MGB Probe (Applied Biosystems), with 10 min activation at 95°C followed by 50 cycles alternating between 95°C (15 s) and 60°C (60 s). Initial PCR reaction volumes were 15 µl with 2 µl template for aquaria samples and pilot field surveys, but were increased to 25 µl with 4 µl template for subsequent surveys to increase detection probability (see rationale under *Pilot testing* above).

Limits of quantification (LOQ) and detection (LOD) were determined for the qPCR assay using the *LoD-calculator* R script by (Klymus et al., 2019). This script uses a curve-fitting approach based on the R *drc* package (Ritz et al., 2015), running all available logarithmic functions and selecting the best-fitting model using *mselect* function in the *drc* package. For the standard curve, a gBlock 10-fold dilution series of 100,000 to 0.1 copies per reaction was tested with $N = 6$ replicates each. The stock concentration of the gBlock standard was quantified with the Qubit High Sensitivity fluorescence-based assay (ThermoFisher) based on three replicates. Data from the 2nd and 3rd quartiles of each dilution series were used in the model, while the remainder were excluded. Best-fitting models from the *LoD-calculator* script were used to report modeled LOQ and LOD, with a 95% LOD probability of detection and a 35% CV for LOQ precision.

2.6 | Droplet digital PCR

The TaqMan assay described above was modified for ddPCR with the Bio-Rad QX200 ddPCR system. Each 22 µl reaction mix contained

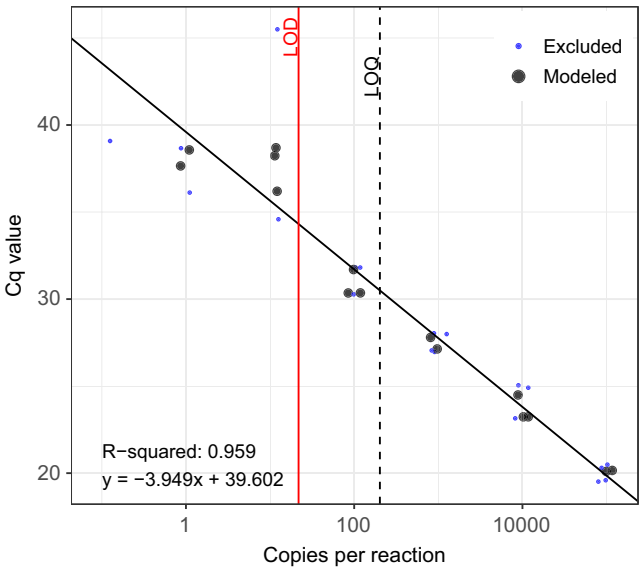

FIGURE 1 Standard curve used to determine the limits of quantification (LOQ; dashed line) and detection (LOD; red line) for the qPCR assay. Data from the 2nd and 3rd quartiles of each dilution series were used in the model ("Modeled"); the remainder were excluded ("Excluded").

1× Bio-Rad ddPCR supermix for probes (no dUTP), 900 nM forward primer, 900 nM reverse primer, 250 nM TaqMan probe, 4 µl template, and 2 µl H_20. PCR reaction mix (20 µl) and Bio-Rad Droplet Generation Oil (70 µl) were added to the appropriate wells of a Droplet Generator DG8 Cartridge (Bio-Rad), covered with DG8 Gaskets (Bio-Rad), and placed in a QX200 Droplet Generator. Resulting droplets (40 µl) were transferred to a 96-well PCR plate (Bio-Rad), which was sealed with pierceable foil. Optimal annealing temperature was determined to be 57°C based on an initial thermal gradient experiment testing temperatures from 54 to 64°C with a gBlock standard. PCR was performed on a C1000 Touch Thermal Cycler (Bio-Rad) with the following final conditions: 1 cycle at 95°C (10 min), 40 cycles alternating between 94°C (30 s) and 57°C (60 s), 1 cycle at 98°C (10 min), and 4°C hold. Droplets were then read on a QX200 droplet reader (Bio-Rad). A manual threshold of 2500 fluorescence units was set for all samples based on positive and negative controls. An average of 14,160 droplets was accepted per reaction. Samples used for occupancy modeling were tested in triplicate ($N = 3$), while samples from field surveys were tested without technical replicates ($N = 1$).

3 | RESULTS

The LOQ of the qPCR assay was modeled at 202 copies per reaction, while the lowest standard with at least 95% positive detection was 100 copies per reaction (Figure 1; $R^2 = 0.96$). The LOD of the assay was modeled at 21.8 copies per reaction, but was estimated as low as 0.2 copies per reaction with eight replicates based on model fit at lower concentrations. These values are within the range of other well-validated qPCR assays (Klymus et al., 2019).

In the abalone aquaria samples, abalone biomass per tank varied between 55 g and 903 g and COI copy number ranged from 4100 to 31,900 copies L^{-1}. A positive relationship was observed between the biomass of abalone in each tank and the number of abalone COI copies detected via qPCR (Figure 2; $R^2 = 0.95$, $p < 0.001$).

At the abalone field restoration sites, abalone biomass based on diver surveys ranged from 8 to 145 g m^{-2} (Figure 3). Although the concentration of abalone eDNA was approximately 100 fold lower than concentrations measured in abalone holding tanks at the hatchery (35–185 copies L^{-1} vs. 4100–31,900 copies L^{-1}), it was positively associated with abalone biomass estimated via diver survey at each site (Figure 3a). Occupancy modeling showed that the probability of abalone eDNA detection in a sample increased with biomass as expected (Table 1, Figure 3b). Probability of detection with ddPCR was higher than with qPCR; at the highest abalone biomass, ddPCR had 32% higher median detection probability, while at the lowest biomass, median detection probability was 89% higher with ddPCR (Table 1, Figure 3b).

We surveyed a total of 80 sites during field surveys over 6 days of sampling (Figure 4a). Average sampling depth was 7.2 m (range = 3.5–12.3 m; Table S1). Eleven (14%) sites had at least one of six positive qPCR replicates, compared with 26 (33%) sites with a single positive ddPCR replicate; seven sites (9%) tested positive using both methods (Figure 4b). Mean eDNA concentration at positive sites determined via ddPCR was 60.3 copies L^{-1} (range = 30–150 copies L^{-1}). Two of the negative (both qPCR and ddPCR) sites showed evidence of partial PCR inhibition, each of them with no amplification in one of the two IPC qPCR replicates. Given the protected status of pinto abalone, geographic coordinates of sites with positive eDNA results are kept confidential.

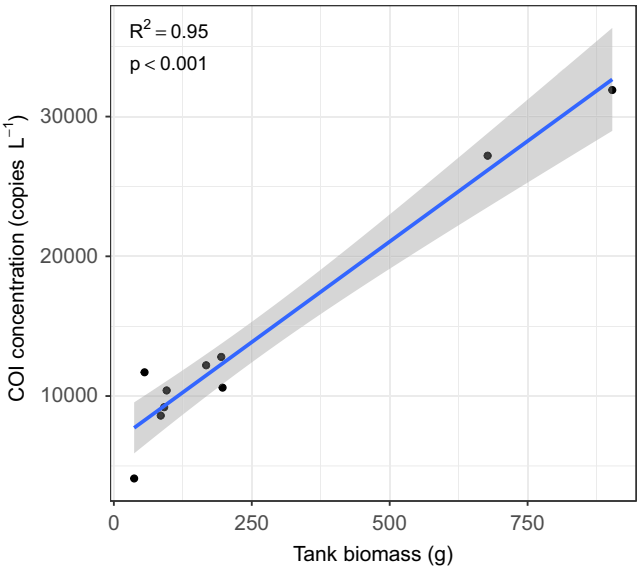

FIGURE 2 Relationship between abalone biomass in aquaria at the restoration facility and the concentration of abalone eDNA, with linear regression trendline and 95% confidence intervals (gray shading).

Cost and time comparisons indicate that pinto abalone eDNA sampling is cost-effective and efficient (Tables 2 and 3). Although qPCR is cheaper on a per reaction basis, ddPCR is cheaper on a per-site basis due to the need for several qPCR technical replicates (Table 2). Time efficiency analysis indicates that eDNA surveys take approximately one tenth of the amount of time as diver surveys on a per-site basis (Table 3). This is due to more sites sampled per survey day and fewer personnel needed for eDNA sampling. However, this analysis does not take into account the very different data obtained by the two methods, namely the richer census and demographic information provided by diver surveys.

Repeated diver surveys had lower coefficients of variation than repeated eDNA surveys (Table 4). Diver surveys conducted in 2011 and 2012 may have had higher CVs due to less experienced surveyors than in subsequent years. It is also worth noting that diver surveys continually detected new individuals (based on tag IDs) in successive surveys, which highlights the cryptic nature of even large, slow-moving snails and helps put the variation in diver survey counts in perspective (Carson et al., 2019).

4 | DISCUSSION

This work establishes eDNA sampling as an effective tool for detection of endangered pinto abalone. Conservation and management of a species rely on effective detection and population monitoring, and as a species becomes more rare, eDNA becomes an increasingly attractive, and potentially necessary, means for population assessment (Jerde et al., 2011; Pikitch, 2018). As restoration efforts expand, this method may become increasingly useful to managers to augment visual diver surveys to monitor abalone populations at restoration sites. With expected increases in the scope and scale of restoration efforts, monitoring of existing wild populations and hatchery-reared outplants will become increasingly onerous with traditional diver surveys. The capability of eDNA techniques to enable surveys at increased spatial scope and relatively low cost may become essential. However, eDNA cannot provide demographic information and is unlikely to fully replace diver surveys, particularly where quantitative census data is necessary. Moreover, although diver surveys are imperfect and have their own associated error, this error is currently lower than that of eDNA surveys. Future improvements in the sensitivity, precision, and accuracy of eDNA technology may change this.

Through widespread, rapid sampling, this work has quantified the extremely low abundance of pinto abalone in Washington waters, corroborating other quantitative data and anecdotal information (Carson & Ulrich, 2019), and further emphasizing the need for conservation and restoration of this species. However, our eDNA surveys of multiple sites in the San Juan Archipelago and Strait of Juan de Fuca uncovered previously unknown areas likely to harbor pinto abalone. These newly identified areas with eDNA signal represent perhaps the most useful application of eDNA, because they provide rapid and low-cost initial scouting work for locations

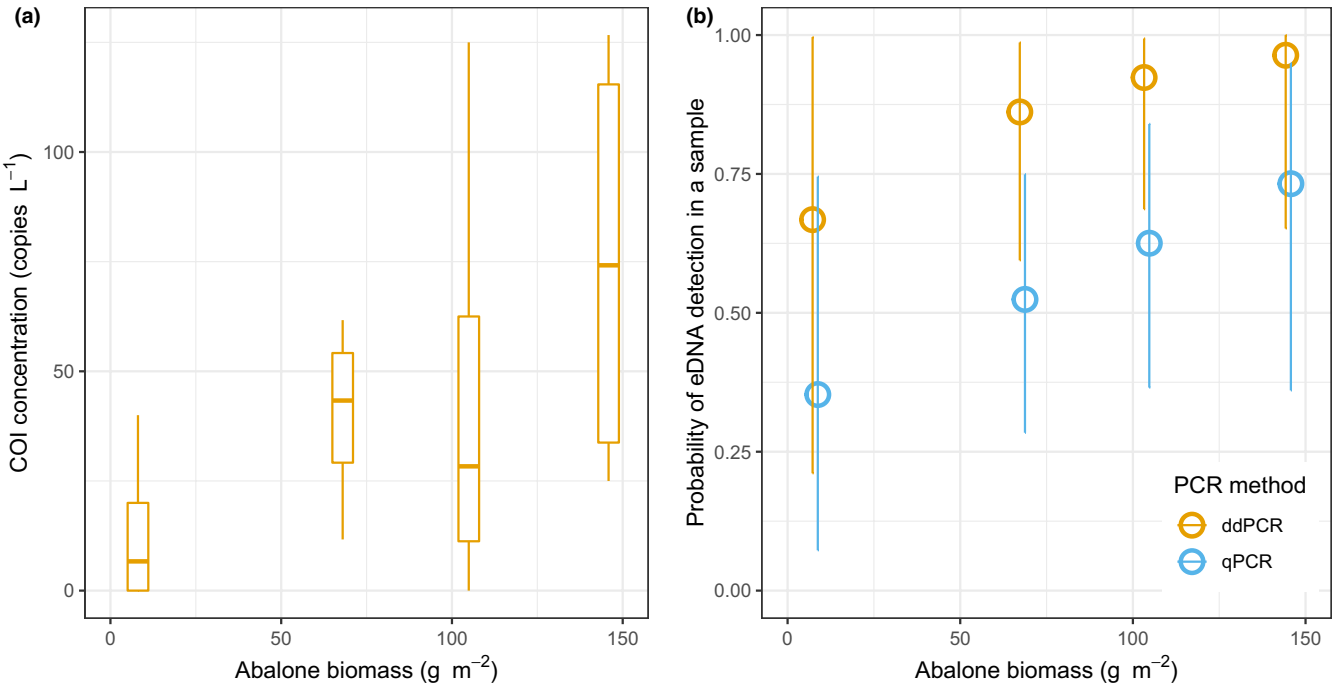

FIGURE 3 (a) Abalone eDNA concentration at each of four different field restoration sites with estimates of abalone biomass based on diver surveys. eDNA concentration was determined via absolute quantification with ddPCR ($N = 4$ sample replicates per site, 3 ddPCR technical replicates per sample). Boxplots represent median plus or minus 75th and 25th percentiles, with whiskers showing 1.5 times the interquartile range above or below 75th and 25th percentiles. (b) Occupancy models of eDNA detection probability in relation to abalone biomass at the four restoration sites, comparing detection probability using qPCR and ddPCR ($N = 4$ sample replicates per site, 3 ddPCR technical replicates, 6 qPCR technical replicates). Points and bars represent median probabilities with 95% credible intervals.

TABLE 1 Posterior summary of occupancy model parameters, showing median values for models based on both qPCR and ddPCR

Site	Diver-estimated biomass (g m^{-2})	Probability of eDNA occurrence at a site (ψ)		Probability of eDNA occurrence in a sample (θ)		Probability of eDNA occurrence in a PCR replicate (p)[a]	
		qPCR	ddPCR	qPCR	ddPCR	qPCR	ddPCR
Omaha	145	0.868	0.872	0.732	0.963	0.825	0.544
Gold	104	0.868	0.872	0.625	0.921	0.825	0.544
Utah	68	0.868	0.872	0.521	0.856	0.825	0.544
Switchback	8	0.868	0.872	0.348	0.678	0.825	0.544

Note: Probability of eDNA occurrence at a site (ψ) and probability of eDNA occurrence in a PCR replicate (p) were fixed in the model, while probability of eDNA occurrence in a sample (θ) was modeled as a function of diver-estimated abalone density at each site.

aSix qPCR replicates vs. three ddPCR replicates per sample.

that can be targeted by divers for abalone broodstock collection. The discovery of new broodstock sources can support conservation efforts by increasing broodstock and family sizes in the hatchery. Large and diverse broodstocks are necessary to maintain genetic diversity among hatchery-reared animals bred for wild stock restoration (Grant et al., 2017). The need to increase broodstock size specifically for pinto abalone restoration in Washington State has been identified based on genomic analyses (Dimond et al., 2022). Current restoration efforts have relied on broodstock from just a few sites in the San Juan Archipelago, and known adult populations are so low that the restoration program may need to bring in broodstock from outside the region. Further eDNA surveys could identify additional wild abalone populations and help reduce the need to consider external broodstock sources.

This study shows that eDNA-based detection of a rare marine invertebrate is surprisingly effective despite potential dilution, degradation, tidal advection, and PCR inhibition in a highly dynamic marine environment. Few studies have evaluated eDNA detection of marine invertebrates; however, two recent studies of abalone are particularly noteworthy. In a large aquarium setting, Martin (2020) found that detectable quantities of eDNA from a single red abalone (*H. rufescens*) permeated a 2 million liter volume within 18 h. On the contrary, Pierce (2020) observed that most *H. rufescens* eDNA decayed within 24 h, with a half-life of 12.77 h. This is in general agreement

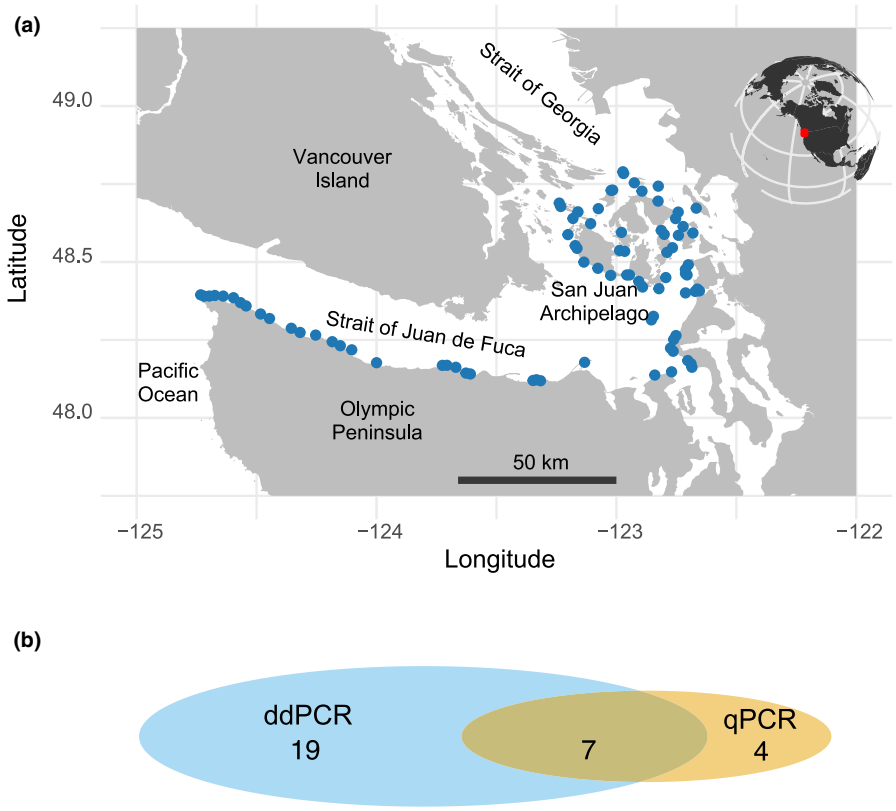

FIGURE 4 (a) Abalone eDNA sampling locations (blue points) in the southern Salish Sea ($N = 80$ sites), with inset globe map showing study area along the coast of North America. (b) Venn diagram showing number of sites with positive abalone eDNA detections by each PCR method.

TABLE 2 Cost comparison of qPCR and ddPCR assays on both a per reaction and per-site basis

	Cost per reaction		Cost per site[a]	
Item	**qPCR**	**ddPCR**	**qPCR**	**ddPCR**
Primers	$0.01	$0.03	$0.08	$0.03
Probe	$0.12	$0.18	$0.72	$0.18
PCR mix	$0.92	$1.29	$5.51	$1.29
IPC reagents	$0.27	NA	$1.63	NA
Plates & seals	$0.10	$0.09	$0.59	$0.09
Droplet generation oil	NA	$0.35	NA	$0.35
Droplet cartridges and gaskets	NA	$1.85	NA	$1.85
Total	$1.42	$3.79	$8.51	$3.79

Note: Values represent 2021–2022 costs in USD.

aCost per site assumes 6 qPCR technical replicates and a single ddPCR reaction.

TABLE 3 Comparison of time per survey and per site for diver surveys and eDNA surveys using qPCR and ddPCR

	Sites per survey day	Personnel per survey	Field hours per survey day	Lab hours per survey day	Total person-hours per survey day	Total person-hours per site
Dive survey	1–3	4–6	9	NA	36–54	12–54
qPCR survey	10–15	2–3	9	5	28–42	1.9–4.2
ddPCR survey	10–15	2–3	9	6	30–45	2–4.5

with other studies on eDNA decay rates in the marine environment (Collins et al., 2018; Jo et al., 2019). Together, these studies suggest that DNA molecules can readily diffuse throughout large water volumes from a single abalone, but that decay and dilution limit the spatial extent and temporal persistence of eDNA. Indeed, these factors likely contribute to the utility of eDNA as a tool, in that it can be used to detect species and distinguish entire marine communities with surprising (tens to hundreds of meters) precision despite advection (Kelly et al., 2018; Port et al., 2016). Albeit with a small sample size of restoration sites, the positive association between abalone biomass

TABLE 4 Comparison of coefficients of variation (CV) for replicate diver versus eDNA surveys

Survey type	Restoration site and survey year	Total elapsed days between surveys	Survey 1	Survey 2	Survey 3	Survey 4	CV (%)
Diver census (number of abalone on plot)	Gold, 2011	18	42	30	17	44	37.5
	Omaha, 2012	25	23	11	12	16	35.1
	Husky, 2016	27	42	27	33	28	21.1
	Baytown, 2016	28	54	51	56	31	24.0
						Mean	29.4
eDNA (abalone COI copies L^{-1})	Gold, 2021	17	15	42	125	0	122.8
	Omaha, 2021	17	37	127	25	112	68.8
	Switchback, 2021	17	13	0	40	0	141.4
	Utah, 2021	17	35	62	12	52	54.6
						Mean	96.9

and eDNA concentration and detection probability we documented suggests that eDNA surveys have the potential to be a quantitative proxy for abalone biomass. This is particularly true for ddPCR, which has higher sensitivity and allows for absolute quantification of extremely low eDNA concentrations.

As with other studies comparing qPCR to ddPCR for eDNA detection (Brys et al., 2021; Doi et al., 2015; Uthicke et al., 2018; Wood et al., 2019), we found that ddPCR outperforms qPCR. Detection probability was higher with ddPCR, particularly at low abalone biomass levels, and ddPCR more than doubled the number of sites with positive eDNA detections. Comparison of time and costs also indicates that ddPCR is affordable relative to qPCR and requires only marginally more time per site. A significant advantage of ddPCR is that it theoretically does not require technical replicates because a single ddPCR reaction is partitioned into thousands of individual replicate reactions. However, considering the extremely low concentrations of abalone eDNA in the natural environment, future work should consider running samples in duplicate or triplicate to increase detection likelihood and provide the most quantitative results. If tested in duplicate, per-site costs of reagents and consumables would still be lower than qPCR costs, and labor time would increase only marginally. Lastly, an additional advantage of ddPCR is its resistance to PCR inhibition relative to qPCR (Dingle et al., 2013; Rački et al., 2014). Although ddPCR may have had increased detections relative to qPCR due to qPCR inhibition, we observed qPCR inhibition in only two reactions out of nearly 1500, suggesting that PCR inhibition was not a major factor in our samples.

Other factors may have contributed to variation in abalone detection, and a few are worth mentioning. Organism size is often positively associated with eDNA shedding rates (Rourke et al., 2022), and this has been documented with red abalone in controlled settings (Pierce, 2020). Each restoration site in our study hosted abalone spanning a range of sizes, and one of the sites (Switchback) received over a thousand juvenile abalone approximately 2 months prior to eDNA surveys. However, we suspect that much of this potential error was accounted for by extrapolating abalone counts to biomass. A second factor that may have played a role in our study is abalone spawning. Pinto abalone in neighboring southern British Columbia spawn during spring and summer (Campbell et al., 2003), and therefore, our eDNA surveys overlapped with spawning season. It is possible that some of the eDNA we captured was from gametes or larvae, which could have led to overestimates of adult abalone occurrence in certain areas. This effect is likely to have been very small during wild abalone surveys due to the extremely low numbers of wild abalone, but it may have been more significant at abalone outplant sites due to the higher abalone densities and multiple sampling days. Future eDNA survey efforts may opt to sample outside of abalone spawning season to avoid this potential source of error.

5 | CONCLUSIONS

Among the challenges of recovery efforts for endangered pinto abalone are costly and time-consuming diver surveys with imperfect abalone detection, and identification of new adult populations that can be used as broodstock for species restoration. This research has validated eDNA techniques for abalone detection in the field, improved our knowledge of abalone distribution in the Salish Sea, and provided a novel toolkit for enhanced management into the future. Future diver surveys will explore areas of eDNA detections to determine whether divers can locate wild pinto abalone nearby. Environmental DNA surveys could be used to monitor the success of abalone outplants, monitor index sites used for population assessments, and identify new areas for broodstock collection and restoration outplants. Regular population assessments are essential for periodic species status reviews, and eDNA sampling could complement or augment these assessments in the future. Given the threatened status of many abalone species throughout the world, the results of this study are likely to be applicable to abalone conservation efforts beyond the study system described here.

AUTHOR CONTRIBUTIONS

JLD conceived and designed the study, collected eDNA samples, contributed to dive surveys, performed laboratory analyses, analyzed data, and wrote the manuscript. BRG contributed to the study design, collected eDNA samples, performed laboratory analyses, analyzed data, and edited the manuscript. JVB contributed to study design and dive surveys, and edited the manuscript. HSC contributed to study design and dive surveys, analyzed data, and edited the manuscript. KS contributed to study design and dive surveys, analyzed data, and edited the manuscript.

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CONFLICT OF INTEREST

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

Raw data underlying the main results of this study are included with the supplementary information.

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SUPPORTING INFORMATION

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