Aquaculture Reports 42 (2025) 102729

Contents lists available at ScienceDirect

Aquaculture Reports

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

Detecting the marine gastropod *Lacuna vincta* at seaweed farms using a novel environmental DNA (eDNA) assay

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ARTICLE INFO

Keywords:
EDNA
Biofouling
Seaweed aquaculture
Snail
Gastropod
Macroalgae
Aquaculture

ABSTRACT

The small marine gastropod *Lacuna vincta* is species of concern for kelp (*Saccharina latissima, S. angustissima*) farmers and processors because snails attach their egg sacs to the blades of cultivated kelp and are difficult to remove. Larval dispersal patterns and post-metamorphic migratory behavior have allowed *L. vincta* to encounter and settle on kelp blades suspended from farms. Snail infestations can force farmers to harvest early or redirect biomass into lower-value products. We created and tested a novel species-specific environmental DNA (eDNA) assay for *L. vincta* throughout two kelp growing seasons in conjunction with traditional visual surveying for snails on farms. We were able to detect and amplify *L. vincta* DNA from environmental field samples interannually and across multiple sites as well as to link the presence of *L. vincta* on farms to the presence of *L. vincta* DNA at farm sites. This and future research will contribute to best management practices for kelp farmers when it comes to choosing lease sites for aquaculture farms and determining when to harvest biomass in the spring.

1. Introduction

The production of algae worldwide has increased 9.2 % between 2018 and 2022, with 36.4 million tonnes of seaweed harvested from marine aquaculture in 2022 (FAO, 2024). Most farmed seaweed comes from East and Southeast Asia, but kelp farming is on the rise in North America and Europe (FAO, 2024). The Maine seaweed industry is responsible for ~50 % of the edible seaweed cultivated in the U.S., which represents over one million wet pounds landed during harvest in 2022, the majority of which comes from farms in Casco Bay (Robidoux and Good, 2023). Biofouling has long been an obstacle for aquaculture, as the introduction of new substrate into any marine setting has the potential to create new habitat, cover from predation, and food source for various invertebrates (Fitridge et al., 2012). Kelp farms are no different, and many studies have quantified their fouling by bryozoans, amphipods, tunicates, hydroids, juvenile bivalves, and epiphytic algae (Førde et al., 2016; Rolin et al., 2017; Forbord et al., 2020; Visch et al., 2020).

Seaweed farms in Maine and globally are generally harvested before summer seawater temperatures are warm enough to support much of this biofouling (Grebe et al., 2019, Biancacci, 2022) however the marine gastropod *Lacuna vincta* (family Littorinidae) has consistently been a hindrance for many kelp farmers in the Gulf of Maine due to its year-round settlement activity and preference for kelps (Johnson and Mann, 1986). The snails are small (on average, adults have a shell length of 3.5–4 mm) (Martel and Diefenbach, 1993) and easy to brush or wash off seaweed blades, but their egg masses are embedded in the blade's surface and are labor and time-intensive for processors to remove. As a result, kelp containing snail eggs is lost revenue for farmers and processors because it can be unusable for the highest value products. According to the lead producer of kelp on the east coast of the US, *L. vincta* is the most problematic organism to the kelp crop observed to date with about 10 % of the crop impacted when there is settlement event (personal communication, Liz MacDonald, Atlantic Sea Farms Seaweed Supply Director).

Lacuna vincta resides primarily on the blades of off-benthic wild kelps and can also travel long distances in the water column as both planktonic larvae and post-metamorphic juvenile snails (Johnson and Mann,

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https://doi.org/10.1016/j.aqrep.2025.102729
Received 7 May 2024; Received in revised form 24 February 2025; Accepted 1 March 2025
Available online 9 March 2025

1986) though little migration occurs once settled as adults (Smith, 1973). Larvae dispersing via ocean currents can achieve distances of over 250 km from their origins even at relatively slow velocities (Martel and Chia, 1991). Post-metamorphic snails extend their feet and antennae and produce mucus threads to drift, two behaviors that reduce sinking rates and greatly boost dispersal during migrations (Johnson and Mann, 1986). Juvenile *Lacuna* snails have been observed employing the foot-raising behavior to "catch a current" when kept in undesirable habitats with no algae present, suggesting that drifting in the water column is a way to find favorable algal habitats that support the feeding and reproductive needs of adult snails (Martel and Diefenbach, 1993). Their ongoing annual reproductive cycle and ability to migrate at various life stages make it difficult to predict *L. vincta* settlement at a particular place and time, however, there are some well-documented details about the species' life history and substrate preferences.

The primary cultivated species of kelp in Maine is *Saccharina latissima*, or sugar kelp (Augyte et al., 2017; Kim et al., 2017). *Saccharina angustissima*, or skinny kelp, is sometimes grown alongside sugar kelp within a single farm. In Maine, kelp farms and their associated gear are deployed at lease sites in the late fall and are fully removed at harvest time in the spring (Grebe et al., 2019). When choosing a lease site for a kelp farm, potential leaseholders are advised to seek a location that is at least 5 m in depth at mean low water to avoid kelp biomass having physical contact with the benthos and, in turn, additional biofouling from benthic organisms (Flavin et al., 2013). While removal of kelp farms before the summer and rigorous surveying prior to lease placement may help deter fouling by other invertebrates, *L. vincta's* planktonic larval stage and juvenile drifting behavior continue to cause problems for farmers.

Observations of egg masses and spawning allowed Martel and Chia (1991) to make successful predictions about the presence of *L. vincta* larvae in plankton tows and the appearance of communities of recently settled juveniles in wild kelp communities. Environmental DNA (eDNA) is another tool that can be used to detect the presence of organisms directly from a water sample by amplifying DNA with quantitative polymerase chain reaction (qPCR), using species-specific primers (Thomsen and Willerslev, 2015). Organisms shed cells and organic matter in the water column as they reproduce, create waste, and die. It is possible to identify organisms from their DNA signatures by filtering and isolating this material from seawater. One major benefit of eDNA sampling is that it bypasses some of the more destructive surveying techniques like trawling and is a safe tool when seeking the presence of rare or endangered species (Eiler et al., 2018, Thomsen et al., 2012). However, the validation of a new eDNA detection study usually requires pairing with a traditional surveying method (LeBlanc et al., 2020, Eiler et al., 2018). In the case of kelp farms, eDNA is beneficial when surveying for biofouling because there is no risk of dislodging kelp from rope lines or otherwise disturbing farms and their gear in any way.

eDNA has been especially useful for surveying a single invasive species in an area (LeBlanc et al., 2020), for measuring intra- or interspecific diversity via metabarcoding techniques (Pawlowski et al., 2022; Pérez-Burillo et al., 2021), and for xenomonitoring (Alzaylaee et al., 2020). The small invasive freshwater New Zealand mud snail *Potamopyrgus antipodarum* has been well-analyzed via eDNA (Goldberg et al., 2013; Clusa et al., 2016; Ponce et al., 2021; Woodell et al., 2021). Ponce et al. (2021) found that eDNA monitoring of this gastropod species was more effective than traditional sampling, despite the snails' patchy distribution and sediment burying behavior. Woodell et al. (2021) used quantitative PCR (qPCR) for qualitative presence/absence data only and were able to detect snails where none had ever been visually identified, but where their presence was later confirmed. These studies suggest that eDNA can be useful in early detection of gastropods, especially for species where it is difficult to identify larvae. *L. vincta* is a very small organism, however, the species employs large and frequent larval migrations and post-metamorphic snails generate copious amounts of mucus. eDNA is a promising tool for the detection of this species within kelp farms so that farmers may make informed decisions about choosing sites for their aquaculture farms and the timing of their seaweed harvests.

The objectives of this study were threefold: 1) To test whether a novel *L. vincta* primer can successfully detect snail DNA from environmental marine samples; 2) To link traditional visual surveys of *L. vincta* with the presence of its DNA in the water column; and 3) To provide evidence that eDNA sampling is an effective tool for early detection of this species.

2. Materials & methods

2.1. Kelp farms

Six (6) kelp farms in southern Maine were examined for *Lacuna vincta.* In our first year of winter fieldwork (January 2022 through May 2022), we surveyed two commercial farms in Casco Bay (A and B in Fig. 1) and two research farms in Saco Bay (E and F in Fig. 1). Farms A and B were located less than 0.5 km apart. In Saco Bay, Farm E was on the windward eastern side of Ram Island and Farm F was along the southern edge of Wood Island (Fig. 1). We continued to collect water samples from these four sites during the summer and fall of 2022 even though farms had been removed for the season. Two additional commercial farms (C and D in Fig. 1) were added for the second year of field work (September 2022 through April 2023) and were less than 1 km from each other. *L. vincta* snails were visually confirmed present at Farms C, D, E, and F and nearby sites prior to this study (Schutt et al., 2023). Temperature loggers (Onset U22–001 and UA-001–64) and dissolved oxygen loggers (Onset U26–001) were put on farms A and B in Casco Bay during both growing seasons (Supplementary Fig. 1).

2.2. Visual inspections of farms

Five to six locations along an outside grow line of each farm were inspected at several spots for *L. vincta* snails and eggs. Because most farms were in use as income-generating businesses for commercial growers, interior grow lines were avoided so as not to cross or snag lines and unintentionally damage the farms. Snails and/or eggs were marked as "present" if any snails or eggs were seen. All Casco Bay work was done during high tides due to tide-dependent boat ramp access, while tides during work in Saco Bay varied. The extensiveness of visual inspections of farms were often dictated by ocean conditions that day.

2.3. Lacuna vincta primer and probe

We used a species-specific cytochrome c oxidase subunit I (COI) primer and probe for *L. vincta* snails that were collected in our study area (location G in Fig. 1). Sequences were forward primer 5'-TGC-TTT-ACT-AGG-GGA-TGA-TCA-GT-3' and reverse primer 5'-CCA-GTC-AGT-TGC-CAA-ATC-CT-3'. The probe was a TaqMan minor groove binder with a nonfluorescent quencher (MGB-NFQ) with the sequence 5'- AAC-AGC-ACA-TGC-CTT-CGT-C-3'. The primers and probe were validated against *L. vincta,* as well as DNA from known non-targets *Littorina littorea* (common periwinkle), *Littorina obtusata* (flat periwinkle), *Littorina saxatilis* (rough periwinkle), *Buccinum undatum* (common whelk), *Nucella lapillus* (dog whelk), *Mytilus edulis* (blue mussel), *Boreochiton ruber* (red northern chiton), *Strongylocentrotus droebachiensis* (green urchin), *Euspira heros* (northern moon snail), *Asterias forbesi* (Forbes' sea star), *Metridium senile* (frilled anemone), *Carcinus maenas* (green crab), and *Saccharina latissima* (sugar kelp). During qPCR, the thermal profile used was 3 minutes at 95° C, then 50 cycles of 10 seconds at 95° C and 30 seconds at 60° C. We defined our limit of detection (LOD) as the threshold point at which 95 % of our standard curve replicates have a quantification cycle (C_q) value (Klymus et al., 2020) and was calculated using R (Github file "Maine-eDNA/qPCR_Limit-of-Detection"). Environmental samples with a DNA concentration below 2.8×10^{-4} copies

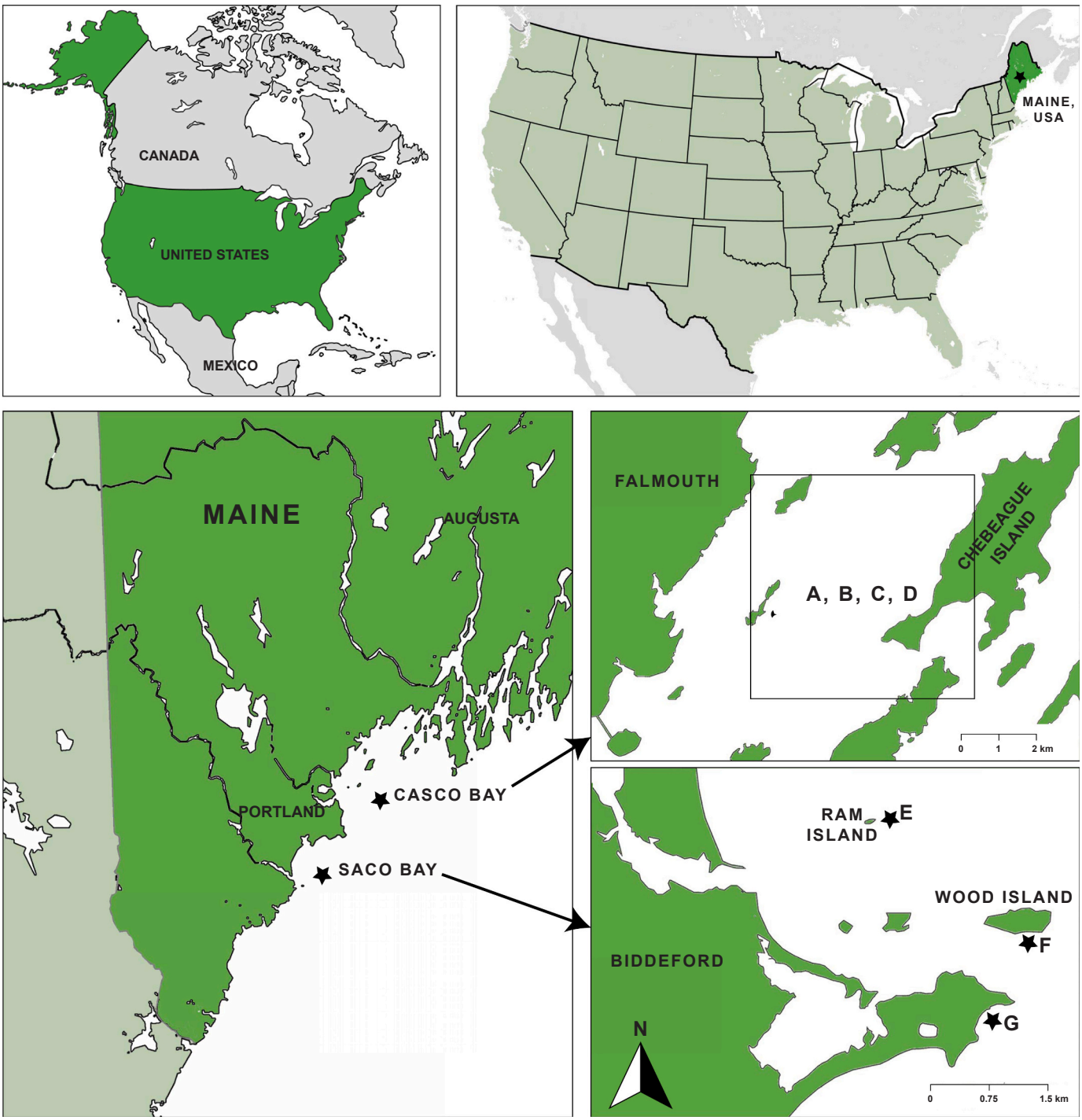

Fig. 1. All research took place in the United States of America (top left) in the northeastern state of Maine (top right). Farm sites A, B, C, and D were all located in western Casco Bay within a 6 km zone, shown by the square in the bottom right (coordinates left out to protect anonymity of commercial farmers); Farms E (43.469779° N, 70.350026° W), F (43.455132° N, 70.336278° W) and snail collection location G (43.444991° N, 70.339867° W) were all in Saco Bay.

per μL, or a C_q of 38, were not included in our analyses, however we did use samples with no recovered DNA in the analysis of our dose-response primer validation experiments.

2.4. Dose-response primer validation experiments

Lacuna vincta snails for validation experiments were collected by hand from a small cove at the northernmost point of Ocean Avenue next to the East Point Sanctuary in Biddeford Pool, Maine (Fig. 1 G). Snails were collected within 48 hours of each experiment and kept in a container within a tank filled with filtered seawater and pumped through a chiller continuously at 11.7–12.8° C at UNE. The container also held a variety of seaweed species collected alongside the snails.

Nine small plastic replicate containers were each filled with one liter of 32 ppt salinity water, made with DI water and Instant Ocean Sea Salt (Spectrum Brands). The plastic replicate containers were suspended in a larger 272 L tank of chilled water so that no water was exchanged, but that the water within each replicate could reach and be kept at 11.7–12.8° C. Nine one-gram pieces of *Fucus distichus*, were weighed out and rinsed thoroughly of any biofouling or epiphytes in deionized plus

Instant Ocean Sea Salt water. Each piece of seaweed was placed in one of the nine plastic containers to test for potentially inhibiting interference. Snails were then added to each container. Three control containers received zero snails, three each received one snail, and three each received ten snails. Snails were left alone for 48 hours, at which time both snails and whole pieces of *F. distichus* were removed. The remaining liter of water from each container was filtered and processed using the methods described below.

2.5. Environmental water samples for eDNA analysis

Immediately following grow-line inspection at a farm, a water sample was taken within 5–10 m of the farm, using a cleaned Niskin bottle at a depth of 2 m. Water from the Niskin was emptied into a 1000 mL pre-marked Nalgene bottle and immediately stored in a clean plastic bag within a cooler full of ice. The Niskin bottle was cleaned between farm sites with one rinse-through of ~500 mL of 10 % bleach and two rinse-throughs of ~500 mL of DI water. For each outing, a field sample of 1000 mL of DI water was run through the Niskin bottle and stored in its own Nalgene in the cooler. This control was done to ensure adequate cleaning practices in the field. Water samples were kept in the cooler until they could be filtered at the lab, which usually took place within three hours of sampling.

2.6. Water sample filtering and storage

All environmental and dose-response validation samples were filtered through 47 mm-diameter white Whatman nitrocellulose membrane filters with a pore size of 0.2 μm. 1000 mL of water from each sample was passed through each filter, except for several instances where too much detritus in the water necessitated that samples be divided across two filters. Using sanitized tweezers, filters were rolled carefully with the DNA-side facing in, placed in 5 mL Eppendorf tubes, and stored at −80° C. Filter cups were cleaned between samples with a 10 % bleach solution and rinsed thoroughly with deionized (DI) water.

2.7. DNA extraction

DNA from all environmental samples, dose-response validation samples, and from *L. vincta* snails were extracted using the Qiagen DNeasy Blood & Tissue kit (Lear et al., 2018) following the manufacturer's instructions with some modifications to increase DNA yield. Modifications included a 3-hour heat block incubation with vortexing every hour, as well as using 80 μL of elution buffer instead of 200 μL. A no-DNA control was included with each set of extractions. The final tubes of extracted DNA were stored at –80° C.

2.8. Quantitative real-time PCR (qPCR)

DNA for qPCR was quantified by extracting DNA from several *L. vincta* snails using the Qiagen DNeasy Blood & Tissue kit and determining concentration with a NanoDrop 2000 Spectrophotometer. qPCR was performed on a Stratagene Mx3005P thermocycler and included serial dilution of 2 μL of *L. vincta* DNA in duplicate to establish a standard curve and two no-template controls. qPCR was performed on all environmental samples in duplicate using 0.2 μL PCR tubes with white wells and optically transparent caps. Each tube contained a super mix containing 10 μL of TaqMan Fast Advanced Master Mix, 0.3 μL each of primer and probe (from a 10 μM stock solution), and 5 μL of extracted DNA per reaction. All environmental samples were spiked with an exogenous internal positive control (ThermoFisher TaqMan IPC Reagents) to detect possible DNA inhibition.

2.9. Statistical Analysis

All eDNA concentrations from environmental samples (field and laboratory) were log-transformed to normalize data for statistical tests (Shapiro Wilk test, $p < 0.05$). For the dose-dependent analysis, a very small constant ($1 *10^{-9}$) was added to all responses before log-transforming. Linear regression was used to analyze results of eDNA concentration as a response to water temperature. A one-way ANOVA was used to test for significance in our dose-response laboratory experiment and continuous data from this is shown as 100 + log(eDNA concentration) in order to better visualize data. A one-way ANOVA was used to test for effects of both kelp farm and wave exposure on eDNA concentration. A Chi-Square Independence Test was used to examine the relationship between the presence or absence of *L. vincta* snails on kelp farms and the presence or absence of *L. vincta* eDNA in the water column. All statistical tests use an alpha value of 0.05 and were conducted using R 4.1.1 GUI 1.77 High Sierra build. Results are presented as mean ± standard error (SE).

3. Results

In a laboratory setting we were able to detect eDNA above the LOD from a single *Lacuna vincta* snail in 1 L of water ($5.6 *10^{-3} \pm 0.001$ ng/μl, $n = 3$) after 48 hours, even with the potentially inhibiting presence of kelp (*Fucus distichus*). Containers with 10 snails had 100-fold more eDNA than those with one snail ($5.7 *10^{-1} \pm 0.2$ ng/μl, $n = 3$) after 48 hours (Fig. 2). No eDNA was detected in the containers with zero snails. More data are needed to fit a curve for the non-linear relationship between containers with one snail and containers with ten snails. There was a significant effect of number of snails on eDNA concentration (One-way ANOVA: $F_{2,15} = 327.6$, $p < 0.001$). A post-hoc Tukey's Honesty Significant Differences (HSD) test showed significant differences between all combinations of treatments ($p < 0.001$).

No amplification of DNA occurred when the *L. vincta* primer was used against known non-targets, as well as for all field controls and extraction controls. IPC results were never more than 1–2C_q values apart within each qPCR run, so external inhibition was ruled out in all environmental and laboratory samples (Thalinger et al., 2021). Our standard curve was a serial dilution that went from 1.52 to 1.52×10^{-5} ng/ul had an r^2 of 0.99, with a slope of –3.501 and an efficiency of 93.0 % (Fig. 3).

A total of 97 separate environmental water samples (not including field controls) were collected across all six sites over the course of two years. Of those, 28 environmental samples contained *L. vincta* eDNA that exceeded our LOD ($2.8x10^{-4}$ copies per μL). There was detection below our LOD in 33 environmental samples, however we cannot draw conclusions from this data and those amounts are not used in our statistical analyses. There was no significant effect of water temperature on eDNA

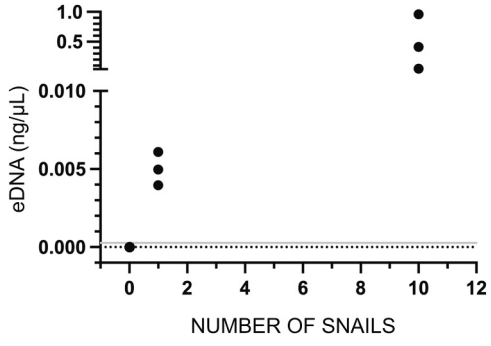

Fig. 2. Dose-response laboratory experiment to detect *L. vincta* DNA from one liter of water. Solid gray line indicates our limit of detection ($2.8 *10^{-4}$ ng/μL) and dotted black line represents zero. No snail DNA was detected when no snails were present, and DNA was detected both when one snail and 10 snails were present. On average, containers with ten snails had 100-fold more eDNA than containers with one snail. There is a non-linear relationship between containers with one snails and containers with ten snails, however more data are needed to fit a model for this relationship.

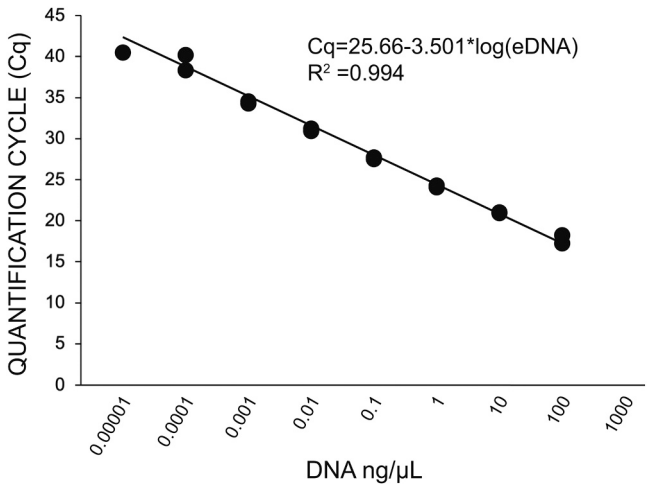

Fig. 3. A representative standard curve used for quantification of eDNA samples for *L. vincta* using a serial dilution from 1.52 to 1.52 × 10^{-5} ng/ul. Standard curve had a slope of −3.501 and an efficiency of 93.0 %.

concentration in our environmental samples ($r^2 = 0.01$, $F = 0.346$; df = 1, 26; $p = 0.562$) (Fig. 4), however we were able to detect *L. vincta* DNA in the water column above our LOD interannually (Fig. 5) regardless of water temperature.

Kelp farms (A through F) had no significant effect on eDNA concentration (One-way ANOVA: $F_{5,22} = 0.765$, $p = 0.585$). We were able to characterize a difference in wave exposure between farms E and F in Saco Bay, however eDNA concentrations between them were not significantly different (One-way ANOVA: $F_{1,11} = 1.712$, $p = 0.217$).

A Chi-square Test of Independence was done that crossed instances of *L. vincta* presence or absence on kelp farms with *L. vincta* eDNA presence or absence in the water column near farms. The test indicated a significant association between the two ($x^2 = 5.76$, df = 1, $p = 0.016$). Recovery of eDNA above our LOD occurred across both field seasons and, except for two instances, coincided with visual presence of *L. vincta* snails on kelp farms (Fig. 5). Positive recovery of eDNA occurs throughout the year, however there is some clustering that happens in the spring and summer months. Approximately 32 % of eDNA recovery occurred when no farms were in the water, the majority of which were between July and September.

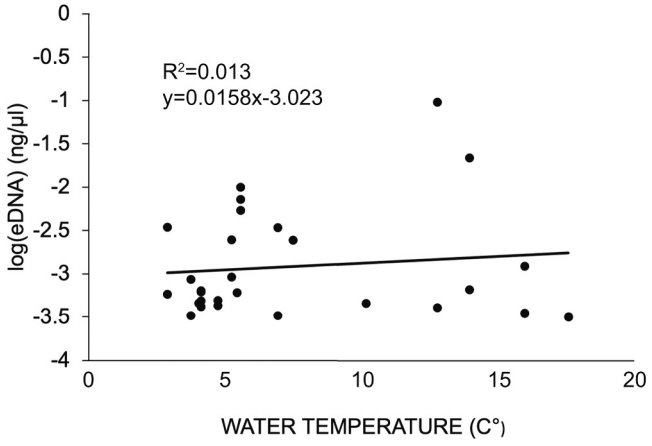

Fig. 4. Linear regression containing all environmental samples from Casco Bay and Saco Bay with *Lacuna vincta* eDNA detected above our limit of detection as a function of seawater temperature. The slope is not significantly different from zero (F=0.346; df=1, 26; p = 0.562).

4. Discussion

During our experiences doing regular field work over the course of 16 months for this study, we learned that *Lacuna vincta* is difficult to quantify in any meaningful way while aboard a boat during the kelp growing season in harsh winter weather. The snail is small and hard to see, and regularly manipulating a kelp grow line without tearing off kelp or entangling grow lines is challenging. Further, sub-freezing temperatures (common during the kelp growing season) can damage or kill kelp blades pulled out of the ocean. Overall, traditional visual methods of surveying for *L. vincta* on kelp farms are unrealistic. Environmental DNA surveys are an novel alternative method to identify presence of *L. vincta.*

Like other marine invertebrates, most of *L. vincta's* life stages are thought to be triggered by cues from the environment, such as water temperature, light intensity, salinity, etc. It is also known that *L. vincta* have spawning events year-round and seem to be tolerant of a range of temperatures. It is not surprising that we see no significant effect of water temperature on eDNA concentration in our environmental samples (Fig. 4). We do, however, see some clustering of positive eDNA recovery in the spring and summer months (Fig 6). Martel and Chia (1991) found *L. vincta* larvae in plankton tows year-round, with significant peaks between April and June, and then again from July to September in British Colombia, Canada. This suggests that a substantial portion of the eDNA we detected may have been from larvae, and not from the juvenile and adult snails that were already living on the kelp farms. Further evidence for this is seen in the nine positive eDNA samples taken during the summer when farms had been removed (Fig. 5) as well as the collection of *L. vincta* snails at non-kelp farm sites in another study in the Gulf of Maine (Schutt et al., 2023). This is promising for eDNA as an early-detection tool for *L. vincta,* however, the addition of plankton tows to the existing methodology outlined in this study is necessary to draw any conclusions about the relationship between eDNA and the presence of *L. vincta* larvae.

We heard from farmers and other industry members that there was historically a larger infestation of snails at Farm A than at Farm B, which are located less than 0.5 km apart in Casco Bay (Fig. 1). Our data, however, show no significant effect of kelp farm on eDNA concentration. The greatest shortcoming of many eDNA studies is the inability to draw conclusions about an organisms' biomass from environmental samples (Lacoursière-Roussel et al., 2016, Beng and Corlett, 2020, LeBlanc et al., 2020, Danziger et al., 2022, Rourke et al., 2022, Xin et al., 2022). *L. vincta* DNA can come from numerous life stages and/or biological shedding: waste, shed dead cells, mucus, larval migrations, etc. Though we show that eDNA above our LOD is detectable in a laboratory setting from a single snail (Fig. 2), we can only speculate about what a similar amount of DNA from an environmental sample might represent. This is the problem we face when comparing eDNA detected at different farm sites. While Farm A may have more settled adults and pose a more serious threat to a farmer's financial profits, Farm B may be situated in a current that regularly has more larvae passing through. Therefore, it is possible that we cannot detect a significant difference in the eDNA from two different farms that are experiencing substantially different snail biomasses on kelp blades.

The settlement abundance and timing of the *L. vincta* snail is difficult to predict due to its ability to travel during multiple life stages, as an adult or juvenile snail. Martel and Diefenbach (1993) demonstrated that juvenile *L. vincta* snails exposed to oscillatory water flows and/or strong currents were more likely to employ mucus threading and foot-raising to migrate to a new location than were adult snails within calm waters. This was corroborated by Duggins et al. (2001) who showed that half of *L. vincta* snails could not adhere to kelp stipes once water flow had increased to only 50 cm s^{-1}. An established population of adult egg-laying snails on a farm in a protected location is more likely to remain there and reproduce than a population located on a farm with a strong current flowing through it, even if that current is carrying a cohort of *L. vincta* larvae. While this helps to explain why we see no

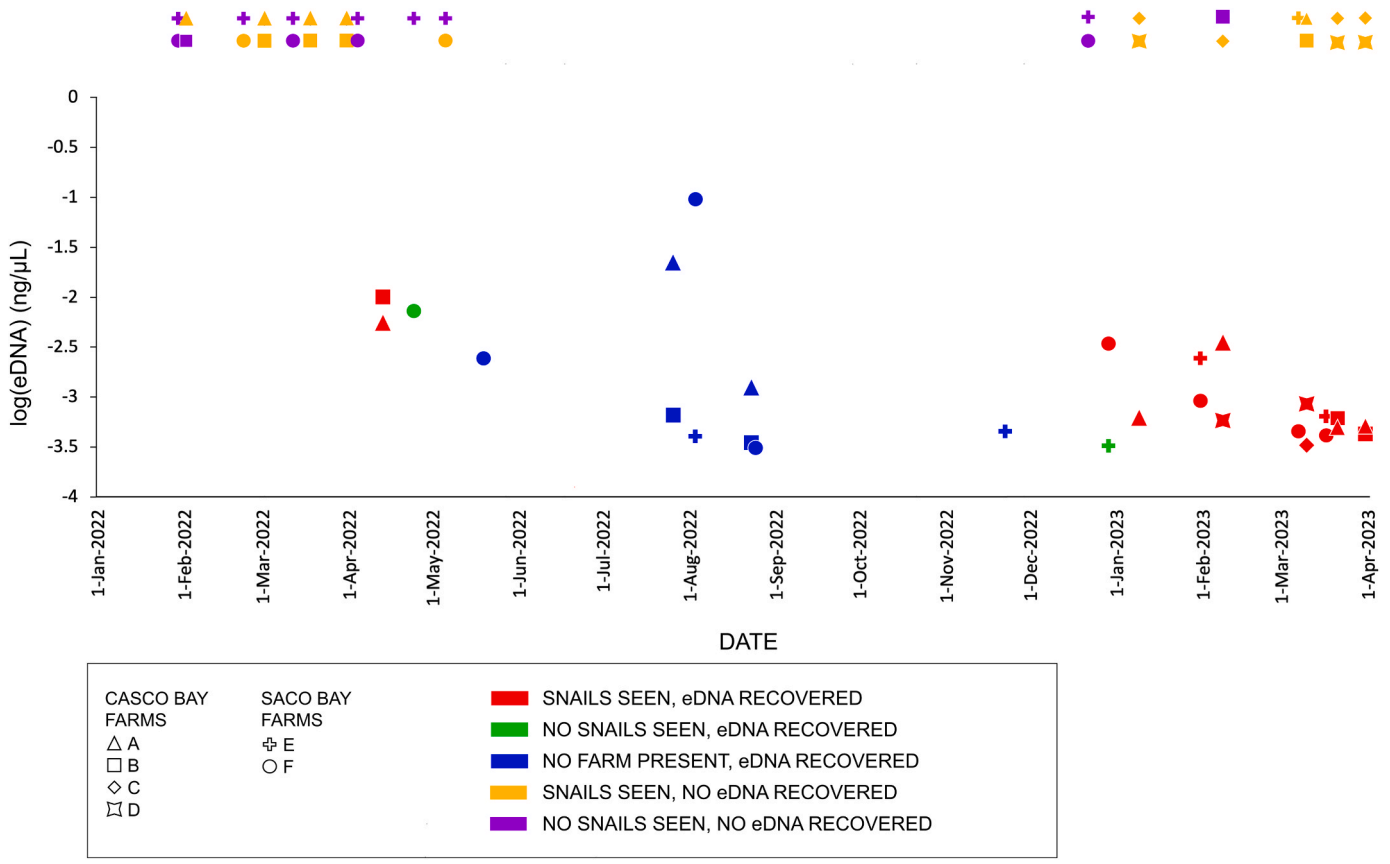

Fig. 5. All environmental samples collected over the course of 16 months, January 2022 through April 2023, from six different kelp farms in Casco Bay and Saco Bay, Maine, USA. Environmental samples were filtered for eDNA and tested with a species-specific primer for the small gastropod *Lacuna vincta*. Graph indicates the days that samples were collected, whether snails were visually seen on farms on those days, if eDNA from snails was recovered, and the concentration of eDNA from recovered samples. On days when no eDNA was recovered from environmental samples, markers along the top of the graph represent the presence or absence of snails.

significant difference in eDNA concentrations between farms, it may also explain why we see no significant difference between farms with differing intensities of wave energy. Farms E and F in Saco Bay (Fig. 1) experience drastically different conditions. Farm E at Ram Island has been used as a proxy studying kelp farms in off-shore conditions and its vulnerability to intense wave energy has been well-characterized (St. Gelais et al., 2022) due to its uninterrupted eastern exposure to the Gulf of Maine. Alternatively, Farm F is on the leeward side of Wood Island and experiences much calmer waters. We expected to see significantly less eDNA at Farm E because fewer snails can withstand the wave energy there. It is possible that larvae in the water column were contributing to the higher-than-expected eDNA concentrations. Our analyses might be affected by the small sample sizes. While our *n* for exposed Farm E was 19, only five of those samples had eDNA concentrations above our LOD. The same is true of Farm F: out of 17 samples, only six surpassed our LOD threshold. More frequent sampling would be required to get a better picture of *L. vincta* presence at exposed and protected sites. Snails were seen at protected Farm F almost twice as often than at exposed Farm E suggesting that, despite no difference in eDNA between the sites, snails are less likely to settle on a farm subjected to constant intense waves and storms.

More snails were seen and more eDNA was detected in the second season of fieldwork than in the first. In the second season, reproductive sorus tissue on the kelp farms in this study appeared earlier than expected around mid-March 2023, whereas in the previous year, no sorus tissue was seen on the same farms before harvest time. In laboratory experiments, *L. vincta* showed a significant feeding preference for sugar kelp's reproductive sporogenous tissue versus its non-reproductive vegetative tissue (O'Brien and Scheibling, 2016). Sugar kelp's peak reproductive periods are variable from year to year and farmed kelp's reproductive cycles generally match that of wild populations (Grebe et al., 2019). As a result, increased biofouling by *L. vincta* and eDNA detection at kelp farms in our second season may be attributable to this variation in the kelp's reproductive cycle.

Significant results of our Chi Square test of independence link the presence and absence of *L. vincta* snails on kelp farms with the presence and absence of its eDNA in the water column. We had 33 instances where *L. vincta* eDNA was suspected, however concentrations did not exceed our LOD threshold. Collecting and filtering a larger volume of seawater for each sample may yield more *L. vincta* eDNA, however we found that even one liter of water in warmer months quickly clogged our filters with detritus and sometimes added hours to the filtration step. In addition to increasing the sensitivity of our eDNA assay, steps that we have considered for future research include testing our primer on *L. vincta* from the Northern Pacific, where anecdotal reports from some Alaskan kelp farmers have shared that they also experience snail biofouling; deployment of loggers that can measure water current velocities at kelp farms or the high-resolution mapping of water movement in specific areas; and a study of *L. vincta* population genomics to identify larval source populations and dispersal patterns in "problem areas".

As the seaweed aquaculture industry grows in the United States, the ablity to perform site-specific detection of biofouling species becomes more important. Our ability to link the presence of *L.vincta* eDNA with the presence of *L. vincta* snails year-round at aquaculture lease sites is promising. This study may serve as a model for other organisms, potentially leading to the development of a primer set for biofouling

organisms specific to aquaculture farms in the Gulf of Maine. We are hopeful that this research and potential future studies contribute to best management practices of *L. vincta* fouling for kelp farmers. eDNA monitoring at farms has the potential to inform farmers when to harvest biomass and where to situate farms early in the aquaculture leasing process.

CRediT authorship contribution statement

Lancaster Emily Rose: Writing – review & editing, Methodology, Conceptualization. **Byron Carrie J.:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Blaine Cara A:** Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Suskiewicz Thew:** Writing – review & editing, Resources, Conceptualization. **Frederich Markus:** Writing – review & editing, Methodology, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Cara A. Blaine reports financial support was provided by The University of Maine Maine Sea Grant. Carrie J. Byron reports financial support was provided by Aquaculture Research Institute. Thew Suskiewicz reports financial support was provided by Maine-eDNA. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Thank you to the supply team and partner farmers of Atlantic Sea Farms, who provided kelp seed for our Saco Bay farms, generously allowed us to do field work on their kelp farms and took the time to share tips and observations. Thank you to Liz MacDonald for ongoing support and identifying a need within the seaweed aquaculture industry. Special thanks to Emilly Schutt for project support, and to Anna Winkler, Sophia Tearman, Abigail Hayne, Aubrey Jane, and Melissa Butler for help on the boat and in the lab. Thank you to Tim Arienti and Chris Gregoricus for boating operations and Saco Bay farm support, and Lindsay Forrette and Michael Galloway for lab supplies and aquaria support. A large contribution to this project came from Geneva York at the University of Maine Environmental DNA Laboratory, who created and optimized our novel *Lacuna vincta* primer. Partial support for Blaine came from the College of Arts and Sciences at the University of New England. Partial funding for this research came from the Aquaculture Research Institute at the University of Maine. This work was in part conducted as a graduate fellowship supported by NOAA to Maine Sea Grant at the University of Maine (Award # NA22OAR4170121). Additional support came from National Science Foundation to Maine EPSCoR at the University of Maine (Award #OIA-1849227).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2025.102729.

Data availability

Data will be made available on request.

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