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Combining Environmental DNA Data With Oceanography, Life History and Ecology for Detecting Climate-Induced Range Shifts

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ABSTRACT

Aim: Tropicalisation and other climate-induced range shifts are rapidly restructuring global biodiversity patterns. The detection of range shifts is often complex and requires big-data approaches. Environmental DNA (eDNA) monitoring is emerging as a powerful method for assessing biodiversity changes at unprecedented spatial and temporal resolutions. While eDNA-based methodologies continue to evolve, the impacts of species traits and eDNA dynamics are rarely measured, though they likely affect our eDNA data interpretation. Here we combine diverse methodologies to better understand processes affecting eDNA data and to elucidate how eDNA dispersal influences the interpretation of eDNA results in a tropicalisation context.

Location: Baja California Peninsula, Mexico.

Methods: We combined semi-quantitative field surveys with eDNA sampling, quantitative PCR assays of different amplicon sizes, assessment of spawning period, and oceanographic modelling. We used as a model system the range-retracting, marine gastropod *Tegula gallina*, which we sampled across a region that is experiencing tropicalisation.

Results: We detected eDNA of *T. gallina* across both its current range (i.e., occupied region) and >250 km beyond the species' range limit (i.e., unoccupied regions). Shorter amplicons were detected more consistently than larger targeted fragments across the unoccupied regions. *Tegula gallina* was likely spawning at the time of eDNA collection, and oceanographic modelling revealed possible transport of eDNA (and early life-history stages) beyond the species' range limit.

Main Conclusions: Our study reveals that eDNA signals can be detected over substantial spatial scales, which can likely be explained by the interaction among spawning period, larval dispersal, and eDNA dispersal. The varying detection sensitivity of the different amplicon sizes may be due to eDNA decay during transport. Our results highlight the need for integrative approaches

combining eDNA detection, life-history traits, field surveys of living organisms, and modelling to uncover the full potential of eDNA data, especially for ecological and conservation applications.

1 | Introduction

The distribution of biodiversity around the world is currently shifting at an unprecedented rate due to anthropogenic impacts (Pecl et al. 2017). To understand such rapid change, big-data methods that detect species range shifts are increasingly needed (Zarzyczny, Rius, et al. 2024). DNA shed by species residing in a particular environment that can be detected with molecular techniques (e.g., molecular tools able to detect environmental DNA or eDNA; Deiner et al. 2017; Gaither et al. 2022) offers a potentially revolutionary approach to track range shifts. Due to the ease of eDNA sample acquisition (e.g., direct water filtration), eDNA studies are increasingly being used for species detection at different spatial scales (Eble et al. 2020; Gaither et al. 2022). Insights from eDNA studies are revolutionising a wide variety of research fields, including conservation biology (McInnes et al. 2017; Sahu et al. 2023), biogeography (DiBattista et al. 2022; Gaither et al. 2022), population genetics (Adams et al. 2019; Andres et al. 2023) and invasion science (Ricciardi et al. 2017). Studies on eDNA are thus shaping our understanding of rapid changes in biodiversity patterns.

Two main approaches are commonly applied to detect species using eDNA data: (1) multispecies detection via metabarcoding (Holman et al. 2019; Miya 2022; Zarcero et al. 2024) and (2) assessment of the presence of a species of interest through quantitative polymerase chain reaction (qPCR) (Hernandez et al. 2020). While metabarcoding efficiently detects numerous species simultaneously, qPCR often targets a single species and can be more sensitive to rare species (Harper et al. 2018; McColl-Gausden et al. 2023). Thus, species-specific qPCR assays are excellent candidate methods for monitoring range shifts of individual species (Zarzyczny, Rius, et al. 2024). However, inaccurate conclusions on range shifts could potentially arise from misinterpreting eDNA results, which could distort our understanding of biodiversity trends and misguide biodiversity conservation efforts.

Although research on eDNA properties has proliferated in recent years (e.g., Collins et al. 2018; Holman et al. 2022), little is known about how eDNA disperses and degrades in situ after being shed, and how this can be effectively measured in the field. Recent research suggests that larger eDNA amplicons may only provide accurate species detection at the local level, whereas smaller amplicons offer the possibility of detecting species over larger spatial scales (Eble et al. 2020). Consequently, research incorporating eDNA fragment size and other eDNA attributes is needed. In addition, oceanographic modelling (such as particle tracking simulations of eDNA) has the potential to enhance our understanding of how eDNA is transported away from source populations (Andruszkiewicz et al. 2019; Holman et al. 2024) and to provide new insights into species detection beyond their range limit.

Life-history traits of species, particularly those linked to early life-history stages, could also play a crucial role in shaping eDNA dispersal across aquatic ecosystems. Characteristics such as spawning period and frequency, as well as larval duration and dispersal potential, are likely to influence the spatial and temporal patterns of eDNA's presence (Crane et al. 2021; Garcia-Vazquez et al. 2021). As eDNA of planktonic larvae can be captured in eDNA surveys (Garcia-Vazquez et al. 2021), we might expect marine species with highly dispersive larvae to contribute to broader eDNA signals than species with limited dispersal capabilities. Our ability to rigorously test these relationships is often limited due to insufficient data on reproductive and larval dispersal traits (Wort et al. 2019; Zarzyczny, Hellberg, et al. 2024). As a result, understanding the interplay between life-history traits and eDNA patterns remains an important yet underexplored research area. A possible way to tackle these limitations could be to combine eDNA data with direct species identification during field surveys, life-history information, and predictive oceanographic modelling. This integrative approach has the potential to enhance the reliability of eDNA data for documenting range shifts.

Despite the increasing use of eDNA-based methods, the suitability of eDNA data for the detection of climate-induced range shifts and broader phenomena such as tropicalisation (Wernberg et al. 2013; Zarzyczny, Rius, et al. 2024) is yet to be assessed (but see Gold et al. 2023). Tropicalisation is a marine phenomenon arising from contemporary climate change (Vergés et al. 2016; Zarzyczny, Rius, et al. 2024) and is underpinned by poleward range expansions of tropical species and range retractions of temperate species (Wernberg et al. 2013; Zarzyczny, Rius, et al. 2024). Tropicalisation has broad-ranging ecological and evolutionary impacts (Zarzyczny, Rius, et al. 2024) that range from altered predator–prey interactions (Fenberg et al. 2023) to ecosystem phase shifts (Mezaki 2012; Vergés et al. 2014) and altered population genetics (Coleman et al. 2020; Zarzyczny, Hellberg, et al. 2024). Moreover, tropicalisation is reshaping latitudinal biodiversity patterns (Zarzyczny, Rius, et al. 2024), with economic impacts such as alteration of fishery catches (Cheung et al. 2013). Surveys based on eDNA detection have the potential to generate a vast amount of information on range shifts, which could significantly advance our understanding of the consequences of tropicalisation.

Here we use a multidisciplinary approach to understand how eDNA dispersal, oceanography, and early life-history traits affect our interpretation of eDNA data in the context of climate-induced range shifts. To do this, we first conducted field surveys (via both in situ species detection and eDNA collection) to detect the range-retracting intertidal gastropod *Tegula gallina*, a species with a well-known historical and contemporary distribution across a tropicalisation hotspot (Zarzyczny, Hellberg, et al. 2024). Subsequently, we ran laboratory tests with qPCR assays targeting two different eDNA amplicon sizes. We also assessed the gonadal ripeness of adults to determine the species' spawning period and to better understand potential eDNA sources. Finally, we conducted oceanographic particle modelling to unveil the role of ocean currents on eDNA dispersal and to unravel any mismatch between the distribution patterns of eDNA and the contemporary species range.

2 | Methodology

2.1 | Study Species and Geographic Distribution

Tegula gallina is an intertidal grazing gastropod, occurring on rocky shores of the north-eastern Pacific coast (Zarzyczny, Hellberg, et al. 2024). Its northern range limit is Point Conception, California (34.4° N), and its southern range limit has undergone a recent (between 1996 and 2022) poleward retraction from Punta Márquez, Baja California Sur (BCS) (24.0° N) to Bahía Magdalena, BCS (24.6° N) (Figure 1A). The historical and modern distributions of this species have been reliably documented, given the substantial investigation of the *Tegula* genus in this region (e.g., Hellberg 1998; Hellberg et al. 2012; Zarzyczny, Hellberg, et al. 2024). Across the species' range, wherever rocky shore habitat is available at the tideline, *T. gallina* creates large aggregates (Zarzyczny, Hellberg, et al. 2024), making its detection through exhaustive surveys relatively easy (Figure 2A,B). High confidence in this species' distribution makes it an ideal candidate for assessing the reliability of species-specific eDNA surveys.

2.2 | Semi-Quantitative Field Surveys of *Tegula gallina*

We conducted all field surveys at low, spring tides between December 2021 and January 2022 (Table S1). We carried out 2-h exhaustive surveys across eight sites along the Baja California Peninsula (Figure 1B). We utilised the SACFOR scale (S = Superabundant, A = Abundant, C = Common, F = Frequent, O = Occasional, R = Rare, and not observed) to obtain semi-quantitative abundances for each surveyed site [see Hiscock 1996 for scale definitions]. We also recorded the abundances of other *Tegula* species using the same method.

2.3 | Sampling of Environmental DNA

We collected seawater samples immediately after conducting the above-mentioned field surveys (Figure 2C). At each site, we randomly selected four sampling points within rockpools and four sampling points at the shoreline away from rockpools. At each sampling point, we collected 50 mL of surface seawater (< 10 cm below the surface) using a sterile 60 mL Luer lock syringe and filtered the water through a 0.22 μm Sterivex filter (Merck Millipore, Massachusetts, USA). This resulted in a total of 400 mL of seawater being filtered through the Sterivex filter (200 mL from rock pools and 200 mL from the shoreline). We repeated this process until we obtained three filter replicates for each site, using new consumables each time. The filters were immediately preserved with 2 mL of ATL lysis buffer (Qiagen, Germany) and were individually stored in sterile plastic bags. All filters were stored inside a box out of direct sunlight until DNA extraction.

In addition to collecting the eDNA samples at each site, we also obtained field'blan' controls (Appendix S1) at sites south of the modern range of *T. gallina*, along the outer Baja California Peninsula (PMZ, CP, and PDC; Figure 1). To do this, we followed

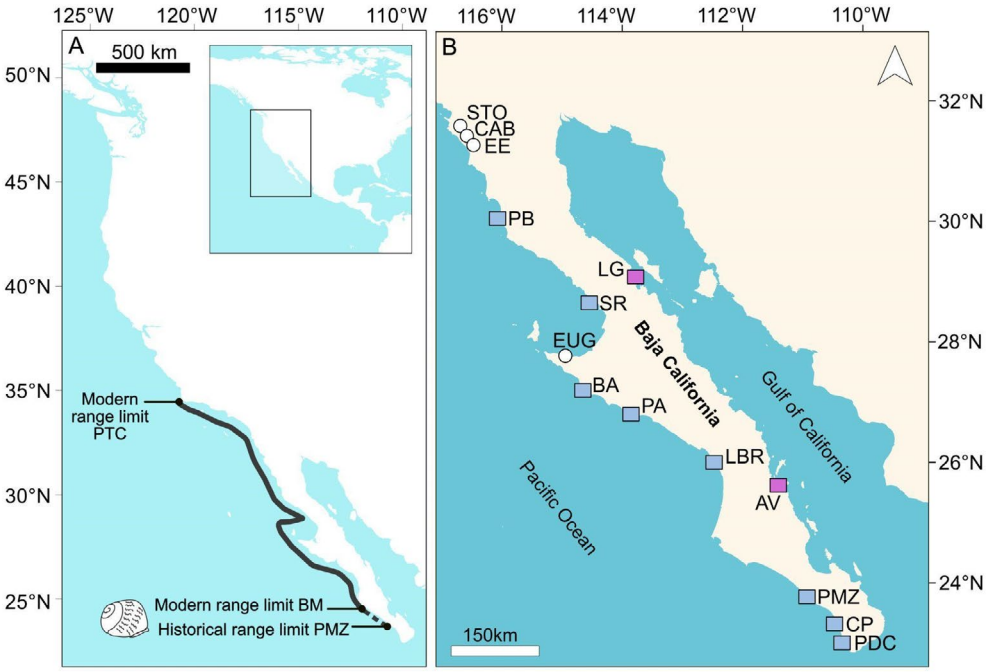


FIGURE 1 | (A) The range extent of the study species *Tegula gallina*, indicating the modern distribution from Point Conception (PTC), California to Bahía Magdalena (BM), Baja California Sur (Zarzyczny, Hellberg, et al. 2024) and the past southern range limit, Punta Marquez (PMZ). (B) The sampling sites are marked with a green rectangle: BA = Bahía Asunción, CP = Cerritos Point, LBR = Las Barrancas, PA = Punta Abreojos, PB = Punta Baja, PDC = Pozo de Cota, PMZ = Punta Marquez, SR = Santa Rosalillita. Pink rectangles mark two negative control sites from the Gulf of California: AV = Agua Verde, LG = La Gringa, which fall out far outside of past and present range extent of *T. gallina*. White circles represent additional sites where samples of *T. gallina* were collected for reproductive condition analysis: CAB = Punta Cabras, EE = Ejido Erendira, EUG = Punta Eugenia, STO = Punta Santo Tomas.

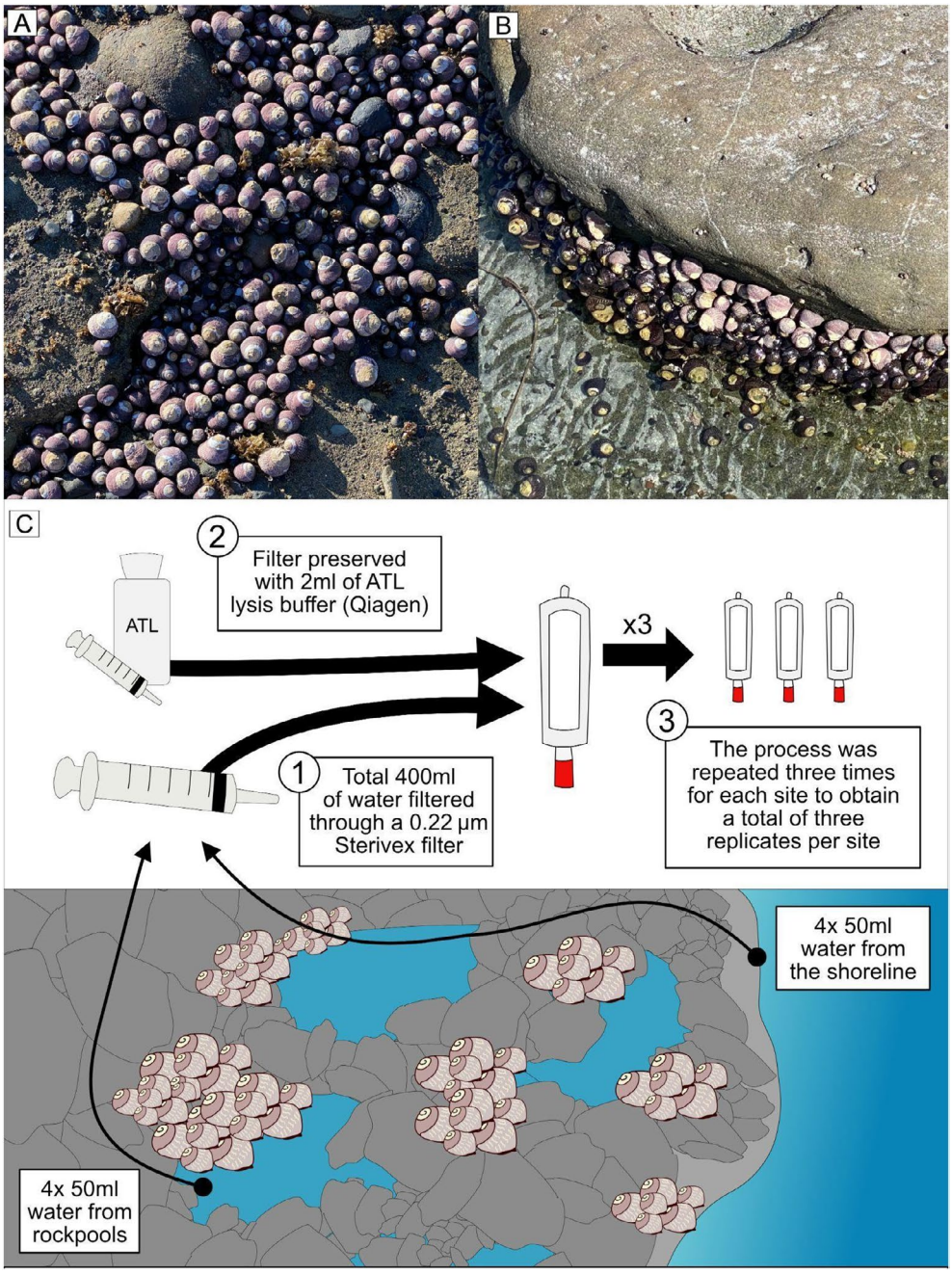


FIGURE 2 | (A, B) Large aggregates of *Tegula gallina* in the high intertidal of the Eastern Pacific coastline; (C) A graphical summary of the eDNA sampling method used: 200 mL of rockpool and 200 mL of shoreline seawater were filtered through a Sterivex filter to obtain a single eDNA sample. This process was repeated three times per study site.

the above-mentioned sampling procedure, albeit instead of filtering sampled water through the Sterivex filter, we used the syringe to push air through. We then preserved the filter with an ATL buffer following the same procedure as with the regular eDNA samples.

To detect the extent of *T. gallina* species range and to account for possible non-target amplifications by our primers (i.e., negative controls), we sampled eDNA from two sites outside both modern and historic ranges of *T. gallina* (in the Gulf of California; Agua Verde and La Gringa; Figure 1) but within the range of its congeners (*T. corteziana* and *T. rugosa*). We also used a previously collected eDNA sample from Sutton Harbour, Plymouth, United Kingdom, where no species in the genus are found (Appendix S1).

2.4 | eDNA Extraction

We performed all eDNA extractions in a PCR-free room. The laboratory was thoroughly cleaned using 10% bleach prior to eDNA extraction, and no other work was permitted in the laboratory during the entire process.

We added 80 µL of Proteinase K to each filter and digested samples overnight at 56°C in a rotating incubator. Following

digestion, we continued with the DNA extraction using the DNeasy Blood and Tissue Spin Column Kit (Qiagen, Germany) as per the manufacturer instructions. Next, we purified the extracted eDNA using OneStep PCR Inhibitor Removal Kit (Zymo Research Corp., USA). We also obtained extraction'blank' by processing a blank sample containing 180 μL of ATL buffer and 20 μL of Proteinase K to test for any contamination during the extraction process (Appendix S1). Finally, using an Invitrogen Qubit 4 Fluorometer, we checked the DNA concentration of all eDNA samples to ensure successful DNA extraction and extraction 'blank' to test for contamination (Table S2).

2.5 | qPCR Assays and Sequencing

We designed *T. gallina* specific primers manually with the aid of Benchling (https://benchling.com/) and Primer Blast (Ye et al. 2012). All primers were developed according to best-practice primer-design guidelines (Robidart et al. 2012; Appendix S2). This process led to a final selection of one forward primer, F-TGCO1-101 (5′ CCAGGAGCATTATTAGGAGACGATCAACT 3′), and two reverse primers, R-TGCO1-194 (5′ GCCATATC AGGTGCTCCTAACATAAGTG 3′) and R-TGCO1-295 (5′ CCA GTTCCTGCCCCTCTTTCAAC 3′). These primer pairs amplified 149 bp and 245 bp size fragments of the COI gene, respectively.

We prepared all qPCR assays in a clean laboratory where no cultures, tissue, PCR products or any equipment which has been in contact with high-concentration DNA was permitted. All consumables and pipettes were UV-treated for 30 min prior to laboratory work, and all reactions were prepared in a UV-cabinet. We added the standards to the reaction plates in a separate laboratory immediately before the qPCR run to reduce the risk of contamination. Standard curves were used to assess the efficiency and sensitivity of the qPCR reaction (Töwe et al. 2010). Given that we developed two sets of qPCR assays, we created two sets of standards, one for each assay, using a method modified from Robidart et al. (2012) (Appendix S3).

We optimised the qPCR assays independently for the two eDNA targets. For both 149 bp and 245 bp fragments (Table S3), we set up 25 μL reactions in 98-well PCR plates (Roche Life Science). Each reaction contained x1 iQ SYBR Green Supermix (Bio Rad), 0.4 μM of forward and reverse primers, 0.1 μg/μl of Bovine Serum Albumin (BSA) (ThermoFisher Scientific) and 1 μL of eDNA sample. We added BSA to reduce the impact of PCR inhibitors.

Samples were run in triplicate to provide technical replication. For each reaction, we ran qPCR standards and negative reaction controls containing 1 μL of PCR-grade water to account for any PCR reaction contamination and background amplification. At the end of each reaction, we conducted a high-resolution melting analysis to test for the presence of non-target amplicons and background amplification. We compared the high-resolution melting curves to the expected modelled curves by uMelt v.3.6.2 Quartz (Dwight et al. 2011). To confirm our assays targeted *T. gallina*, we amplified and sequenced a subset of positive amplicons using Taq Polymerase and Sanger sequencing (Eurofins Genomics; Table S4). We conducted all qPCR reactions using the Roche LightCycler 96 (Roche Life Science).

We determined the limit of detection (LOD) and the limit of quantification (LOQ) individually for each plate run to account for interplate variation (Ruijter et al. 2015). We defined the LOD as the cycle of quantification (Cq) of any negative control included on the plate. When no background amplification occurred, we considered every positive reaction to be positive, as long as high-resolution melting demonstrated amplification of the target fragment. Where only one of three technical replicates produced a positive result, we repeated the reaction in triplicate. If the second reaction produced three negative results, we considered the sample as negative. However, if at least one of the technical replicates produced another positive result, we considered the sample as positive. The LOQ refers to the standard concentration that is accurately and reliably quantified in triplicate across all standard curves (Klymus et al. 2020). We determined the LOQ based on the amplification kinetics for each individual assay.

2.6 | Reproductive Condition

We assessed the gonadal maturity of *T. gallina* from samples collected between 1992 and 2004 to determine the spawning season of *T. gallina*. Whole snails were collected from five sites ranging from Ejido Erendira (31.26° N) to Punta Eugenia (27.85° N; Figure 1B; Table S1). Shells were cracked to remove the visceral mass and allow for gonadal inspection. The gonads were qualitatively assessed and categorised into reproductive conditions as either 1—Absent (no visible gonad tissue); 2—Indeterminate (possible signs of gonad development but insufficient for sampling or sex determination); 3—Developing (definite gonad tissue present, and sex could be determined, though the gonad was modest in size and not easily separated from digestive gland); 4—Ripe (gonads were mature and abundant, clearly distinguishable from digestive gland and easy to sample).

2.7 | Oceanographic Modelling

To assess the most likely origin of the detected eDNA fragments and the extent of ocean current-mediated connectivity along the Baja California peninsula, we conducted particle tracking simulations using TRACMASS (Döös et al. 2013) in the operational Mercator global ocean analysis and forecast system model (Global Ocean Physics Reanalysis 2023). Such particle tracking analyses enabled us to test whether eDNA could be transported to sites where *T. gallina* is absent.

The Mercator model is based largely on the current real-time global forecasting CMEMS system, which incorporates the NEMO ocean model (Madec et al. 2023) driven at the surface by ERA5 re-analyses (Hersbach et al. 2020). Observations are assimilated, including satellite sea surface temperature, in situ temperature, and salinity vertical profiles. The Mercator model output used includes temperature, salinity, currents, sea level, and mixed layer depth on a standard regular grid at 1/12° (approximately 8 km) horizontal resolution and on 50 standard depth levels, at 6-hourly temporal resolution.

For the three southernmost sampling sites on the outer peninsula (Figure 1B), where *T. gallina* is absent (Zarzyczny, Hellberg, et al. 2024), we ran site particle tracking backwards for 35 days using seven separate particle releases, one every 6 h, covering the 48-h period around the date of sampling. In addition, we ran particle tracking forwards from Bahia Magdalena (southern range limit of this species; Zarzyczny, Hellberg, et al. 2024) from 9th of December 2021 for 53 days to account for potential effects of spawning and/or larval dispersal, as well as eDNA dispersal during the sampling period (December 2021–January 2022), using the same model parameters. For each tracking experiment, particles were distributed evenly over the model grid square closest to the sampling site up to 10 m in depth, with each particle representing $10^4 m^3$ of water, resulting in a total of ~600,000 particles for each experiment.

3 | Results

3.1 | Field and eDNA Detection of *Tegula gallina*

Tegula gallina was common, abundant, or superabundant at all sites sampled across its modern range (26.0° N—29.9° N); while it was not detected using field surveys at sites further south along the outer peninsula (Table 1). At the sites where we observed *T. gallina*, we also detected both eDNA target amplicons in all three field replicates (Table 1). Using a subset of eDNA samples and Sanger sequencing, we verified that these amplicons came from *T. gallina* (Table S4). We detected no contamination in any of the field or extraction'blank', and recorded no non-target amplification in our control samples from Agua Verde, La Gringa, or Sutton Harbour (Appendix S1).

For the standard curve analysis, we excluded the 10^0 dilution and used the 10^1 dilution as the LOQ (Table S5), as dilutions from 10^1 to 10^7 exhibited consistent and linear amplification, providing a dynamic range for quantification. At sites where *T. gallina* was "abundant" or "superabundant", we were able to consistently quantify the gene copy number present in the sample using the larger 245 bp amplicon target (Table 2). Although we detected *T. gallina* eDNA at Las Barrancas (Table 1) where this species is "common", we were not able to quantify the gene copy number present within the sample (Table 2).

Quantification of the gene copy number using the smaller 149 bp amplicon target was less reliable. Using the smaller 149 bp amplicon fragment, we were only able to consistently quantify gene copy number for Punta Abreojos, where *T. gallina* was superabundant, as the gene copy number for all other sites was below the LOQ (Table 2).

Notably, our qPCR assays consistently detected eDNA of *Tegula gallina* over 250 km south of the nearest known source population (Figure 1; Table 1). Specifically, we detected (albeit could not reliably quantify; Table 2) the larger eDNA fragment (245 bp) beyond the species' range limit at Punta Marquez (2/3 samples), Cerritos Point (1/3 samples), and Pozo de Cota (2/3 samples). However, we were only able to confirm the sequence identity as *T. gallina* for Punta Marquez. We were not able to obtain any amplicons using Taq polymerase for the 245 bp fragment for Cerritos Point or Pozo de Cota, and therefore, we did not obtain any sequences for those samples. For the shorter amplicon (149 bp), while the data were not quantitative, we were able to detect the shorter eDNA fragment at all surveyed southern sites, and confirmed the sequence identity as *T. gallina* for all positive samples (Tables 1 and 2; Table S4). Specifically, we detected the shorter fragment at Punta Marquez (3/3 samples), Cerritos Point (2/3 samples), and Pozo de Cota (3/3 samples).

3.2 | Reproductive Condition

We assessed the gonad condition of 62 individuals collected from five sites (Table 3) to better understand the reproductive cycle of *T. gallina*. Ripe individuals were found during the cooler months (April and November). Meanwhile, in June, all individuals had either no gonads visible, or had indeterminate or developing gonads. The findings of the gonadal assessment reveal that *T. gallina* likely spawns in the cooler months in the southern portion of the range, in contrast to the northern populations found in California, which spawn in the summer months (Paine 1971; Moran 1997; Sato 2001). Consequently, we can consider the possibility that *T. gallina* was spawning during our eDNA sample collection (December and January).

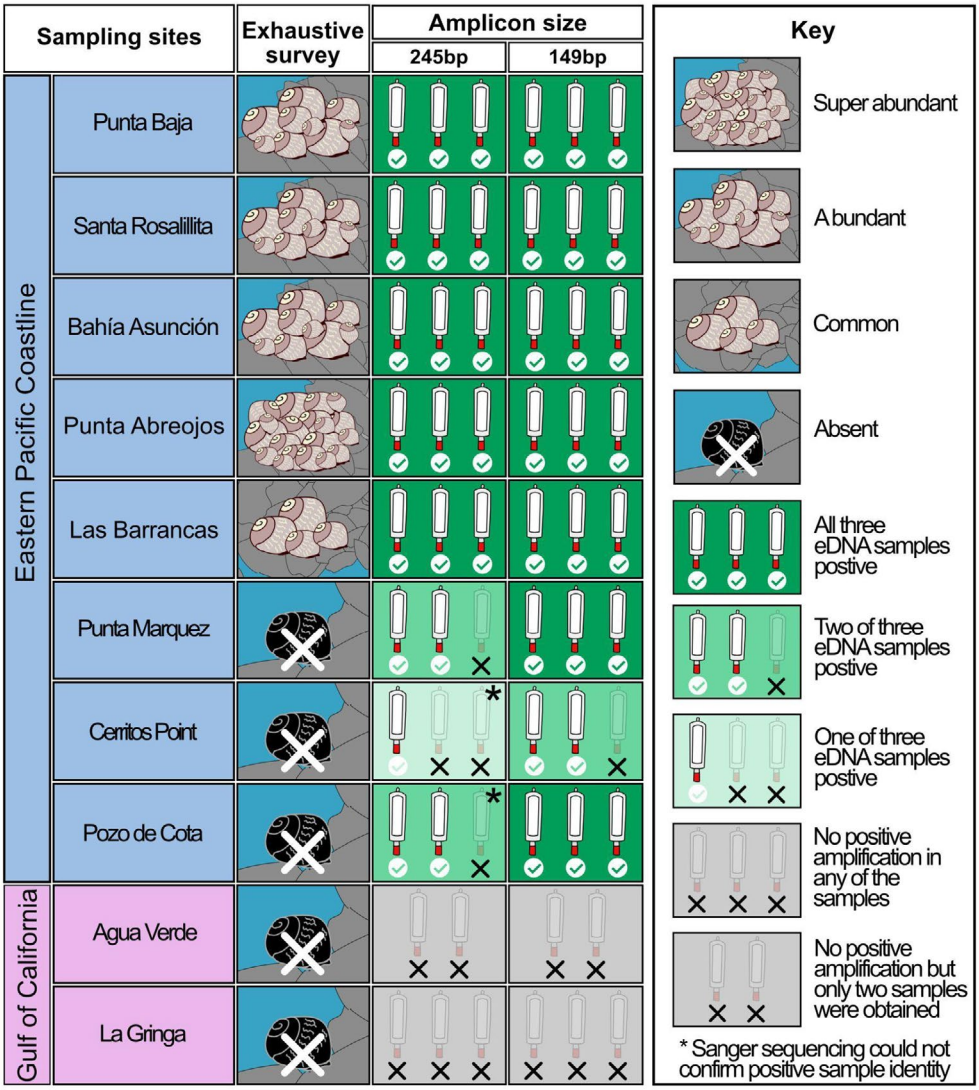
3.3 | Oceanographic Modelling

We performed particle tracking simulations to determine whether water (and eDNA or larvae borne within it) could reach sites beyond the range limit of *T. gallina* (i.e., Punta Marquez, Cerritos Point and Pozo de Cota, Figure 1). As expected, none of the four particle tracking simulations identify water (or eDNA particles) connectivity from the two (control) Gulf sites (Agua Verde or La Gringa), which fall well beyond (~864 km and ~1632 km of coastline, respectively) the range of *T. gallina*, and any of the Eastern Pacific sites within the modelled time period. This result is supported by our eDNA survey results, which exhibit no detection of *T. gallina* DNA in any of the Gulf samples.

Backward particle tracking revealed that most of the water reaching Punta Marquez, Cerritos Point, and Pozo de Cota within 35 days originated from south of each site and from offshore (Figure 3A–C). However, some eDNA transport may be possible from just south of Bahia Magdalena (current southern range limit) to Punta Marquez (historical southern range limit and closest surveyed site to current range limit; Figure 3A). Backward tracking for the two southernmost sites, Cerritos Point and Pozo de Cota, revealed that no eDNA particles could have been transported from occupied sites within 35 days.

Given that Bahia Magdalena is the nearest source population to the southern unoccupied sites, we ran a forward tracing particle model to test whether any particles from this area could reach the southern sites, and within what time frame. The simulation revealed that water (and suspended eDNA) carried from Bahía Magdalena could reach Punta Marquez, Cerritos Point, and Pozo de Cota in 42.25, 47.50, and 47.75 days respectively (Figure 4).

TABLE 1 | Detection of *Tegula gallina* using an exhaustive survey, and eDNA surveys targeting two different sizes of partial COI gene fragments (149 and 245 base pairs).



Note: The sampling sites appear from north to south along the Pacific coast of Baja California (from top to bottom) except the last two, which are from the Gulf of California (Figure 1). Exhaustive surveys were carried out using the semi-quantitative SACFOR scale (Superabundant, Abundant, Common, Frequent, Occasional, Rare). A sample was considered as positive if at least two technical qPCR replicates returned a positive result. Note that three eDNA samples were obtained for all sites, apart from Agua Verde, where the third sample was excluded from analysis due to unsuccessful eDNA extraction. The asterisk (*) refers to samples which returned a positive result using qPCR, but Sanger sequencing could not confirm amplicon identity.

4 | Discussion

Our integrative approach combining field surveys, oceanographic modelling, reproductive biology data, and eDNA analysis provides valuable insights into interpreting eDNA signals for range-shifting species. We reveal that eDNA can be detected over large spatial scales (> 250 km), which can likely be explained by an interaction between spawning period, larval dispersal, and subsequent eDNA dispersal. Our findings highlight the importance of understanding the early life-history stages of the studied species, especially when interpreting range shifts using eDNA data. Moreover, we stress the need to integrate multiple data sources to correctly interpret eDNA data, particularly regarding eDNA's spatial resolution and the influence of biological and physical processes in shaping eDNA distribution.

4.1 | Interpreting eDNA Detections Beyond Established Range Limits

Whilst we reliably detected our target species' presence from eDNA samples across the confirmed species range, our eDNA data also showed detection of *T. gallina* over 250 km south (Pozo de Cota) from the nearest possible source population (Bahia Magdalena). Had this eDNA detection pattern been encountered without prior knowledge of the species' range, this result could have led to an incorrect interpretation of the species distribution, with potential conclusions of recent population expansion or colonisation. Whilst we must consider the possibility that a more southern population of *T. gallina* exists, which was not captured in our field surveys, we expect this to be highly unlikely. Punta Marquez (Figure 1; 23.95° N) was the historical southern range limit for this

TABLE 2 | The estimated gene copy number of the two fragment sizes in each sample based on the standard curve for each plate.

			149 bp Fragment				245 bp Fragment			
			LOQ		Sample quantification		LOQ		Sample quantification	
Sample	Site	Exhaustive survey	Gene copy	Error	Gene copy	Error	Gene copy	Error	Gene copy	Error
PB1	Punta Baja	Abundant	12.11	2.63	216.60	17.22	12.78	4.99	160.80	6.29
PB2			32.13	33.93	Below LOQ		20.70	4.40	21.22	5.14
PB3			33.36	29.83	Below LOQ		20.70	4.40	37.96	16.44
SR1	Santa Rosalillita	Abundant	33.36	29.83	174.00	19.91	20.70	4.40	180.00	23.52
SR2			32.13	33.93	Below LOQ		20.70	4.40	27.17	9.44
SR3			12.11	2.63	114.40	7.40	12.78	4.99	123.80	25.25
BA1	Bahía Asunción	Abundant	12.11	2.63	19.09	4.80	12.78	4.99	14.64	5.70
BA2			33.36	29.83	Below LOQ		20.70	4.40	56.71	7.25
BA3			32.13	33.93	Below LOQ		20.70	4.40	45.71	9.37
PA1	Punta Abreojos	Super Abundant	12.11	2.63	148.50	20.74	12.78	4.99	124.50	23.58
PA2			32.13	33.93	339.70	5.74	20.70	4.40	709.70	59.24
PA3			33.36	29.83	314.40	85.63	20.70	4.40	315.10	88.10
LBR1	Las Barrancas	Common	13.28	4.26	Below LOQ		20.70	4.40	Below LOQ	
LBR2			12.11	2.63	Below LOQ		12.78	4.99	13.80	6.59
LBR3			32.13	33.93	Below LOQ		20.70	4.40	22.70	5.73
PMZ1	Punta Marquez	Not Detected	12.11	2.63	Below LOQ		12.78	4.99	16.31	4.07
PMZ2			33.36	29.83	27.28	18.86	13.13	7.07	Below LOQ	
PMZ3			32.13	33.93	Below LOQ				Negative	
CP1	Cerritos Point	Not Detected			Negative		12.78	4.99	Below LOQ	
CP2			32.13	33.93	Below LOQ				Negative	
CP3					Negative				Negative	
PDC1	Pozo de Cota	Not Detected			Negative		20.70	4.40	Below LOQ	
PDC2			12.11	2.63	Below LOQ				Negative	
PDC3			32.13	33.93	Below LOQ		13.13	7.07	Below LOQ	

Note: The Limit of Quantification (LOQ) was estimated based on the lowest quantifiable standard dilution of 10^1.

species (Zarzyczny, Hellberg, et al. 2024). In 1996, the site was surveyed by M. E. Hellberg, who observed only large individuals and no recruits of *T. gallina* (Anonymised, unpublished data). Since 1996, we have surveyed Punta Marquez in 2017, 2022 (Zarzyczny, Hellberg, et al. 2024; this study) and 2024 (Anonymised, unpublished data), and have not detected any *T. gallina* during exhaustive surveys (indicating range retraction of this species). As the rocky shore habitat is largely absent or fragmented between Bahia Magdalena and Punta Marquez, with the coastline between the rocky shore sites dominated by sandy beaches (Fenberg and Rivadeneira 2019), it is unlikely that an unsampled *T. gallina* population could be present. A small rocky shore is present at Punta Conejo (24.05° N), just north of Punta Marquez (~19 km), but *T. gallina* were not detected there in 2018 (Zarzyczny, Hellberg, et al. 2024). Consequently, our combined approach highlights the importance of integrating eDNA data with other robust data sources to correctly interpret eDNA results.

Transport of eDNA particles beyond live organisms' range has been documented in other marine taxa. For example, using qPCR, Andruszkiewicz et al. (2019) detected Northern anchovy eDNA that, based on particle modelling, likely originated up to 40 km away from their sampling location. Meanwhile, using digital droplet PCR (ddPCR), Kutti et al. (2020) detected eDNA of the cold water coral *Lophelia pertusa* at all ten of their surveyed reefs, when in fact, in field studies, *L. pertusa* was only observed in surveys at five of those reefs. The authors were able to demonstrate using particle modelling that eDNA could be transported to unoccupied reefs ~28 km away from the source, over a 2-week

TABLE 3 | Reproductive assessment of *Tegula gallina* individuals.

Sampling site	Collection date	Reproductive condition	
Punta Santo Tomas	April 1994	Absent	0/13
		Indeterminate	0/13
		Developing	0/13
		Ripe	**13/13**
Punta Cabras	April 1994	Absent	0/8
		Indeterminate	0/8
		Developing	0/8
		Ripe	**8/8**
Ejido Erendira	June 1992	**Absent**	**3/4**
		Indeterminate	**1/4**
		Developing	0/4
		Ripe	0/4
Punta Baja	June 1992	**Absent**	**8/23**
		Indeterminate	**4/23**
		Developing	**11/23**
		Ripe	0/23
Punta Eugenia	November 2004	Absent	0/13
		Indeterminate	0/13
		Developing	**3/13**
		Ripe	**10/13**

Note: Reproductive condition was classified as: Absent (no visible gonad tissue); Indeterminate (possible signs of gonad development but insufficient for sampling or sex determination); Developing (definite gonad tissue present, and sex can be determined, though the gonad is modest in size and not easily separated from digestive gland); or Ripe (gonads are mature and abundant, clearly distinguishable from digestive gland and easy to sample). Fractions refer to the number of individuals of a specific reproductive condition, out of the total number of individuals collected for each site. Detected reproductive conditions are indicated in **bold** for each sampling site.

period (Kutti et al. 2020), explaining the presence of eDNA at other sites.

Our oceanographic particle modelling revealed that theoretically, eDNA from *T. gallina* shed at Bahia Magdalena could reach all southern sites within ~42–47 days—a time frame which largely exceeds the expected persistence of eDNA in marine waters, which typically range from hours to a few days (Collins et al. 2018; Holman et al. 2022; Weltz et al. 2017), and over a distance much greater than what was observed by Andruszkiewicz et al. (2019) or Kutti et al. (2020). This temporal and spatial mismatch strongly suggests that the eDNA of *T. gallina* detected over 250 km south of the southernmost population is unlikely to be from local shedding, but rather reflects transient dispersal of other forms of genetic material—potentially from planktonic larvae (Garcia-Vazquez et al. 2021).

4.2 | Transient Dispersal of Genetic Material

Our gonadal assessment revealed that *T. gallina* populations in Baja California likely spawn during the cooler months—contrasting with earlier studies of Californian populations, which spawn in the summer (Paine 1971; Moran 1997; Sato 2001). This latitudinal difference in reproductive timing may reflect local environmental cues such as temperature and photoperiod, which are known to affect the spawning of marine invertebrates (Boolootian 1964; Lawrence and Soame 2004).

Research on spawning and larval duration of rocky shore invertebrates from Baja California is limited, with much of the life histories being inferred from studies on congeneric species (Fenberg and Rivadeneira 2019; Zarzyczny, Hellberg, et al. 2024). While the larval duration of *T. gallina* is unknown, like its sister species *T. funebralis, T. gallina* is expected to be a broadcast spawner (Paine 1971; Moran 1997; Sato 2001). Moreover, population genetic analyses of *T. gallina* by Zarzyczny, Hellberg, et al. 2024) revealed high genetic connectivity among sites across Baja California. In contrast, other rocky shore species inhabiting the same coastline, such as *Lottia conus* and *L. strigatella*—with an expected planktonic larval duration of 5–14 days—show clear phylogeographic structure (Zarzyczny, Hellberg, et al. 2024). Although such genetic breaks may arise from multiple factors, they are broadly consistent with more limited gene flow (Palumbi 1994; Hellberg 2009). The absence of similar genetic structure in *T. gallina* suggests greater connectivity between the sites, which could reflect a higher capacity for gamete or larval dispersal. Furthermore, considering that gametes and planktonic larvae are influenced by water advection, their dispersal likely mirrors the eDNA particle modelling observed in this study. Taking this evidence together, it is plausible that larvae or gametes released from the known range could be transported southward by coastal currents (as demonstrated by the oceanographic modelling), shedding eDNA along the way. This process offers a biologically plausible explanation for the eDNA detected beyond the observed adult range, consistent with transient dispersal rather than local establishment.

Whilst the presence of eDNA shed by larvae (or DNA directly from larvae or gametes) during sampling is plausible, uncertainty remains due to limited knowledge about larval duration and dispersal ecology (Zarzyczny, Hellberg, et al. 2024). This underscores the need for caution when interpreting eDNA detections beyond known ranges, particularly for species with poorly understood life histories. Where possible, integrating biological and phenological information into eDNA studies can help prevent misinterpretation of presence signals and improve the reliability of biodiversity monitoring in marine ecosystems.

4.3 | The Role of eDNA Fragment Size in Detection Sensitivity and Spatial Resolution

To our knowledge, our study is the first to compare the detection of different eDNA fragment sizes across a range shift gradient. Our findings demonstrate that target amplicon size can influence eDNA results, with smaller target amplicons being more consistently detected at greater distances from the eDNA source. While both 145 bp and 245 bp fragments were detected using qPCR across the outer coast of Baja California, the larger amplicon was only amplified in a subset of samples and failed to yield sequence data from samples at the southernmost sites. These results align with prior studies showing that larger DNA fragments degrade more rapidly than smaller ones (Jo et al. 2017), and thus are less likely to persist over long distances or extended time periods (Collins et al. 2018; Holman et al. 2022).

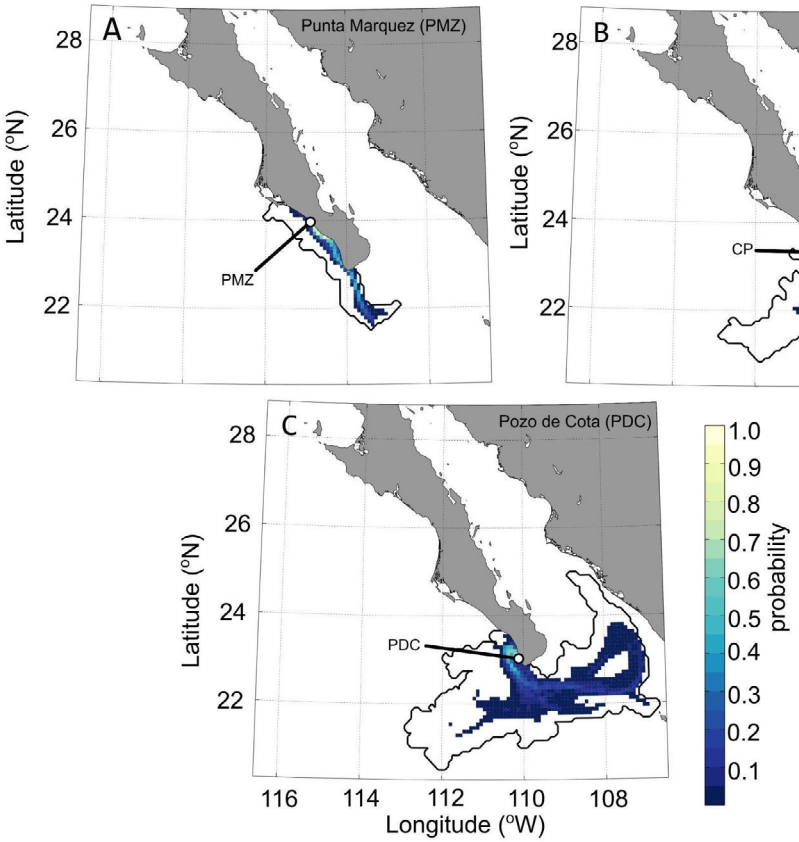


FIGURE 3 | Normalised particle density maps for particle tracing simulations for (A) Punta Marquez (PMZ), (B) Cerritos Point (CP), and (C) Pozo de Cota (PDC), 35 days backwards in time. The colour scale depicts the probability of source sites of the water. The probability was calculated as the number of unique particles recorded in a grid square during the tracing experiment divided by the total number of particles released. The black contour depicts 0% probability, and is the limit of potential particle dispersal within the specified time period. Particle tracking simulations were conducted using TRACMASS (Döös et al. 2013) with ocean data taken from the CMEMS global ocean eddy-resolving reanalysis model GLORYS12V1 (Global Ocean Physics Reanalysis 2023).

While eDNA persistence beyond 58 days has been reported in cold water systems (Strickler et al. 2015), such longevity is unlikely under the conditions present in our study region. The relatively warm, subtropical waters, high UV radiation, and high wave exposure along the southern Baja California coast suggest that relatively rapid eDNA degradation is to be expected (Collins et al. 2018; Joseph et al. 2022; Ottoni et al. 2017; Strickler et al. 2015). Consequently, the detection of *T. gallina* eDNA at sites far beyond the adult distribution is more plausibly explained by the transient dispersal of eDNA from recently released biological material, such as gametes or larvae, rather than the passive presence of shed DNA from adults. This interpretation is consistent with our earlier findings that reproductive activity is likely occurring during the winter months when eDNA sampling took place, and with dispersal modelling suggesting passive particles could travel southwards over 42–48 days. It is therefore reasonable to hypothesise that if we sampled eDNA from seawater collected in the summer (i.e., outside of the spawning period), we would not detect eDNA of *T. gallina* at the southernmost sites.

As DNA degrades, the DNA molecules are broken down into smaller fragments (Ottoni et al. 2017; Swango et al. 2006). Large, intact fragments are more likely to be present closer to the source population (i.e., eDNA which has been shed most recently), while smaller, degraded fragments may persist farther from the source. Consequently, detection of the longer 245 bp fragment may offer more spatially specific information on the species distribution and perhaps even species abundance, as eDNA gene copy was more consistently quantifiable. Meanwhile, shorter fragments offer greater detection sensitivity, which may be beneficial when the assays are targeted at the detection of rare species rather than determining species ranges.

Studies that compare the effectiveness of different eDNA applications frequently find the method that targets smaller genetic markers to be more sensitive to eDNA detection. For instance, Wood et al. (2019) found that qPCR and droplet digital PCR (ddPCR) targeting a 90 bp region of the COI marker gene of *Sabella spallanzanii* yielded more detections than longer amplicons (~300 bp region of COI and ~400 bp region of the 18S rRNA marker gene) used in metabarcoding. Similarly, McColl-Gausden et al. (2023) found qPCR to be more sensitive to eDNA detection when targeting a 57 bp fragment of the mitochondrial control region than metabarcoding targeting a ~140 bp region of the mitochondrial 12S rRNA gene. These findings, together with ours, suggest that fragment size influences not only detection probability but also the spatial and ecological interpretation of eDNA data.

This observation also points to the challenges of accurate interpretation of eDNA results, especially without prior knowledge

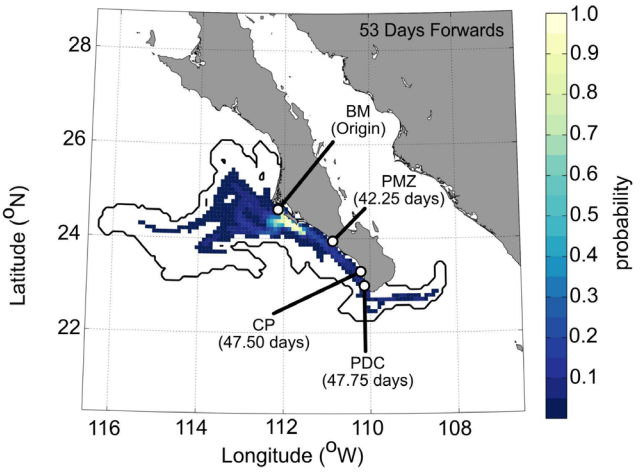


FIGURE 4 | Normalised particle density maps for particle tracing simulations for 53 days forwards in time. The colour scale depicts the probability of finding water from the release site (BM) at remaining sampling sites (PMZ, CP, and PDC) where BM = Bahia Magdalena, CP = Cerritos Point, PDC = Pozo de Cota, PMZ = Punta Marquez. The numbers in brackets refer to the number of days it took for water to travel from the release site (BM), to the three sampling sites. The black contour depicts 0% probability, and is the limit of potential particle dispersal within the specified time period. Particle tracking simulations were conducted using TRACMASS (Döös et al. 2013) with ocean data taken from the CMEMS global ocean eddy-resolving reanalysis model GLORYS12V1 (Global Ocean Physics Reanalysis 2023).

of species distribution. If we consider a species as present only if the qPCR result is positive in all three field samples in our study, detection of the larger amplicon better reflects the actual southern range limit of *T. gallina*. However, if we consider a species as present if the qPCR result is positive for one or two field samples, we would conclude that the species range extends beyond the actual recorded southern limit of *T. gallina*. While these results may not apply universally, they suggest that when using eDNA to understand the current geographic ranges of intertidal *T. gallina*, triplicate detection of the larger amplicon is required.

Taken together, our results highlight two important considerations. First, the eDNA target fragment size significantly influences both the sensitivity and spatial resolution of eDNA detection. Secondly, sequencing remains essential for verifying qPCR results, especially when detections occur at the edges of known species ranges. As eDNA continues to be adopted for biodiversity monitoring and range shift assessment, advancing our knowledge on the impacts of marker selection for eDNA surveys will be crucial and has broad implications, given that currently many key universal metabarcoding and qPCR primers target short (< 200 bp) fragments of marker genes (Gold et al. 2021; Hernandez et al. 2020; Miya 2022).

4.4 | Implications for Monitoring Range Shifts

eDNA approaches offer an exciting opportunity to obtain extensive datasets across large spatial scales, making eDNA surveys an invaluable method for a range of applications, including invasive species monitoring (Ricciardi et al. 2017), biogeography (West et al. 2021), and conservation biology (Sahu et al. 2023). Despite its demonstrated utility in these fields, eDNA data potential remains underexplored for documenting tropicalisation and other climate-induced range shifts (Zarzyczny, Rius, et al. 2024). Moreover, as most tropicalisation research has focused on large, charismatic species or entire ecosystems, such as corals (Cant et al. 2022; Zarzyczny et al. 2022), macroalgal forests (Vergés et al. 2014), and mangroves (Cavanaugh et al. 2019), often neglecting cryptic or small taxa like gastropods, eDNA has the potential to address this gap by enabling simultaneous detection of multiple species, including those that are otherwise difficult to survey.

Our study reveals that eDNA can be detected over substantial spatial scales (> 250 km), which can likely be explained by an interaction between spawning time, larval dispersal and subsequent eDNA dispersal. In the case of *T. gallina*, we found that eDNA likely originated from larvae or gametes transported southward during the species' winter spawning period. We therefore highlight the importance of understanding both life-history traits of species of interest and the oceanography of the studied area when utilising eDNA for biodiversity monitoring. Additionally, we emphasise that the design of qPCR and metabarcoding assays for eDNA requires careful consideration, as the target fragment size could significantly influence assay sensitivity, leading to species detection beyond its true distribution. Consequently, interpretation of eDNA qPCR results (especially when targeting < 200 bp fragments) to understand present-day distributions requires careful consideration of life history, eDNA decay rates and local transport dynamics.

Our results suggest that eDNA dispersal over vast distances may be driven by larval movement and early life-history stages' DNA, emphasising the need for integrative approaches that combine eDNA analysis, life-history traits, field surveys, and modelling to uncover the full potential of eDNA for ecological and conservation applications. Whilst our study focused on a tropicalisation example, our results highlight the potential of our integrative approach for a wide array of research fields such as macroecology, conservation biogeography, and invasive science.

Author Contributions

K.M.Z. conceived the initial concept, led study and manuscript write up. K.M.Z., J.C.R. and M.R. designed the reseach plan. K.M.Z. and P.B.F. conducted the fieldwork and collected eDNA samples, with in country support and local expertise from D.A.P.-G. K.M.Z. conducted the laboratory work under the guidance of J.C.R. and M.R. S.T.W. supported laboratory study design and troubleshooting. M.E.H. collected *Tegula gallina* specimens and conducted the reproductive condition assessment. A.F. conducted the oceanographic modelling, with expert input from A.C.N.G. All authors were involved in result interpretation and contibuted to the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Relevant data regarding field surveys is available in the supporting information. Sequences generated in this study are available from Genbank, accession numbers PV053641—PV053651.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ddi.70064.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** ddi70064-sup-0001-Appendix.docx. **Data S2:** ddi70064-sup-0002-TablesS1-S5.xlsx.