Fall 12-20-2024

Integrating Life History and Environmental DNA (eDNA) Data to Enhance Detection of Sea Scallop (Placopecten magellanicus) Populations

Phoebe Jekielek
University of Maine, phoebe.jekielek@maine.edu

INTEGRATING LIFE HISTORY AND ENVIRONMENTAL DNA (eDNA) DATA TO ENHANCE DETECTION OF SEA SCALLOP (*Placopecten magellanicus*) POPULATIONS

By

Phoebe Jekielek

B.A. Boston University, 2003

Dual M.S. University of Maine, 2012

A DISSERTATION

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Doctor of Philosophy

(in Ecology and Environmental Science)

The Graduate School

The University of Maine

December 2024

Advisory Committee:

Heather Leslie, Professor of Marine Sciences, University of Maine, Advisor

Nichole Price, Senior Research Scientist, Bigelow Laboratory, Co-Advisor

Damian C. Brady, Professor of Oceanography, University of Maine

Shannon Meseck, Chief of NOAA Aquaculture Systems and Ecology Branch

Roger Mann, Professor of Molluscan Ecology, Virginia Institute of Marine Science

INTEGRATING LIFE HISTORY CHARACTERISTICS AND ENVIRONMENTAL DNA (eDNA) FOR ENHANCED DETECTION OF SEA SCALLOP (P*lacopecten magellanicus*) POPULATIONS

By Phoebe Jekielek

Dissertation Advisor: Dr. Heather Leslie

An Abstract of the Dissertation Presented
In Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
(in Ecology and Environmental Science)
December 2024

The sea scallop (*Placopecten magellanicus)* fishery is the largest and most valuable wild scallop fishery in the world. Offshore, it is among the most lucrative federal fisheries in the U.S. and supports a highly profitable near-shore fishery in Maine. The sustainability of wild capture fisheries for sea scallops are uncertain and aquaculture efforts are developing in response. Areas where both wild and aquacultured populations of the same species co-occur offer a unique opportunity to explore interactions among these populations and to develop new and innovative ways to monitor and, potentially, manage them. This dissertation investigates the patterns and underlying dynamics of variation in the reproductive ecology of wild and cultured shellfish populations and provides quantitative measures in support of the application of environmental DNA (eDNA) tools to detecting life history events of sea scallops.

Environmental DNA (eDNA) provides a potential solution to the challenges of monitoring, detecting, and quantifying commercially important species with complex life histories. It has the potential to be used for adult stock assessments, larval transport models, and

to estimate recruitment potential, provided patterns in eDNA occurrence and their significance are well understood. To determine the capacity for eDNA to be applied in natural systems, groundtruthing of these tools in laboratory settings is needed. eDNA approaches - like metabarcoding and quantitative polymerase chain reaction (qPCR) assays - may help disentangle the complex ecology of sea scallops and other marine invertebrates by providing a direct approach for species identification and enumeration of gametes and larvae in the water column. These relationships have not been validated for sea scallop eggs or larvae and have not been tested in the field over wild scallop beds or on scallop aquaculture farms. We also do not know how sampling at different depths and points in time influences one's ability to distinguish eDNA from adults vs. gametes and larvae. As scallop aquaculture continues to expand alongside the existing wild scallop fishery in Maine, there is a need to understand the consequences of farming scallops at a large scale and explore and develop novel methods for monitoring and potentially managing commercially important shellfish populations like sea scallops.

Chapter 1 provides an overview of the existing biological, ecological, and management landscapes for sea scallops in New England and, specifically, along the coast of Maine, and highlights current knowledge, information gaps, challenges, and applications of eDNA methods.

In Chapter 2, we compared the morphometrics of farmed and wild scallops at three locations in Penobscot Bay, Maine, to determine spawning synchronicity in farmed and wild scallops and if they allocate energy differently to their reproduction and growth. Our main objectives were to (1) identify the progression and onset of spawning events, (2) compare reproductive investment, (3) compare morphometrics (gonad, meat, total viscera, and shell masses), and (4) explore differences in energy allocation between farmed and wild scallops. The spawning timing and magnitude are highly variable in both wild and cultured populations of sea

scallops, but generally occur at similar time periods in each year. Overall, farmed scallops in this study invested more energy in soft tissues (gonads, viscera, meat) whereas wild scallops invested more energy in shell across all size classes. Larger meat yields from farmed scallops offer a significant potential return on investment for scallop growers, while their larger gonads suggest an increased potential for reproductive output with ecological ramifications for both aquaculture and wild harvest industries. These results shed light on the complex interplay between aquaculture and the natural environment, highlighting the need to further investigate the ecological consequences of cultivation on sea scallop populations and develop new and innovative ways to do so.

In Chapter 3, considering these knowledge gaps, we aim to (1) quantify relationships between scallop larval density and DNA copy number, (2) quantify eDNA shedding and degradation rates of scallops, and (3) relate these rates to the biomass of non-spawning scallops in mesocosms. Through lab-based larval collections and dilution series, we established a significant linear relationship between scallop larval density and gene copy values, identifying an average value of 3.41×10^7 gene copies per larvae. Using mesocosm-based controlled lab experiments, we determined that gene copy quantities generally increased with increasing adult scallop biomass through time. Together, the results of these experiments support interpretation of eDNA signals generated by larval and adult scallops and inform sampling practices that use eDNA to monitor biological processes, particularly in the context of ecosystem-based fisheries management of sea scallops.

In Chapter 4, using scallop aquaculture farms and wild scallop beds as research sites, we used gene copy number, determined through qPCR primers, GSIs, and plankton tows to evaluate the capacity of eDNA tools to detect life history events of sea scallops and the spatial and

temporal variability in these signals. The objectives of this work are to (1) determine the ability of eDNA tools to successfully detect sea scallop DNA in the field, (2) evaluate spatial (across depth and across sites) and temporal (across spawning seasons) differences in sea scallop eDNA distribution, and (3) evaluate the use of eDNA methods to detect biological processes, such as sea scallop spawning and larval presence. The available scallop qPCR probe and primers successfully detected scallop eDNA on scallop aquaculture farms and above a wild well-characterized, deeper scallop bed. There was temporal (across weeks) and spatial (across sites and depths) variation in these signals on farms and above wild beds. With one exception, associations between larval density and gene copy were not found at farms in any sampling year. Scallop eDNA was detected at all depths, but not during all sampling events, above the well characterized wild scallop bed. Scallop eDNA was detected at all depths - sometimes at high concentrations - at a site lacking scallops, suggesting that transport of eDNA and quantifying stochasticity in 'background' signals is an important consideration in future studies. Scallop eDNA signal increased at wild population sites and across depths after maximum GSI were observed and during the time of assumed peak larval presence from 30-45 days after spawning.

In Chapter 5, I review the outcomes of this work and provide direction and recommendations for future research, highlighting a need to evaluate the ecological interactions between wild and farmed sea scallop industries. I suggest evaluating interactions through the lens of the connections between environmental variability and life histories of scallops as a necessary step in planning for the future of this resource and the potential fitness impacts in wild and farmed populations. Lastly, I express an urgent need for continued ground truthing of eDNA tools, a move toward standardization of methods, and an evaluation of the relevance of laboratory-based experiments to field-based applications and monitoring.

DEDICATION

This dissertation is dedicated to the many people who have worked with me, laughed with me, cried with me, cooked with me, argued with me, lived with me, swam with me, ran with me, sang with me, learned with me, and cared for me over the past four years through the ups and downs of figuring it all out. And my cat, Danger Cat (Deece), who has no concept of his involvement but was essential to my success. It takes a village, even for us adults.

ACKNOWLEDGEMENTS

I thank my committee, H. Leslie, N. Price, D. Brady, S. Meseck, and R. Mann for their continued support, encouragement, phone calls, zoom calls, discussions and many reviews and revisions of my dissertation. Thank you to the Hurricane Island Center for Science and Leadership for their commitment and collaboration to this work through staff support, vessel support, communication and outreach, the time and space to complete this work in the midst of a job, and for continuing to believe in and support me. Specifically, thank you to M. Maier, C. Huntsberger, L. Williams, S. Poratti, S. Burgess, E. Martin, C. Finger, and J. Fogg for all the help with collections, dissections, filtering and lugging all of the things that it takes to do research, both as part of my PhD and our own research endeavors that we dreamed up. Thank you to Jeff Chace, the man who showed me how to have a good attitude in the face of any situation, that laughter is a medicine, and that solutions-based thinking can be a way of life not just work. Thank you to the many sea scallop fishermen and farmers that agreed to collaborate on this work, shared their own data, or simply let me ask them questions for a few hours; especially Marsden Brewer, Andrew Peters, Alex de Koning, Stewart Hunt, Nate Perry, Tad, Dan, and Peter Miller, Jim Wotton, and Curtis Haycock. The knowledge they hold and are willing to share are essential to this body of work, my future work interests, and the resilience and success of Maine's natural marine resources. Thank you to the many funding sources that have supported this work and my ideas - Maine Sea Grant, The Builder's Initiative, the Davis Conservation Foundation, and the National Science Foundation award #OIA-1849227 to Maine EPSCoR at the University of Maine. Thank you to the VAST community of friends, co-workers, collaborators, family, and animals that have supported me over the years - I am an independent woman who would not be here without the love from you on which I depend.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

This dissertation focuses on the patterns and underlying dynamics of variation in the reproductive ecology of wild and cultured shellfish populations. We use newly developed environmental DNA (eDNA) tools and lab-based experiments to quantify the relationships between larval counts and gene copy number and DNA generation rates in the commercially important sea scallop, *Placopecten magellanicus.* In parallel with traditional techniques like gonadal somatic indices (GSIs) and microscopy of plankton tows, we ground truth the potential application of eDNA techniques to monitoring and quantifying biological processes in the field, such as spawning and larval transport. Using this combination of field observations and lab-based experiments in partnership with scallop farmers, wild harvesters, scientists, and management partners along the coast of Maine, we explore the role that eDNA tools may play in understanding the ecology of economically valuable species and how these methods may be used in future management of these species. In this chapter, we provide important contextual information on eDNA as well as sea scallop life history and the sea scallop wild fisheries and aquaculture sectors in the northeastern US to set the stage for the data-focused chapters that follow. We conclude by detailing the significance of each chapter to come, in the context of marine fisheries ecology and management.

1.1 SEA SCALLOP LIFE HISTORY

Wild populations of sea scallops (*Placopecten magellanicus)* are distributed on the continental shelf from Newfoundland to North Carolina and generally found in depths ranging from 18-100 m. There is evidence of sea scallops being found in waters as shallow as two m and as deep as 384 m, but this is not regular (see Hart and Chute 2004), and they generally avoid

depths with higher temperatures. Adult sea scallops are found in aggregations, called beds, corresponding to areas of suitable temperature, food availability, substrate, and oceanography (Thouzeau et al. 1991; Stokesbury and Himmelman 1993).

Sea scallops are highly fecund broadcast spawners that sexually mature around 40 mm in shell height and increase gamete production with size (MacDonald & Thompson 1985; Parsons et al. 1992). Scallops exhibit both complete (synchronous) and protracted spawning events that vary spatially on annual and semi-annual cycles and are driven by environmental conditions (Langton et al. 1987; Smith & Rago 2004; Thompson et al. 2014). Generally, the gametogenic cycle of scallops in more southerly populations from Georges Bank to the mid-Atlantic Bight exhibit semi-annual spawning in spring and fall (Schmitzer, Dupaul & Kirkely 1991), whereas more northerly populations from Maine to the Canadian Maritimes exhibit annual spawning events in late summer or early fall (Dadswell and Parsons 1992; Thompson et al. 2014). During spawning events, scallops aggregate into groups, increasing the chance of successful fertilization and facilitating the synchrony of spawning (Dadswell & Parsons 1992; Barber & Blake 1991; Bayer et al. 2019).

Once adult sea scallops have spawned and successful fertilization has occurred, they proceed through a planktonic cycle lasting 30-45+ days (Culliney 1974; Figure 1). Larvae are planktonic and planktivorous, exhibiting vertical migration behaviors and utilization of different water column temperatures throughout their development (Culliney 1974; Manuel et al. 1996).

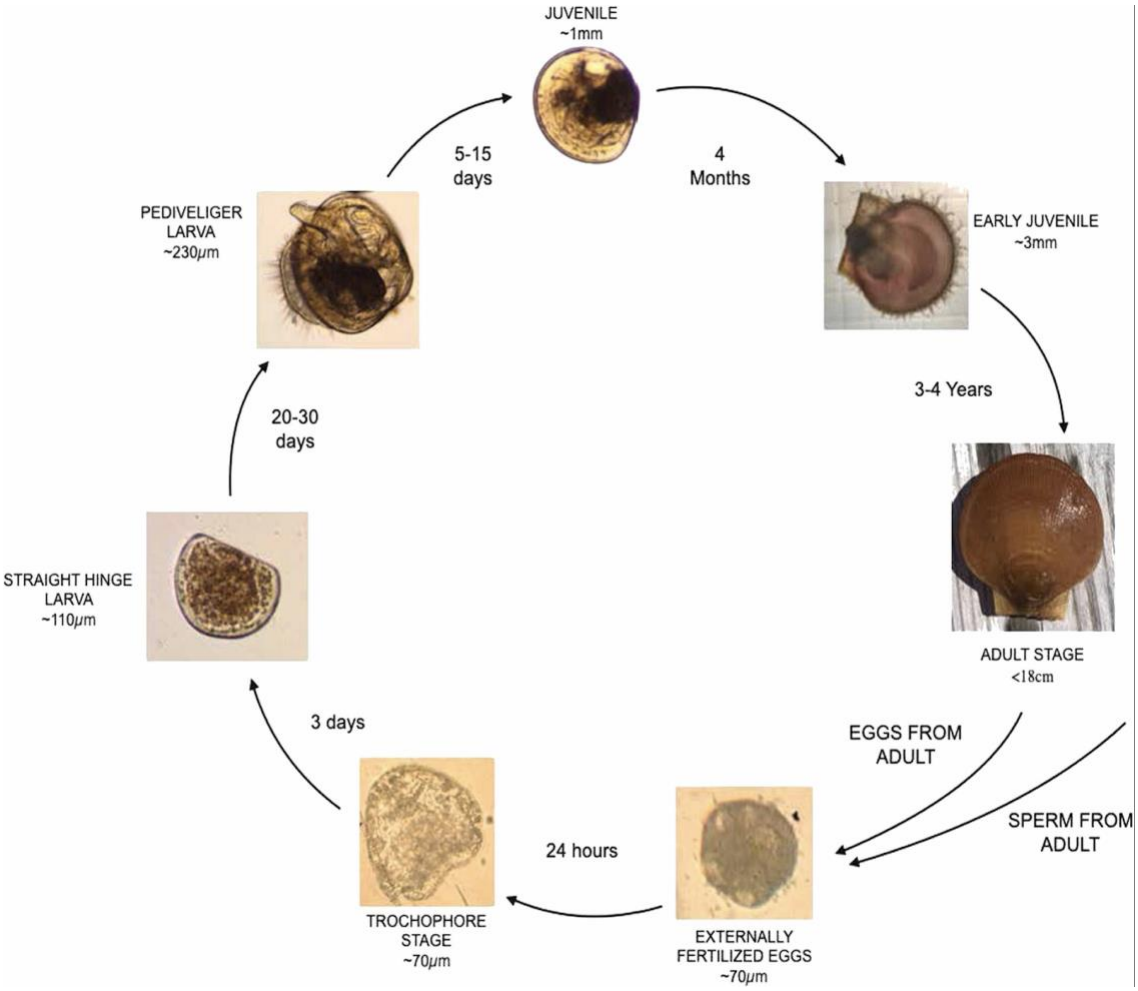


Figure 1: Sea scallop life cycle. Adapted from Ishaq et al. 2023, after Culliney 1974.

Development time throughout the planktonic stages and settlement to the benthos is influenced by oceanography, temperature, food availability, and habitat suitability, and scallops can delay settlement for up to one month (see Mann 1988; Stewart and Arnold 1974; Culliney 1974). These biological processes proceed within a dynamic oceanographic context, which presents challenges to understanding larval dynamics and has implications for management of these commercially important species (Morgan 2001).

Adding complexity to the life history sea scallops is the maintenance of swimming ability throughout their life history. In early benthic stages of their life, sea scallops use a swimming

response to escape predation, move to more suitable habitat, and find conspecifics (Shumway and Parsons 2016). As they grow and find suitable habitat, the likelihood of swimming decreases with age and size as their shell thickens and they are less vulnerable to predators. Scallops do, however, maintain the capacity to swim throughout their life due to the unique monomyarian arrangement of their tonic and phasic adductor muscles (Lafrance et al. 2003; Sturm, Pierce and Valdez 2006), influencing their ability to swim for reproductive, habitat or food access purposes.

1.2 SCALLOP FISHERIES AND AQUACULTURE IN THE NORTHEAST U.S.

The sea scallop fishery is the largest and most valuable wild scallop fishery in the world and among the most lucrative federal fisheries in the U.S., generating $478 million in commercial landings in 2022 (NOAA, 2024). Federally managed fisheries occur from the mid-Atlantic Bight to the Northern Gulf of Maine management zone, spanning approximately 1,300 miles of coastline, and occur from three miles offshore out to the US exclusive economic zone two hundred miles offshore. Maine's state-managed scallop fishery generated $9.3 million dollars in 2023 and demands one of the highest state average prices per meat pound (~$14.12/lb in 2023; MEDMR 2024). The state-run fishery consists of highly productive shallow inshore aggregations managed in three zones along the coast and utilizes rotational closed areas with open/closed areas changing annually. State-managed fisheries consist of both drag and dive harvest methods and engage 301 individual boats and an additional 31 divers. However, the sustainability of both the state and federal wild capture fisheries for scallops are uncertain in the face of climate impacts (Hare et al. 2016, Tanaka et al. 2020).

Aquaculture is viewed as a potential mitigation strategy for wild fisheries changes and losses in the coming decades and, more immediately, as a strategy for fishery-dependent communities in Maine to diversify the species, products and employment opportunities available

(GMRI 2016, Maine Economic Recovery Plan 2020, Britsch et al. 2021, although also see Stoll et al. 2019). Aquaculture, the farming of aquatic organisms, has become the fastest growing food sector globally and accounts for 59 percent of total fisheries production valued at 312.8 billion USD in 2022, a 25% increase from 2018 (FAO, 2024). There are approximately 730 different species of fish, molluscs, crustaceans, amphibians, plants and algae cultivated in fresh and marine waters as part of this growth, with marine species (mariculture) representing approximately 37.4 percent of all aquaculture production (FAO, 2024). The growth of aquaculture is fueled by decreases in wild fisheries resources, expanding global trade and incomes, and urbanization driving the need for more secure food resources globally (see Naylor et al., 2021). This growth also impacts to the social-ecological systems within which it is functioning, e.g., through the creation of jobs and ecosystem services.

In Maine, scallop aquaculture has been in its infancy since the early 2000s, but has recently seen an increase in interest, investment and profit generation (Fitzgerald 2021, ME DMR 2024). Extensive knowledge and technology exchange between well-established scallop aquaculture efforts in Japan and the burgeoning industry here in Maine has bolstered the adoption of scallop aquaculture in Maine. Scallops are grown in vertically-suspended lantern nets or ear-hung from drop lines that are hung from longlines submerged 6-10 m below the water surface (Figure 2). Currently there are four farms growing and selling scallops into seafood markets, three that collect and sell spat to other farms, and an additional 99 leases that list sea scallops as a potential product, but not the primary resource, to grow on their farm (Jekielek, pers. obv.).

Figure 2: Scallop lantern nets hanging from a long line (photo: jjchicolino.com)

Scallops are unique among bivalves in Maine in that there is a strong wild commercial harvest and an aquaculture industry is developing in parallel. Experts see potential for industry expansion even though both wild and cultured industries rely on sourcing seed from wild populations (Fitzgerald 2021). As successful shellfish culture consists of seed collection, grow-out, and harvest, reliance on a variable wild set is a potential bottleneck for consistent culture production in the emerging scallop aquaculture industry in Maine (Fitzgerald 2021). The co-location of farms within wild management zones offers unique opportunities for research

questions addressing issues within and among scallop populations and industries, specifically pertaining to the potential biological and ecological effects of raising scallops in aquaculture. This co-location of industries and populations also requires new and innovative approaches to monitoring and management.

1.3. eDNA AS A TOOL FOR POPULATION ASSESSMENT AND FISHERIES MANAGEMENT

Genetic material captured in aqueous or other environments, i.e., environmental DNA, (eDNA) can be quantified to provide ecological inferences (Thomsen & Willerslev 2015; Benoit et al. 2023). This material includes whole genomes of intact microorganisms (e.g., gametes or larvae) to free DNA fragments shed from cells of larger organisms. The steps involved in eDNA methodologies include collection of the sample from aqueous environments, extraction of DNA from the collected sample and then analysis of the DNA.

Quantitative polymerase chain reaction (hereafter qPCR) and metabarcoding eDNA methods are applied in terrestrial and marine ecosystems around the world - from coral reefs to arctic climes (Doyle et al. 2017; Fukumori et al. 2024). The qPCR process uses species-specific probes and primers to quantify the amount of organismal DNA in a sample (Vadopalas et al. 2006; Bayer, Countway & Whale 2019). These assays are used to monitor endangered and at-risk species (Marques et al. 2023; Skinner et al. 2021), commercially important species (Wang et al. 2021), life history events (Tillotson et al. 2018; Troth et al. 2021), and pathogens (Gomes et al. 2017). Metabarcoding methods, which detect the diversity of all DNA in a sample, have been applied to monitor commercial fisheries (Afzali et al. 2020), to track changes in community composition (Carvahlo et al. 2024), to understand food web dynamics (Tournayre et al. 2020), and to evaluate microbiomes (Ishaq et al. 2023). When combined, qPCR and metabarcoding

methods can evaluate species-specific populations while providing a deeper contextual understanding of the more complex structure of the ecological community (Yu et al. 2022).

The use of eDNA in monitoring biodiversity, species invasions, and presence or absence of commercially important species in dynamic marine environments is growing due to its less-invasive nature, low cost, and timely results (Merten et al. 2023; Uthicke et al. 2022; Allen, Nielsen, Peterson & Lockwood 2021). By virtue of its taxon specificity, low cost, and consistent accessible sampling approaches, eDNA science offers capacity for wider research participation (Creer et al., 2016), shared sampling effort with participants in commercial natural resource industries (Larson et al. 2020), and deeper data integration to address the scales and complexities of natural resources. However, the method is not without its limitations and questions remain about the application of eDNA tools for management purposes.

Biological and environmental factors can influence the availability and longevity of eDNA in a system. The detection and degradation rates of eDNA can vary seasonally, experience high sampling variability between sites, and may be species-specific (Pierce 2020, Troth et al. 2021). eDNA has the potential to be used for adult stock assessments, larval transport models, and to estimate recruitment potential, if these patterns in eDNA occurrence and their significance were understood (Alexander et al 2021, Kirtane 2021). This frequent disconnect makes stock assessments challenging, especially for organisms with complex life histories, such as scallops and other broadcast spawning invertebrates.

Quantitative eDNA assays for sea scallops have been developed based on ITS gene fragments (Bayer et al. 2019). Bayer and colleagues (2019) established that gene copy number, determined through quantitative PCR (qPCR) primers and size fractionation protocols, can be used as a proxy for sea scallop gamete number, specifically sperm, in the water column.

Understanding larval supply of sea scallops is further challenged in that their microscopic gametes and larvae cannot be distinguished taxonomically from other bivalves, and are difficult to track in situ (Pechenik, 1999). eDNA approaches may finally disentangle the supply-side of sea scallop recruitment ecology by providing a direct approach for species distinction and enumeration of gametes or larvae in the water column.

1.4 OVERVIEW OF THE CHAPTERS TO COME

In Chapter 2, I ask if the morphometrics of farmed and wild scallops differ? Partnering with scallop aquaculture farmers, I sampled sea scallops from three farms and from wild beds in Penobscot Bay, Maine, to determine if farmed and wild scallops allocate energy differently to their reproduction and growth. Our main objectives were to (1) indicate the progression and onset of spawning events, to (2) compare reproductive investment, to (3) compare morphometrics (gonad, meat, total viscera, and shell masses), and to (4) explore differences in energy allocation between farmed and wild scallops. We hypothesized that spawn timing and magnitude will differ between farmed and wild populations within years and between years and sites, that reproductive investment between farmed and wild scallops will differ, that farmed scallops will have larger gonad, meat, total viscera and shell masses than wild scallops, and that these populations will allocate energy to meat and gonads differently over time. Integrated studies of wild and cultured scallop populations will help us better understand the potential roles aquaculture farms can play in supporting healthy and resilient coastal ecosystems and fisheries.

Chapter 3 describes laboratory experiments that I conducted to quantify the relationships among scallop larval abundance and DNA copy number and controlled mesocosm experiments to determine the eDNA shedding and degradation rates of different biomasses of non-spawning scallops in mesocosms. The main objectives of this work are to (1) quantify relationships

between scallop larval density and DNA copy number, (2) quantify eDNA shedding and degradation rates of scallops, and (3) relate these rates to the biomass of non-spawning scallops in mesocosms. We expected to find positive linear relationships of larval densities with gene copy number. We expected that the mean amount of DNA shed over the lifetime of the experiment to increase with increasing scallop biomass and the average shedding rate (copies/hour/gram) to increase with increasing biomass. We expected eDNA degradation to occur more quickly at lower biomasses than at higher biomasses. Together, the results of these experiments support interpretation of eDNA signals generated by larval and adult scallops and inform sampling practices that use eDNA to monitor biological processes, particularly in the context of ecosystem-based fisheries management of sea scallops.

Chapter 4 compares traditional monitoring methods, such as gonadosomatic indices and microscopy of plankton tows, to field applications of eDNA tools for monitoring spawning and larval transport on scallop aquaculture farms. I also implemented a vertically stratified sampling design to evaluate the spatial and temporal variation in eDNA signal above a wild scallop bed. The objectives of this work are to (1) determine the ability of eDNA tools to successfully detect scallop DNA in the field, (2) evaluate spatial (across depth and across sites) and temporal (across spawning seasons) differences in scallop eDNA distribution, and (3) evaluate the use of eDNA methods to detect biological processes, such as scallop spawning and larval transport. We hypothesized that eDNA tools will successfully detect scallop DNA in the field and that detected signals will vary spatially and temporally among different sites where scallops are living on scallop aquaculture farms and in wild populations. We also hypothesized that eDNA tools will successfully detect biological processes as evidenced by differences in detectable signal pre-, during, and post-spawning, maximum gene copy values occurring during larval transport season,

increased vertical distribution of the signal above wild beds during spawning and larval transport season, and positive correlations between gene copy number and bivalve larval counts from plankton tows. eDNA approaches may help disentangle the complex ecology of sea scallops and other marine invertebrates by providing a direct approach for species identification and enumeration of gametes and larvae in the water column.

In the Conclusions, Chapter 5, I bring together the key messages from each of these chapters. I suggest best practices for eDNA research to come, including providing recommendations on designing and implementing monitoring protocols for commercially important species with complex life histories.

CHAPTER 2

FARMED AND WILD SEA SCALLOP ENERGY ALLOCATION TO REPRODUCTION AND GROWTH IN PENOBSCOT BAY, MAINE

2.1 ABSTRACT

The Atlantic sea scallop (*Placopecten magellanicus*) fishery ranks among the most economically valuable marine fisheries in the United States (~$479M in 2022). Recent declines in wild catch and projections for increased demand for sea scallops are supporting the development of a scallop aquaculture industry in Maine. However, the effects of raising sea scallops in aquaculture remain largely unexplored. Here, we assess biological and ecological impacts of sea scallop aquaculture on farmed scallops by comparing the morphometrics of cultured and wild sea scallops in Penobscot Bay, Maine. In 2020-2022, we sampled farmed scallops from lantern nets at three aquaculture sites in Penobscot Bay, Maine, and collected wild scallops via SCUBA from beds adjacent to each farm. During each sampling event, we measured shell heights and shell, adductor, gonad, and total viscera masses. We found that farmed scallops have significantly larger adductor, gonad, and viscera masses compared to wild scallops within the largest size classes 80 – 110 mm. Conversely, wild scallops have significantly larger shell masses. Generally, gonadosomatic indices are greater in farmed scallops than in wild scallops and spawning time is variable in both populations. Larger meat yields from farmed scallops offer a significant potential return on investment for scallop growers, while larger gonads suggest an increased potential for reproductive output with ecological ramifications for both aquaculture and wild harvest industries. These results shed light on the complex interplay between aquaculture and the natural environment, highlighting the potential ecological and economic consequences of sea scallop cultivation.

2.2 INTRODUCTION

The Atlantic sea scallop (*Placopecten magellanicus,* hereafter "scallop") fishery is the largest and most valuable wild scallop fishery in the world, and is also among the most lucrative fisheries in the United States, generating $479 million in 2022 (NOAA 2024). Scallops are harvested in deep (up to 300 m), offshore federal waters from Cape Hatteras, NC, to Newfoundland, Canada, and in shallow (up to 10m) inshore state waters along the Maine coast (NOAA 2024). In 2023, Maine's state-managed fishery brought in over $9.3 million dollars and yielded one of the highest state average prices for meat per pound (~$14.12/lb in 2023; MEDMR 2024). Although this fishery is increasing in value, the catch is variable from year to year and remains a fraction of the large harvests recorded in the 1980s (MEDMR 2023).

Scallop aquaculture has emerged as a new industry along the coast of Maine, given the variability of the state and federal scallop fisheries and a forecasted increase in demand (Fitzgerald 2021). Aquaculture could supplement the wild scallop market in Maine, potentially doubling the current volume and total value of sea scallop harvests by 2030 while maintaining a price premium and extending the availability of consumable products year-round (Cole, Langston & Davis 2016; GMRI 2016). This emerging industry increases the diversity of available products: from meats alone to whole live, roe on, and meats on the half shell, consequently increasing the potential marketability of scallops (Fitzgerald 2021). Scallop aquaculture generated ~$585,000 in 2023 due to both whole scallop and shucked meat sales - a 400% increase in value from 2022 and a 700% increase from 2021 (MEDMR 2023). However, the economic viability of this emerging industry remains uncertain. Low engagement, despite high interest, demonstrates that high labor costs, low mechanization, market volatility, concerns over seed procurement, and regulatory roadblocks challenge industry expansion (Fitzgerald

2021). Of the 99 farms in Maine that are permitted to grow scallops, only five farms do so at any scale to generate revenue.

Sea scallops are highly fecund broadcast spawners that sexually mature at a small size of around 40mm and increase gamete production with size (Macdonald & Thompson 1985; Langton, Robinson & Schick 1987; Parsons et al. 1992). Fecundity is lower and spawning is less seasonally pronounced in deepwater (170-180 m) scallop populations from the Gulf of Maine than in populations of similar size classes in shallower waters (13 - 20 m) in coastal Maine (Barber, Getchell, Shumway & Schick 1988). Scallops exhibit both complete (synchronous) and protracted spawning events that vary spatially on annual and semi-annual cycles and are driven by environmental conditions (Langton, Robinson & Schick 1987; Parsons et al. 1992; Thompson, Stokesbury & Inglis 2014; Smith & Rago 2004). On Georges Bank, scallops experience two spawning events in a year, whereas scallops along the coast of Maine spawn once a year during the late summer (Kirkley & DuPaul 1991; Thompson 2014; Bayer 2016). Temperature maxima and large temperature fluctuations coincide with peak spawning (Bonardelli, Himmelman & Drinkwater 1996). In the wild, scallops aggregate into groups during spawning events, which facilitates synchrony of spawning and increases the chance of successful fertilization (Dadswell & Parsons 1992; Barber & Blake 1991; Bayer, Countway & Wahle 2019). Conditions that favor successful external fertilization, including high densities, large population sizes, and close proximity of fecund individuals (Levitan 1998), are also those likely to be found on scallop aquaculture farms.

Scallop aquaculture is practiced throughout the world but is new to the United States. For instance, in Mutsu Bay, Japan, many Yesso scallop (*Patinopecten Mizuhoopecten yessoensis*) farms have thrived since the 1930s with no hatchery, suggesting that the large number of farms

within the relatively closed Mutsu Bay has sustained ample seed production to support the industry (Fitzgerald, 2021). In Canada, scallop aquaculture of the hybrid Qualicum scallop (*Patinopecten caurinus x yessoensis*) occurs on West coast while efforts on the East coast focus on raising sea scallops and Northern bay scallops (*Argopecten irradians*). Growing practices differ across the world, but most farmed sea scallops are grown in vertically-suspended lantern or pearl nets hung from horizontal long lines submerged 6-10m below the water surface, rather than on the benthos as in the wild. Consequently, cultured scallops experience environments that differ substantially from those of their wild relatives. Therefore, we hypothesize that the different growing conditions experienced by wild and farmed scallops affects their biology as well as their interactions with other scallops and the broader ecosystem.

Generally, scallops living in shallow water have faster shell growth and more pronounced somatic growth than those living in deeper water, which suggests that scallops grown in aquaculture may experience conditions more favorable for growth (Macdonald and Thompson 1988). Additionally, farmed scallops in Newfoundland exhibited greater rates of growth, greater somatic weights, and greater total production than wild scallops of the same age (Macdonald 1986). In the Gulf of St. Lawrence, farmed scallops had larger somatic tissues and greater clapping response to predators than wild scallops but the wild scallops had stronger shells and more intense escape responses (Lafrance, Cliche, Haugum & Guderley 2003). However, Kleinman et al. (1996) showed that growth rates and adductor muscle condition index were higher in bottom-reared scallops than in suspended cultured scallops. Collectively, these studies suggest farmed scallops may experience conditions more favorable for growth and reproduction than wild scallops, but none explore this possibility more deeply.

Maine's growing scallop aquaculture industry is situated in a rapidly changing marine ecosystem. The Gulf of Maine (GOM) has warmed faster than 99% of the world's oceans at a rate of 0.04°C/yr since 1982, four times faster than the global average (Thomas et al. 2017; Pershing et al. 2021). In addition, the GOM is projected to experience conditions that inhibit calcification (aragonite saturation state < 1.5) for most of the year by 2050 (Siedlecki et al. 2021). These changes have the potential to affect reproduction, recruitment, fecundity, and distribution of benthic marine populations, both farmed and wild, in positive and negative ways, and there are consistent knowledge gaps in understanding of these effects (Holden et al. 2019). Sea scallops are highly vulnerable to climate change with recent work suggesting deleterious effects of rising CO_2 and temperature on sea scallop respiration rate, growth rate, feeding rates, among other bioenergetics, but results are variable between studies and call for further evaluations of scallop responses to changing environments (Pousse et al. 2023; Cameron, Grabowski, and Ries 2022; Rheuban et al. 2018; see also Hare et al. 2016; Cooley et al. 2015).

As scallop aquaculture continues to expand alongside the existing wild scallop fishery in Maine, there is a need to understand the consequences of farming scallops at a large scale. Aquaculture farms can provide *in-situ* experimental sites to explore interactions between wild and farmed scallop populations while also providing access to monitor environmental variables governing those interactions. Integrated studies of wild and cultured scallop populations will help us better understand the potential roles aquaculture farms can play in supporting healthy and resilient coastal ecosystems and fisheries. We compared the morphometrics of farmed and wild scallops at three locations in Penobscot Bay, Maine, to determine if farmed and wild scallops allocate energy differently to their reproduction and growth. Our main objectives were to (1) identify the progression and onset of spawning events, (2) compare reproductive investment, (3)

compare morphometrics (gonad, meat, total viscera, and shell masses), and (4) explore differences in energy allocation between farmed and wild scallops. We hypothesize that a) spawn timing and magnitude will differ between farmed and wild populations within years and between years and sites, that b) reproductive investment between farmed and wild scallops will differ, that c) farmed scallops will have larger gonad, meat, total viscera and shell masses than wild scallops, and that d) these populations will allocate energy to meat and gonads differently over time.

2.3 MATERIALS AND METHODS

2.3.1 Farmed scallop sampling and site characterization

To determine spawning timing, reproductive investment and compare the morphometrics of farmed scallops we sampled scallops from scallop aquaculture farms in Penobscot Bay, Maine, USA. We sampled farmed scallops at three sites: North Haven Farm (44.17571° N, 68.81849° W), a 0.5-acre site with a depth of 9-15 m mean low water (MLW); Hurricane Island Farm (44.03965° N, 68.89103° W), a 3-acre site with a depth of 6-9 m MLW; and Stonington Farm (44.143611° N, 68.704444° W), 3.2-acre site with a depth of 16-21 m (Fig. 3). Both the North Haven and Hurricane Island Farms have a gently sloping bottom consisting primarily of gravel and mud, whereas the Stonington farm consists of soft mud. All collections from farmed and wild populations occurred under Maine Department of Marine Resources special licenses ME 2020-61-04, ME 2021-16-03, and ME 2022-38-02.

Figure 3. Farm and wild scallop collection sites. Collection sites (black pins) of farmed Atlantic sea scallops (*Placopecten magellanicus*) in the Penobscot Bay, Maine, USA, in 2020−2022. Red circles represent the areas in which we harvested wild scallops for comparison.

At each farm, two experimental lantern nets with 12-mm mesh were suspended in the water column along a horizontal line sitting 5-10 m below the surface (Fig. 4). Each net had a diameter of 50cm, a height of 200cm, and consisted of 10 levels spaced 20 cm apart. Nets were stocked with scallops from the inventory available at the host farm. Stocking density followed the industry standard of 30% coverage within each level of the lantern net which was calculated by determining the area available in the net based on the size class of the scallops to be stocked. Experimental nets were stocked with scallops of similar size classes on aquaculture farms in each year with scallops resident to each farm so as to not confound the impact of site on the morphometrics of the sampled scallops. Size classes varied between years and were based on

what was available at a given farm. A minimum of 20 farmed scallops were collected for dissections during each sampling event. Scallops were sampled biweekly in June-July and weekly in August-September to collect data during the pre-spawning and spawning season, respectively. As weather permitted in 2020-2021, monthly sampling of scallops occurred during the post-spawning season (October-June). A total of 2,862 farmed and 1,015 wild scallops were collected in 2020–2022 (Fig. 5).

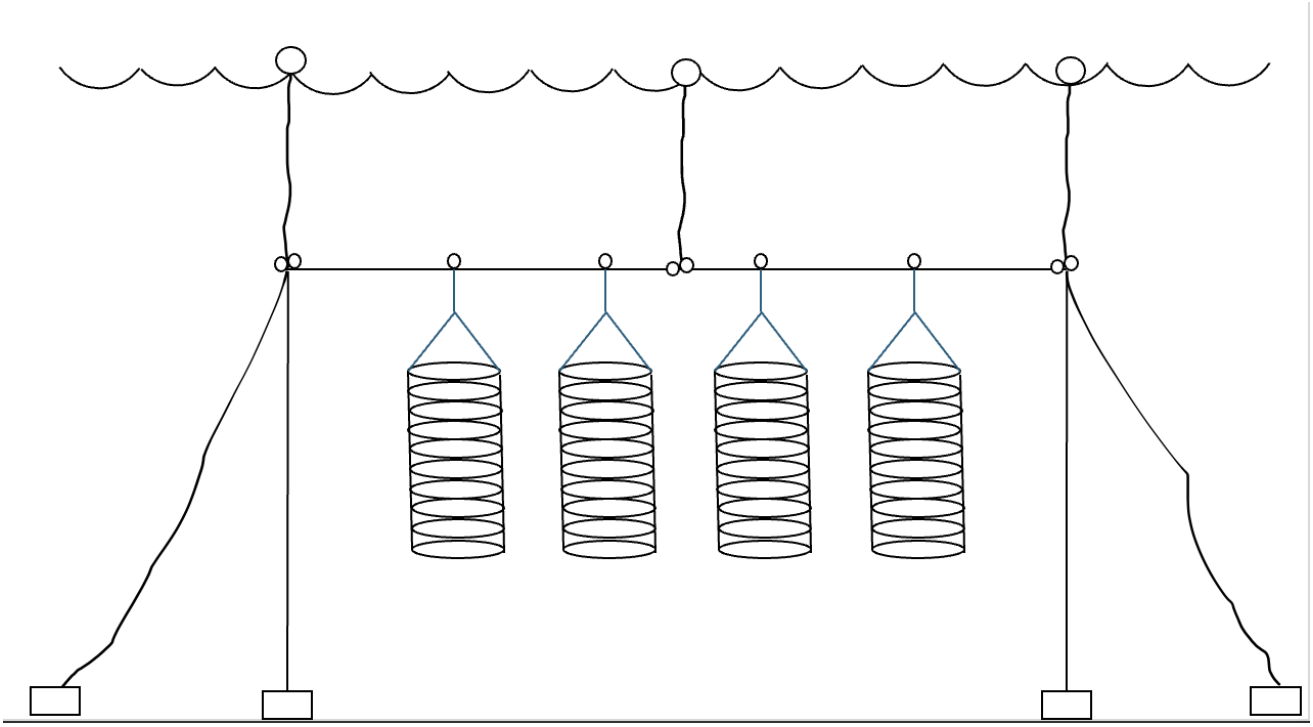


Figure 4. Aquaculture farm schematic. Schematic of the general Atlantic sea scallop aquaculture farm arrangement of lantern nets for farmed Atlantic Sea Scallops (*Placopecten magellanicus*) in Penobscot Bay, Maine, 2020−2022.

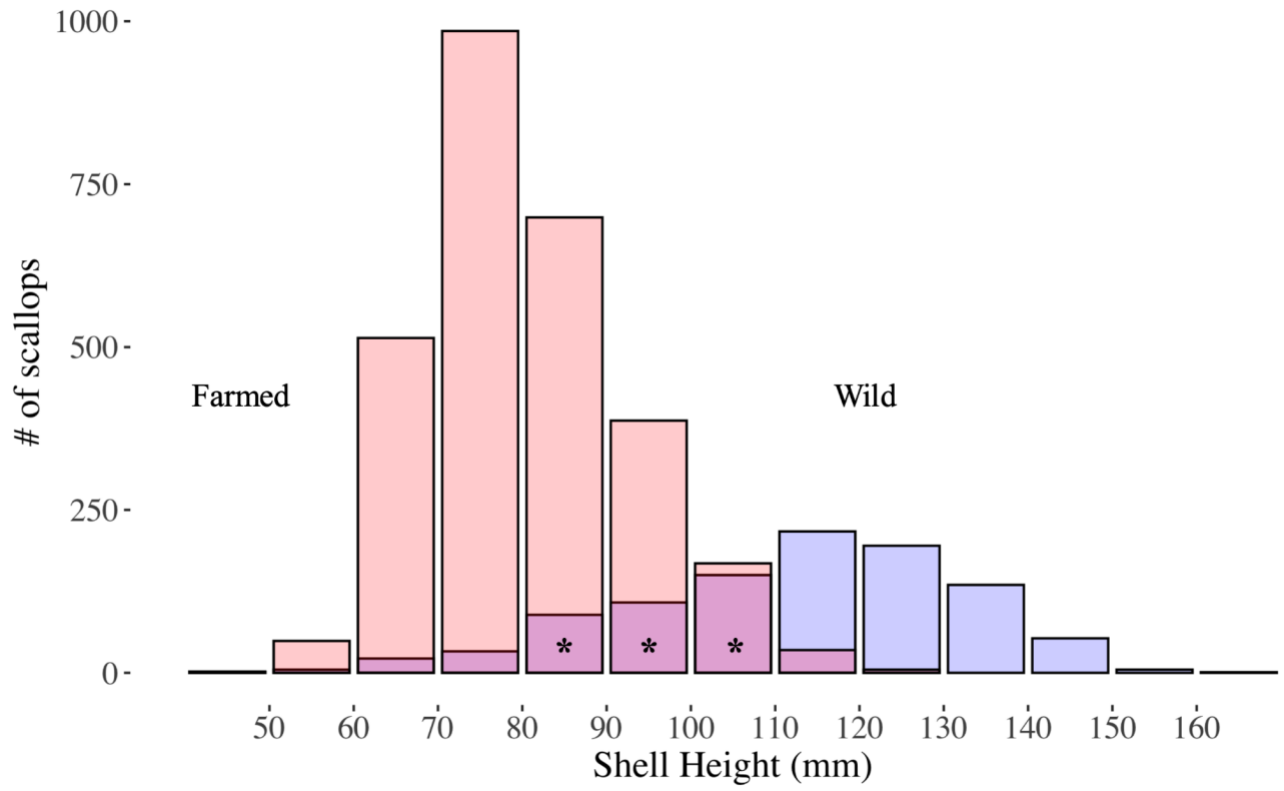


Figure 5. Sampling frequency of scallops. Samples of farmed (n = 2,862) and wild (n = 1,015) Atlantic sea scallops harvested in Penobscot Bay, Maine, 2020−2022. We used the three largest overlapping size classes (80-89, 90-99, 100-109 mm height), denoted with an asterisk (*), to compare morphometric data in Figure 5.

2.3.2 Wild scallop sampling

To determine spawning timing, reproductive investment and compare the morphometrics of wild scallops we received a special license from the Maine Department of Marine Resources to collect wild scallops within the Isle au Haut Bay and Lower Penobscot Bay rotational management areas. Wild scallops were sampled on SCUBA in 10-20 m of water within a 3.3-kilometer radius of each farm from a substrate that was predominantly composed of shell hash, sand, and cobble. A total of 20 wild scallops were haphazardly sampled from wild beds adjacent

to each of the farms at each sampling event, focusing on animals of similar size to those deployed in experimental nets for the most direct comparison between wild and cultured populations. Wild specimens were sampled during the same intervals as farmed scallops, with increased effort in June-October to more directly compare morphometrics between farmed and wild scallops during the spawning season.

2.3.3 Biological data collection

All farmed and wild scallops were processed at the Hurricane Island Center for Science and Leadership (44.036156 North, -68.889392 West). Shells were cleaned of biofouling and shell height (SH) was recorded to the nearest millimeter using Vernier calipers. The entire intact viscera was separated from the dorsal and ventral shells using a shucking knife and then the gonad and adductor muscle (hereafter meat) were separated from the remainder of the viscera using dissecting scissors and forceps. The wet weight of the cleaned shells, entire viscera, meat, and gonad (including the crystalline stylus but not the foot) separately were measured to the nearest 0.01 g (Fig. 6) on a Fristaden Digital Precision Analytical Balance Lab Scale 1000G X 0.01G. We calculated the total mass of each scallop by summing the total viscera mass and the cleaned shell mass.

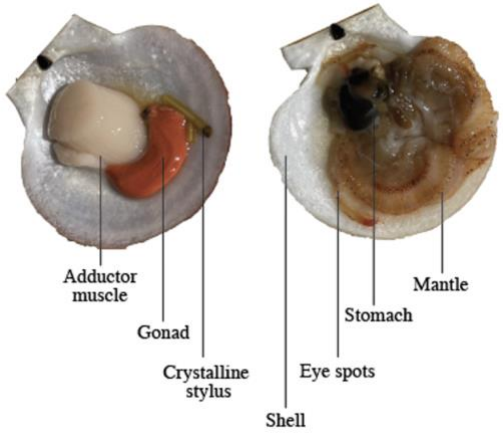


Figure 6. Biological components of a dissected Atlantic sea scallop.

2.3.4 Data analysis

We first calculated the gonadal somatic index (hereafter GSI, %), which is the ratio of the gonad to the total viscera weight ($weight_{gonad}$ / $weight_{viscera}$) **100*, as an indicator for spawning and reproductive investment (Dadswell & Parsons 1992; Thompson et al. 2014; Bayer et al. 2016, 2018, 2019). The timing of spawning events in farmed and wild populations was determined by examining the relationship between GSI and week of the year, where spawning events are indicated by a maximum seasonal GSI value followed by a decrease in mean GSI. Prior to conducting the following statistical analyses, we defined weeks 32-38 as spanning the highest GSI measurements in both farmed and wild populations during the three-year study (Fig. 5).

To test our second hypothesis that reproductive investment, as indicated by GSI, differs between farmed and wild scallops, we compared the GSI from farmed and wild scallops during weeks 32-38 during each year and between size classes using Mann-Whitney U tests, which account for unequal sample sizes. Data were determined to be non-normal using Shapiro-Wilk tests. The GSI measurements across all sizes (60 - 119mm), in size classes of 10-mm increments, collected for wild and farmed scallops were compared.

We used Mann-Whitney U tests to test our third hypothesis that farmed scallops have different somatic tissue and shell masses compared to wild scallops from three different size classes (shell heights: 80-89, 90-99, and 100-109 mm) collected during weeks 32-38 over a three-year period. These three size classes represent the highest sampling frequency and the size classes important to management with 80-100mm size classes representing “seed” scallops to recruit to the wild fishery and 101-110mm representing legal size scallops for harvest.

To test our fourth hypothesis that farmed and wild scallop allocate energy differently, we fit linear models to the mophometric data to assess the relationship between the individual somatic tissues (meat mass, gonad mass, shell mass, viscera mass) and the total mass of wild and farmed scallops in three size classes (shell height: 80-89, 90-99, 100-109 mm) during the spawning season (weeks 32-38) each year in 2020–2022. The total mass of each scallop was calculated by summing the viscera mass and the cleaned shell mass. We also included an interaction term in our models to allow our slopes for wild and farmed scallops to vary, testing the hypothesis that the relationship between somatic tissue mass and total mass changes depending on whether scallops are wild or farmed. A greater slope for either wild or farmed scallops suggests a relative difference in energy allocation to the growth of that tissue. We reported the coefficient of determination (R^2), slope (β_1), and how likely a difference in slopes is due to chance (p) for wild and farmed scallops.

Lastly, we used the local regression model function LOESS (LOcal Polynomial RegrESSion Fitting) in ggplot (Cleveland et al. 1992) to explore the smoothed relationships (and 95% CI) among means of gonad mass, meat mass, and time to investigate the timing in which when scallops allocate energy to reproduction and growth of their meat, respectively.

We conducted all analyses in R and tested for statistical differences between groups using a significance level (α) of 0.05 (2023.12.0, R Core Team 2023).

2.4 RESULTS

2.4.1 Gonadosomatic indices (GSI) differed among wild and farmed populations

Farmed and wild scallops show differences in spawning time and magnitude within years and between years. Peak spawn timing, as indicated by peak mean GSI followed by a continuous decline in mean GSI, did not differ more than one week within years and between years and

occurred in weeks 35 - 37 (late August and early September) in each year (Fig. 7). Peak spawn timing between scallop aquaculture sites was no more than two weeks different between sites within years. The magnitude of spawning, as indicated by maximum mean GSI, was greater for wild scallops ($\bar{x}$= 21.5 ± 6.9; W=210909, p<0.0001) than farmed scallops ($\bar{x}$= 18.1 ±7.3) in 2020 (W=17872, p<0.0001) and 2022 (W = 31248, p=0.0004), but not 2021 (W=22277, p=0.3161) (Fig. 7), but there was no difference between wild and farmed scallops in any size class (Fig. 8). Farmed populations of scallops had consistently lower GSIs than wild scallops with maximum mean GSI never exceeding 25% whereas wild populations exceeded 25% in 2020 and 2022 (Figure A.1). There were no differences in GSIs of wild and farmed scallops across all size classes (Fig. 8; Table A1).

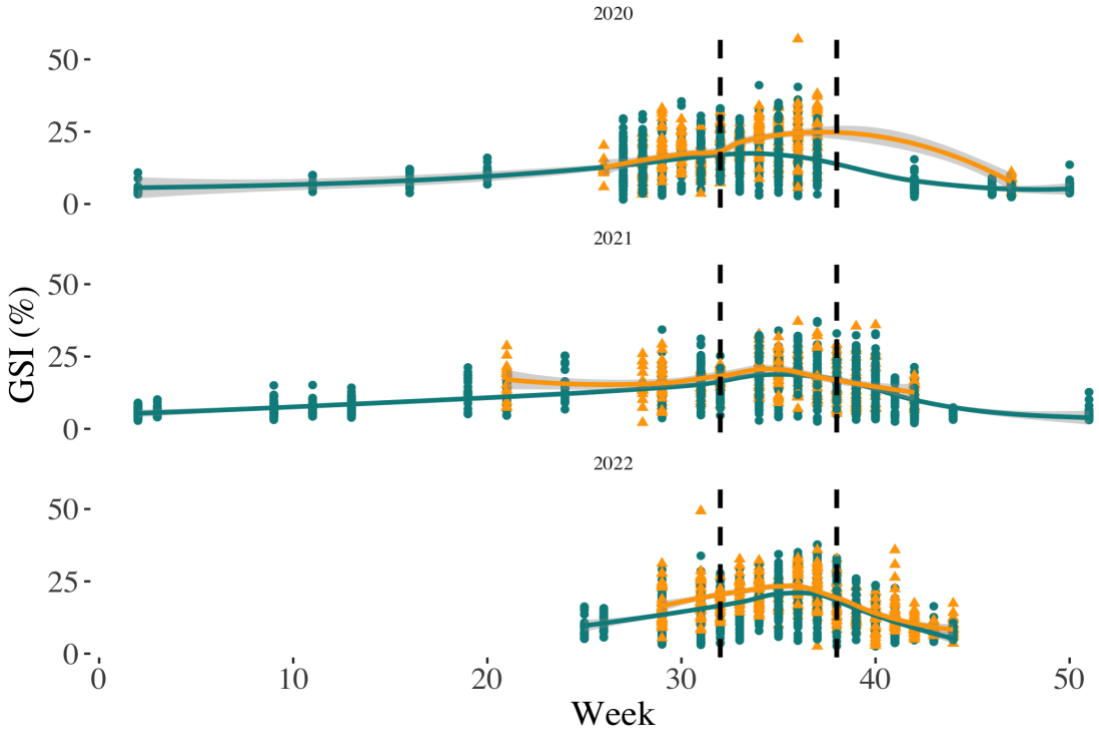


Figure 7. Gonadosomatic indices of wild and farmed sea scallops over time in each year. Mean GSI by year for farmed (green) and wild (yellow) sea scallops (n=4,082) in the Penobscot Bay, Maine, 2020−2022. Dash lines denote weeks 32-38 (n=1,640). Yellow and green lines track

mean GSI values in farmed (green) and wild (yellow) populations with 95% confidence intervals.

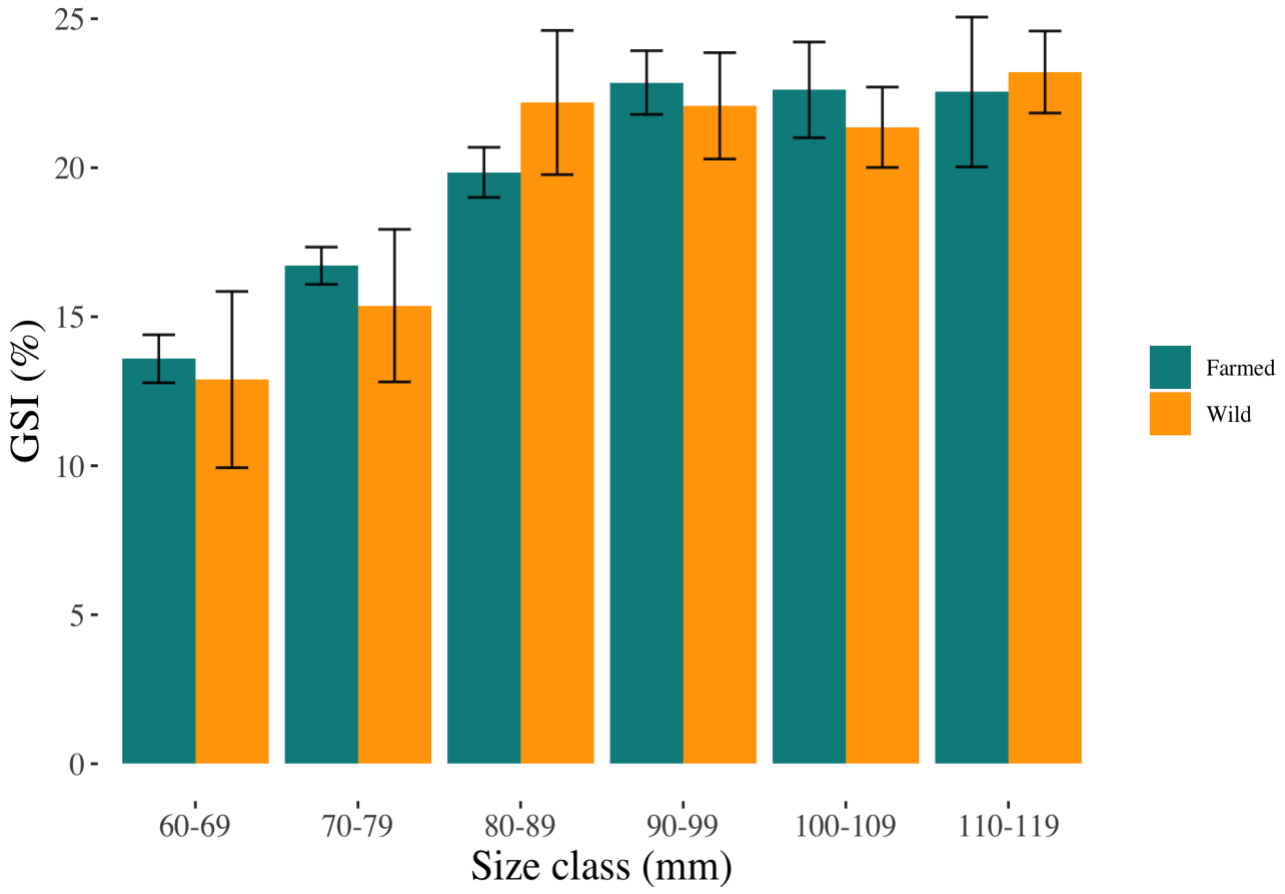


Figure 8. Gonadosomatic indices of wild and farmed scallops across size classes. A comparison of the gonadosomatic index (GSI) for farmed (*n*=1,136) and wild (*n*=504) Atlantic sea scallops at different size classes during the spawning season of this study (weeks 32-38).

2.4.2 Scallop Morphometrics

The meats, gonads, and viscera of farmed scallops (*n*=489) were larger than wild (*n*=167) scallops during the spawning season for all three size classes (*n*=656), except for gonads in the

80-89 size class as determined by Wilcoxon tests (Fig. 9, Table A.2). In contrast, wild scallop shells had greater masses than farmed scallops, for all size classes (Fig. 9, Table A.2).

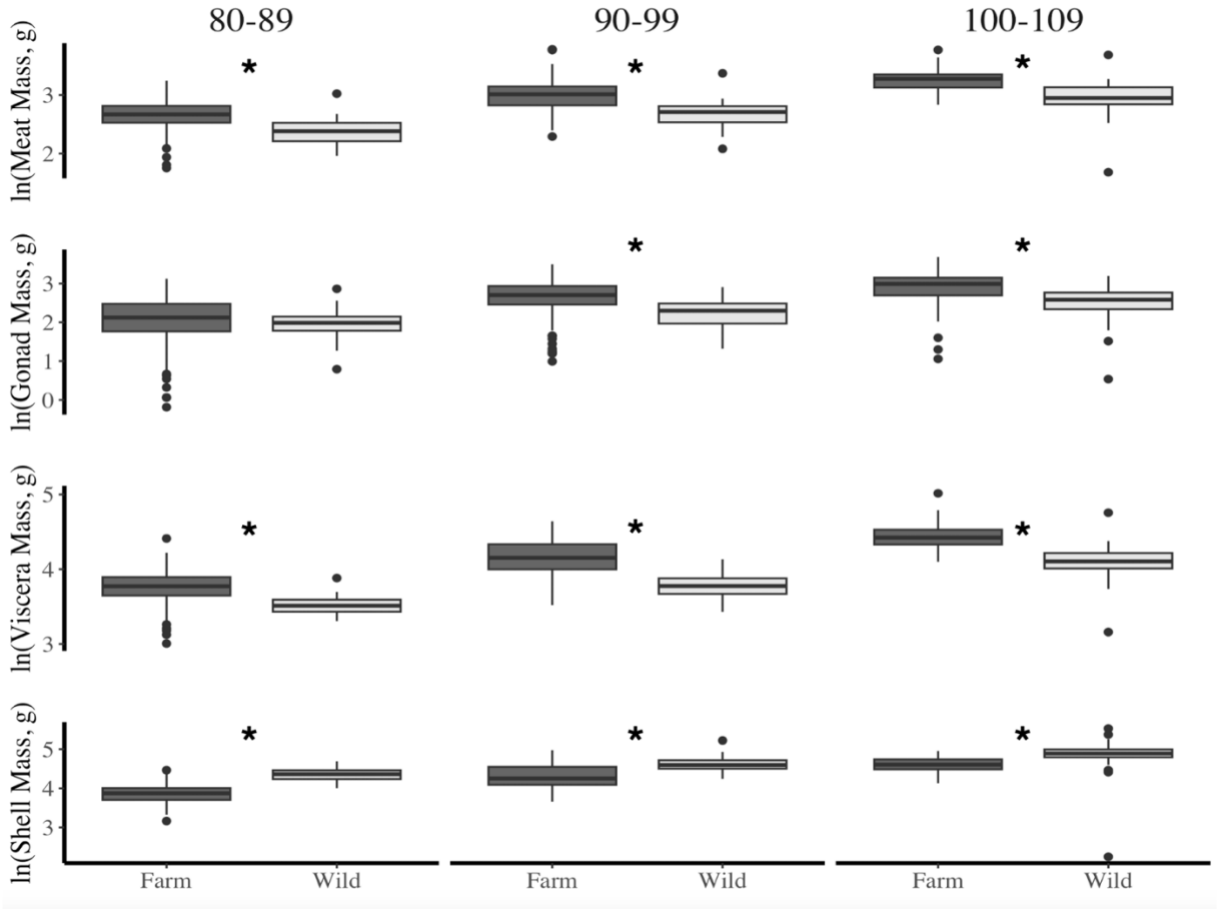


Figure 9. Morphometric comparisons of wild (n = 167; light box) and farmed (n=489; dark box) Atlantic sea scallops for three different size classes (shell height: 80-89, 90-99, 100-109 mm) collected during weeks 32-38 in the Penobscot Bay, Maine, 2020−2022. Asterisks denote pairs that are significantly different (α=0.05); the remaining pair is not different (Table A.2).

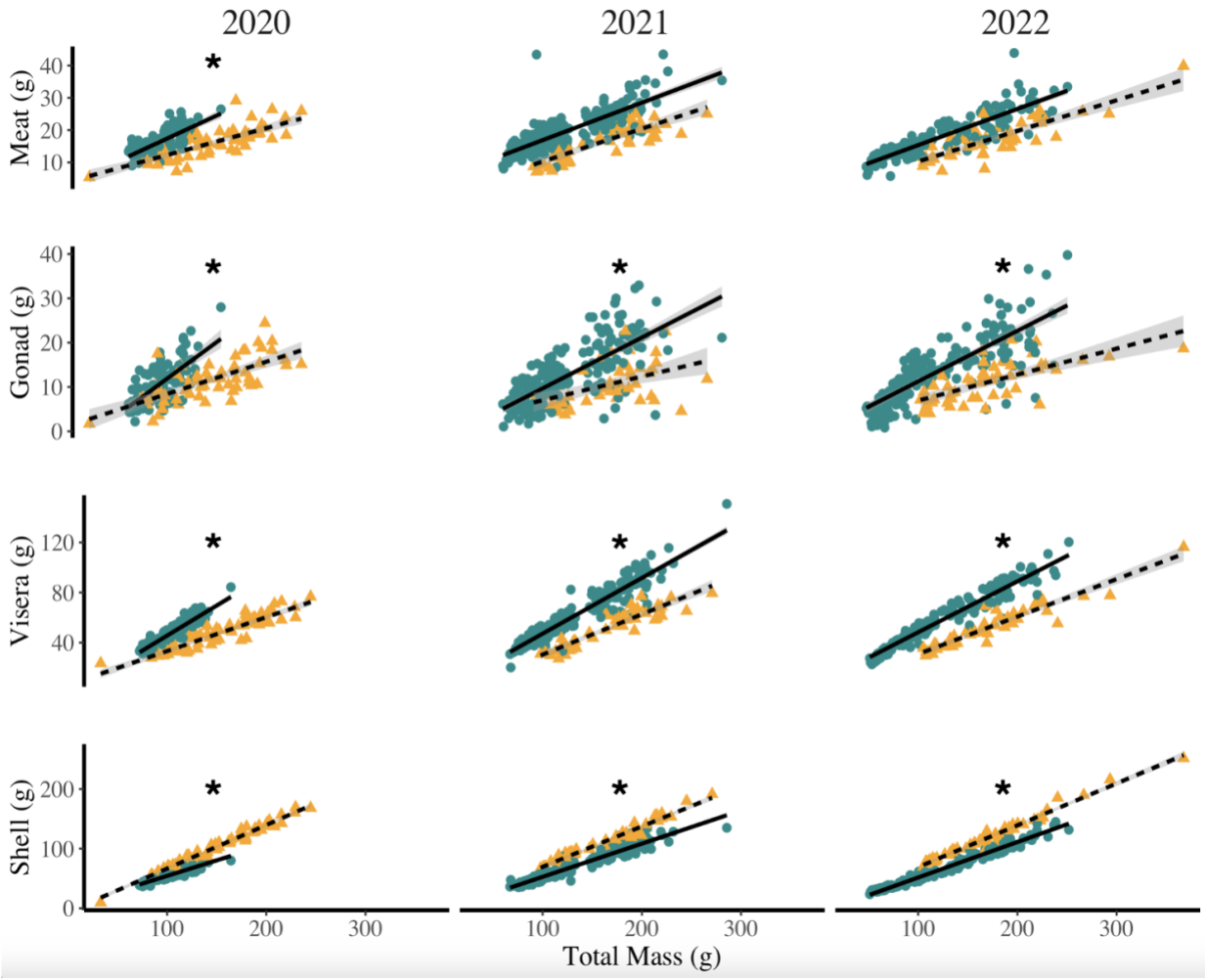


Figure 10. Sea scallop component mass relationships. Linear relationship (and 95% CI) between the scallop component mass (g) and total mass (g) of three size classes (shell height: 80-89, 90-99, 100-109 mm) of farmed (green; n = 167) and wild (orange; n = 489) Atlantic sea scallops collected during weeks 32-38 in the Penobscot Bay, Maine, 2020−2022. An asterisk (*) denotes slopes that are significantly different.

Farmed and wild scallops allocated energy differently, and each also varied in energy allocation across seasons. The ratio of scallop soft tissue mass (meat, gonad, viscera) to overall mass was greater for farmed scallops than wild scallops, with the exception of meat weight for

2021 and 2022 (Fig. 10, Table A.3). Shell mass to overall mass was less for farmed scallops than wild scallops (Fig. 10, Table A.3). We also found that the change in somatic tissue mass and total mass was greater in farmed than wild scallops, whereas shell growth was greater in wild than farmed scallops (Fig. 10; Table A.3).

Lastly, both wild and farmed scallops have increasing meat masses following decreasing gonad masses and farmed scallops appear to dedicate more energy to growing adductor muscle (meat) than wild scallops following peak spawning (Fig. 11).

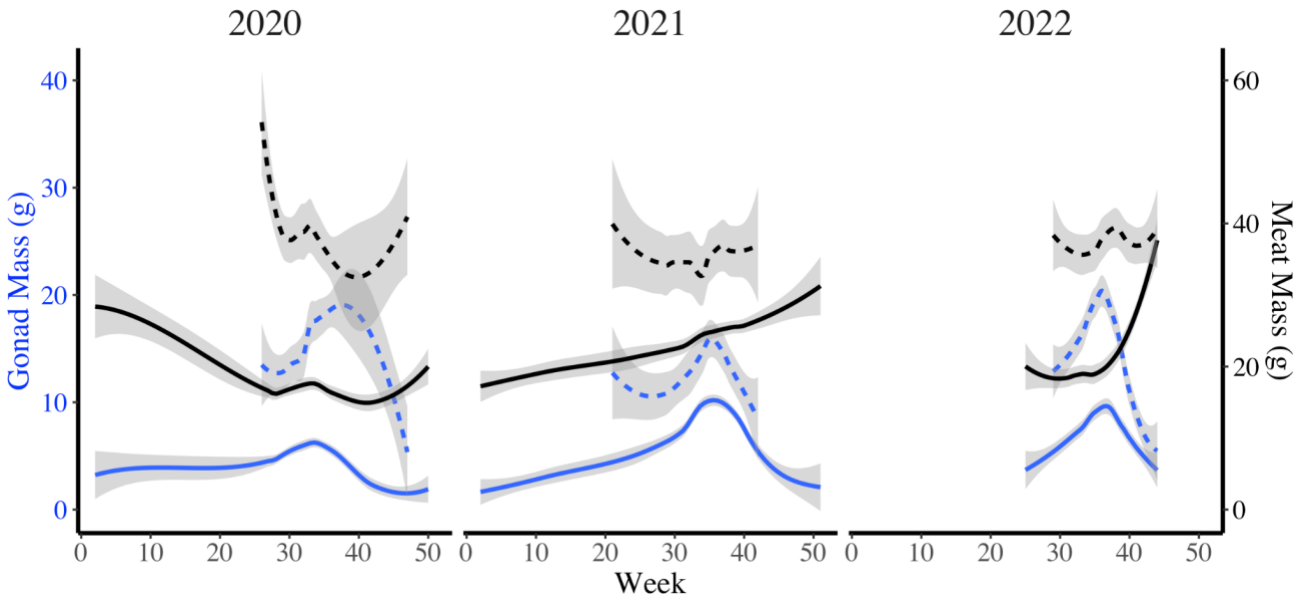


Figure 11. Farmed and wild gonad and meat masses over time. The relationship (smoothed line and 95% CI) between mean gonad (blue) and meat (black) masses for farmed (solid line) and wild (dashed) Atlantic scallops (*n*=3,877) over time in the Penobscot Bay, Maine, 2020−2022.

2.5 DISCUSSION

2.5.1 Spawn timing and magnitude

This dataset represents the most comprehensive collection along the Maine coast of wild and cultured sea scallop GSIs to date and suggests that spawn timing is variable along the coast. The timing and magnitude of spawning are highly variable in both wild and farmed populations of sea scallops in this study, but generally occur at similar time periods in each year (Fig. 7). Generally, within the northern range of sea scallops (Maine and Canada), spawning occurs on an annual cycle, whereas it occurs semi-annually in the southern portions of its range on Georges Bank and the mid-Atlantic Bight (DuPaul et al. 1989; Thompson et al. 2014). Wild and farmed populations of sea scallops in this study had significant differences in maximum GSI between sites. Long-term data on scallop spawning cycles in Maine is lacking. We see similar patterns in populations of farmed and wild sea scallops in Penobscot Bay in this study with differences in overall GSIs and spawn timing of a few weeks. Based on GSI data from multiple studies in the Damariscotta River, Maine, spawning has occurred in wild populations as early as mid-July (Bayer 2016) and in farmed populations of scallops in Casco Bay in late July (NP *pers. comm*.). With a larval period of 35-40+ days, drastic differences in timing of spawning may impact spat distribution and settlement along the coast and has implications for seed collection in the aquaculture industry.

Magnitude and timing of spawning is influenced by environmental variability and can impact the likelihood of a population responding with synchronous versus protracted spawning ("dribble") events (Langton, Robinson & Schick 1987). The high variability of GSI indicates lower synchrony in populations and points to the presence of protracted spawning events whereas low variability indicates a higher likelihood of synchronicity of spawning and resultant

increased fertilization success due to the higher numbers of gametes in the water together (Parsons et al. 1992). Our data suggest a higher likelihood of protracted spawning events due to the high variability of GSI values in wild and cultured populations.

2.5.2 Reproductive investment

As predicted by hypothesis b), we found that farmed scallops invest more energy into reproduction than wild scallops, as indicated by higher GSIs in the majority of farmed populations (Figure 9). The GSIs of farmed scallops are larger than those of their wild counterparts for all size classes with the exception of 80-89mm and 110-119mm size classes. Due to low sampling numbers of wild populations at smaller size classes, these relationships could not be evaluated for significance and require additional research. The GSI is a good approximation of reproductive output without specifically calculating zygote production or additional egg-specific biological data (egg diameter, quality, etc., Parsons et al. 1992). Therefore, using the GSI can support a clearer understanding of the potential reproductive output of an entire population or a specific area for management purposes. Macdonald (1986) found higher reproductive output and effort in farmed than wild scallops in Newfoundland, but this is the sole comparative study for this work. Newfoundland is at the northern range for *P. magellanicus* and the overall somatic and gonad weights are smaller as compared to populations in the Gulf of Maine. The larger reproductive investment from small size scallops on aquaculture farms, as compared to existing literature on GSIs of wild scallops, might influence the potential for aquaculture farms to provide ecologically significant larval output to surrounding wild populations, especially at smaller size ranges. Scallop farmers are attempting to create a new market for a smaller, whole-scallop product ranging from 50-65mm. With these size classes contributing more to reproduction, in comparison to their wild counterparts, and with the high

densities and populations of scallops on aquaculture farms, even the increased output from smaller size classes could contribute to increased larval supply from farms, but this warrants further investigation.

2.5.3 Morphometrics and energy allocation

As predicted by hypothesis c), farmed scallops in this study invested more energy in soft tissues whereas wild scallops invested more energy in hard tissue (shell) across all size classes. There are significant differences in growth rates (mm/day) between scallops grown in different seasons, depth, gear types and stocking densities in aquaculture operations (Grecian, Parsons, Dabinett & Couturier 2000; Coleman et al. 2021). Wild scallops from shallow water in Newfoundland and New Brunswick, Canada, showed increased shell and somatic growth in comparison to those found in deeper waters, with some site-specific variability (Macdonald and Thompson 1988). Scallops from bottom and above-bottom environments in Nova Scotia showed no difference in growth rates while soft tissues of scallops grown off-bottom had 40% larger soft tissues masses than those above-bottom (Emerson et al. 1994). The differences in conditions of deep and shallow water environments are more variable for other species, such as blue mussels, where mussels from shallow sites had larger shell masses and longer shell heights than those from deepwater sites and is potentially more connected to physical disturbance from storms and resulting responses from runoff (Murray, Gallardi & Mills 2019; Gallardi et al. 2017). Wild populations of scallops are distributed from Newfoundland to North Carolina and generally found in depths ranging from 18-100m. There is evidence of them being found in waters as shallow as 2m and as deep as 384m, but this is not typical (see Hart and Chute, 2004). Farmed scallops in this study were raised in “shallow” water in that they inhabited nets hung in 5-10m of water, unlike their deeper-water counterparts in the wild that inhabited 10m and deeper, so these

results are similar to what we might expect in wild populations of scallops from shallow water, but that is beyond the scope of this study.

Overall, the biological indices of individual soft tissues compared to the total mass of all soft and hard tissues was greater for the cultured scallops than wild scallops we sampled. Generally, farmed scallops invest more energy into reproduction than wild scallops as indicated by higher GSIs in the majority of size classes (Fig. 8), significantly larger gonad masses (Figure 9), and steeper slopes in tissue indices (Figure 10). There are seasonal differences in soft tissue generation and contraction, the latter of which generally follows the energetically demanding process of gametogenesis (Figure 11; Robinson et al. 1981). We also observe depletion of energetic reserves available for adductor growth that occurs in many scallop species during gametogenesis and spawning (Barber and Blake 1981; Brokordt and Guderley 2004). The growth environment can have large impacts on energy allocation. The farm-influenced environment may increase the organic matter available for consumption by scallops, potentially resulting in faster growth and increased tissue masses as seen in mussels grown within a salmon farm when compared to those grown outside (Lander et al. 2012).

Differences in soft tissue allocation can produce ecological impacts that affect energetic reserves of other tissues, predator responses, and recovery time from predator avoidance or other physiologically stressing activities. Larger soft tissue masses in cultured scallops resulted in greater numbers of escape claps, longer clapping time and quicker recuperation after an escape response as compared to wild scallops (Lafrance et al. 2003). Scallops assessed by Lafrance et al. (2003) differed in size classes (25-51mm) from those in our study (60-109mm) and shell masses did not differ significantly between cultured and wild scallops, but wild scallops did have significantly stronger shells when tested (Lafrance et al. 2003). Most likely, the larger shell

masses in wild scallops in this study are due to thicker shells, although we did not evaluate this, which can affect the vulnerability of cultured scallops to predators if used in seeding efforts to enhance wild populations. For scallops grown in aquaculture, the impacts of gametogenesis on meat growth, the benefits of larger meats on predator avoidance, or the risk of having thinner shells may be less realized because scallops are not at risk of predation and also do not need to move to find mates as is necessary in wild populations. As scallop aquaculture continues to grow along the coast, increased reproductive investment in and gametic output from farmed populations could result in increased larval output and subsequent larval supply to wild and farmed industries.

Larger meat yields from aquaculture-raised scallops also offer a potentially significant return on investment for scallop growers, while larger gonads suggest an increased potential for reproductive output with ecological ramifications for both aquaculture and wild harvest industries. These results shed light on the complex interplay between aquaculture and the natural environment, highlighting the need to further investigate the ecological consequences of sea scallop cultivation.

These results have important implications for stock assessment and management of fisheries, the potential implications of aquaculture on scallop biology and ecology, and the imminent impacts of a changing climate. Data on reproduction (e.g. monitoring GSI, for example) in wild populations of scallops, at both federal and state fisheries levels, is not widely used as part of management plans or data collection efforts. More recently, there has been an increase in funding for projects to develop reference points in order to understand spawning stock biomass (SSB), determine spawning occurrence, and calculate reproductive output (NOAA 2024). These projects are also working to establish standardized data collection methods for

monitoring reproduction and the biological sampling required for the process. In Maine, reproductive monitoring is not part of the existing survey methods and this research provides the most temporally comprehensive evaluation of reproduction, although the spatial resolution is small. Scallop aquaculture farms provide a space to conduct these collections and, in part, increase our understanding of these dynamics along the coast. Farms are perhaps more accessible than wild populations and could serve as an initial monitoring effort. Eventually, farmed scallops may have to be considered as part of the potential spawning stock biomass for Maine state-managed fisheries, though this is not yet taken into account.

The results from this study suggest that scallops in Penobscot Bay experience protracted spawning events due to the multiple decreases in GSI throughout the spawning season. This could have impacts on fertilization success resulting from a decrease in synchronous spawning, although that has not been evaluated. Aquaculture scallop spawning events may potentially strengthen wild stock-recruitment dynamics in Penobscot Bay, which may feed back into the success of both wild harvest and farming by bolstering seed production. Factors influencing variability in spawn duration and timing in wild and farmed scallop populations may have implications for seed collection in the aquaculture industry. Because aquaculture farms aggregate large, sexually mature individuals in small areas, the recipe for successful fertilization, they could be supporting increased fertilization events and may have similar effects as closed areas and thus could increase sea scallop larval production rates from farms.

Long-term scallop fishery closures have shown multiple direct and indirect benefits to target populations and are currently being explored as a management tool for local populations in Maine (Beukers-Stewart et al., 2005; Cleaver et al., *in prep*). On Georges Bank, areas that were closed to fishing from 1994 to 1999 showed some of the highest densities and largest sea

scallops ever observed on Georges Bank (Stokesbury 2002). Given a known exponential relationship between shell height and gonad mass and greater average shell height in fishing closures on Georges Bank, Bayer et al. (2018) implies that closed regions may be producing substantially more larva per individual scallop than areas open to fishing. Tian et al. (2009) showed that scallop larval dispersion and retention are determined primarily by current systems and locations of adult spawning populations. Because aquaculture farms are generally closed to harvest from other fisheries, are aggregating individuals in small areas, and are still influenced by the same environmental variables governing the wild populations, they may have similar effects as closed areas and allow unique access to explore this and other biological and ecological questions.

Maine's growing scallop industries are situated in a rapidly changing marine ecosystem, the impacts of which are still being determined. Environmental variability and stress have been shown to have negative effects on physiology, egg production, function, and fertilization success in adult marine invertebrates and these effects may be heritable (Pilditch and Grant, 1999; Foo and Byrne, 2017; Pousse et al., 2020). Adult scallops are susceptible to changes in temperature and food availability and their metabolism is tightly coupled to these variables, impacting somatic and gonadal production and resulting in decreases in overall production at high temperatures and low food availability (Pilditch and Grant 1999). Lafrance et al. (2003) suggest that more favorable temperatures and better food supply for suspension culture scallops resulted in larger somatic tissues and higher muscle energetic contents than wild counterparts. Most Maine scallop growers use vertically-suspended lantern nets that are hung from lines submerged 6-10m below the water surface. Therefore, unlike their wild counterparts, scallops in lantern nets are growing within the water column rather than on the benthos, potentially experiencing

different temperature, flow and food supply that result in differences in biological responses; these relationships need further evaluation.

Cultured scallops are exposed to a variety of disturbances during their culture period that their wild counterparts do not experience. As cultured scallops grow, they are graded using an automated grading machine and then sorted between lantern nets to maximize growth rate and farm production. Scallops also undergo cleaning using pressurized water machines once or twice a year to remove biofouling from shells and gear. This sorting, grading, and cleaning process utilizes automated grading and cleaning systems, often exposing scallops to the air for long periods of time and significant movement of scallops among machinery, nets, and vessels. Yesso scallops subjected to abrupt mechanical shock showed decreases in metabolic processes producing amino acids (Tian et al. 2021), while extended air exposure results in hypoxic stress, influences downstream farm production, and may result in reproductive impacts or mortality (Christopherson et al 2008). For these reasons, the farming process itself may induce stress in cultured scallops and, consequently, lead to individual physiological and population scale survival and fitness effects resulting in observable differences in the responses of wild and cultured populations to environmental variability and change.

Farmed scallops may be more resilient to environmental variability than wild counterparts, but not in all cases. Scallops in aquaculture are exposed to more variability in flow, temperature and food availability, including different food types, as a result of growing up in the water column. They may experience more abrupt changes on a more regular basis and definitely do so during the maintenance activities that take place on farms. Perhaps this increased variability will actually increase the resilience of cultured scallops in the face of climate change. Scallops exposed to temperature shifts for short time periods (3h) did not elicit heat shock

protein responses whereas those exposed for longer periods (24h) did (Brun et al. 2008). More recently, studies evaluating the effects of stress experienced by parents suggest that stress exposure can actually increase the tolerance of offspring to environmental variability and that abrupt environmental changes have different effects than more gradual stresses (see Leung, Harvey and Russel 2021 and Foo, Deaker & Byrne 2021). Understanding the effects of environmental variability on parental, and subsequent offspring, fitness are essential to planning for the impacts of climate change. These works also highlight the importance of studies evaluating the effects of climate variability on gametes and how they may experience climate variability differently. For example, sperm requires less energy than eggs to create, so what does this mean both for the experience of the parents and the resulting impact on the gamete and are there sex-related imbalances in responses to change. Or, regardless of parental experience, are there differences in effects of climate variability between eggs and sperm? Eggs from species of two echinoderms both experienced decreases in jelly coats of eggs, which help increase fertilization likelihood during reproduction, when exposed to lower pH (Foo, Deaker & Byrne 2018).

There is much more work to be done to understand implications for gametes. Additionally, populations may vary substantially in their genetic makeup and may differ in their responses to environmental variability (Owen and Rawson 2013). The genotypic variation in sea scallop populations from Western, Midcoast, and Eastern coasts of Maine differ substantially - where populations in the western Gulf are homogeneous, those in the East are differentiated, and those in the Midcoast are different from either West or East. Understanding the phenotypic variation and responses to environmental variability in these diverse populations will inform our

comprehension of the implications of moving sea scallop spat to disparate environments along the coast and the potential impacts of climate change.

2.5.4 Future Work

Further research should be conducted to understand the association between scallop farms, fertilization success, and potential larval output. Future work quantifying larval output from farms can be incorporated into biophysical models to further understand how aquaculture may be influencing population connectivity of wild and cultured populations. Coast-wide efforts should be made to monitor reproduction on scallop aquaculture farms as well as in wild populations. These efforts would require coordination between fishermen, farmers, managers, and researchers to ensure the success of wide-spread data collection efforts but would provide a consistent data stream while deepening relationships between these entities. Additional work should also address the impacts of environmental variability on farmed and wild populations of scallops and the biological and ecological implications of this change in both lab and field settings.

CHAPTER 3

QUANTIFYING eDNA OF SEA SCALLOP LARVAE AND ADULTS IN A LABORATORY SETTING

3.1 ABSTRACT

Environmental DNA (eDNA) can offer a non-invasive, cost-effective and efficient method for monitoring aquaculture and commercial fisheries populations to inform sustainable fisheries management practices. eDNA tools must be thoroughly ground truthed to determine best practices for their appropriate application. While quantitative eDNA assays for sea scallops (*Placopecten magellanicus*) have been developed and calibrated for sperm and dockside conditions, we lack quantification rates of scallop eDNA generation and degradation, and calibration for other life stages. Here we applied qPCR methods to quantify the eDNA signals from different life stages and densities of scallops in laboratory settings. We conducted multiple larval dilution experiments to establish a linear relationship between larval numbers and resultant gene copy numbers, establishing an average estimate 3.09×10^7 gene copies per individual. We also conducted a controlled mesocosm experiment to quantify eDNA shedding rates of scallops and relate these rates to different biomasses of non-spawning scallops in mesocosms. There is a significant relationship between biomass and peak gene copy values as determined by biexponential five parameter (5p) modeling. This is the first experiment to our knowledge that evaluates DNA shedding rates and identifies relationships to biomass and larval concentration in sea scallops. These relationships will help to inform field sampling efforts and interpreting data from natural experiments.

3.2 INTRODUCTION

Environmental DNA (eDNA) has the potential to be used for adult stock assessments, larval transport models, and to estimate recruitment potential, provided patterns in eDNA occurrence and their significance are well understood (Alexander et al 2021, Kirtane 2021). Sampling designs and species-specific applications require thorough and vigorous ground truthing and evaluation of eDNA tools to determine their limitations, ensure their accuracy, and refine their applications (Rojahn et al. 2023). A continuous challenge in determining appropriate uses for eDNA tools is to understand the sources of eDNA, the rates at which that DNA is generated and degraded, and the processes influencing both.

Environmental DNA originates from many sources in marine systems and is influenced by biological, chemical and physical processes. Organismal DNA is generated by whole live organisms (e.g., zooplankton) and considered to be high quality because the origin of DNA is clearly known (Rodriguez-Ezpeleta et al. 2020). Linear relationships between gene copy number and numbers of whole live organisms, such as copepod nauplii or bivalve sperm, have been established using quantitative PCR methods and applied to assess food availability and reproductive processes in dynamic marine systems (Jungbluth, Goetze & Lenz 2013; Bayer, Countway & Wahle 2019). Extra-organismal DNA sources include (i) biologically shed materials (e.g. scales, tissue, or waste), (ii) biologically active propagules (e.g. gametes), and (iii) DNA resulting from physical or chemical cell lysis or extrusion that is free or adsorbed onto another surface (e.g., sand) (see Rodriguez-Ezpeleta et al. 2020 for review). Detection of extra-organismal sources of DNA are more variable because of the diversity of extra-organismal sources and the interactions of this DNA with the environment. Depending on the target organism, many eDNA samples from field collections often consist of a complicated mixture of

both organismal DNA and extracellular DNA resulting from complex biological and physical environmental interactions. Therefore, it is essential to have a foundational understanding of the potential sources of organism-specific DNA when developing research questions and methodologies.

An understanding of the "ecology of DNA" (the origin, state, transport and fate) in marine systems is necessary for the appropriate development and application of eDNA tools and the interpretation of sampling results (Barnes and Turner 2016, Figure 12). In marine and aquatic systems, the generation, degradation, transport and retention of DNA is influenced by biotic and abiotic environments, organismal biology and life history, and hydrological characteristics of the system (Stewart 2019; Harrison et al., 2019). DNA generation, or more appropriately shedding, rates are impacted by factors such as stress, age, diet and temperature and primarily originates from organismal excretion, secretion, and decomposition (Harrison, Sunday & Rogers 2019). Shedding rates can differ significantly between species and are highly influenced by life history events, such as spawning (Kirtane et al. 2021; Troth et al. 2021). Shedding rates also are influenced by life history phase and DNA signals can increase with larval size and developmental stage (Clemmensen 1994; Doyle, McKinnon & Uthicke 2017). Therefore, the presence of different developmental stages (i.e., trochophore larvae) or sizes of larvae in a sample can complicate the interpretation of eDNA results. Organismal morphologies, such as the presence or absence of a shell or exoskeleton, size, and activity levels also impact the detection of DNA shedding (Pierce 2020; Wood et al. 2020; Sassoubre et al. 2016). For species-specific applications, a clear understanding of the shedding rates and the DNA quantities from potential sources of DNA for the species of interest is required to successfully apply eDNA as a tool.

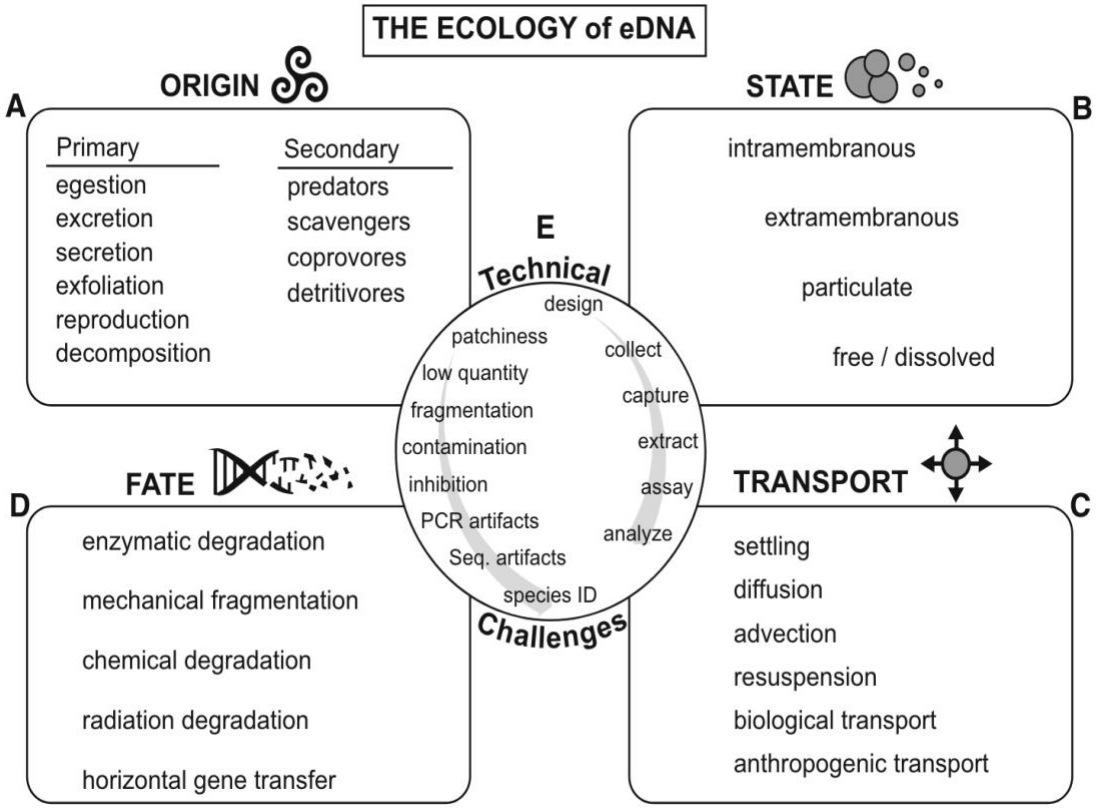


Figure 12. The ecology of eDNA. Knowledge of the processes and properties within four domains of eDNA ecology (a–d) and key technical challenges (e) can guide eDNA conservation and research applications. Reprinted with permission from Barnes and Turner (2016).

Generation and degradation rates in different systems and environmental influences can vary. Degradation rates are generally higher in marine systems than in freshwater systems with temperature as a key driving factor over pH or dissolved oxygen (McCartin et al. 2022; Lamb et al. 2022). Physiochemical changes, such as temperature and salinity, may affect eDNA shedding or detection, but changes in tides have little to no impact on community composition (Kelly et al. 2018). The breakdown, dilution and dispersal of eDNA in marine environments, which is influenced by both species life histories and environmental impacts, can limit research

applications (Ruppert et al. 2018). Bacteria and light may also influence the longevity of eDNA in the water column, and thus limit detectability, especially of motile animals (Dejean et al. 2011; Strickler et al. 2015; Tsuji et al. 2017; Salter 2018). Physical effects, such as stirring of sediments, may lead to an increase in detectable eDNA resulting from long-term DNA settling or decreased degradation in sediments (Turner et al. 2015). All these factors interact to influence the availability and longevity of eDNA in a system, subsequently impacting the application of such genetic tools (McCartin et al. 2022; Joseph et al. 2022).

For eDNA to be applicable to monitoring and managing commercially important marine species, these challenges need to be evaluated on a species-level and the impacts of life history taken into consideration. Our focus here is on sea scallops, one of the leading fisheries on the US Atlantic coast (as detailed in Chapter 1). Quantitative eDNA assays for sea scallops specifically have been developed based on ITS gene fragments (Bayer et al. 2019). Bayer and colleagues (2019) established that gene copy number, determined through quantitative PCR (qPCR) primers and size fractionation protocols, can be used as a proxy for sea scallop gamete number, specifically sperm, in the water column. These relationships have not been validated for sea scallop eggs or larvae. As noted in Bayer et al. (2019), the attempts to develop a linear relationship between cell counts and gene copy numbers for eggs were unsuccessful. We also lack quantification of generation and degradation rates of scallop eDNA in a laboratory setting.

In light of these knowledge gaps, the main objectives of this work are to (1) quantify relationships between scallop larval density and DNA copy number, (2) quantify eDNA shedding and degradation rates of scallops, and (3) relate these rates to the biomass of non-spawning scallops in mesocosms. We expect to find positive linear relationships of larval densities with gene copy number and an increase in the mean amount of DNA shed over the

lifetime of the experiment with increasing scallop biomass. We expect the average shedding rate (copies/hour/gram) to increase with increasing biomass and DNA degradation to occur more quickly at lower biomasses than at higher biomasses. Together, the results of these experiments support interpretation of eDNA signals generated by larval and adult scallops and inform sampling practices that use eDNA to monitor biological processes, particularly in the context of ecosystem-based fisheries management of sea scallops.

3.3 MATERIALS AND METHODS

3.3.1 Larval dilution experiment

Sea scallop larvae at the straight-hinged stage (aged 4 days) were collected from the Mook Sea Farm hatchery in Walpole, ME (43.976462, -69.558282) at a density of 319 larvae/mL. A total of 100 ml of concentrated larvae (31,900 total) were collected and diluted in 1500 ml of 1µm filtered and UV-sterilized seawater. One liter of the filtered and UV-sterilized seawater also was collected to determine the background scallop DNA signal within the filtered seawater.

From the diluted well-mixed 1,600 ml larval sample, a 200ml subsample was collected in a 250ml hydrochloric acid-washed container and serially diluted four times before filtering onto 0.2 um, 47 mm diameter Whatman Supor filters. This dilution and collection process was repeated 4 times.

3.3.2 Adult shedding and degradation experiment

To evaluate the shedding and degradation rates of sea scallop eDNA we conducted controlled mesocosm experiments from April 3-6, 2024. We used the large mesocosms in the flowing seawater suite at the Bigelow Laboratories for Ocean Sciences in East Boothbay, ME (43.854360, -69.629100). Prior to the experiment, the 2,460L tanks (0.61m x 2.44m)

were filled with 500L of 10% bleach solution and all tank surfaces were cleaned in order to remove any prior eDNA signals. This solution was pumped through the recirculation pipes for 10 minutes and the bleach solution was drained from the tanks. Tanks and recirculation pipes were then thoroughly rinsed with freshwater and 500L of recirculated freshwater for 10 minutes and then filled with 1 μm-filtered and UV-sterilized seawater to a starting volume of 2261 L in each tank.

Scallops were sourced from the experimental research scallop aquaculture farm at the University of Maine Darling Marine Center (69.583237 W, 43.930808 N) in Walpole, ME, approximately three miles upriver from Bigelow. All scallops originated from wild spat procured from wild-set spat bags and were within the 65-85 mm size class. This size class is equivalent to the size of marketable farmed scallops and wild-fished "seed" scallops. Two days prior to the experiment, we cleaned scallops of all biofouling and held them in open circulation flowing seawater tanks at Bigelow to control for disturbance effects from cleaning and transport to the laboratory. To decrease the chance of a spawning event occurring unexpectedly and confounding the experiment, the scallops were sorted to verify that they did not have ripe gonads.

The filled mesocosms were cooled to the ambient temperature of 6-7 ℃ to simulate the home environment and decrease chances of stress on the scallops. This temperature was maintained throughout the entirety of the experiment using an Aqua Logic Cyclone Drop-In Chiller and recorded at each sampling time. Water was recirculated in the mesocosms using an Iwaki America MD 100-RT pump at a rate of 20 gpm resulting in full tank turnover approximately every 33 minutes. In addition to the built-in recirculation system, water was circulated in the tank using two Hydor Koralia circulation pumps to ensure mixing

throughout the entire mesocosm. Each tank was equipped with an air stone to maintain sufficient oxygen levels for scallops. To decrease potential starvation effects on eDNA shedding rates and simulate field conditions as best as possible, scallops were batch fed an equal mixture of *Rhodomonus* (red algae), *Pavlova* (brown microalgae), and *Thalassiosira* (diatom) plankton to maintain a 50,000 - 100,00 cells/mL density at total of 3 times over the duration of the shedding experiment at Time 0 (prior to introduction of the scallops), 12 hours and 24 hours. The exception is that the highest biomass tank of scallops was drip fed, rather than batch fed, because of the high density of scallops in the tank.

A total of six mesocosms were used for this experiment: five experimental tanks and one control tank. Prior to introducing the scallops, source water samples were taken from each tank to establish baseline DNA signals prior to the start of the experiment. This collection also counted as the "Time 0" sampling event. Scallops were randomly selected from the holding tanks and distributed in five lantern nets ranging from high to low densities, representing high to low biomasses of scallops and distributed between the five experimental tanks (Table 1). I estimated the biomass of scallops in each tank based on average meat weight of scallops within the 65-85 mm size class from previously collected data on farmed scallops at the Darling Marine Center experimental research farm. The control tank contained an empty lantern net with identical pump, circulation, and temperature set up. To determine eDNA shedding rates, the water in the tanks was sampled at 1, 2, 4, 8, 16, and 32 hours.

At each sampling time for each experimental tank, three sterilized and labeled 500mL samples bottles were triple rinsed with water from the recirculation pipe, filled, and placed on ice until all sampling for that time point was concluded. Samples were then

filtered onto 0.2µm, 47mm Supor filters following methods from Bayer, Countway, and Wahle (2019). Filters were rolled into 2 ml sample tubes and frozen at -20 ℃ until extraction. All bottles were sterilized between sampling events in a 10% bleach bath and thoroughly rinsed using first fresh water and then deionized water in triplicate. All filter components and forceps were also sterilized between filtrations in 10% bleach bath and thoroughly rinsed in fresh water and deionized water in triplicate.

Table 1: Mesocosm experimental set up. Tank IDs, numbers of scallops, and total biomass per tank (n=320).

Density	**High**			**Low**		**Control**
Tank ID	A	B	C	D	E	F
Scallops/level	16	8	4	2	1	0
Total scallops/tank	160	80	40	20	10	0
Biomass (g)	1,260	560	280	140	7	0

Water quality data was collected using a YSI EXO Sonde to monitor temperature, oxygen, and chlorophyll levels in each of the tanks at 0, 2, 8, and 32 hours. The sonde was held just below the surface of the tank and then lowered to the bottom of the tank to ensure each tank was well-mixed. To protect the integrity of the sensors we could not rinse the Sonde using a 10% bleach solution and was instead rinsed with deionized water between each tank data collection and sampling started with the control tank followed by ordered sampling from lowest density tanks to highest density tanks.

Due to the power outage at 32 hours after the initiation of the experiment, we had to modify the degradation experiment. To conduct a modified degradation experiment, 54 L of water from each of the experimental tanks was transferred to individual sterilized 75 L tanks in a water table. Air stones were added to the new experimental tanks and water temperatures were maintained using a flow-through bath of filtered seawater within the water table. Initiation of the modified experiment occurred 5.5 h after the power outage and an initial sample was taken from each of the larger experimental mesocosms during the transfer to the smaller tanks in order to represent a Time 0 sample for the modified experiment. The sampling and filtering procedures were repeated at 2, 12, 18, 24 and 36 hours and all disinfecting procedures, as described above, were followed between each sampling event.

3.3.3 DNA Extraction and qPCR processing

DNA extraction from the larval dilution experiment took place at the University of Maine Coordinated Operating Research Entities (CORE) molecular lab in Orono, ME, using the PowerWater DNA extraction and purification kit (Qiagen) following the modified methods outlined in Bayer et al. 2019. Thermal cycling of these samples also took place at the CORE and the methods followed those of Bayer et al. (2019).

DNA extraction and thermal cycling of the adult scallop shedding and degradation rate experiments took place at the University of New Hampshire Collaborative Core Wet Lab in Durham, NH. DNA was extracted using the Qiagen DNeasy 96 PowerSoil Pro Kit (Cat# 47017) following a modified manufacturer protocol. Frozen filters were transferred to Qiagen PowerWater DNA Bead Tubes (Cat# 14900-50-NF-BT) with 800 ul of solution CD1 from the PowerSoil Kit. Tubes were homogenized on an HG-400 MiniG homogenizer (Cole-Parmer) at

1200 RPM for 10 minutes. This was repeated three times for 30 minutes of bead-beating. Tubes were then stored at –20 °C until extraction. Tubes were thawed, centrifuged at 4121 x g for 6 minutes, and 700 ul of supernatant was transferred to collection tubes. From there, extraction followed the manufacturer's protocol starting with the addition of buffer CD2. DNA concentration was measured with a Qubit Flex Fluorometer (Invitrogen Cat# Q33327) using the 1x dsDNA High-Sensitivity assay (Invitrogen Cat# Q33231).

3.3.4 Analysis of larval eDNA data

Using the standard curve that generated from a linearized plasmid dilution series (after Bayer et al. 2019), the Cq values (qPCR threshold cycle number) resulting from cell-based DNA samples were converted to gene copy numbers to investigate the relationship between gene copy number and egg or larval abundance using linear regressions and estimate gene copy values per larvae. The contribution of DNA from the source water was determined from qPCR analysis and subtracted from the calculations. Linear regressions were used to evaluate the relationship between larval concentrations and resulting gene copy values from the dilution series.

3.3.5 Analysis of adult scallop eDNA data

We first evaluated data from each tank for extreme outliers following methods outlined by Klymus et al. (2015) because eDNA often has large variability, often associated with heterogeneously distributed particles containing eDNA. The JMP (version Pro 16.0) program was used to identify outliers as points 1.5 times the interquartile range and those points were removed prior to calculating average eDNA shedding rate for each tank. Using Shapiro-Wilk tests, the distribution of gene copy data was determined to be non-normal. Prior to transformation, mean copies L^{-1} were calculated for each biomass level. Kruskall-Wallis H tests

were used to evaluate for statistical differences in gene copy numbers among biomasses and the nonparametric Dunn's pairwise test was used to determine differences between biomasses.

DNA generation and degradation rates were evaluated by fitting the data for each biomass over time to a series of exponential growth and decay models, respectively, to determine if rates and peak DNA generation concentrations were related to initial scallop biomass. We used the best fit model to visualize temporal patterns in gene copy values and identify peak DNA generation points, time at which peak generation occurred, and asymptotic equilibrium for each biomass level. For degradation rates, the goal was to determine if degradation rates scaled with initial biomass. Using AICc and BIC criteria and r-squared values, a biexponential five-parameter decay model (Biexponential 5p) was determined to best characterize the time-dependent response of DNA generation and degradation for each biomass. This model allows us to capture both the fast and slow decay components of DNA generation and decay, providing a more nuanced understanding of its behavior over time. The model is defined by the following equation:

$$y(t) = a - (b \cdot e^{-c \cdot t} + d \cdot e^{-f \cdot t})$$

where:

- a represents the asymptote, indicating the value that $y(t)$ approaches as time increases.
- b and d are scale factors that modulate the contribution of the two exponential decay components.
- c and f are the decay rates for the first and second exponential terms, respectively.
- t represents time, measured in hours.

The maximum gene copy values were determined from the generation curve by applying the model output to the above equation and applying individual hours to the equation, rather than just the hours at which sampling occurred. This provided a prediction of gene copies generated at each hour time point and allowed us to identify the maximum gene copies and the time at which they occurred. To determine the time at which the asymptote was reached in the degradation process, we again applied the model outputs from the degradation to the above equation and solved for each hour.

To determine if shedding rate increases with adult scallop biomass, we calculated mean shedding rate, eDNA per unit biomass in copies/hour/gram (C/h/g), following methods from Ruiz-Ramos et al. (2024), where S *(shedding rate)* $= F/b$, where F = mean eDNA flux (C/h), and b = the total scallop biomass in grams (g).

3.4 RESULTS

3.4.1 Larval dilution experiment

As predicted, in the larval dilution experiment, higher larval concentrations were associated with higher gene copy numbers (Linear regression: $F = 159$; $r^2=0.90$; $p < 0.0001$; Fig. 13). The larval concentrations in the dilution series explained 90% of the variation in gene copy value. One data point became negative after being adjusted for gene copy number contribution from source water and was removed from the analysis. With the exception of the removed data point, all samples in the larval dilution series had gene copy values greater than 8.7×10^7. The spread of the data points around the trendline indicates variability among the replicates. The estimated average gene copies per individual larvae was 3.09×10^7 (Table 2).

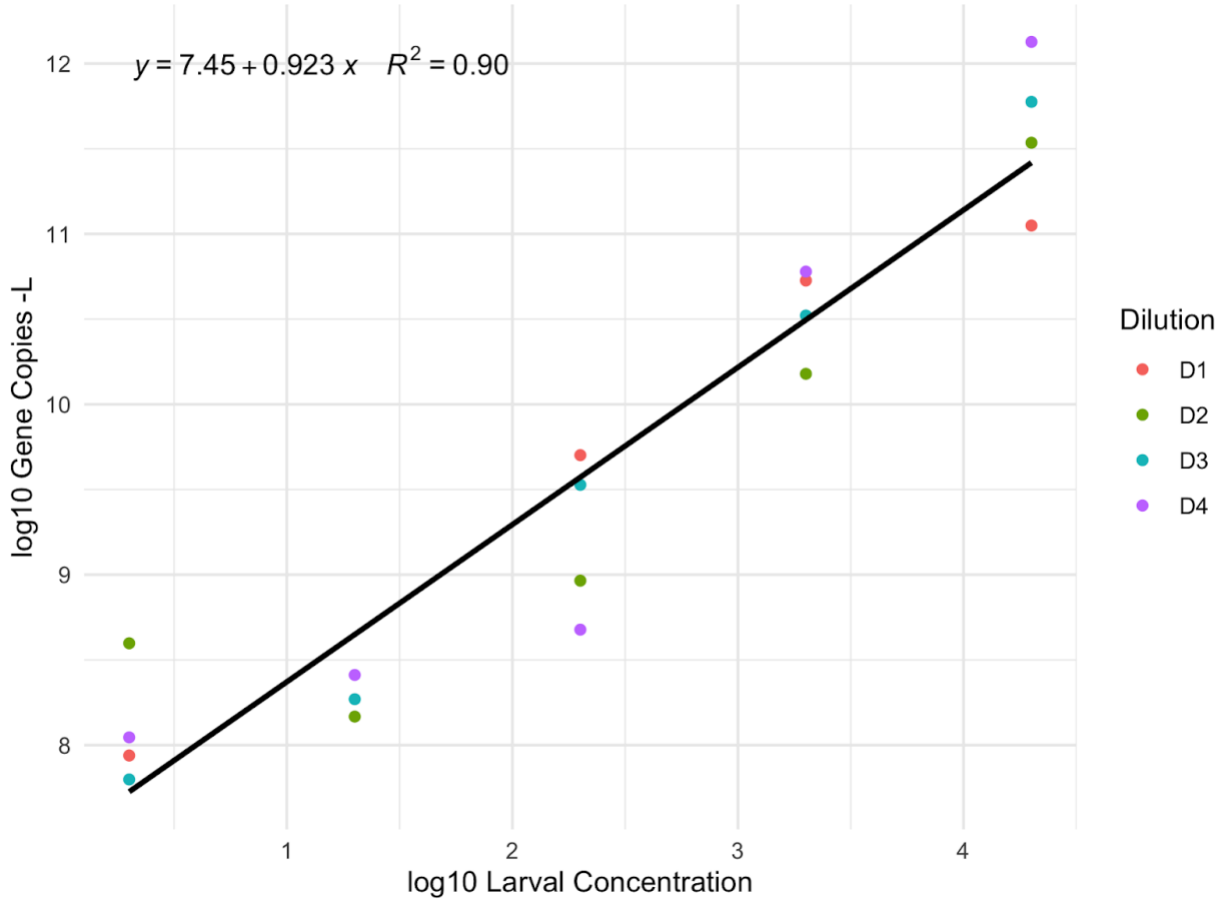


Figure 13: Relationship between larval concentration and gene copies. Larval concentration and gene copy number are linearly related, as demonstrated by this linear regression of scallop larvae concentrations and gene copies L^{-1}. The four replicate dilutions (D1-D4) are annotated in different colors. All values were log-10 transformed.

Table 2: Estimated larval concentrations of dilution series. Estimated larvae per dilution, gene copies per replicate of each series in the dilution, average gene copy values, standard deviation of the average, and gene copies/larvae calculated from each larval dilution series (n=4).

Larvae/ Dil	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average	St Dev	copies/larvae
20000	1.12E+11	3.43E+11	5.96E+11	1.34E+12	5.99E+11	5.35E+11	2.99E+07
2000	5.33E+10	1.51E+10	3.32E+10	6.01E+10	4.04E+10	2.04E+10	2.02E+07
200	5.03E+09	9.23E+08	3.37E+09	4.76E+08	2.45E+09	2.14E+09	1.23E+07
20	NA	1.47E+08	1.86E+08	2.58E+08	1.97E+08	5.62E+07	9.85E+06
2	8.69E+07	3.96E+08	6.29E+07	1.11E+08	1.64E+08	1.56E+08	8.21E+07

3.4.2 Adult shedding and degradation experiment

Patterns of DNA generation for different biomass levels are variable (Fig. 14). All biomass treatments show initial peaks in DNA concentration, but the magnitude and timing of these peaks differ among biomass levels. Peak DNA concentrations occurred within the first 10 hours of data collection across biomasses, with the exception of the 70g biomass which occurred at the 16-hour sampling event as determined by the highest average gene copy values occurring at this time.

For each biomass, r-squared values are higher for degradation results in comparison to the generation results. With the exception of the 560g biomass, degradation approached asymptotic values within the first 10-16 hours of the experiment (Figure 14). Gene copy number decreased for all biomasses over time with the highest biomass (1120 g) having the lowest

percent change between mean gene copies at Time 0 and Time 6 (Table 3). The 140g biomass level experienced an 88% decrease in gene copies from Time 0 to Time 6.

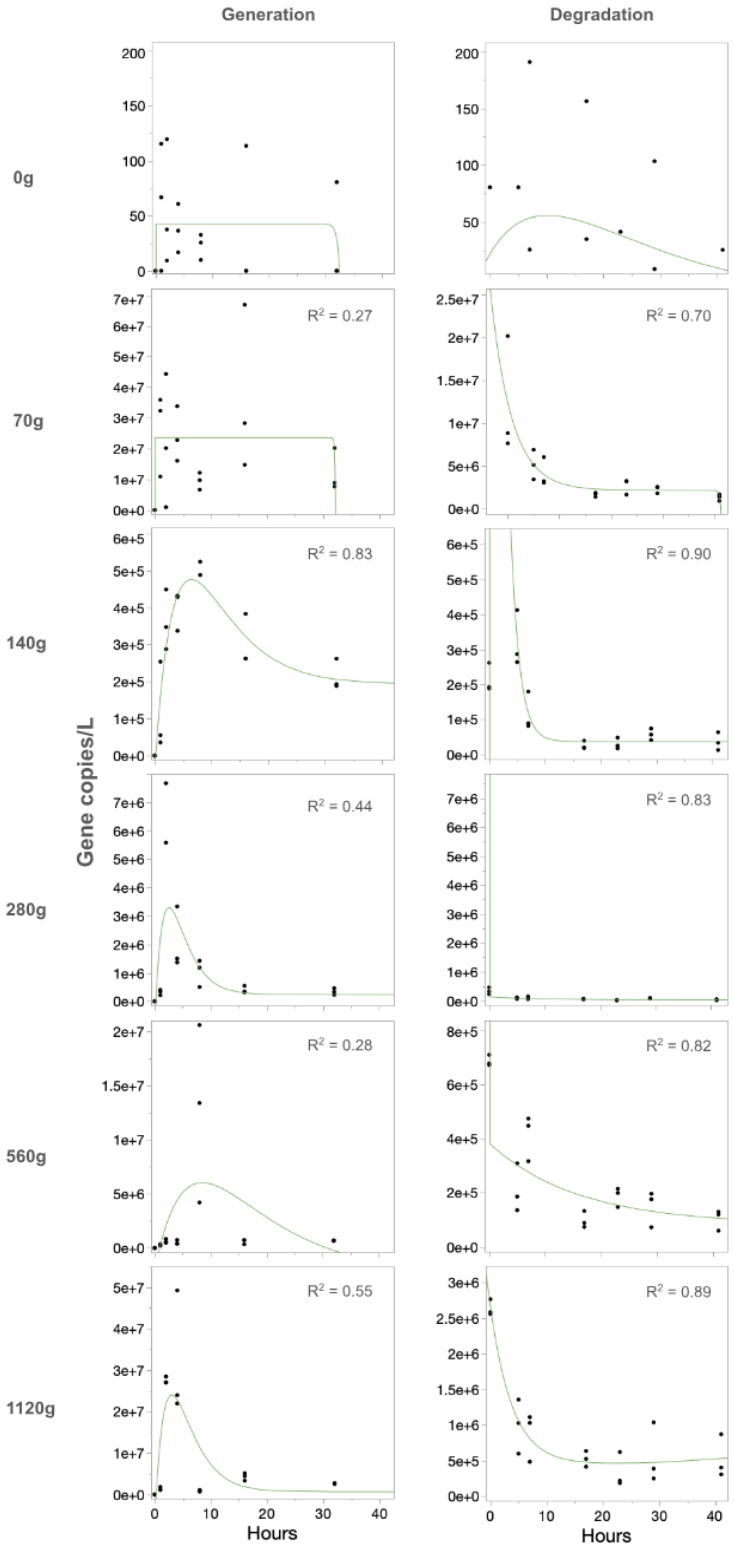


Figure 14 (previous page): Biexponential 5p growth and decay models. Relationships between DNA generation (left column) and degradation in Gene copies L^{-1} over time (hours) for different biomass (grams) levels in mesocosms. Data for controls is included (biomass = 0). Average gene copy L^{-1} for each sampling replicate at each time point is shown. Note that all scales are not the same for generation and degradation for each biomass and r-squared values are included in the figure for each biomass.

Table 3: Decreasing mean gene copies over time. Mean gene copies L^{-1} decreased from Time 0 to Time 6 for each of the biomass levels of the degradation experiment as indicated by the Kruskall-Wallis H test results and the percent change in mean gene copy number.

Biomass (g)	**T0 Mean Copies L^{-1}**	**T6 Mean Copies L^{-1}**	**H**	**df**	**p**	**Percent Change**
0	29.27	9.22	2.48	5	0.78	68%
70	5145415.27	1316583.61	48.67	5	0.0001	74%
140	321059.98	36643.05	55.73	5	0.0001	88%
280	88376.11	34620.01	55.41	5	0.0001	60%
560	209746.84	102925.18	50.25	5	0.0001	50%
1120	994230.91	527551.90	29.80	5	0.0001	46%

Overall mean shed gene copies L^{-1} values varied among different biomass levels (Kruskall-Wallis H test: $H= 223$, $df = 5$, $p < 0.0001$) and generally showed an increasing trend with biomass (Fig. 15 , Table 3). Unexpectedly, the lowest biomass of 70g (10 scallops) had the highest mean copies L^{-1}, while the remaining mean copies L^{-1} values increased with increasing

biomass. Estimated shedding rates generally increase from low biomass to high biomass, with the exception of the 70 g biomass (Table 4). Standard deviations for the estimated shedding rates were high, indicating the high variability in the values within each biomass level. Biomass explains 26% of the variation in peak gene copy values as predicted by the model (Linear regression: $F = 181$, $r^2 = 0.395$, $p = 0.001$) with the 70g biomass included and 93% of the variation with the 70g biomass excluded (Fig. 16). All biomasses reached peak gene copy generation values prior to the 10-hour sampling event (Table 5).

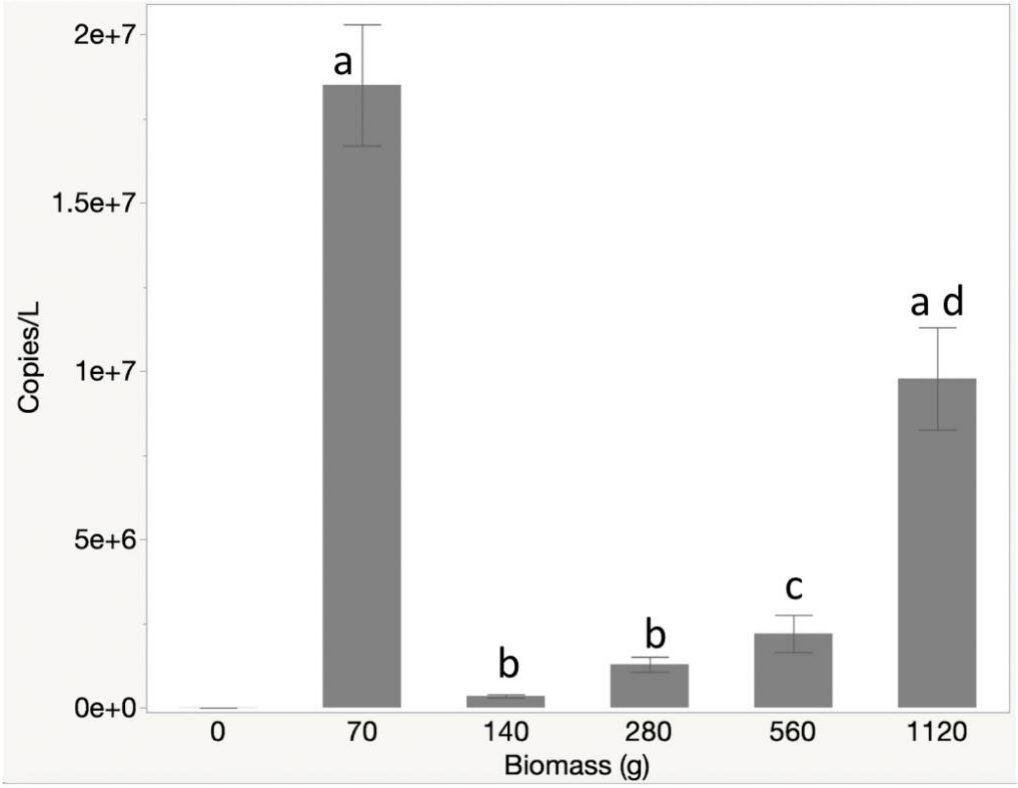


Figure 15: Overall average of gene copies per biomass. With the exception of the 70g (n = 88) level, total mean gene copies L^{-1} generated over the duration of the experiment increase with biomass for 140 (n = 84), 280 (n = 80), 560 (n = 84) and 1120 (n = 84) levels in mesocosms. Standard error is indicated and the letters a-d indicate significant differences (as indicated by Dunn's pairwise tests) among the biomass levels.

Table 4: Estimated biomass shedding rates. Estimated shedding rates (gene copies/hour/gram) and standard deviation for each biomass level.

Biomass (g)	Copies/h/g	St. Dev
70	9559.3	7302.4
140	90.2	96.7
280	169.1	231.9
580	143.3	300.3
1260	294.12	371.1

Table 5: Peak predicted gene copies from biexponential 5p models for each biomass. Upper and lower confidence intervals and hours at peak gene copy generation included.

Biomass (g)	Peak Gene Copies	Upper CI	Lower CI	Hours
0	42.93	NA	NA	1.14
70	2,3697,044	31,888,440	15,505,649	0.79
140	476,904.5	541,550.1	412,258.8	6.32
280	3,311,854	4,689,123	1,934,585	2.52
560	6,069,150	9,754,001	2,384,299	8.4
1260	24,087,434	31,818,156	16,356,712	2.87

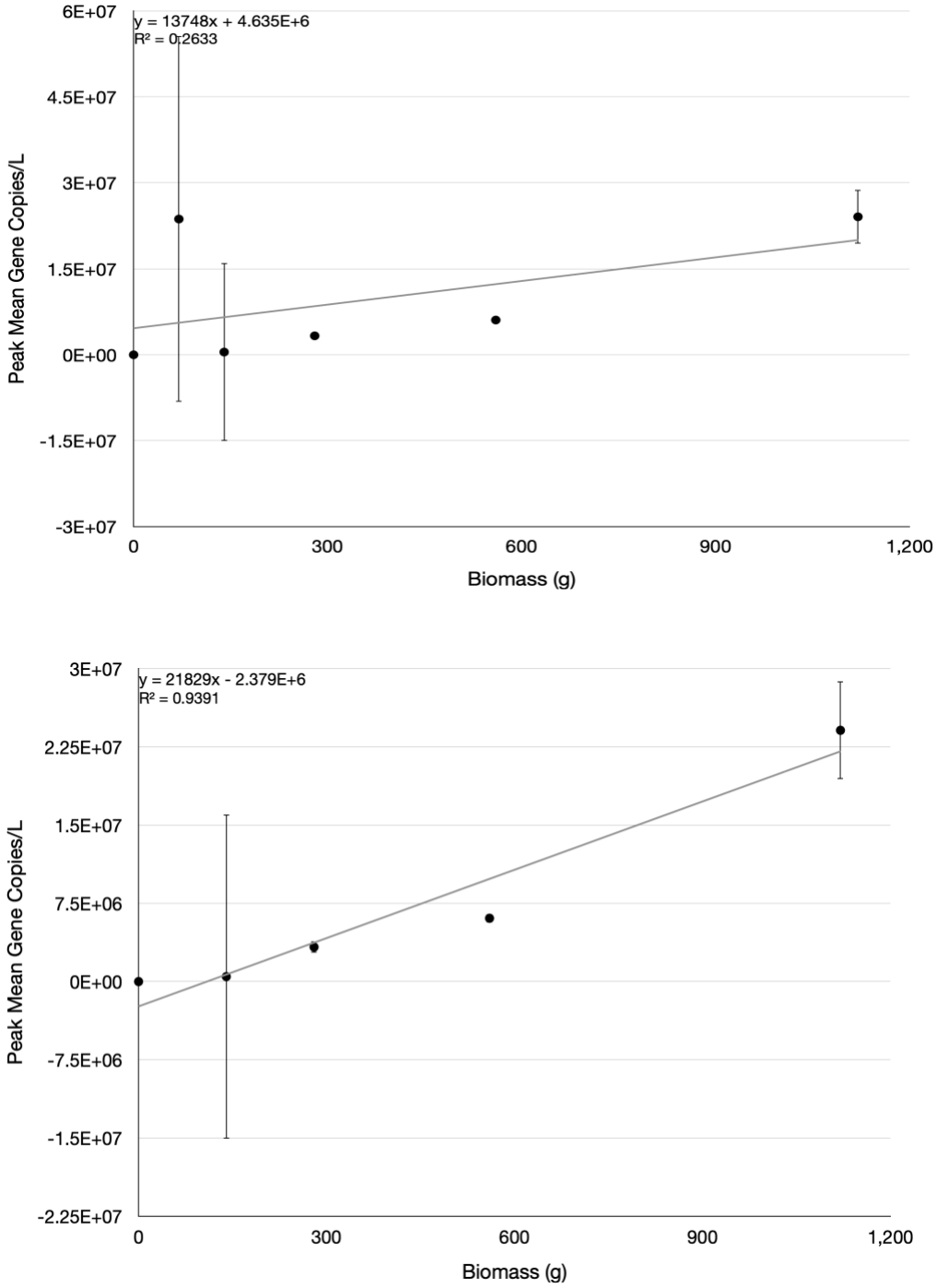


Figure 16: Peak gene copy and biomass relationships. Biomass and peak mean gene copies L^{-1} are linearly related as demonstrated by the linear regression of biomass (g) and Copies L^{-1} (Linear regression: $F = 181$, $df = 1$, $p < 0.0001$) with the 70g biomass included (top panel) and the 70g biomass excluded (bottom panel).

3.5 DISCUSSION

To determine the capacity for eDNA to be applied in natural systems, ground truthing of these tools in laboratory settings is needed. There may be high variability in detection because of the diversity of eDNA sources and the interactions of this DNA with the environment. Depending on the target organism, many eDNA samples from field collections often consist of a complicated mixture of both organismal DNA and extracellular DNA resulting from complex biological and physical environmental interactions. Laboratory experiments do not fully simulate these systems, but are an essential step in understanding of the dynamics of eDNA.

Our larval dilution series successfully established a significant linear relationship between scallop larval density and gene copy values, identifying an average value of 3.41×10^7 gene copies per larva. The high variation among the replicates at the same dilution factor could be the result of differing concentrations of larvae in each 200mL starting stock and following dilutions - a difference of four larvae is an order of magnitude change based on the estimated gene copy value per larvae we calculated in this study. Variation in gene copy numbers also can occur from differences in DNA quantities based on larval size or developmental stage because of increasing cell numbers during growth. Clemmesen (1994) determined increasing DNA amounts with increasing larval size of herring while Doyle, McKinnon & Uthicke (2017) found that different developmental stages, from fertilized egg to competent brachiolaria, of crown of thorns sea stars had significantly different DNA concentrations. Our estimates of DNA per individual are similar to other invertebrate larval DNA estimates, such as the crown of thorns seastar (Doyle, McKinnon & Uthicke 2017), but vary greatly from individual larval estimates from the bivalve *Mytilus edulis* (Alexander et al. 2021). If there are different developmental stages or even different sizes of larvae in a sample, this could contribute to the mismatch between eDNA

detection and larval counts, but this trend does not always hold true and may be species- or life history stage-specific (Vadopalas, Bouma, Jackals & Friedman 2006). Although our larvae were received from Mook Sea Farm at four days of development, we have no data on the size and frequency of those larvae. We do not know the differences in DNA content of scallop developmental stages or sizes, which could also be impacting the amount of DNA detected. This effect is most likely minimal due to the small size of the larvae at this time of development, but cannot be ruled out.

Gene copy generally increased with increasing adult scallop biomass through time, with the exception of the unexpectedly high DNA values at the lowest biomass level of adult scallops (Fig. 15). Although it has been documented in other mollusc species that lower biomasses can shed equal amounts or even more DNA than larger biomasses of the same species (Ruiz-Ramos et al. 2024), these differences did not span multiple magnitudes of differences as ours did in the 70g biomass tank. The high amounts of DNA in the 70g tank could be from multiple sources. The first is DNA shed from a scallop that died during the experiment and was degrading in the tank. We did not confirm mortalities at the conclusion of the experiment, but dead tissue can contribute to increasing the DNA content (Kamorof and Goldberg 2018) and is shed at higher rates after death than from live organisms (Tillotson et al. 2018). Another source of this DNA could be from spawning individuals. Although we visually confirmed that no individuals in this experiment indicated readiness to spawn, scallops may have been induced to spawn in less than ideal spawning conditions due to the stress from moving scallops for the experiment or even from the aeration from the air stone. Movement and aeration are common stressors for scallops and are even used to induce spawning in scallops (Bayer et al. 2016). With scallop sperm having

high gene copy values (Bayer et al. 2019), this could contribute to the high gene copy values in the tank although we did not attempt to determine this during the experiment.

Lastly, we need to consider the impact of feeding activity and fecal generation on the eDNA values in mesocosms. Feeding activity has been found to increase DNA generation rates across species with different fecal generation and, therefore, different feeding activity depending on the source and amount of food (Klymus et al. 2015). We did not specifically assess fecal generation in this study; however, the mesocosms were acting as highly mixed systems and thus impact of this input likely would be standardized throughout the experiment (and for all the other biomass levels). Differences in feeding behavior and fecal generation could account for some of the variability in the timing of eDNA peak values between biomass levels, especially as it relates to increases in eDNA detection after feeding. But, it was not a goal of this study to account for the differences in fecal generation or feeding behavior between biomasses of scallops and is an area for additional work in future mesocosm experiments. Experiments exploring the interactions of these factors in dynamic systems and replicating potential field conditions should be prioritized to fully evaluate the complexities of eDNA detection with environmental variability and organismal behavior.

Differences in feeding activity and fecal generation also could account for some of the variability we see in shedding rates and the overall pattern of eDNA generation between biomasses. Our results are similar to ranges of eDNA shedding rates for freshwater mussels (Ruiz-Ramos et al. 2024) and we also see the phenomenon of higher shedding rates at lower biomasses that Ruiz-Ramos and colleagues observed. Because all of the animals were within the same size range, we cannot assume that these effects are from differences in eDNA generation based on size (Yates et al. 2021). We do not know if scallops could be filtering and sequestering

eDNA in the filtration process, but this phenomenon and its impact on eDNA detection of different species has been documented in other marine species (Mariani et al. 2019; Friebertshauser et al. 2019). Therefore, we cannot rule it out as a potential influence in our detection and quantification of shedding rates in this experiment. Ruiz-Ramos (2024) suggested that decreases in shedding rates at higher biomasses could be due to a decrease in activity because of the increased densities of organisms, but this was in relation to fish species decreasing activity at higher densities. Scallops are not very active as they are sessile invertebrates; however, they are also generally solitary individuals and have been documented to "knife" each other in lantern nets at higher densities, essentially clasping onto one another and cutting the soft tissues (Fitzgerald 2021). Rather than decreasing the shedding rate, this would potentially increase the shedding rate, so we do not think this is occurring in our nets.

Feeding has been shown to significantly increase shedding rates in organisms (Klymus et al. 2015). All tanks, with the exception of the highest biomass tank, were batch fed. Although the amounts of food for each tank were different, they were scaled to be equal based on the densities of the scallops in each tank. Therefore, we would expect to see similar patterns of variability across biomasses in relation to feeding if this were occurring. Feeding times were intentionally offset from sampling times (after sampling occurred) to attempt to miss any spikes in eDNA generation due to feeding behavior. This could account for the spikes in eDNA values at the 16-hour sampling event for the 70g, but this pattern was not seen in the other tanks who were also batch fed.

Temperature did not fluctuate during this experiment; however, the impact on temperature on shedding rates varies between species. For example, differences in temperature did not significantly affect shedding rate for different species of carp or crown of thorns larvae

(Klymus et al. 2015; Kwong et al. 2021), but significantly increased with increasing temperature for tench in mesocosm experiments (Herve et al. 2023); however, eDNA can persist for longer periods of time at temperatures comparable to those in this experiment (i.e. </= 10°C, McCartin et al. 2022) and may be influencing the overall detection and persistence of eDNA in this experiment. The metabolism of sea scallops is significantly impacted by temperature fluctuations and could impact the generation rates of scallops in different environments (Pilditch and Gant 1999).

Our goals with this study were to (1) quantify relationships between scallop larval density and DNA copy number, (2) quantify eDNA shedding rates of adult scallops and (3) relate these rates to different biomass levels of non-spawning scallops in a laboratory setting. We were successful in accomplishing these objectives but there is much work to be done in order to relate these findings to natural systems.

In addition to bringing these experiments into the field, there also are still outstanding questions related to eDNA and the biology and ecology of sea scallops and other benthic marine invertebrate species that could be illuminated by laboratory studies. For example, a laboratory mesocosm experiment modeled after the ones reported here to determine potential differences in DNA content, and resultant gene copy numbers, of different larval stages and sizes would be useful, given that we know from Zhang et al. (2015) that DNA content of loaches differs with life history stage. It also would be helpful to assess the contribution of eggs to eDNA detection. Large scallops can produce upwards of 50 million eggs in a single spawn (Langton, Robinson & Schick 1987), and both laboratory and field assessments of egg detection via eDNA would advance our understanding of the species reproductive ecology as well as provide key information for sustainable management of these populations. This may be a challenge as it has

been found that mussel eggs have significantly less DNA than sperm (Dave Ernst, *pers. comm*.). Finally, the impacts of biotic and abiotic factors on eDNA generation in scallops also warrants further study, both in the lab and the field. Scallops live in dynamic marine environments spanning fluctuations in temperature, depth, food supply, and flow. Evaluating the influences of these factors is an essential next step to further evaluating eDNA signals in the field and need to be taken into consideration for field applications.

CHAPTER 4

eDNA METHODS CAN DETECT TEMPORAL AND SPATIAL VARIABILITY OF SCALLOP DNA IN FARMED AND WILD POPULATIONS

4.1 ABSTRACT

Environmental DNA (eDNA) is potentially a non-invasive, cost-effective and efficient method for monitoring aquaculture and commercial fisheries populations to inform sustainable fisheries management practices. eDNA tools must be thoroughly ground truthed to determine best practices for their appropriate application. Using sea scallop aquaculture farms and a vertically-stratified sampling design above a wild sea scallop bed in Penobscot Bay, Maine, we evaluated the temporal and spatial variability in scallop eDNA signal. The available scallop qPCR probe and primers successfully detected scallop eDNA on scallop aquaculture farms, above a wild well-characterized, deeper scallop bed, and at a site lacking sea scallops and established high temporal and spatial variation in this signal. Seasonal gene copy number per liter seawater maxima on sea scallop farms did not occur after peak scallop spawning, as indicated by GSI values, and did not occur in tandem with maximum counts of bivalve larvae with one exception of one farm site. Sea scallop eDNA was detected at all depths above a wild scallop bed and at a site lacking scallops, indicating that transport of eDNA and quantifying stochasticity in 'background' signals is an important consideration in future studies. The scallop eDNA signal increased at both wild population sites and across depths after maximum GSI were observed during the time of assumed peak larval presence from 30-45 days after spawning. The high spatial and temporal variability in scallop DNA detection supports the need for carefully constructed sampling designs that are informed by organismal life history traits and patterns and

the physical oceanographic characteristics of local waters to best apply eDNA tools to monitoring commercially important species.

4.2 INTRODUCTION

The sea scallop (*Placopecten magellanicus*) fishery is the largest and most valuable wild scallop fishery in the world and among the most lucrative fisheries in the U.S (NOAA 2024). The fishery focuses on wild harvest of offshore populations in federal waters from the mid-Atlantic to the US-Canada border. The Northern Gulf of Maine (NGOM) federal management area is managed separately from the rest of the federal stock and spans the coast from NH to Maine, including Maine's state-managed fishery. Maine's wild sea scallop fishery is highly lucrative and demands one of the highest state average prices per meat pound (~$14.12/lb in 2023; Maine DMR 2024). This predominantly wild capture fishery consists of highly productive shallow inshore aggregations managed in three zones along the coast and utilizes rotational closed areas with open/closed areas changing annually (ME DMR 2024). Declines in commercial landings of sea scallops since the 1980s and increasing demand have supported the rise in sea scallop aquaculture, which has been identified as one of the most promising avenues for further developing Maine's shellfish aquaculture sector (Cole, Langston & Davis 2016). Thus, the wild and farmed sea scallop sectors occur side-by-side along the coast of Maine.

Understanding larval supply, connectivity and the dynamics governing the processes of reproduction, larval dispersal, and settlement of sea scallops and other commercially important marine invertebrates with complex life histories is a continuing ecological challenge and is also relevant to their management (Cowan & Sponagle 2009; Munroe et al. 2018; Close et al. 2024). Sea scallops are highly fecund broadcast spawners that engage incomplete (i.e., synchronous male and female release of gametes) and protracted spawning events (Langton, Robinson &

Schick 1987). These events vary spatially and temporally on annual and semi-annual cycles and are often driven by environmental conditions (Parsons et al. 1992; Smith & Rago 2004; Thompson, Stokesbury & Inglis 2014). Sea scallops in the NGOM generally spawn on an annual cycle occurring from July through September, whereas populations on Georges Bank experience semi-annual spawning in the spring and fall (Parsons et al. 1992; Thompson, Stokesbury & Inglis 2014). Along the coast of Maine, spawning occurs from July through September with the specific timing of these events varying in both wild and farmed populations (Chapter 2; Bayer et al. 2016). The reproductive development of sea scallops can be measured as the gonadosomatic index (GSI), the ratio of wet gonadal mass to the total wet body mass without the shell (Langton, Robinson & Schick 1987; Parsons and Dadswell 1992). GSI serves as a proxy for per capita gamete production. It has been a long-used tool for monitoring reproductive timing in *P. magellanicus* to indicate onset and progression of spawning, which is indicated by a sudden decrease in the GSI (Langston, Robinson & Schick 1987; Parsons et al. 1992; Bayer, Countway & Whale 2019; Parsons et al. 1992). Monitoring reproduction is not currently a part of management efforts for either wild or farmed sea scallop fisheries in Maine, and the federal fishery has only recently begun collecting these data.

The sea scallop planktonic larval period lasts anywhere from 35-40+ days and is considered to be the life stage at which most mortality occurs. At this life stage larvae are planktonic and planktivorous, exhibiting vertical migration behaviors and utilization of different water column temperatures throughout their development (Culliney, 1974; Manuel et al. 1996). Development time throughout the planktonic stages, and settlement to the benthos, is influenced by temperature and food availability and sea scallops can delay settlement for up to one month (Stewart and Arnold 1974; Culliney 1974). Larval dispersion and retention during this vulnerable

period is determined primarily by currents and the locations of adult spawning populations (Tian et al. 2009). At the end of this pelagic larval period, competent sea scallop larvae recruit to the benthic phase. This life stage is commonly termed “spat” and also is the life stage captured for grow-out on aquaculture farms (Culliney 1974; Truesdell 2014).

Biophysical models and empirical plankton and larval sampling can be used to investigate these important life history phases in scallops and other marine invertebrates (Tremblay et al. 1994; Munroe et al. 2018; Chen et al. 2021); however, sea scallop gametes and larvae are difficult to distinguish taxonomically from other bivalves in common methods such as plankton tows, and also are difficult to track *in situ* (Pechenik 1999). To monitor adult sea scallops, researchers have used dredge (Stokesbury, O’Keefe & Harris 2016), drop camera (Stokesbury and Bethoney 2020), and dive surveys (Bethoney et al. 2019). These methods are time-consuming, financially expensive, and, in the case of dredge surveys, potentially harmful to marine habitats (NOAA 2024). In summary, innovative approaches to detecting, quantifying, and monitoring the larval and adult stages and life history processes of commercially-important marine species are needed.

Environmental DNA (eDNA) provides a potential solution to the challenges of monitoring, detecting and quantifying commercially important species with complex life histories. eDNA ranges from whole genomes of intact microorganisms (like gametes or larvae) to free DNA fragments shed from cells of larger organisms. This genetic material from aqueous or other environments can be quantified to provide ecological inferences of diversity, species distributions, or community structures (Thomsen & Willerslev 2015; Goldberg, Strickler & Pilliod 2015; Creer et al. 2016; Deiner et al. 2017). By virtue of its taxon specificity, low cost, and associated consistent accessible sampling approaches, eDNA offers capacity for wider

research participation, shared sampling effort with industry, and deeper data integration to replace or, more likely, complement existing monitoring and survey methods for both adult and larval populations (Creer et al. 2016).

eDNA approaches such as metabarcoding and quantitative polymerase chain reaction (qPCR) assays may help disentangle the complex ecology of sea scallops and other marine invertebrates by providing a direct approach for species identification and enumeration of gametes and larvae in the water column. Compared to traditional sampling techniques (e.g., trawling, gill netting, dredging) to determine marine biodiversity or populations, eDNA methods require less dependence on specialized taxonomic expertise or field gear, which can result in faster sampling, cost-effectiveness, efficiency and accuracy (Rourke et al. 2021; Herve et al. 2022). Recent applications have successfully verified presence or absence of species in comparison to trawl surveys (Kirtane et al. 2020), established relationships between species densities and eDNA signals (Skinner et al. 2019) and related larval densities of marine corals to eDNA detections (Doyle, McKinnon & Uthicke 2017). However, challenges for applications, such as the breakdown or dilution of eDNA in marine environments, can limit research as can the tendency of eDNA to be broadly dispersed over time (Ruppert et al. 2018). Other limitations of eDNA sampling include its inability to directly assess biomass or abundance, age, sex and behaviors, (Kirilchik et al. 2018). And, eDNA testing is relatively new for macro organisms; standardization across collection, laboratory and data analysis processes is still needed (note, however, that microbiologists have been using related nucleic acid technologies for decades, e.g., Karl et al. 1988).

Quantitative eDNA assays for sea scallops have been developed based on ITS gene fragments (Bayer, Countway, and Wahle 2019). Bayer et al. (2019) established that gene copy

number, determined through quantitative PCR (qPCR) primers and size fractionation protocols, can be used as a proxy for sea scallop gamete number, specifically sperm, in the water column. They demonstrated a close agreement between sperm cell count and eDNA copy number *in situ*, using a laboratory calibration, but did not expand the study to include larvae.

Using scallop aquaculture farms and wild scallop beds as research sites, the objectives of this work are to (1) determine the ability of eDNA tools to successfully detect sea scallop DNA in the field, (2) evaluate spatial (across depth and across sites) and temporal (across spawning seasons) differences in sea scallop eDNA distribution, and (3) evaluate the use of eDNA methods to detect biological processes, such as sea scallop spawning and larval presence. We hypothesize that eDNA tools will successfully detect sea scallop DNA in the field and that detected signals will vary spatially and temporally among different sites where sea scallops are living on aquaculture farms and in wild populations. We also hypothesize that eDNA tools will successfully detect biological processes as evidenced by differences in detectable signal pre- , during, and post-spawning, maximum gene copy values occurring during time periods when we would expect to see larvae present, increased vertical distribution of the signal above wild beds during spawning and timing of larval presence, and positive correlations between gene copy number and bivalve larval counts from plankton tows.

4.3 METHODS AND MATERIALS

4.3.1 Scallop farm characteristics and field sampling

To determine spatial (site) and temporal (weeks to months) differences in sea scallop eDNA we conducted field sampling at experimental sites on sea scallop aquaculture farms June - October in 2020 and 2021. Sampling on sea scallop farms was conducted in collaboration with three sea scallop aquaculture farms within Penobscot Bay, ME (Fig. 1). The North Haven farm

(NH) (44.17571° N, 68.81849° W) is a 15-acre site with a depth of 9-15 m at mean low water (MLW) with a gently sloping bottom consisting primarily of gravel and mud (DMR Permit: HOG MC). The Stonington farm (ST) (44.14328° N, 68.70747° W) is a three-acre lease site with a depth of 17-21 m at MLW with a predominantly muddy bottom (DMR Permit: PEN AI). The Hurricane Island farm (HI) (44.03965° N, 68.89103° W) is a three-acre site with a depth of 6-9 m at MLW with a gently sloping bottom consisting primarily of sand and gravel (DMR Permit: PEN HIX).

In 2020, sampling at farm sites occurred at weekly intervals from weeks 29 through 37 and weeks 33 through 41 in 2021, as weather and other conditions permitted. In 2020, our initial sampling effort planned for 2020 was delayed and then cut short at week 37 by the COVID pandemic due to limited access to the facilities and vessels at Hurricane Island and at partnering sea scallop aquaculture farms. In 2021, samples collected in weeks 23-32 (June and July) from scallop farms were unable to be analyzed due to a freezer malfunction and sample thawing.

All farms utilized vertical lantern nets hanging from horizontal long lines spaced 1-2 meters apart along the line. Sampling depth (depth of the lantern nets) varied at each farm based on the specifics of the farm infrastructure and design: depths were 5, 7, and 9 m at HI, NH, and ST farms, respectively. eDNA Sampling occurred from a motorized vessel owned by the Hurricane Island Center for Science and Leadership that was tied to a stationary buoy at each farm site. At each farm, a sterilized and weighted 4L Niskin bottle was deployed to the depth of the farm-based lantern nets to collect water samples. Samples were taken within 1m to the side of the nets on each farm. Upon retrieval, the water was funneled into a 4L opaque bottle that was previously sterilized, rinsed with deionized water, and then triple rinsed with sample water and

put on ice until filtration within ~ 2 hrs. The Niskin bottle and funnel were sterilized with 10% bleach solution and rinsed with deionized water between sampling at each farm site.

For filtering, each 4L sample was further divided into three, 1L replicates that were gravity- filtered through 0.2 μm Supor filters installed in 47 mm, in-line filter holders (Pall Laboratory) for later extraction of DNA; the remaining 1L was discarded. All filters were rolled loosely with two, bleach-cleaned forceps, placed in a labeled 2 mL cryovial and frozen at -20C until later DNA extraction processes. All filtering equipment was sterilized with 10% bleach solution and rinsed with fresh and deionized water between filtering efforts.

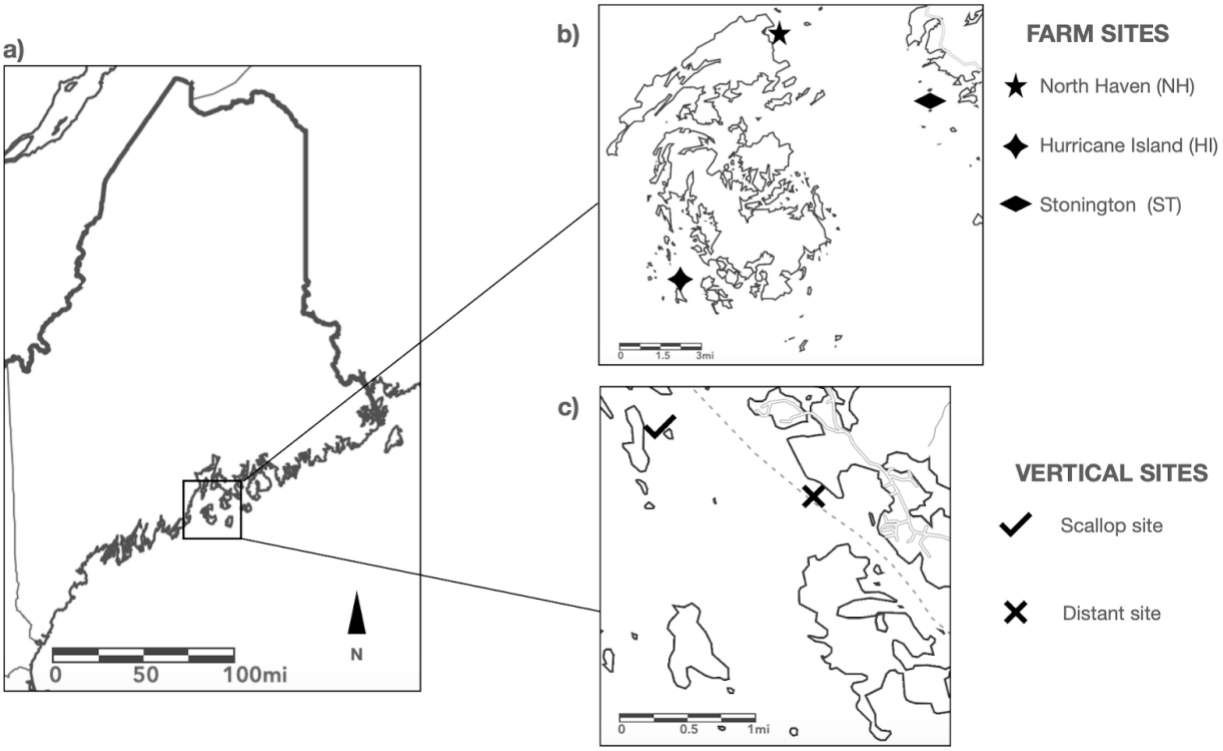


Figure 17: Sampling sites in Penobscot Bay. Locations of research efforts in Penobscot Bay, ME (a) at sea scallop aquaculture Farm Sites at Hurricane Island, North Haven, and Stonington (b) and Vertical Sites with scallops and lacking scallops (c). Maps were made in ArcGIS.

4.3.2 Vertical sampling site characteristics and field sampling effort

We sampled above a well-characterized wild sea scallop bed and another site lacking sea scallops in 2023 in Penobscot Bay, Maine, to evaluate seasonal variation in the vertical distribution of scallop eDNA in wild populations (Fig. 1c). This well-characterized wild scallop bed (hereafter "scallops site") was sampled May-November, 2023. A geographically proximate (1.9 km) yet distant site (hereafter "distant site") with no observed scallops was sampled (Fig. 1c). The sea scallop population at the wild bed was estimated to be 0.86 scallop/m^2 with a sandy bottom and shell hash habitat based on SCUBA surveys conducted by Jekielek and Hurricane Island staff in 2022. SCUBA surveys consisted of one 200 m^2 belt transect (2m in width by 100 m in length) where scallops were counted along the entire length of the transect and an estimate of length was collected for each scallop located along the transect. The distant site habitat consisted of deep mud and supported no scallop populations (0 scallop/m^2) as confirmed by site monitoring via SCUBA in previous years. Both sites had a depth of 18 m at mean low water.

eDNA sampling occurred from a motorized vessel owned by the Hurricane Island Center for Science and Leadership that was anchored at the site coordinates for each event. Sampling occurred at slack low tide, or as near to as possible at wild bed and control sites, with the wild bed prioritized to decrease the influence of active tidal flow on sampling efforts and results. At the wild bed, samples were collected at five uniformly distributed depths from the bottom to the surface: at 17 m (1 m above the bottom), 13 m, 9 m, 5 m, and 1 m below the surface. At the distant site, samples were collected at three depths: 17 m, 9 m and 1 m below the surface. At each depth, a sterilized 4L Niskin bottle was deployed from a boat to the corresponding sample depth. Sample collection, equipment sterilization, and filtering methods for the vertical sampling effort were identical to those outlined above for the farm sampling.

4.3.3 Environmental data collection

Environmental data also were collected at all farm, wild bed, and distant sites at each sampling event. At each site, we conducted a single profile cast from surface to bottom using a YSI EXO2 Sonde to collect salinity, temperature (°C), dissolved oxygen (mg/L), and chlorophyll-*a* (rfu) data. We collected data on the downcast to 1 m above the bottom at a rate of every 10 seconds. The Sonde was factory-calibrated in April 2020 and in May 2021 and lab-calibrated in August 2020 and July 2021.

4.3.4 Gonadosomatic indices and plankton tows

To evaluate the relative utility of eDNA methods to detect sea scallop spawning timing as compared to traditional methods, we collected farmed sea scallops for dissections and subsequent calculations of gonadosomatic indices (GSI) using the methods described in Chapter 2. In 2020 and 2021, adult farmed sea scallop (males and females) collections occurred in coordination with eDNA sampling efforts (see Methods, Chapter 2). In 2023, adult (males and females) wild sea scallop collections occurred from wild beds adjacent to (within 1.6 km) of the vertical scallop site. Twenty scallops were collected via SCUBA for dissection and GSI calculation every two to four weeks throughout the vertical sampling effort for a total of six times. We opportunistically sampled 20 wild sea scallops via SCUBA in 10-20 m of water from a substrate that was predominantly composed of shell hash, sand, and cobble. We attempted to collect 10 male and 10 female individuals at each collection, but it was not always possible to identify the sex at the time of collection.

To evaluate the use of eDNA methods to detect larval abundance, we conducted vertical plankton tows at farm, scallop, and distant sites to quantify the presence or absence of bivalve larvae. At each sampling event at each site, we deployed a Sea Gear 60 μm plankton net with a

3:1 mouth ratio and weighted filtering cod end to within 1m of the bottom and vertically retrieved the net to the surface for a single tow. Scallop eggs are generally 60-65µm and larvae are all larger through their development (Culliney 1974). The net was equipped with a Sea Gear mechanical flow meter (model MF 315). At the surface, the contents of the net were concentrated in the cod end and transferred to a 50mL sample tube with denatured 90% EtOH for later analysis.

We enumerated previously preserved plankton samples from each sampling event via a Nikon SMZ745 dissecting microscope set to 30x magnification. Each sample was split to ¼ of the sample using a Wildco Folsom plankton splitter and filtered through a 60 µm filter to preserve any eggs and/or larvae from the sample. Deionized water was used to dilute the sample to a reasonable density for viewing through a microscope. The unused portions of the sample were returned to their original containers for future processing. Two milliliters of the sample were transferred into a Bogorov counting chamber for microscopy using a pipette. For the 2020 samples, all straight-hinge bivalve larvae of sizes 70-90 um were counted and photographed. In 2021 and 2023 larval samples, all larvae of straight-hinge stage and above were counted. Continuing through the entire sample, we processed 2 ml at a time to a clean racetrack until the whole sample has been processed.

4.3.5 DNA Extraction

For all sampling efforts, DNA was extracted using the PowerWater DNA kit (Qiagen) with a slight modification of the sample lysis step following methods described in Bayer, Countway and Wahle (2019). Frozen Supor filters were transferred to 5 ml Power-Water bead tubes containing pre-loaded silica particles. One ml of heated (55°C) PW1 lysis buffer was added to the bead tubes. The 5 ml tubes were transferred to a 5 ml sample-tube adapter (MoBio) and

loaded onto a MM400 Retsch Mixer Mill for sample bead-beating for 30 minutes. Additional sample processing followed the Qiagen PowerWater protocol exactly. TaqMan qPCR was performed on the extracted samples with the previously developed *P. magellanicus* probe and primers using 3 µl aliquots of each purified DNA sample in triplicate (Bayer, Countway and Wahle 2019).

Using the standard curve ($y = –1.44 \ln(x) + 39.50$) that was generated from the previously-developed and ground truthed linearized-plasmid dilution series from Bayer, Countway and Wahle (2019), we translated the Cq values (qPCR threshold cycle number) resulting from DNA samples to gene copy numbers and further calculated gene copies L^{-1} for analysis.

4.3.6 Data analysis

We used JMP Pro 17.0 statistical software to visualize and statistically analyze GSI data for farm and vertical sampling efforts. GSI data from all years and sites was tested for normality using Shapiro-Wilk tests, equal variance using Levene's tests, and outliers using the Quantile Range method to identify outliers beyond 1.5 quantiles from the data. We determined the distribution of gene copy data to be non-normal and to have unequal variances for all years and sites. We identified the timing of spawning events in farmed and wild populations by assuming peak spawning activity occurred at the point when the highest documented GSI value was reached and then followed by a continuous decrease in mean GSI (Dadswell & Parsons 1992; Thompson et. al. 2014). We used Kruskall-Wallis H tests to evaluate for statistical differences in GSI among sampling weeks and then used the nonparametric Dunn's pairwise tests to determine statistical differences between sampling weeks to identify weeks over which spawning occurred.

To estimate a total larval count from each individual plankton tow, we first had to determine the amount of water sampled. The distribution of flow meter readings was right skewed and appeared to have two separate groups, one group represented times when the flowmeter propeller was spinning properly, while the other represented times when the propeller was not spinning properly. Since the flowmeter data indicated there were times that the propeller was not spinning properly, and that would eliminate a large segment of the dataset, we decided to calculate the volume of water filtered using the depth of each vertical tow. We assumed the tow started approximately 1 m from the bottom and went straight up to the surface. We multiplied the length of the tow by the size of the net opening (0.196 $m^{\wedge 2}$) to estimate the amount of water in cubic meters that each sample was filtered from. This assumes that the boat was largely in the same place during the tow because it was anchored for the entirety of the sampling effort. We then divided the number of larvae counted by the split to get the number of larvae in the whole sample. We divided that by the number of cubic meters filtered to get the larvae per cubic meter. We used linear regression to explore relationships between larval counts and gene copy numbers.

We used JMP Pro 17.0 statistical software to visualize and statistically analyze scallop eDNA data (gene copies L^{-1}). Farm and vertical sampling data were evaluated for normality using Shapiro-Wilk tests and were determined to be non-normal. We removed any samples that did not have a date, site, or gear type in the metadata. Because of the high variability in quantified DNA from tank samples, we evaluated eDNA data from each year for extreme outliers following methods outlined by Klymus et al. (2015). We identified outliers as points 1.5 times the interquartile range and removed those points prior to calculating average eDNA shedding rate for each tank to account for potential variation in the distribution of eDNA containing particles in the tank. The eDNA data were non-normal. Therefore, we used Kruskall-

Wallis H tests to evaluate for statistical differences in gene copy numbers over time and among aquaculture farms, to evaluate differences in gene copy number among scallop and distant sites in the vertical sampling experiment, and to test for differences among depths and over time. We then used the nonparametric Dunn's pairwise tests to compare differences in gene copy between weeks on sea scallop farms to identify peaks in signals and between depths at wild and control beds in the vertical experiment.

For each aquaculture site, we selected out and averaged the data from the depth of the long line to the estimated bottom of the net. Data was averaged from 5-7m for HI, from 6-8m for NH, and from 8-10m for ST. Average values and standard error were plotted for each variable at each site in each year to visualize environmental variability at each site.

4.4 RESULTS

4.4.1 Individual scallop aquaculture farms

Gene copy numbers per liter of seawater sampled were variable across the weeks sampled in each year, and across scallop farms with maximum gene copies being recorded in different weeks at each farm site in 2020 and 2021 (Fig. 18). In 2020, there were differences in mean gene copy values among the sites (Kruskall-Wallis H tests: $H = 8.19$; $df = 2$; $p = 0.0167$) with the HI farm site having lower overall mean gene copy values than the NH site (Dunn's pairwise test: $z = 2.54$; $p = 0.03$). In 2021, we found differences in mean gene copy values among the sites (Kruskall-Wallis H tests: $H = 7.6$; $df = 2$; $p = 0.0224$), with the nonparametric Dunn's pairwise tests identifying a significant difference between ST and NH farms only ($z = -2.72$; $p = 0.02$).

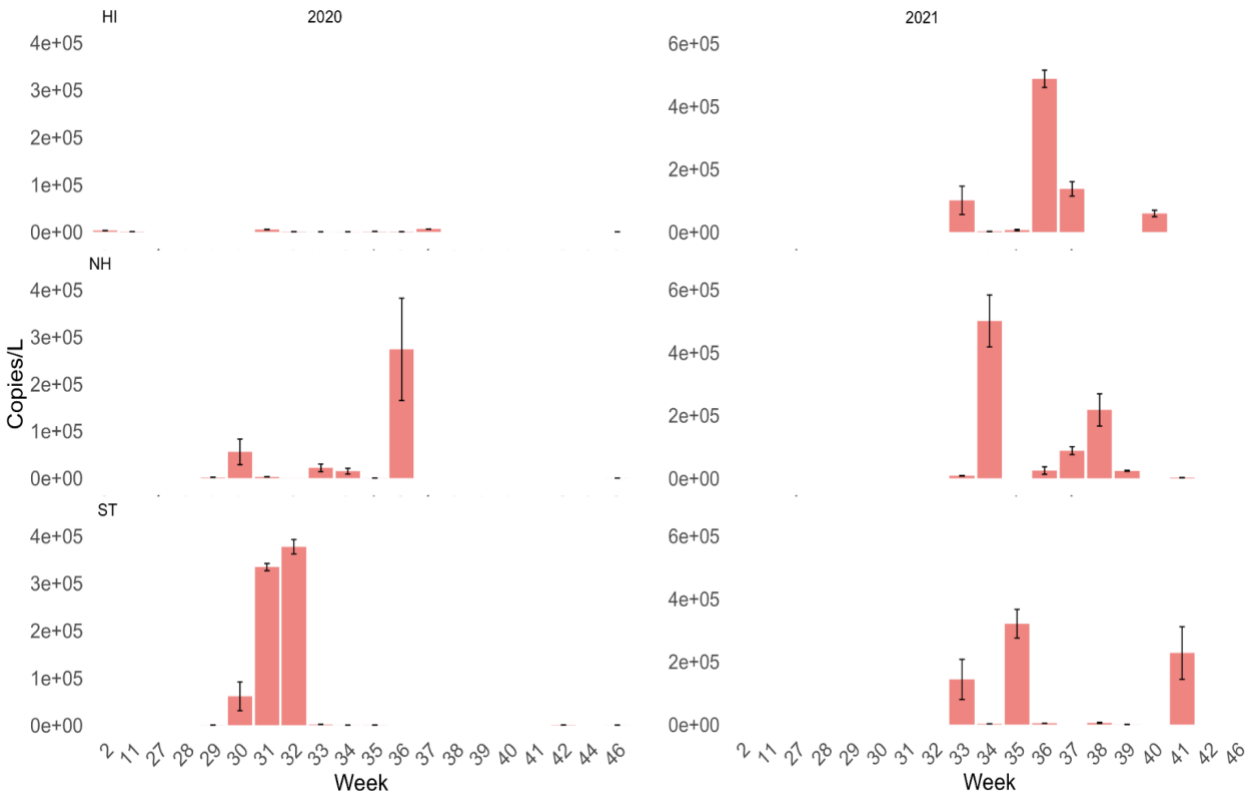
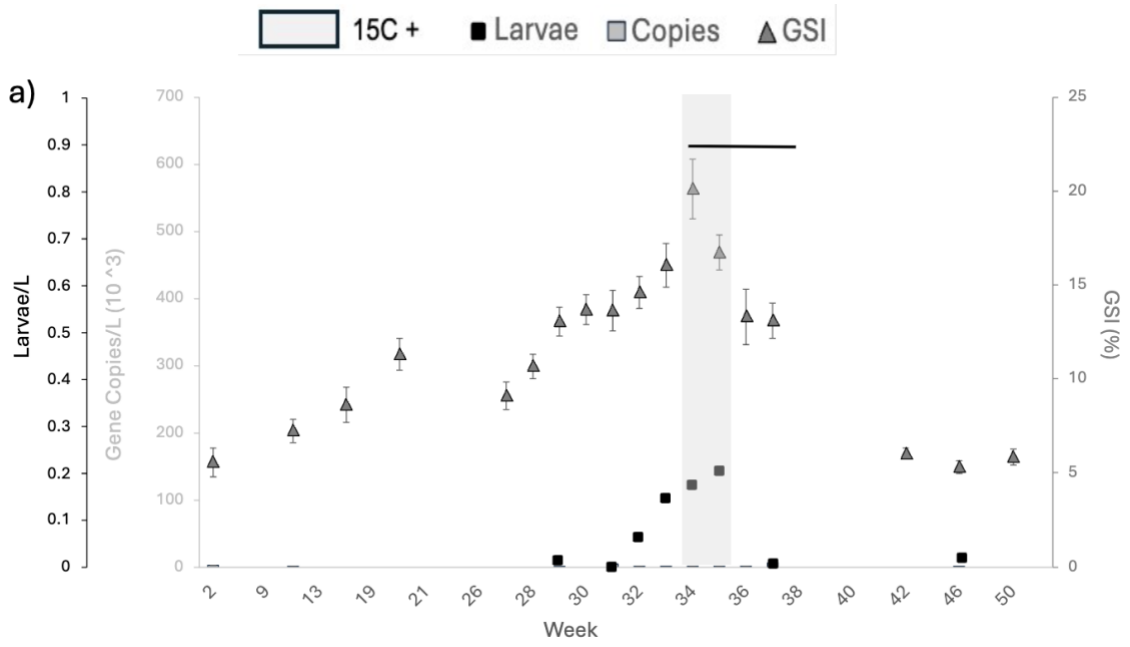


Figure 18: Temporal and spatial gene copy variation on Hurricane Island (HI), North Haven (NH) and Stonington (ST) farms. Weekly mean copies/L (+/- SE) at each farm site in 2020 and 2021.

Maximum gene copy values did not occur in the week following maximum GSI values for any farm sites in 2020 or 2021 (Figs. 19 & 20). In 2020, maximum gene copy values varied in timing from two weeks before to four weeks after maximum GSI values at farms, which occurred at week 34 at HI and ST sites and at week 32 at the NH site (Fig. 19). Maximum gene copy values did not correspond to maximum larval counts at any sites, with the exception of the ST farm site, in 2020 (Fig. 19). Larval count explains 76% of the variation in gene copy numbers at the ST site (linear regression: $F = 12.77$; $df = 4$; $p = 0.02$). Gene copy and larval counts were not associated at the HI and NH sites. The greatest GSI value occurred at week 34

at both HI and ST sites an at week 32 at the NH site. There were no differences in weekly GSI values between greatest GSI values and other weeks, with the exception of week 42, indicating that maximum GSI values were reached and followed by a period of spawning as indicated by continuous decreasing GSI values. Spawning was also initiated and occurred during the 15°C time period at HI and NH sites (Figure 19).



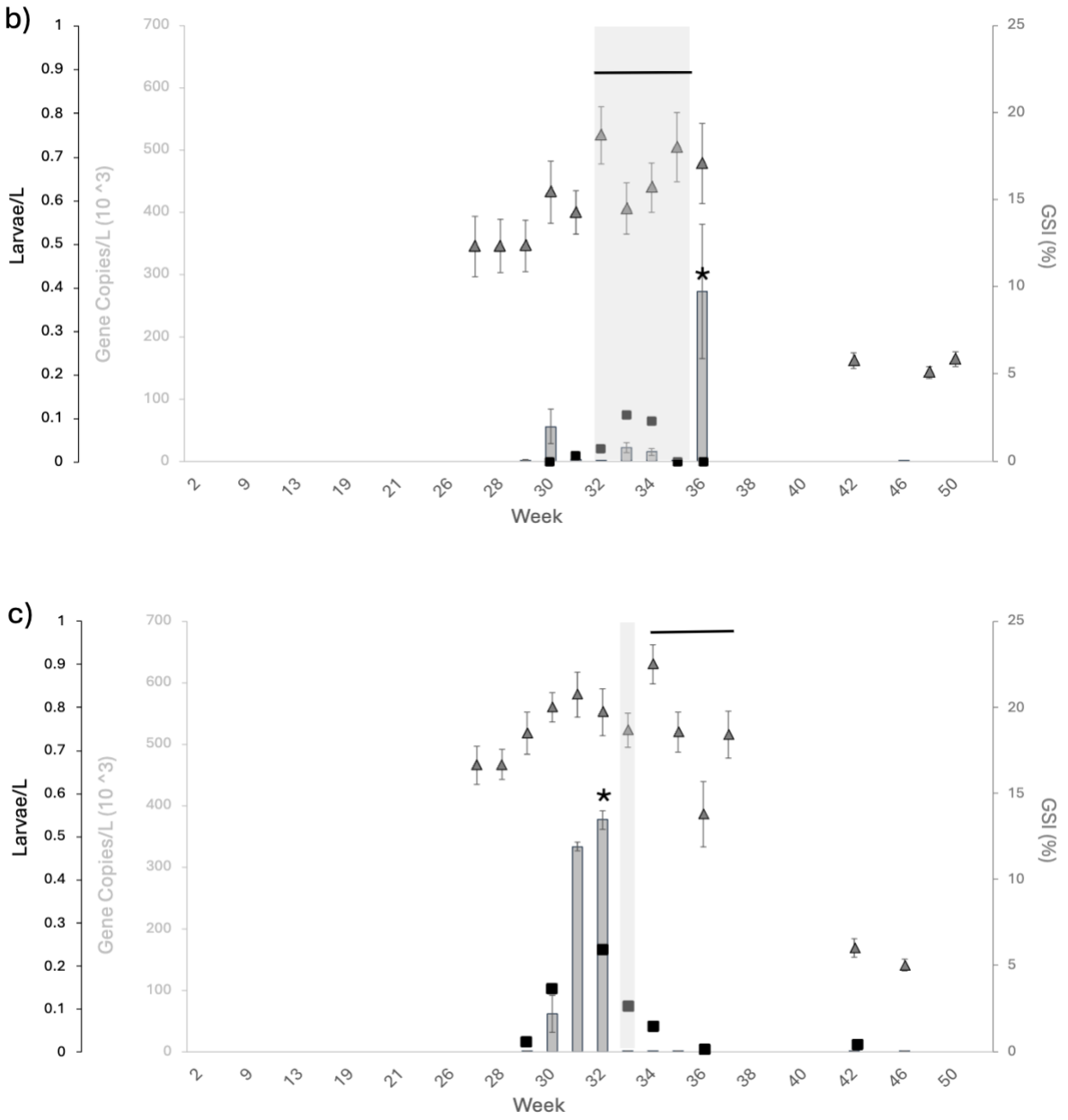
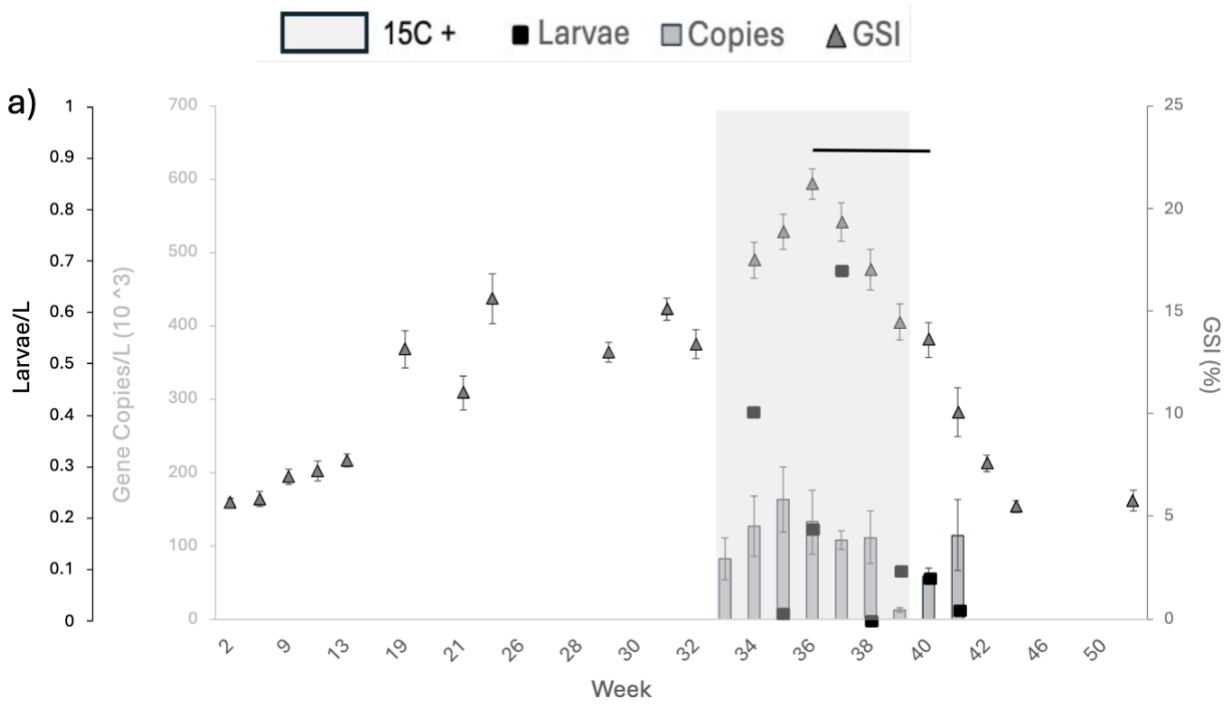


Figure 19: Weekly gene copies, GSI, and plankton tows in 2020. Weekly mean gene copy values (gray bars), mean GSI (gray triangles), and mean bivalve larvae L^{-1} (black squares) at HI (a), NH (b), and ST (c) farm sites in 2020. Shaded area indicates dates where average temperature is 15°C+ the net depth at each site. Black horizontal bars indicate spawning periods. Asterisks indicate significant peaks in eDNA values.

In 2021, maximum gene copy and highest GSI values occurred during the same week at the ST farm site while maximum gene copy occurred at one and two weeks prior at the HI and NH sites, respectively (Fig. 20). In 2021, maximum gene copy values and larval counts occurred during the same week only at the ST farm site (Fig. 20c). There were no associations between gene copy values and larval counts at any of the sites in 2021. The 15℃+ average temperature window occurred for a minimum of 7 weeks at each site in 2021. The highest GSI value occurred at week 36 at both HI and NH sites and at week 35 at the ST site. There were no differences in weekly GSI values between greatest GSI values and other weeks, with the exception of week 42, indicating that maximum GSI values were reached and followed by a period of spawning as indicated by continuous decreasing GSI values. Spawning was also initiated and occurred during the 15℃ time period at all sites (Figure 20).



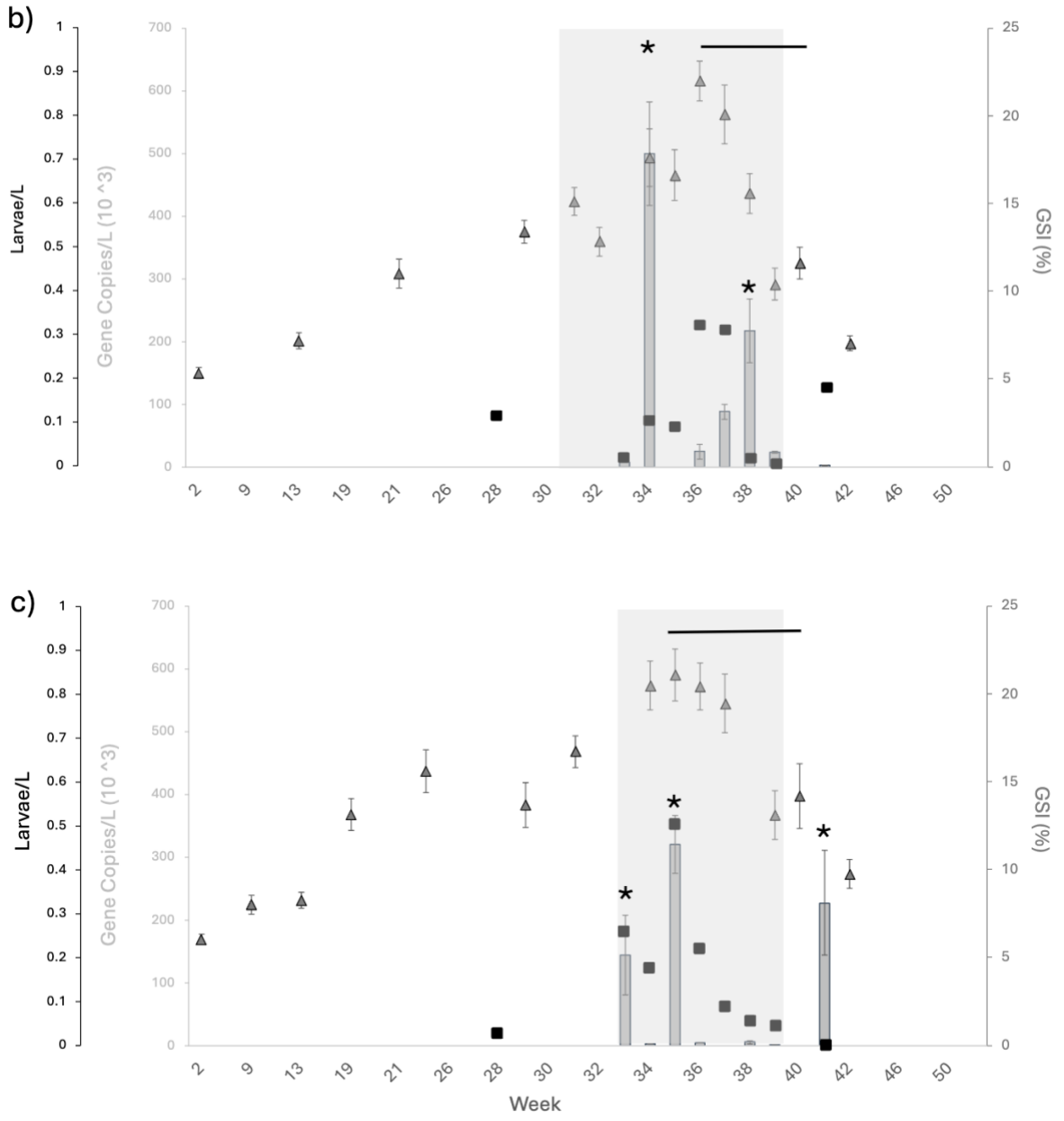
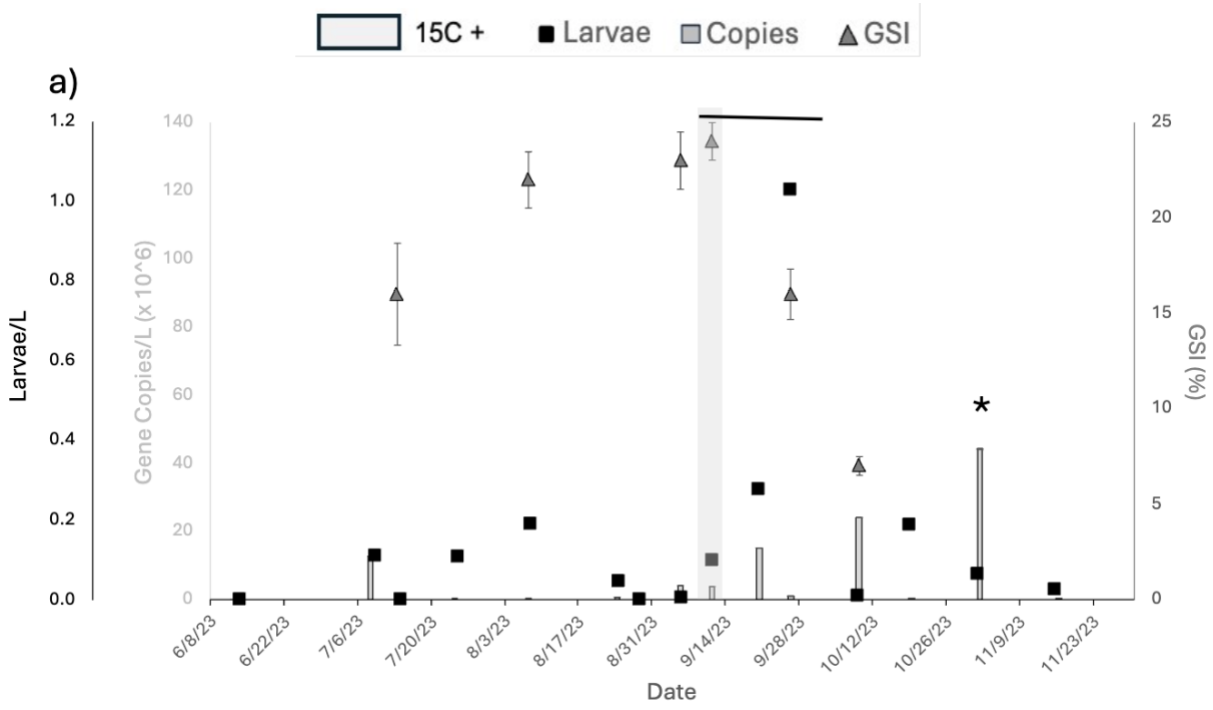


Figure 20: Weekly gene copies, GSI, and plankton tows in 2021. Weekly mean gene copy values (gray bars), mean GSI (gray triangles), and mean bivalve larvae L^{-1} (black squares) at HI (a), NH (b), and ST (c) farm sites in 2021. Shaded area indicates dates where average temperature is 15°C+ the net depth at each site. Black horizontal bars indicate spawning periods. Asterisks indicate significant peaks in eDNA values.

4.4.2 Detection of DNA above a wild scallop population and at a distant site

eDNA tools successfully detected scallop DNA above a wild scallop bed (Fig. 21). Scallop DNA also was detected above a control site lacking a wild scallop population. There was no difference in overall mean gene copy values between the wild and distant site (Kruskall-Wallis: H = 2.6; -1.6; p = 0.11). Maximum gene copy values did not occur on the same date as maximum larval counts at the wild bed or distant site (Fig. 21). There was no relationship between larval counts and gene copy values at either site (linear regressions, p>0.05). The 15°C+ average temperature window was only reached for one week at both sites.



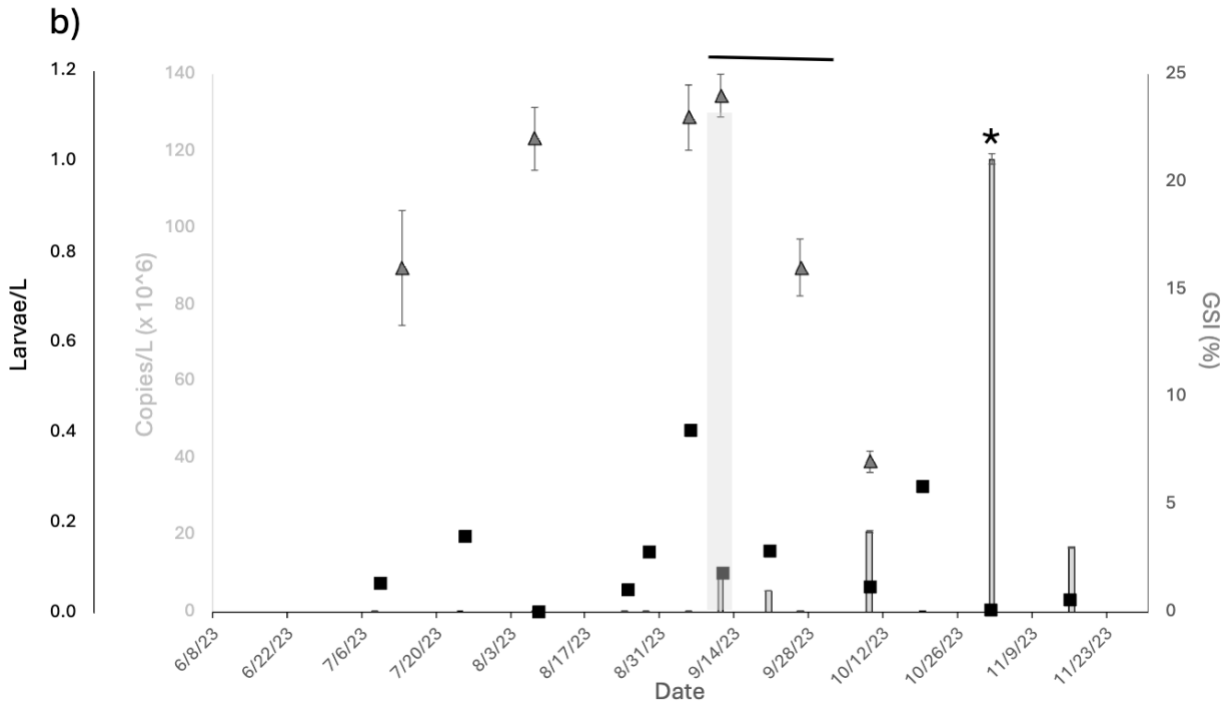


Figure 21: Weekly gene copies, GSI, and plankton tows above a wild scallop bed in 2023. Weekly mean gene copy values (gray bars), mean GSI (gray triangles), and mean bivalve Larvae L^{-1} (black squares) at the wild (a) and distant (b) sites in 2023. Dates of temperature maximum (dotted line) and pH minimum (dashed line) are also indicated. Shaded area indicates dates where average water column temperature is 15°C+ at each site. Black horizontal bars indicate spawning periods. Asterisks indicate significant peaks in eDNA values.

The highest GSI value from wild sea scallop beds occurred on September 14 at both sites (Figure 21). There were no differences in weekly GSI values between greatest GSI values and other weeks until October, indicating that maximum GSI values were reached and followed by a period of spawning as indicated by continuous decreasing GSI values. Peak spawning was also initiated and occurred during the 15°C time period (Figure 21). From this, we deduce that scallops were spawning throughout the remainder of September and into October 2023. Overall

scallop gene copy values were higher at wild bed and distant sites after maximum GSI values occurred (i.e. beginning of peak spawning; Figs. 21 & 22, Table 1). The vertical distribution of the signal also increased above wild beds during the spawning and larval transport seasons. Prior to spawning, the highest signal was detected in the deepest sampling depth, nearest the bottom, while after spawning, the signal detection across depths increased at each site (Fig. 22).

Table 6: Table of pre- and post-spawn means at wild and distant sites. Post-spawn gene copy values were higher than the pre-spawn values at both the wild bed and distant sites, as indicated by the Kruskall-Wallis H test results above.

Site	Pre-spawn Mean	St. Error (+/-)	Post-spawn Mean	St. Error	H	df	p
Wild	100,259	22,924	179,535	18,787	6.88	1	.0088
Distant	16,353	3,702	248,817	42,684	11.34	1	.0008

Maximum scallop DNA gene copy values occurred during potential larval transport times at both wild and distant sites (Fig. 22). The maximum scallop DNA signals were detected at mid-water depths (i.e. 13 m) at the wild site, and nearest the surface at the distant site, with the distant site maximum occurring three weeks prior to the wild site maximum (Fig. 22). The greatest variability, as indicated by standard error, also was detected at this sampling date for both wild and distant sites with the variability in the distant site the highest across all sampling events and depths.

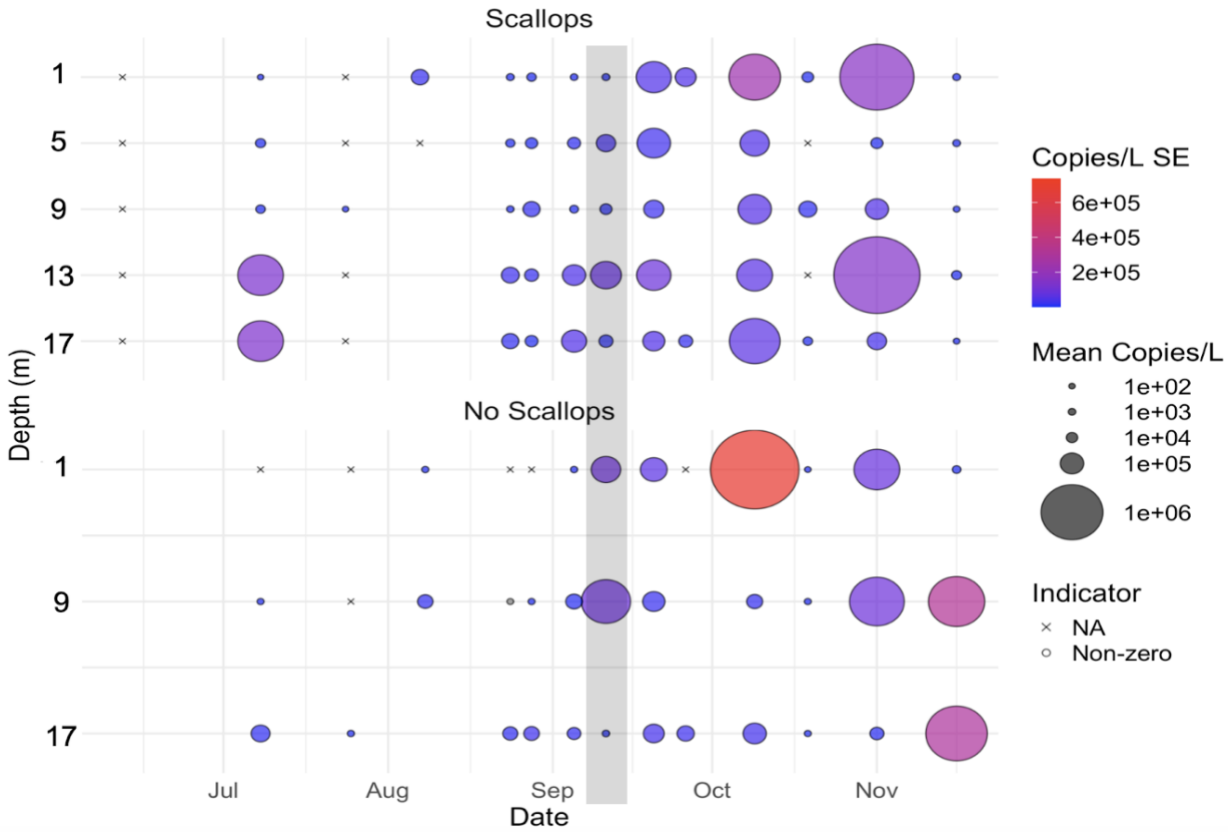


Figure 22: Bubble plot of vertical sampling above a wild sea scallop bed. Bubble plot of gene copy values at different depths at the wild bed and distant sites in 2023. Gene copy L^{-1} values are indicated by circle size and variability (standard error) is indicated by circle color. "Xs" indicate undetectable quantities of eDNA at a sampling event. The gray box indicates the sampling week in which peak GSI occurred. The wild bed site estimated scallop density is 0.86 scallops m^{-2}.

4.5 DISCUSSION

The available scallop qPCR probe and primers successfully detected scallop eDNA on scallop aquaculture farms and above a wild well-characterized, deeper scallop bed. There was temporal (across weeks) and spatial (across sites and depths) variation in this signal over the two-year sampling period. Scallop eDNA also was detected at a site otherwise lacking scallop

populations and 1.9kms from the wild scallop bed. Contrary to our hypotheses, seasonal maxima of gene copy number per liter seawater sampled did not occur after peak scallop spawning, as indicated by GSI values, and did not occur in tandem with maximum counts of bivalve larvae at any site in 2020 or 2021, with the exception of one farm site. In this one instance, larval count explained 76% of the variation in gene copy at the ST site in 2020, but no other associations between larval density and gene copy were found at the other farms in any sampling year. Scallop eDNA was detected at all depths, but not during all sampling events, above the well characterized wild scallop bed. Scallop eDNA also was detected at all depths - sometimes at high concentrations - at a site lacking scallops, suggesting that transport of eDNA and quantifying stochasticity in 'background' signals is an important consideration in future studies. Scallop eDNA signal increased at both wild population sites and across depths after maximum GSI were observed and during the time of assumed peak larval presence from 30-45 days after spawning.

Seasonal variation in species-specific eDNA signal is not uncommon and is often related to biological (i.e., reproduction or larval transport; Troth et al. 2019; Tillotson et al. 2018; Doyle, McKinnon & Uthicke 2017), physical (i.e., oceanography; Harrison, Sunday & Rogers 2019), and chemical processes (i.e., degradation; Mauvisseau et al. 2022). Dynamic marine environments present challenges for collection and interpretation of data due to high spatial and temporal variability in signal detection. Distance from the organism can have significant implications for detection and quantification of signals with 79% of positive detections occurring within 30m of the signal source (Murakami et al. 2019; Allan et al. 2021). Physical dynamics, such as currents and stratification, can also influence detection, especially in extremely high dynamic coastal areas or low dynamic environments like the deep ocean. In highly stratified systems, eDNA methods have been used to determine behavior of deep sea fishes and identify

depth partitioning by invertebrates in kelp forest systems (Canals et al. 2021; Monuki, Barber and Gold 2021). The physical dynamics of the system and the life history and behavioral dynamics of the organisms of interest should be given equal consideration in eDNA sampling efforts.

The scallop DNA signal detected at farms, the wild bed, and the distant sites across years was higher during spawning and larval transport season in comparison to collections occurring in winter months, but limited winter collections occurred beyond 2021 to further support this. There is little evidence of spawning occurring during winter months in Maine, but scallop populations on Georges Bank experience spring and summer spawns (Thompson, Inglis & Stokesbury 2014). Fishermen consistently observe scallops with full gonads during the winter wild harvest season (P. Jekielek, *pers. observ.*). There is potential for additional spawning to occur outside of the summer season in Maine but this has not yet been observed. GSI values and variation from our limited winter sampling does not support the possibility of additional spawning.

Scallop DNA values generally increased with an increase in GSI (Figures 19 & 20), but eDNA maxima occurred prior to peak spawning events at farms rather than after. This increasing eDNA detection could be a result of protracted or dribble spawning events taking place on scallop farms and in wild populations. The variance in GSIs of both farmed and wild populations is greater during summer and gonad ripening months than in the non-spawning (winter) months (Figures 19 & 20), suggesting that scallops are progressing towards spawning, and potentially spawning, at different times both within and between populations. Although there is a clear peak in the GSI, spawning is most likely occurring in local populations (see Chapter 2) at different rates prior to this peak and could be responsible for the pattern of increasing gene copy values as

maximum GSI values are approached. Signal detection resulting from spawning activity or from larval transport from more distant populations cannot be ruled out.

Scallop eDNA signal was not always higher on scallop aquaculture farms or above a wild scallop bed in comparison to distant sites. Regardless of the high densities of scallops on farms and sampling directly beside scallop lantern nets, scallops may not be generating highly detectable amounts of DNA when they are not actively spawning (see Chapter 3). Shelled organisms, such as crabs or mollusks, do not generate DNA at the rates of other non-shelled organisms and are generally more difficult to detect even when visually present (Crane et al. 2021; Pierce et al. in prep). Even if scallops are actively spawning, this signal could be carried away via currents if the timing of our sampling does not match up or if there is not a constant gradient of gamete concentration around the nets due to variability in gamete release or local hydrodynamics (Lotterhos and Levitan 2010).

Although the linear relationships between scallop eDNA signal and larval counts were generally not significant or strong at scallop farms, above a wild bed with scallops, or at the distant site without scallops, with the exception of the ST farm site in 2021, bivalve larvae were present during the sampling and most likely influenced the signal being detected (Figs. 19, 20, 21). Bivalve larvae are historically challenging to identify from one another in plankton samples (Garland & Zimmer 2002). We were unable to distinguish scallop larvae from other potential species of bivalve larvae such as mussels, clams, or oysters using microscopy. Blue mussels (*Mytilus edulis)* generally spawn in July in Maine (Newell et al. 1982) and, with a larval period that can last from 3 - 5 weeks (Wang & Widows 1991), the likelihood of mussel larvae in our samples is high throughout July and August although we could not distinguish between mussel and scallops. This could potentially skew any predictable relationships between peak sea scallop

gene copy number and peak larval counts. Additional eDNA applications, both metabarcoding and qPCR, to remaining plankton samples could better parse out the presence of additional bivalve species and clarify the proportion of sea scallop larvae in samples in comparison to other potential bivalve species.

With the average gene copy value of a single larva quantified as 3.09×10^7 (see Chapter 3), a single larva being captured in any single liter of water sampled could significantly increase the overall signal. This could explain the sometimes high variability (i.e., standard error value) seen in average gene copy values if one of the replicate 1L samples from the Niskin had a larva and the other two did not. With the low numbers of larvae L^{-1} present in samples across sites and sampling designs, ranging from .01 to 1 larvae L^{-1}, this possibility should be considered. Different life stages of larvae can also have different amounts of DNA. Doyle, McKinnon & Uthicke (2017) found that different developmental stages, from fertilized egg to competent brachiolaria, of crown of thorns sea stars had significantly different DNA concentrations. If there are different developmental stages (i.e. trochophore larvae) in addition to larvae, this could contribute to the mismatch between eDNA detection and larval counts. Again, the potential mismatch of sampling at the "right time" to capture a larva remains a challenge.

The spatial and temporal variability in eDNA signal is further illustrated in the multi-depth sampling above a wild bed and a distant site lacking scallops. Although there were no scallops present at the distant site, we detected scallop signals above this site at each sampling event but the DNA signal was not detected at all depths at each sampling event (Figure 7). Prior to spawning, the signal detected at the depth nearest the bottom, 17 m, was highest at both the wild bed and distant site, although it was higher and detected across all depths above a wild bed. Mean gene copy values at wild and distant sites pre-spawning were lower than mean gene copy

values post-spawning (i.e. after peak GSI values). The signal and its detection increased across all depths and at all sampling events after this time at the wild bed and distant site. Maximum gene copy values at wild bed and distant sites occurred in October and November sampling events and were detected in mid-water depths at each site. The time frame of these maximum detections suggests that this signal may be a result of larval presence because scallops have a planktonic larval period ranging from 30 - 45 days, which is supported by our peak GSI occurring in mid-September but not supported through corresponding maximum larval counts, as expected (Culliney 1974). This could be another effect of hydrodynamics interacting with sample timing and the patchiness of individual larvae throughout the water column.

DNA signals from surrounding populations may also be transported into our sampling area as a result of more distant scallop populations located throughout the highly dynamic hydrography of Penobscot Bay and the island archipelago. Sampling at a determined tidal cycle, such as slack low or high tide could control for some of this variability. Sampling at farms did not occur at the same time from week to week and the tides were different at almost every farm site sampling event because we were coordinating with scallop farmers to access scallop farms and sampling often occurred around their schedules. Tidal cycles are not likely to have a large effect on detection of the diversity of DNA collected at a site, but may influence the species-specific signal quantified within a single location as a result of a mismatch between sampling time, tidal dynamics, and biological processes (Kelly et al 2018).

In Penobscot Bay where this study occurred, the ST and NH farms were located in the Eastern Bay, while the HI and wild bed sites were located in the Western Bay. These two sections of the Bay are characterized by differences in flow dynamics - the Western Bay experiences outflows of water near the surface and the bottom and inflows at mid-water depths;

the Eastern Bay outflows in the upper water column and inflows at greater depths (Xue et al. 2000). This diversity in flow may be impacting the signal detected across sites and across sampling regimes and potentially transporting DNA, in the form of gametes, tissue, or larvae from other scallop populations in the bay or from further Eastern populations transported as part of the Eastern Maine Coastal Current. This is most likely one reason for scallop DNA being detected at distant sites where we expected less of a signal in comparison to scallop farms or wild beds.

The eDNA signals were also highly variable among sampling events at individual farms with gene copy values decreasing in orders of magnitude from one sampling event (i.e. week) to the next in both years (Figures 19 & 20). Although there was variability in the signal, we did not see this pattern of magnitude-level differences during the 2023 vertical sampling events at the wild bed or distant sites until well after spawning season and into larval transport season. Inhibition of DNA signal is another potential challenge as a result of probe and primer competition by unknown non-target DNA and natural inhibitor molecules from samples, but this should be minimal because the Qiagen extraction kit includes an inhibitor removal technology (Bayer, Countway & Whale 2019). Inhibition could be occurring due to increased biomass from plankton blooms or runoff from coastal areas increasing turbidity, which has been shown to impact eDNA signal detection, and cannot be ruled out (Jane et al. 2014; Stoeckel et al. 2021).

eDNA has the potential to be used for adult stock assessments, larval transport models, and to estimate recruitment potential, if these patterns in eDNA occurrence and their significance were understood (Alexander et al 2021, Kirtane 2021). For stock assessments and estimates of recruitment potential, it could reduce the need for more invasive surveys, such as dredging or netting, and can inform understanding of species-specific larval distributions. Indeed, integration

of validated eDNA science into existing biophysical models predicting where and how many larvae will disperse could allow more accurate quantification of recruitment dynamics and exchange between populations. The specific life-histories of organisms play an important role in understanding the appropriate applications for eDNA tools and require substantial ground truthing in both laboratory and field settings to determine the appropriate application for any given species (Rojahn et al. 2023). If successful, carefully constructed sampling designs could allow this eDNA tool to be used both for adult stock assessments and to estimate recruitment potential.

Additional work is needed to clarify spatial and temporal variability of eDNA in this and, no doubt, other species. In the future, we are interested in using metabarcoding and qPCR methods of the remaining plankton samples to conduct additional comparisons with larval counts. Our studies were conducted in relatively shallow waters. Repeating a vertical sampling design in deeper waters, above larger scallop populations and at increased distance from shore would provide a more comprehensive understanding of the variability in these signals and the physical dynamics acting at larger scales.

4.6 CONCLUSIONS

We successfully detected sea scallop DNA on scallop aquaculture farms and above wild beds, and also at control sites. There is high spatial and temporal variability in scallop DNA detection influenced by biological and physical factors. Knowledge generated through this study of scallop life history, the region and site-specific oceanographic conditions, and documented variability in detection among different habitats and sampling methods can support future applications of eDNA tools to monitor populations of commercially important species.

CHAPTER 5

SYNTHESIS

5.1 REVIEW OF MAJOR FINDINGS

The goal of this dissertation was to evaluate eDNA tools in order to progress our understanding of their appropriate applications to monitoring natural populations of commercially-important species. A thorough understanding of the biology and ecology of the species of study, here the sea scallop (*Placopecten magellanicus*), is essential to evaluating new technologies for monitoring these populations. The application of eDNA tools to develop relationships between larval concentrations and densities of adult scallops to resulting gene copy numbers in laboratory settings provided novel data to inform field applications and interpret resulting data. The monitoring of biological processes, namely spawning and larval transport, in farmed and wild scallop populations is the first application of eDNA tools for this species in a natural setting and reveals key insights to the spatial and temporal variability of these processes.

Chapter 1 provided an overview of the importance of scallop populations, from ecological and economic perspectives, at national and local scales and outlined the unique life history characteristics that make this species a challenge to study. It also reviews the development of scallop aquaculture in coastal Maine and highlights the possible interactions of wild and farmed scallop populations. Lastly, this chapter introduces environmental DNA and its potential as a tool for conservation and management.

In Chapter 2 we found that farmed scallops have significantly larger adductor, gonad, and viscera masses compared to wild scallops within the largest size classes 80–110mm, while, conversely, wild scallops have significantly larger shell masses. The timing of spawning in the populations is variable on farms and in the wild, occurring up to three weeks apart. Larger meat

yields from farmed scallops offer a significant potential return on investment for scallop growers, while their larger gonads suggest an increased likelihood for reproductive output with ecological ramifications for both aquaculture and wild harvest industries at local scales. These results shed light on the complex interplay between aquaculture and the natural environment, highlighting the need to further investigate the biological, ecological, and economic consequences of sea scallop cultivation and its interactions with the wild fishery. Furthermore, monitoring of life history events in commercially important species can inform the application of environmental DNA tools for scientific research and, potentially, management efforts.

In Chapter 3, we applied qPCR methods to quantify the eDNA signals from different life stages and densities of scallops in laboratory settings. We collected a known concentration of scallop larvae from Mook Sea Farm in Walpole, Maine and conducted multiple larval dilution experiments to establish a linear relationship between larval numbers and resultant gene copy numbers, establishing an estimate for individual larval DNA quantities. We also conducted a controlled mesocosm experiment to quantify eDNA shedding rates of scallops and relate these rates to different biomasses of non-spawning scallops in mesocosms. The shedding rates (copies/h/g) of sea scallops are comparable to those found in studies on freshwater mussels, although these data are the first of their kind for sea scallops. There is a significant relationship between biomass and gene copies that explains 39% of the variation in the signal. Regardless, this is the first experiment to my knowledge that evaluates eDNA shedding rates and relationships to biomass in sea scallops. These relationships will help to inform experimental design for field sampling efforts and interpreting data from natural experiments.

In Chapter 4, we used scallop aquaculture farms and wild scallop beds as research sites to detect scallop DNA in the field, evaluate spatial and temporal differences in eDNA distribution,

and determine the use of eDNA methods to detect biological processes, such as spawning and larval transport. Although eDNA tools were successful at detecting scallop DNA in farmed and wild populations, maximum detections did not occur one week after peak spawning in farmed and wild populations of scallops or during maximum counts of bivalve larvae from plankton tows, as expected. Using a vertical sampling design above a wild bed, we detected eDNA at all depths above a wild scallop bed and at a control site distant from the wild bed and lacking scallops. Scallop DNA signal increased at both sites and across depths after maximum GSI were observed and during expected peak larval transport, as evidenced by gene copy maxima occurring on the same sampling date, for both wild bed and distant sites. eDNA tools revealed the prevalence of scallop DNA throughout the water column over time where we expect to see it (at scallop aquaculture farms and above wild beds) and also where we do not (above distant benthic sites without scallops).

The results from this work identify areas for future research and areas of concern for the application of eDNA tools for monitoring populations with complicated life histories, such as sea scallops. As eDNA continues to grow in popularity among the scientific and management communities, we need to recognize the shortcomings of these methods and where they can and should be applied in order to best inform and support their application for management efforts.

5.2 FUTURE WORK AND RECOMMENDATIONS

5.2.1 Scallop aquaculture and wild population interactions

Scallop aquaculture farms offer known age structures and aggregate high densities of mature individuals (7-13/m^2, Hurricane Island) within areas of just two to three acres. Wild populations on Georges Bank (1/m^2, Thorzeau 1991) and the Maine coast (0.56/m^2, Bethoney 2019) are generally much less dense in comparison and cover much larger areas on the order of

kilometers (Stokesbury 1995).The conditions for successful external fertilization - high densities, large populations, and close proximity of spawning individuals - are likely to be found on farms as a result of culturing methods (Levitan 1998). Aquaculture farms aggregate sexually mature individuals in small areas suggesting they may have similar effects as fishing refugia (or marine protected areas) and thus could increase sea scallop larval production rates.

The likelihood of successful fertilization in broadcast spawning populations can be very low and is influenced by biotic and abiotic factors at various scales. Factors that can interfere with fertilization processes include variability in the life spans of sperm and eggs, the influence of flow on the rates at which gametes interact, non-viability of embryos due to polyspermy, and a decrease in the likelihood of gamete mixing as a result of asynchronous spawning (Levitan, 1995; Whale and Gilbert, 2002; Bayer et al., 2016). In contrast to benthic-dwelling wild populations inhabiting depths of 10 m and below, cultured populations are grown in lantern nets suspended 6-10 m below the surface in the hydrographically complex water column. Scallop aquaculture farms in Maine are generally located in exposed areas experiencing high tidal flows with highly variable directions and velocities (Xue et al 2000). Suspended aquaculture can aid in reduction of flow and increased turbulent stirring which increases the potential for eggs and sperm to interact (Grant and Bacher, 2001; Crimaldi and Browning, 2004). Because aquaculture farms aggregate large, sexually mature individuals in small areas in the complex water column, they may increase the rate of successful fertilization and, subsequently, have similar effects as closed areas via an increase in sea scallop larval production rates. If larvae are dispersed from farms to nearby wild populations, aquaculture may play a critical role in influencing the overall productivity of this commercially important species. Understanding of this dynamic is gaining importance as aquaculture efforts continue to expand at state and federal levels.

Maximizing the access that scallop aquaculture farms provide to evaluate fertilization success and resultant larval supply will provide useful information to inform both the established wild fishery and the growing scallop aquaculture industry. Fertilization experiments deployed on farms would verify if there is increased fertilization success occurring and provide data to further develop biophysical models exploring larval production and transport. Variables, such as egg size, are well-documented in early literature (Culliney, 1974; Langton et al., 1987, and others), but recent data is lacking and there is little data to inform the potential effects of aquaculture practices on the fitness of scallops in recent literature. This work would also inform our understanding of population connectivity in Maine scallops populations and the influence scallop aquaculture farms may have in maintaining, expanding, or impeding that connectivity and the fitness of scallops.

5.2.2 Scallops in a changing ocean environment

Maine's growing aquaculture industry and wild capture fisheries are situated in a rapidly changing marine ecosystem in the Gulf of Maine (Pershing et al. 2021) that has low buffering capacity (Gledhill et. al 2015). The Gulf of Maine (GoM) has warmed faster than 99% of the world's oceans at a rate of 0.04°C/yr since 1982, four times faster than the global average (Thomas et al. 2017; Pershing et al. 2021). Climate change impacts and coastal acidification are likely to affect calcified benthic marine populations' reproduction, recruitment, fecundity, and distribution although there are consistent knowledge gaps in our understanding of these effects (Hare et al. 2016; Gledhill et al. 2015). Sea scallops have been assessed to be at higher risk than other Atlantic species (Cooley et al. 2015; Rheuban et al. 2018). Establishing biological monitoring programs to attribute population level changes to management programs or shifting environmental conditions will help us better understand how to manage the important, lucrative

wild capture and aquaculture fisheries in concert. Evaluating the connections between environmental variability and life histories of scallops is a necessary step in planning for the future of this resource. A deeper understanding of the variables controlling spawning and their frequency would provide a clearer picture of larval dynamics at multiple scales. Incorporating monitoring of gonadal development and spawning timing along the coast would be an important first step in this direction.

Environmental variability and stress have been shown to have negative effects on physiology, egg production, function, and fertilization in marine invertebrates and may be heritable traits (Pilditch and Grant 1999; Foo and Byrne 2017; Pousse et al. 2020). Other scallop species show significant physiological and biomineralogical phenotypic variability in response to punctual stress conditions with negative impacts on survivorship over the long term (Ramajo et al. 2020). Additionally, populations along the coast of Maine can vary substantially in their genetic makeup and may differ in their responses to environmental variability (Owen and Rawson 2013). In Maine, cultured scallops are sourced from wild populations along the entire coast, which offers a unique opportunity to explore potential differences in biological and physiological responses to environmental variability from different seed sources and evaluate effects of culture practices on scallops' responses to environmental variability.

Cultured scallops are exposed to a variety of disturbances during their culture period that their wild counterparts do not experience. As cultured scallops grow, they are graded using an automated grading machine and then sorted between lantern nets to maximize growth rate and farm production. Scallops also undergo cleaning using a pressure-washing machine once or twice a year. This sorting, grading, and cleaning process utilizes automated grading and cleaning systems, often exposing scallops to the air for long periods of time and significant movement of

scallops among machinery, nets, and vessels. Sea scallops subjected to abrupt mechanical shock showed decreases in metabolic processes producing amino acids (Tian et al. 2021), while extended air exposure results in hypoxic stress, influences downstream farm production, and may result in reproductive impacts or mortality (Christopherson et al 2008). For these reasons, the farming process itself may induce stress in cultured scallops and, consequently, lead to individual physiological and population scale survival and fitness effects resulting in observable differences in the responses of wild and cultured populations to environmental variability and change.

5.2.3 Continued ground truthing and applications for eDNA methods and tools

eDNA sampling efforts support faster sampling, cost-effectiveness, efficiency and accuracy in comparison to their well-established existing monitoring counterparts like surveys, plankton tows, or SCUBA diving collections (Rourke et al. 2021). These methods offer independence from specialized field gear and taxonomic expertise and increase access for non-specialist participation in data collection. eDNA methods continue to need additional ground truthing and evaluations of applications, especially if using species-specific qPCR methods rather than community-level methods like metabarcoding. Specifically, given eDNA testing is relatively new, there is still a general need to promote standardization across collection, testing and assessment processes and to evaluate the relevance of laboratory-based experiments to field-based applications and monitoring.

The lack of standardization of methods for eDNA sampling and analysis is becoming more prevalent as eDNA applications continue to expand. There is a lack of standardized methodology, geographical bias of applications, incomplete reference sequence databases, and provision of methodological details across the eDNA and newly-emerging eRNA study

landscapes (Bunholi, Foster & Casey 2023). The variability in collection and analysis methods, such as water filtration volume, filter materials and pore size, extraction methods, and bioinformatic pipelines, from study to study is high. With factors such as particle size (Brandao-Dias et al. 2023), fragment length, pH, microbial activity and biofilms affecting the detection, persistence, and regulation of DNA in aquatic and marine systems (see review of Joseph et al. 2022), a movement toward standardization of sampling methods would inform these affects across species and systems (Geerts et al. 2018).

The translation of results from laboratory experiments to field applications is another area requiring additional research. The controlled mesocosm experiments conducted as part of this dissertation had high variation in the results even within the short time frame. There are few studies that pair controlled laboratory experiments with a complementary field application to ground truth the results, but successes have been found in validating laboratory detection of four species of freshwater fish in experimental ponds (Davison et al. 2016) and for the monitoring of endangered frogs in Himalayan regions (Saeed et al. 2022). Rojahn et al. (2023) had near perfect detection of turtle species in the lab but had sub-optimal detection in the field, even in areas of known presence, and suggested that field evaluations be conducted on a species-by-species basis to determine limitations and error rates. Developing workflows that include thorough lab to field applications would benefit the development of eDNA methods for applications to any species and these workflows should be standardized in some capacity so that the results are comparable across species and, potentially, across systems.

5.3 CONCLUDING REMARKS

This dissertation applies knowledge and monitoring of the life history characteristics of a commercially important species to the evaluation of eDNA as a tool for surveying populations of sea scallops. Further validation of eDNA methods against existing monitoring methods, such as gonadosomatic indices and plankton tows, is necessary to assess their appropriate application for research and monitoring. This dissertation is unique in that it directly compares eDNA methods to traditional methods on aquaculture farms and in wild populations of sea scallops and identifies temporal and spatial variation of eDNA signals in these environments. At this time eDNA tools should not be used to conduct stock assessments or population surveys of sea scallops for management purposes as additional work should be done to establish biomass and eDNA relationships in wild settings. eDNA tools could be used to monitor life history processes, like spawning, in wild and farmed populations but should not yet be used to understand larval distribution or supply until methods are developed to identify scallop plankton versus other bivalve plankton from plankton tows to establish clearer relationships between eDNA and larval counts. Here I provide the first record of DNA generation and degradation rates for sea scallops in mesocosms and relate larval concentrations to gene copies to determine a gene copy value per individual larvae. I also identify temporal patterns in sea scallop DNA prior to and post spawning and larval transport season. These applications inform appropriate applications of eDNA tools for monitoring commercially important species with complex life histories like the sea scallop.

REFERENCES

Alexander, Jenna L., et al. “Improving Quantification of Bivalve Larvae in Mixed Plankton Samples Using QPCR: A Case Study on Mytilus Edulis.” *Aquaculture*, vol. 532, Feb. 2021, p. 736003, https://doi.org/10.1016/j.aquaculture.2020.736003.

Allan, Elizabeth Andruszkiewicz, et al. “Modeling Characterization of the Vertical and Temporal Variability of Environmental DNA in the Mesopelagic Ocean.” *Scientific Reports*, vol. 11, no. 1, Oct. 2021, p. 21273, https://doi.org/10.1038/s41598-021-00288-5.

Allen, Michael C., et al. “Terrestrial EDNA Survey Outperforms Conventional Approach for Detecting an Invasive Pest Insect within an Agricultural Ecosystem.” *Environmental DNA*, vol. 3, no. 6, 2021, pp. 1102–12, https://doi.org/10.1002/edn3.231.

Barber, Bruce J., et al. “Reduced Fecundity in a Deep-Water Population of the Giant Scallop *Placopecten Magellanicus* in the Gulf of Maine, USA.” *Marine Ecology Progress Series*, vol. 42, no. 3, 1988, pp. 207–12, https://www.jstor.org/stable/24825653.

Bastos Gomes, Giana, et al. “Use of Environmental DNA (EDNA) and Water Quality Data to Predict Protozoan Parasites Outbreaks in Fish Farms.” *Aquaculture*, vol. 479, Oct. 2017, pp. 467–73, https://doi.org/10.1016/j.aquaculture.2017.06.021.

Bayer, Skylar. “Measuring fertilization in populations of sea scallop (Placopecten magellanicus): Developing and testing methods in the laboratory and field.” The University of Maine, 2017.https://www.proquest.com/openview/1945cb1c18db397697c5373438bd4d63/1?pq-origsite=gscholar&cbl=18750. Accessed 12 Jan. 2022.

Bayer, Skylar R., et al. “Developing an EDNA Toolkit to Quantify Broadcast Spawning Events of the Sea Scallop *Placopecten magellanicus*: Moving beyond Fertilization Assays.” *Marine Ecology Progress Series*, vol. 621, July 2019, pp. 127–41, https://doi.org/10.3354/meps12991.

Beng, Kingsly C. and Richard T. Corlett. “Applications of Environmental DNA (eDNA) in Ecology and Conservation: Opportunities, Challenges and Prospects.” *Biodiversity & Conservation*, vol. 29, no. 7, June 2020, pp. 2089–121, https://doi.org/10.1007/s10531-020-01980-0.

Benoit, Natalie P., et al. “Using QPCR of Environmental DNA (eDNA) to Estimate the Biomass of Juvenile Pacific Salmon (*Oncorhynchus* Spp.).” *Environmental DNA*, vol. 5, no. 4, 2023, pp. 683–96, https://doi.org/10.1002/edn3.422.

Bethoney, N. David, et al. "A comparison of drop camera and diver survey methods to monitor Atlantic sea scallops (*Placopecten magellanicus*) in a small fishery closure." *Journal of Shellfish Research* 38.1 (2019): 43-51.

Bonardelli, J. C., et al. “Relation of Spawning of the Giant Scallop, *Placopecten magellanicus*, to Temperature Fluctuations during Downwelling Events.” *Marine Biology*, vol. 124, no. 4, Feb. 1996, pp. 637–49, https://doi.org/10.1007/BF00351045.

Bourque, Danielle A., et al. “Environmental DNA Tracks Zooplankton Population Dynamics in Experimental Mesocosms.” *Environmental DNA*, vol. 5, no. 5, 2023, pp. 880–93, https://doi.org/10.1002/edn3.347.

Brandão-Dias, Pedro F. P et al. “Particle Size Influences Decay Rates of Environmental DNA in Aquatic Systems.” *Molecular Ecology Resources* 23.4 (2023): 756–770. Web.

Breton, Brie-Anne A., et al. “Testing the Effectiveness of Environmental DNA (EDNA) to Quantify Larval Amphibian Abundance.” *Environmental DNA*, vol. 4, no. 6, 2022, pp. 1229–40, https://doi.org/10.1002/edn3.332.

Bunholi, Ingrid V., Nicole R. Foster, and Jordan M. Casey. "Environmental DNA and RNA in aquatic community ecology: Toward methodological standardization." *Environmental DNA* 5.6 (2023): 1133-1147.

Buxton, Andrew S., et al. “Seasonal Variation in Environmental DNA in Relation to Population Size and Environmental Factors.” *Scientific Reports*, vol. 7, no. 1, Apr. 2017, p. 46294, https://doi.org/10.1038/srep46294.

Cameron, Louise P., et al. “Effects of Elevated PCO2 and Temperature on the Calcification Rate, Survival, Extrapallial Fluid Chemistry, and Respiration of the Atlantic Sea Scallop *Placopecten magellanicus*.” *Limnology and Oceanography*, vol. 67, no. 8, 2022, pp. 1670–86, https://doi.org/10.1002/lno.12153.

Canals, Oriol, et al. “Vertical Stratification of Environmental DNA in the Open Ocean Captures Ecological Patterns and Behavior of Deep-Sea Fishes.” *Limnology and Oceanography Letters*, vol. 6, no. 6, 2021, pp. 339–47, https://doi.org/10.1002/lol2.10213.

Capo, E., Spong, G., Königsson, H., & Byström, P. “Effects of Filtration Methods and Water Volume on the Quantification of Brown Trout (Salmo Trutta) and Arctic Char (Salvelinus Alpinus) EDNA Concentrations via Droplet Digital PCR.” *Environmental DNA*, vol. 2, no. 2, 2020, pp. 152–60, https://doi.org/10.1002/edn3.52.

Carmichael, R. H., Shriver, A.C., & Valiela, I. “Changes in Shell and Soft Tissue Growth, Tissue Composition, and Survival of Quahogs, *Mercenaria Mercenaria*, and Softshell Clams, *Mya Arenaria*, in Response to Eutrophic-Driven Changes in Food Supply and Habitat.” *Journal of Experimental Marine Biology and Ecology*, vol. 313, no. 1, Dec. 2004, pp. 75–104, https://doi.org/10.1016/j.jembe.2004.08.006.

Chen, C., Zhao, L., Gallager, S., Ji, R., He, P., Davis, C., ... & Bethoney, D.. “Impact of Larval Behaviors on Dispersal and Connectivity of Sea Scallop Larvae over the Northeast U.S. Shelf.” *Progress in Oceanography*, vol. 195, July 2021, p. 102604, https://doi.org/10.1016/j.pocean.2021.102604.

Cleaver, C., S. Arnold, S. Asci, S. Bayer, N. D. Bethoney, T. Cheney, C. Guenther, P. Jekielek, I.Miller, K. Stokesbury, R. Wahle, E. Waterman, C. Wilson & J. Wotton. The effect of a small-scale closed area on *Placopecten magellanicus* in Maine. *Marine & Coastal Fisheries*. (Revising)

Clemmesen, C. “The Effect of Food Availability, Age or Size on the RNA/DNA Ratio of Individually Measured Herring Larvae: Laboratory Calibration.” *Marine Biology*, vol. 118, no. 3, Feb. 1994, pp. 377–82, https://doi.org/10.1007/BF00350294.

Close, H., Lambert, G., Robins, P., & Gimenez, L. "Connectivity between populations of the scallop *Pecten maximus* in the Irish Sea and the implications for fisheries management." *Frontiers in Marine Science* 10 (2024): 1274136.

Cole, A., A. Langston, and C. Davis. *Maine Aquaculture Economic Impact Report. Report for the University of Maine Aquaculture Research Institute.* 2016, https://umaine.edu/aquaculture/wp-content/uploads/sites/134/2017/01/Aquaculture- Econ-Report. pdf.

Coleman, S., Cleaver, C., Morse, D., Brady, D. C., & Kiffney, T. “The Coupled Effects of Stocking Density and Temperature on Sea Scallop (*Placopecten magellanicus*) Growth in Suspended Culture.” *Aquaculture Reports*, vol. 20, July 2021, p. 100684, https://doi.org/10.1016/j.aqrep.2021.100684.

Cooley, S. R., Rheuban, J. E., Hart, D. R., Luu, V., Glover, D. M., Hare, J. A., & Doney, S. C. “An Integrated Assessment Model for Helping the United States Sea Scallop (Placopecten Magellanicus) Fishery Plan Ahead for Ocean Acidification and Warming.” *PLOS ONE*, vol. 10, no. 5, May 2015, p. e0124145, https://doi.org/10.1371/journal.pone.0124145.

Cowen, Robert K., and Su Sponaugle. “Larval Dispersal and Marine Population Connectivity.” *Annual Review of Marine Science*, vol. 1, no. 1, 2009, pp. 443–66, https://doi.org/10.1146/annurev.marine.010908.163757.

Crane, L. C., Goldstein, J. S., Thomas, D. W., Rexroth, K. S., & Watts, A. W. “Effects of Life Stage on eDNA Detection of the Invasive European Green Crab (*Carcinus Maenas*) in Estuarine Systems.” *Ecological Indicators*, vol. 124, May 2021, p. 107412, https://doi.org/10.1016/j.ecolind.2021.107412.

Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W. K., & Bik, H. M. “The Ecologist’s Field Guide to Sequence-Based Identification of Biodiversity.” *Methods in*

Ecology and Evolution, vol. 7, no. 9, 2016, pp. 1008–18, https://doi.org/10.1111/2041-210X.12574.

Culliney, John L. "Larval Development of the Giant Scallop *Placopecten magellanicus* (Gmelin)." *The Biological Bulletin*, vol. 147, no. 2, Oct. 1974, pp. 321–32, https://doi.org/10.2307/1540452.

Dadswell, Michael J., and G. Jay Parsons. "Exploiting Life History Characteristics of the Sea Scallop, *Placopecten magellanicus*, from Different Geographical Locations in the Canadian Maritimes to Enhance Suspended Culture Grow-Out." *Journal of Shellfish Research,* vol. 11, no. 2, 1992, pp. 299 - 305.

Davies, K. T. A., Gentleman, W. C., DiBacco, C., & Johnson, C. L. "Semi-Annual Spawning in Marine Scallops Strengthens Larval Recruitment and Connectivity on Georges Bank: A Model Study." *Marine Ecology Progress Series*, vol. 516, Dec. 2014, pp. 209–27, https://doi.org/10.3354/meps10975.

Davison, P.I., Créach, V., Liang, W.-.-J., Andreou, D., Britton, J.R. and Copp, G.H. (2016), "Laboratory and Field Validation of a Simple Method for Detecting Four Species of Non-native Freshwater Fish Using eDNA." J Fish Biol, 89: 1782-1793. https://doi.org/10.1111/jfb.13086

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., ... & Bernatchez, L. "Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities." *Molecular Ecology*, vol. 26, no. 21, 2017, pp. 5872–95, https://doi.org/10.1111/mec.14350.

Doyle, J. R., McKinnon, A. D., & Uthicke, S. "Quantifying Larvae of the Coralivorous *Seastar Acanthaster* Cf. Solaris on the Great Barrier Reef Using QPCR." *Marine Biology*, vol. 164, no. 8, Aug. 2017, p. 176, https://doi.org/10.1007/s00227-017-3206-x.

Emerson, C. W., Grant, J., Mallet, A., & Carver, C. "Growth and Survival of Sea Scallops *Placopecten Magellanicus*: Effects of Culture Depth." *Marine Ecology Progress Series*, vol. 108, 1994, pp. 119–32, https://doi.org/10.3354/meps108119.

Fisheries, NOAA. "Atlantic Sea Scallop | NOAA Fisheries." *NOAA*, 11 Apr. 2024, https://www.fisheries.noaa.gov/species/atlantic-sea-scallop.

---. "Fisheries Economics of the United States: Data and Visualizations | NOAA Fisheries." *NOAA*, 13 Nov. 2023, https://www.fisheries.noaa.gov/data-tools/fisheries-economics-united-states-data-and-visualizations.

Fitzgerald, D. "Maine Scallop Aquaculture Report." Gulf of Maine Research Institute, 2021.

Fonseca VG, Davison PI, Creach V, Stone D, Bass D, Tidbury HJ. The Application of eDNA for Monitoring Aquatic Non-Indigenous Species: Practical and Policy Considerations. *Diversity*. 2023; 15(5):631. https://doi.org/10.3390/d15050631

Foo, S. A., Deaker, D., & Byrne, M. "Cherchez La Femme – Impact of Ocean Acidification on the Egg Jelly Coat and Attractants for Sperm." *Journal of Experimental Biology*, vol. 221, no. 13, July 2018, p. jeb177188, https://doi.org/10.1242/jeb.177188.

Friebertshauser, R., Shollenberger, K., Janosik, A., Garner, J. T., & Johnston, C. "The Effect of Bivalve Filtration on eDNA-Based Detection of Aquatic Organisms." *PLOS ONE*, edited by Hideyuki Doi, vol. 14, no. 11, Nov. 2019, p. e0222830, https://doi.org/10.1371/journal.pone.0222830.

Fukumori, K., Kondo, N. I., Kohzu, A., Tsuchiya, K., Ito, H., & Kadoya, T. "Vertical eDNA Distribution of Cold-Water Fishes in Response to Environmental Variables in Stratified Lake." *Ecology and Evolution*, vol. 14, no. 3, 2024, p. e11091, https://doi.org/10.1002/ece3.11091.

Gallardi, D., Mills, T., Donnet, S., Parrish, C. C., & Murray, H. M. "Condition and Biochemical Profile of Blue Mussels (*Mytilus edulis* L.) Cultured at Different Depths in a Cold Water Coastal Environment." *Journal of Sea Research*, vol. 126, 2017, pp. 37–45, https://doi.org/10.1016/j.seares.2017.07.001.

Geerts AN, Boets P, Van den Heede S, Goethals P. A search for standardized protocols to detect alien invasive crayfish based on environmental DNA (eDNA): A lab and field evaluation. Ecological Indicators. 2018 Jan 1;84:564-72.

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. "Moving Environmental DNA Methods from Concept to Practice for Monitoring Aquatic Macroorganisms." *Biological Conservation*, vol. 183, Mar. 2015, pp. 1–3, https://doi.org/10.1016/j.biocon.2014.11.040.

Grecian, L. A., Parsons, G. J., Dabinett, P., & Couturier, C. "Influence of Season, Initial Size, Depth, Gear Type and Stocking Density on the Growth Rates and Recovery of Sea Scallop, *Placopecten magellancius*, on a Farm-Based Nursery." *Aquaculture International*, vol. 8, no. 2, Mar. 2000, pp. 183–206, https://doi.org/10.1023/A:1009298631346.

Hare, J.A., Morrison, W.E., Nelson, M.W., Stachura, M.M., Teeters, E.J., Griffis, R.B., Alexander, M.A., Scott, J.D., Alade, L., Bell, R.J. and Chute, A.S. "A Vulnerability Assessment of Fish and Invertebrates to Climate Change on the Northeast U.S. Continental Shelf." *PLOS ONE*, vol. 11, no. 2, Feb. 2016, p. e0146756, https://doi.org/10.1371/journal.pone.0146756.

Hernández, J. C., Brito, A., Cubero, E., García, N., Girard, D., González-Lorenzo, G., & Falcón, J. M. "Temporal Patterns of Larval Settlement of *Diadema antillarum* (Echinodermata:

Echinoidea) in the Canary Islands Using an Experimental Larval Collector." *Bulletin of Marine Science*, vol. 78, no. 2, Mar. 2006, pp. 271–79.

Hervé, Alix, Isabelle Domaizon, Jean-Marc Baudoin, Tony Dejean, Pierre Gibert, Alice Valentini, et al. "Do Temperature and Population Size Structure Influence the Quantity of Fish EDNA in Water?" *Frontiers in Environmental Science*, vol. 11, July 2023, https://doi.org/10.3389/fenvs.2023.1193393.

Hervé, Alix, Isabelle Domaizon, Jean-Marc Baudoin, Tony Dejean, Pierre Gibert, Pauline Jean, et al. "Spatio-Temporal Variability of EDNA Signal and Its Implication for Fish Monitoring in Lakes." *PLOS ONE*, vol. 17, no. 8, Aug. 2022, p. e0272660, https://doi.org/10.1371/journal.pone.0272660.

Ishaq, S.L., Hosler, S., Dankwa, A., Jekielek, P., Brady, D.C., Grey, E., Haskell, H., Lasley-Rasher, R., Pepperman, K., Perry, J. and Beal, B. "Bacterial Community Trends Associated with Sea Scallop, *Placopecten magellanicus*, Larvae in a Hatchery System." *Aquaculture Reports*, vol. 32, Oct. 2023, p. 101693, https://doi.org/10.1016/j.aqrep.2023.101693.

Iversen, L.L., Kielgast, J. and Sand-Jensen, K. "Monitoring of Animal Abundance by Environmental DNA — An Increasingly Obscure Perspective: A Reply to Klymus et al., 2015." *Biological Conservation*, vol. 192, Dec. 2015, pp. 479–80, https://doi.org/10.1016/j.biocon.2015.09.024.

Jeunen, G.J., Lamare, M.D., Knapp, M., Spencer, H.G., Taylor, H.R., Stat, M., Bunce, M. and Gemmell, N.J.. "Water Stratification in the Marine Biome Restricts Vertical Environmental DNA (EDNA) Signal Dispersal." *Environmental DNA*, vol. 2, no. 1, 2020, pp. 99–111, https://doi.org/10.1002/edn3.49.

Manuel, J.L., Gallager, S.M., Pearce, C.M. and Manning, D.A. "Veligers from Different Populations of Sea Scallop *Placopecten magellanicus* Have Different Vertical Migration Patterns." *Marine Ecology Progress Series*, vol. 142, Oct. 1996, pp. 147–63, https://doi.org/10.3354/meps142147.

Joseph, C., Faiq, M. E., Li, Z. & Chen, G. "Persistence and Degradation Dynamics of EDNA Affected by Environmental Factors in Aquatic Ecosystems." *Hydrobiologia*, vol. 849, no. 19, Nov. 2022, pp. 4119–33, https://doi.org/10.1007/s10750-022-04959-w.

Jungbluth, M. J., Goetze, E. & Lenz, P. H. "Measuring Copepod Naupliar Abundance in a Subtropical Bay Using Quantitative PCR." *Marine Biology*, vol. 160, no. 12, Dec. 2013, pp. 3125–41, https://doi.org/10.1007/s00227-013-2300-y.

Kamoroff, C. & Goldberg, C.S. "An Issue of Life or Death: Using eDNA to Detect Viable Individuals in Wilderness Restoration." *Freshwater Science*, vol. 37, no. 3, 2018, pp. 685–96, https://doi.org/10.1086/699203.

Karl, D.M., Taylor, G.T., Novitsky, J.A., Jannasch, H.W., Wirsen, C.O., Pace, N.R., Lane, D.J., Olsen, G.J., Giovannoni, S.J. "A Microbiological Study of Guaymas Basin High Temperature Hydrothermal Vents." *Deep Sea Research Part A. Oceanographic Research Papers*, vol. 35, no. 5, May 1988, pp. 777–91, https://doi.org/10.1016/0198-0149(88)90030-1.

Kelly, R. P., Gallego, R. & Jacobs-Palmer, E. "The Effect of Tides on Nearshore Environmental DNA." *PeerJ*, vol. 6, Mar. 2018, p. e4521, https://doi.org/10.7717/peerj.4521.

Kirkley, J. & DuPaul, W. "Temporal Variations In Spawning Behavior Of Sea Scallops, *Placopecten magellanicus* (Gmelin, 1791), In The Mid-Atlantic Resource Area." *Journal of Shellfish Research*, vol. 10, no. 2, Jan. 1991, pp. 389–94, https://scholarworks.wm.edu/vimsarticles/1281.

Kirtane, A., Wieczorek, D., Noji, T., Baskin, L., Ober, C., Plosica, R., Chenoweth, A., Lynch, K., Sassoubre, L. "Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Commercially Harvested Fish Species and Comparison between EDNA Detection and Trawl Catches." *Environmental DNA*, vol. n/a, no. n/a, https://doi.org/10.1002/edn3.236. Accessed 3 Nov. 2021.

Kleinman, S., Hatcher, B. G., Scheibling, R. E., Taylor, L. H. & Hennigar, A. W. "Shell and Tissue Growth of Juvenile Sea Scallops (*Placopecten magellanicus*) in Suspended and Bottom Culture in Lunenburg Bay, Nova Scotia." *Aquaculture*, vol. 142, no. 1, June 1996, pp. 75–97, https://doi.org/10.1016/0044-8486(95)01165-X.

Kwong, S. L. T., Villacorta-Rath, C., Doyle, J. & Uthicke, S. "Quantifying Shedding and Degradation Rates of Environmental DNA (EDNA) from Pacific Crown-of-Thorns Seastar (*Acanthaster Cf. solaris*)." *Marine Biology*, vol. 168, no. 6, May 2021, p. 85, https://doi.org/10.1007/s00227-021-03896-x.

Lafrance, M., Cliche, G., Haugum, G. & Guderley, H. "Comparison of Cultured and Wild Sea Scallops *Placopecten magellanicus*, Using Behavioral Responses and Morphometric and Biochemical Indices." *Marine Ecology Progress Series*, vol. 250, 2003, pp. 183–95, https://doi.org/10.3354/meps250183.

Lamb, P. D., Fonseca, V. G., Maxwell, D. L. & Nnanatu, C. C. "Systematic Review and Meta-Analysis: Water Type and Temperature Affect Environmental DNA Decay." *Molecular Ecology Resources*, vol. 22, no. 7, 2022, pp. 2494–505, https://doi.org/10.1111/1755-0998.13627.

Lambert, Y., and Dutil, J. D. "Can Simple Condition Indices Be Used to Monitor and Quantify Seasonal Changes in the Energy Reserves of Cod (*Gadus morhua*)?" *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 54, no. S1, Jan. 1997, pp. 104–12, https://doi.org/10.1139/f96-149.

Langton, R. W., Robinson, W. E. & Schick, D. “Fecundity and Reproductive Effort of Sea Scallops *Placopecten magellanicus* from the Gulf of Maine.” *Marine Ecology Progress Series*, vol. 37, no. 1, 1987, pp. 19–25, https://www.jstor.org/stable/24825487.

Larson, Eric R., et al. “From EDNA to Citizen Science: Emerging Tools for the Early Detection of Invasive Species.” *Frontiers in Ecology and the Environment*, vol. 18, no. 4, 2020, pp. 194–202, https://doi.org/10.1002/fee.2162.

Lawson Handley, L., et al. “Temporal and Spatial Variation in Distribution of Fish Environmental DNA in England’s Largest Lake.” *Environmental DNA*, vol. 1, no. 1, 2019, pp. 26–39, https://doi.org/10.1002/edn3.5.

Lemos, D., Garcia-Carreño, F. L., Hernández, P., & Del Toro, A. N. "Ontogenetic variation in digestive proteinase activity, RNA and DNA content of larval and postlarval white shrimp *Litopenaeus schmitti*." *Aquaculture* 214, no. 1-4, 2002, pp. 363-380.

Leonard, J & Cordoba-Aguilar, A. *The Evolution of Primary Sexual Characters in Animals*. Oxford University Press, 2010.

Littlefair, Joanne E., et al. “Thermal Stratification and Fish Thermal Preference Explain Vertical EDNA Distributions in Lakes.” *Molecular Ecology*, vol. 30, no. 13, 2021, pp. 3083–96, https://doi.org/10.1111/mec.15623.

Lotterhos, K. E., Levitan, D. R., Traits, G. “Gamete release and spawning behavior in broadcast spawning marine invertebrates.” *The evolution of primary sexual characters in animals.* 2010 Jul 16;99:120.

Lundquist, C. J.,m and Botsford, L. W. “Model Projections of the Fishery Implications of the Allee Effect in Broadcast Spawners.” *Ecological Applications*, vol. 14, no. 3, 2004, pp. 929–41, https://doi.org/10.1890/02-5325.

MacDonald, B. A., and R. J. Thompson. “Intraspecific Variation in Growth and Reproduction in Latitudinally Differentiated Populations of the Giant Scallop *Placopecten magellanicus* (Gmelin).” *Biological Bulletin*, vol. 175, no. 3, 1988, pp. 361–71, https://doi.org/10.2307/1541727.

MacDonald, Ba. “Production and Resource Partitioning in the Giant Scallop *Placopecten magellanicus* Grown on the Bottom and in Suspended Culture.” *Marine Ecology Progress Series*, vol. 34, 1986, pp. 79–86, https://doi.org/10.3354/meps034079.

Marques, Virginie, et al. “Optimizing Detectability of the Endangered Fan Mussel Using eDNA and DdPCR.” *Ecology and Evolution*, vol. 14, no. 1, 2024, p. e10807, https://doi.org/10.1002/ece3.10807.

Maruyama, Atsushi, et al. “The Release Rate of Environmental DNA from Juvenile and Adult Fish.” *PLoS ONE*, edited by Matthias Stöck, vol. 9, no. 12, Dec. 2014, p. e114639, https://doi.org/10.1371/journal.pone.0114639.

Mauvisseau, Quentin, Alfred Burian, et al. “Influence of Accuracy, Repeatability and Detection Probability in the Reliability of Species-Specific EDNA Based Approaches.” *Scientific Reports*, vol. 9, no. 1, Jan. 2019, p. 580, https://doi.org/10.1038/s41598-018-37001-y.

Mauvisseau, Quentin, Lynsey R. Harper, et al. “The Multiple States of Environmental DNA and What Is Known about Their Persistence in Aquatic Environments.” *Environmental Science & Technology*, vol. 56, no. 9, May 2022, pp. 5322–33, https://doi.org/10.1021/acs.est.1c07638.

McCartin, Luke J., et al. “Temperature Controls EDNA Persistence across Physicochemical Conditions in Seawater.” *Environmental Science & Technology*, vol. 56, no. 12, June 2022, pp. 8629–39, https://doi.org/10.1021/acs.est.2c01672.

McGarvey, Richard, et al. “Spatial and Parent-Age Analysis of Stock–Recruitment in the Georges Bank Sea Scallop (*Placopecten Magellanicus*) Population.” *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 50, no. 3, Mar. 1993, pp. 564–74, https://doi.org/10.1139/f93-065.

Mcgarvey, Richard, et al. “Statistics of Reproduction and Early Life History Survival of the Georges Bank Sea Scallop (*Placopecten Magellanicus*) Population.” *Journal of Northwest Atlantic Fishery Science*, vol. 13, Dec. 1992, pp. 83–99, https://doi.org/10.2960/J.v13.a7.

Merten, Véronique, et al. “Arctic Nekton Uncovered by EDNA Metabarcoding: Diversity, Potential Range Expansions, and Pelagic-Benthic Coupling.” *Environmental DNA*, vol. 5, no. 3, 2023, pp. 503–18, https://doi.org/10.1002/edn3.403.

Miranda, Leandro. “Ovarian Weight as an Index of Fecundity, Maturity, and Spawning Periodicity.” *Journal of Fish Biology*, Jan. 1997, https://www.academia.edu/87614211/Ovarian_weight_as_an_index_of_fecundity_maturity_and_spawning_periodicity.

Monuki, Keira, et al. “eDNA Captures Depth Partitioning in a Kelp Forest Ecosystem.” *PLOS ONE*, vol. 16, no. 11, Nov. 2021, p. e0253104, https://doi.org/10.1371/journal.pone.0253104.

Morgan, S.G. “The Larval Ecology of Marine Communities.” In Marine Community Ecology, edited by M. D. Bertness, S. D. Gaines, and M. E. Hay, 159–82. Sunderland, MA: Sinauer Associates, Inc., 2001.

Moushomi, Rashnat, et al. “Environmental DNA Size Sorting and Degradation Experiment Indicates the State of Daphnia Magna Mitochondrial and Nuclear EDNA Is Subcellular.”

Scientific Reports, vol. 9, no. 1, Aug. 2019, p. 12500, https://doi.org/10.1038/s41598-019-48984-7.

Munroe, Daphne M., et al. "Modeling Larval Dispersal and Connectivity for Atlantic Sea Scallop (*Placopecten magellanicus*) in the Middle Atlantic Bight." *Fisheries Research*, vol. 208, Dec. 2018, pp. 7–15, https://doi.org/10.1016/j.fishres.2018.06.020.

Murakami, Hiroaki, et al. "Dispersion and Degradation of Environmental DNA from Caged Fish in a Marine Environment." *Fisheries Science*, vol. 85, no. 2, Mar. 2019, pp. 327–37, https://doi.org/10.1007/s12562-018-1282-6.

Murphy, H.M., and Jenkins, G.P. "Observational Methods Used in Marine Spatial Monitoring of Fishes and Associated Habitats: A Review." *Marine and Freshwater Research*, vol. 61, no. 2, Feb. 2010, pp. 236–52, https://doi.org/10.1071/MF09068.

Murray, Harry M., et al. "Effect of Culture Depth and Season on the Condition and Reproductive Indices of Blue Mussels (*Mytilus edulis* L.) Cultured in a Cold-Water Coastal Environment." *Journal of Shellfish Research*, vol. 38, no. 2, Aug. 2019, pp. 351–62, https://doi.org/10.2983/035.038.0215.

NOAA, et al. "Ocean and Coastal Acidification off New England and Nova Scotia." *Oceanography*, vol. 25, no. 2, June 2015, pp. 182–97, https://doi.org/10.5670/oceanog.2015.41.

NOAA. 2024. "2024 Sea Scallop Research Set-Aside Projects Selected". *NOAA Fisheries.* https://www.fisheries.noaa.gov/new-england-mid-atlantic/science-data/2024-sea-scallop-research-set-aside-projects-selected#project-summaries. Accessed October 23, 2024.

Parsons, G. J., et al. "Intra-Annual and Long-Term Patterns in the Reproductive Cycle of Giant Scallops Placopecten Magellanicus (Bivalvia: Pectinidae) from Passamaquoddy Bay, New Brunswick, Canada." *Marine Ecology Progress Series*, vol. 80, no. 2/3, 1992, pp. 203–14, https://www.jstor.org/stable/24826607.

Pazos, Antonio J., et al. "Stereological Studies on the Gametogenic Cycle of the Scallop, *Pecten maximus*, in Suspended Culture in Ria de Arousa (Galicia, NW Spain)." *Aquaculture*, vol. 142, no. 1, June 1996, pp. 119–35, https://doi.org/10.1016/0044-8486(95)01247-8.

Pechenik, Jan A. "On the Advantages and Disadvantages of Larval Stages in Benthic Marine Invertebrate Life Cycles." *Marine Ecology Progress Series*, vol. 177, Feb. 1999, pp. 269–97, https://doi.org/10.3354/meps177269.

Peñarrubia, Luis, et al. "Validated Methodology for Quantifying Infestation Levels of Dreissenid Mussels in Environmental DNA (EDNA) Samples." *Scientific Reports*, vol. 6, Dec. 2016, p. 39067, https://doi.org/10.1038/srep39067.

Pershing, Andrew J., et al. “Climate Impacts on the Gulf of Maine Ecosystem: A Review of Observed and Expected Changes in 2050 from Rising Temperatures.” *Elementa: Science of the Anthropocene*, vol. 9, no. 1, Aug. 2021, p. 00076, https://doi.org/10.1525/elementa.2020.00076.

Phillips, B. F., and J. D. Booth. “Design, Use, and Effectiveness of Collectors for Catching the Puerulus Stage of Spiny Lobsters.” *Reviews in Fisheries Science*, vol. 2, no. 3, Jan. 1994, pp. 255–89, https://doi.org/10.1080/10641269409388559.

Pierce, Emily. “Quantifying the Emanation and Decay of Environmental DNA from Three Marine Molluscs.” *Capstone Projects and Master’s Theses*, Apr. 2020, https://digitalcommons.csumb.edu/caps_thes_all/839.

Poirier, Luke A., et al. “Sink before You Settle: Settlement Behaviour of Eastern Oyster (*Crassostrea Virginica*) Larvae on Artificial Spat Collectors and Natural Substrate.” *Aquaculture Reports*, vol. 13, Mar. 2019, p. 100181, https://doi.org/10.1016/j.aqrep.2019.100181.

Pousse, Emilien, et al. “Juvenile Atlantic Sea Scallop, *Placopecten magellanicus*, Energetic Response to Increased Carbon Dioxide and Temperature Changes.” *PLOS Climate*, vol. 2, no. 2, Feb. 2023, p. e0000142, https://doi.org/10.1371/journal.pclm.0000142.

Rheuban, Jennie E., et al. “Projected Impacts of Future Climate Change, Ocean Acidification, and Management on the US Atlantic Sea Scallop (*Placopecten magellanicus*) Fishery.” *PLOS ONE*, vol. 13, no. 9, Sept. 2018, p. e0203536, https://doi.org/10.1371/journal.pone.0203536.

Richard, Pierre, et al. “Effect of Starvation on RNA, DNA and Protein Content of Laboratory-Reared Larvae and Juveniles of Solea Solea.” *Marine Ecology Progress Series*, vol. 72, no. 1/2, 1991, pp. 69–77, https://www.jstor.org/stable/24825429.

Rodriguez-Ezpeleta, Naiara, et al. “Trade-Offs between Reducing Complex Terminology and Producing Accurate Interpretations from Environmental DNA: Comment on ‘Environmental DNA: What’s behind the Term?’ By Pawlowski et al., (2020).” *Molecular Ecology*, vol. 30, no. 19, 2021, pp. 4601–05, https://doi.org/10.1111/mec.15942.

Rojahn, Jack, et al. “Does Mesocosm Validation of Environmental DNA Methods Translate to Natural Environment Monitoring Applications? A Case Study Detecting a High-Profile Invader; the Red Eared Slider Turtle, *Trachemys scripta elegans*, in Australia.” *Conservation Genetics Resources*, vol. 16, no. 1, Mar. 2024, pp. 63–71, https://doi.org/10.1007/s12686-023-01333-3.

Rourke, Meaghan L., et al. “Environmental DNA (EDNA) as a Tool for Assessing Fish Biomass: A Review of Approaches and Future Considerations for Resource Surveys.” *Environmental DNA*, vol. 4, no. 1, 2022, pp. 9–33, https://doi.org/10.1002/edn3.185.

Saeed, M., et al. Development and validation of an eDNA protocol for monitoring endemic Asian spiny frogs in the Himalayan region of Pakistan. *Sci Rep* **12**, 5624 (2022). https://doi.org/10.1038/s41598-022-09084-1

Sakata, Masayuki K., et al. "Detection and Persistence of Environmental DNA (EDNA) of the Different Developmental Stages of a Vector Mosquito, Culex Pipiens Pallens." *PLOS ONE*, vol. 17, no. 8, Aug. 2022, p. e0272653, https://doi.org/10.1371/journal.pone.0272653.

Sánchez, Ana, et al. "Identification and Quantification of Two Species of Oyster Larvae Using Real-Time PCR." *Aquatic Living Resources*, vol. 27, no. 3/4, July 2014, pp. 135–45, https://doi.org/10.1051/alr/2014012.

Sanderson, M., Garcia, L., and Davis, F.. "Sea Scallop Growth and Reproduction Research to Support Improved Resource Management." Report to the Sea Scallop Research Set Aside Program. 2022.

Sansom, B. J. and Sassoubre, L. M. "Environmental DNA (EDNA) Shedding and Decay Rates to Model Freshwater Mussel EDNA Transport in a River." *Environmental Science & Technology*, vol. 51, no. 24, Dec. 2017, pp. 14244–53, https://doi.org/10.1021/acs.est.7b05199.

Sassoubre, Lauren M., et al. "Quantification of Environmental DNA (EDNA) Shedding and Decay Rates for Three Marine Fish." *Environmental Science & Technology*, vol. 50, no. 19, Oct. 2016, pp. 10456–64, https://doi.org/10.1021/acs.est.6b03114.

Schick, Daniel F., and Scott C. Feindel. *"Maine Scallop Fishery: Monitoring and Enhancement." Final Report to the Northeast Consortium*. Sept. 2005, 73pp.

Schmitzer, A. C., duPaul, W. D., & Kirkley, J. E., "Gametogenic Cycle of Sea Scallops (*Placopecten magellanicus* (Gmelin, 1791)) in the Mid-Atlantic Bight. *Journal of Shellfish Research*, vol. 10, no. 1, 1991, pp. 220-228.

Siedlecki, SA, et al. "Projecting Ocean Acidification Impacts for the Gulf of Maine to 2050: New Tools and Expectations." *Elementa: Science of the Anthropocene*, vol. 9, no. 1, May 2021, p. 00062, https://doi.org/10.1525/elementa.2020.00062.

Skinner, Marc, et al. "A Mesocosm Comparison of Laboratory-Based and on-Site EDNA Solutions for Detection and Quantification of Striped Bass (*Morone saxatilis*) in Marine Ecosystems." *Environmental DNA*, vol. 2, no. 3, 2020, pp. 298–308, https://doi.org/https://doi.org/10.1002/edn3.61.

Smith, S. and P. J. Rago. "Biological Reference Points for Sea Scallops (*Placopecten magellanicus*): The Benefits and Costs of Being Nearly Sessile." *Canadian Journal of*

Fisheries and Aquatic Sciences, vol. 61, Apr. 2004, pp. 1338–54, https://doi.org/10.1139/f04-134.

Stoeckle, Bernhard C., et al. "A Systematic Approach to Evaluate the Influence of Environmental Conditions on EDNA Detection Success in Aquatic Ecosystems." *PLOS ONE*, vol. 12, no. 12, Dec. 2017, p. e0189119, https://doi.org/10.1371/journal.pone.0189119.

Stoeckle, Bernhard C., et al. "Influence of Stream Characteristics and Population Size on Downstream Transport of Freshwater Mollusk Environmental DNA." *Freshwater Science*, vol. 40, no. 1, Mar. 2021, pp. 191–201, https://doi.org/10.1086/713015.

Stokesbury, Kevin D. E., et al. "Fisheries Sea Scallop, *Placopecten magellanicus*." *Developments in Aquaculture and Fisheries Science*, edited by Sandra E. Shumway and G. Jay Parsons, vol. 40, Elsevier, 2016, pp. 719–36, https://doi.org/10.1016/B978-0-444-62710-0.00016-X.

Stokesbury, Kevin D. E., et al. "High Densities of Juvenile Sea Scallop (*Placopecten magellanicus*) on Banks and Ledges in the Central Gulf of Maine." *Journal of Shellfish Research*, vol. 29, no. 2, July 2010, pp. 369–72, https://doi.org/10.2983/035.029.0212.

Stokesbury, Kevin DE, and N. David Bethoney. "How Many Sea Scallops Are There and Why Does It Matter?" *Frontiers in Ecology and the Environment*, vol. 18, no. 9, 2020, pp. 513–19, https://doi.org/10.1002/fee.2244.

Taberlet, Pierre, et al. "Environmental DNA." *Molecular Ecology*, vol. 21, no. 8, 2012, pp. 1789–93, https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Takahara, Teruhiko, et al. "Estimation of Fish Biomass Using Environmental DNA." *PLOS ONE*, vol. 7, no. 4, Apr. 2012, p. e35868, https://doi.org/10.1371/journal.pone.0035868.

Tettelbach, St, et al. "Priming the Larval Pump: Resurgence of Bay Scallop Recruitment Following Initiation of Intensive Restoration Efforts." *Marine Ecology Progress Series*, vol. 478, Mar. 2013, pp. 153–72, https://doi.org/10.3354/meps10111.

Thomas, Andrew C., et al. "Seasonal Trends and Phenology Shifts in Sea Surface Temperature on the North American Northeastern Continental Shelf." *Elementa: Science of the Anthropocene*, edited by Jody W. Deming and Ken Drinkwater, vol. 5, Aug. 2017, p. 48, https://doi.org/10.1525/elementa.240.

Thompson, Katherine J., et al. "Identifying Spawning Events of the Sea Scallop *Placopecten magellanicus* on Georges Bank." *Journal of Shellfish Research*, vol. 33, no. 1, Apr. 2014, pp. 77–87, https://doi.org/10.2983/035.033.0110.

Thomsen, Philip Francis, et al. "Environmental DNA from Seawater Samples Correlate with Trawl Catches of Subarctic, Deepwater Fishes." *PLOS ONE*, vol. 11, no. 11, Nov. 2016, p. e0165252, https://doi.org/10.1371/journal.pone.0165252.

Thomsen, Philip Francis, and Eske Willerslev. "Environmental DNA – An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity." *Biological Conservation*, vol. 183, Mar. 2015, pp. 4–18, https://doi.org/10.1016/j.biocon.2014.11.019.

Tian, Rucheng C., et al. "Dispersal and Settlement of Sea Scallop Larvae Spawned in the Fishery Closed Areas on Georges Bank." *ICES Journal of Marine Science*, vol. 66, no. 10, Dec. 2009, pp. 2155–64, https://doi.org/10.1093/icesjms/fsp175.

Tillotson, Michael D., et al. "Concentrations of Environmental DNA (DNA) Reflect Spawning Salmon Abundance at Fine Spatial and Temporal Scales." *Biological Conservation*, vol. 220, Apr. 2018, pp. 1–11, https://doi.org/10.1016/j.biocon.2018.01.030.

Tremblay, M.J., et al. "Drift of Sea Scallop Larvae *Placopecten magellanicus* on Georges Bank: A Model Study of the Roles of Mean Advection, Larval Behavior and Larval Origin." *Deep Sea Research Part II: Topical Studies in Oceanography*, vol. 41, no. 1, Jan. 1994, pp. 7–49, https://doi.org/10.1016/0967-0645(94)90061-2.

Tremblay, M. J., L. D. Meade, and G. V. Hurley. "Identification of Planktonic Sea Scallop Larvae (*Placopecten magellanicus*) (Gmelin)." *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 44, Apr. 2011, pp. 1361–66, https://doi.org/10.1139/f87-162.

Troth, Christopher R., et al. "Seasonality, DNA Degradation and Spatial Heterogeneity as Drivers of EDNA Detection Dynamics." *Science of The Total Environment*, vol. 768, May 2021, p. 144466, https://doi.org/10.1016/j.scitotenv.2020.144466.

Tsuji, Satsuki, et al. "Water Temperature-Dependent Degradation of Environmental DNA and Its Relation to Bacterial Abundance." *PLOS ONE*, edited by Hideyuki Doi, vol. 12, no. 4, Apr. 2017, p. e0176608, https://doi.org/10.1371/journal.pone.0176608.

Tsuji, Satsuki, and Naoki Shibata. "Identifying Spawning Events in Fish by Observing a Spike in Environmental DNA Concentration after Spawning." *Environmental DNA*, vol. 3, no. 1, Jan. 2021, pp. 190–99, https://doi.org/10.1002/edn3.153.

Urban, P., et al. "Scaling from eDNA to biomass: Controlling allometric relationships improves precision in bycatch estimation." *ICES Journal of Marine Science*, *80*(4), 2023, pp. 1066-1078.

Uthicke, Sven., et al. "Developing an Effective Marine EDNA Monitoring: EDNA Detection at Pre-Outbreak Densities of Corallivorous Seastar (*Acanthaster* Cf. *Solaris)*." *Science of The*

Total Environment, vol. 851, Dec. 2022, p. 158143, https://doi.org/10.1016/j.scitotenv.2022.158143.

Uthicke, Sven, et al. "Spawning Time of *Acanthaster Cf. solaris* on the Great Barrier Reef Inferred Using QPCR Quantification of Embryos and Larvae: Do They Know It's Christmas?" *Marine Biology*, vol. 166, no. 10, Sept. 2019, p. 133, https://doi.org/10.1007/s00227-019-3582-5.

Vadopalas, Brent, et al. "Application of Real-Time PCR for Simultaneous Identification and Quantification of Larval Abalone." *Journal of Experimental Marine Biology and Ecology*, vol. 334, no. 2, July 2006, pp. 219–28, https://doi.org/10.1016/j.jembe.2006.02.005.

Vautier, Marine, et al. "A Quantitative EDNA-Based Approach to Monitor Fish Spawning in Lakes: Application to European Perch and Whitefish." *Fisheries Research*, vol. 264, Aug. 2023, p. 106708, https://doi.org/10.1016/j.fishres.2023.106708.

Wang, Xiaoyan, et al. "Assessment of Fishery Resources Using Environmental DNA: The Large Yellow Croaker (Larimichthys Crocea) in the East China Sea." *Fisheries Research*, vol. 235, Mar. 2021, p. 105813, https://doi.org/10.1016/j.fishres.2020.105813.

Wang, W.X., Widdows, J. 1991. "Physiological response of mussel larvae *Mytilus edulis* to environmental hypoxia and anoxia." *Marine Ecology Progress Series*, 70: 223–236.

Wood, Susanna A., et al. "Release and Degradation of Environmental DNA and RNA in a Marine System." *Science of The Total Environment*, vol. 704, Feb. 2020, p. 135314, https://doi.org/10.1016/j.scitotenv.2019.135314.

Yates, Matthew C., et al. "The Relationship between EDNA Particle Concentration and Organism Abundance in Nature Is Strengthened by Allometric Scaling." *Molecular Ecology*, vol. 30, no. 13, 2021, pp. 3068–82, https://doi.org/10.1111/mec.15543.

Yates, Matthew C., et al. "Integrating Physiology and Environmental Dynamics to Operationalize Environmental DNA (EDNA) as a Means to Monitor Freshwater Macro-Organism Abundance." *Molecular Ecology*, vol. 30, no. 24, 2021, pp. 6531–50, https://doi.org/10.1111/mec.16202.

Yates, Matthew C., et al. "Meta-Analysis Supports Further Refinement of EDNA for Monitoring Aquatic Species-Specific Abundance in Nature." *Environmental DNA*, vol. 1, no. 1, 2019, pp. 5–13, https://doi.org/10.1002/edn3.7.

Yu, Zeshu, et al. "Comparison of Species-Specific QPCR and Metabarcoding Methods to Detect Small Pelagic Fish Distribution from Open Ocean Environmental DNA." *PLoS ONE*, vol. 17, no. 9, Sept. 2022, p. e0273670, https://doi.org/10.1371/journal.pone.0273670.

Zeng, Yangqing, and Huiping Yang. “Review of Molluscan Bivalve Condition Index Calculations and Application in Northern Quahogs *Mercenaria mercenaria*.” *Aquaculture Research*, vol. 52, no. 1, 2021, pp. 23–36, https://doi.org/10.1111/are.14866.

Zhang, Y. L., et al. “Ontogenetic Changes in RNA, DNA and Protein Contents of Chinese Loach, *Paramisgurnus dabryanus* (Dabry de Thiersant, 1872), Larvae and Juveniles.” *Journal of Applied Ichthyology*, vol. 31, no. 5, 2015, pp. 876–82, https://doi.org/10.1111/jai.12808.

APPENDIX A: STATISTICS FOR MORPHOMETRIC COMPARISONS OF SEA SCALLOPS

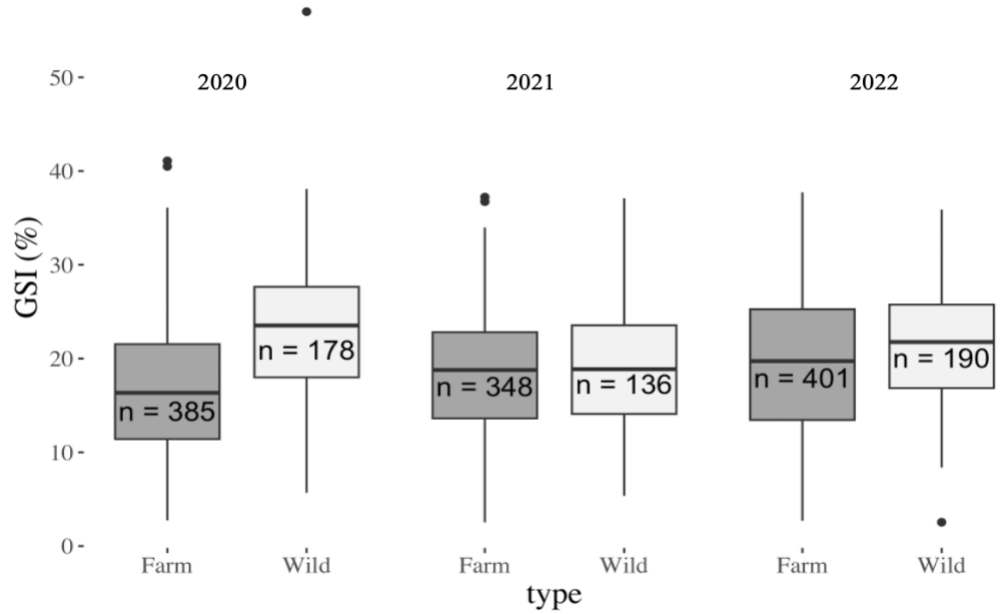


Figure A1. Gonadosomatic index (GSI) for farmed (dark) and wild (light) scallops (n=1,640) by year in the Penobscot Bay, Maine, 2020−2022.

Table A1. Results of statistical tests used to compare the gonadosomatic index (GSI) for farmed (n=1,136) and wild (n=504) Atlantic sea scallops during the spawning season (weeks 32-38) in the Penobscot Bay, Maine, 2020−2022 (from Fig. 7). Mann-Whitney U tests were used for the first four size class because the data were non-normal and t-tests were used to compare the two largest size classes because the data were normal.

Size class	W / t	p
	W	
60-69	1016.5	0.859
70-79	4449	0.415
80-89	5202	0.152
90-99	3346	0.349
	t	
100-109	1.171	0.243
110-119	-0.458	0.652

Table A2. Results of Mann-Whitney U tests to compare the mass (g) for wild (n=167) and farmed (n=489) Atlantic sea scallops for three different size classes collected during the spawning season (weeks 32-38) in the Penobscot Bay, Maine, 2020−2022 (from Fig. 8). Asterisk denotes a pair that is not significantly different (α=0.05), whereas all the remaining pairs are different.

Mass by size (g)	W	p
80-89		
Meat	10,104	7.90E-13
Gonad	7,112.5	*5.55E-02
Viscera	10,338	3.66E-14
Shell	452	1.59E-22
90-99		
Meat	5,240.5	8.34E-13
Gonad	4,842.5	4.94E-09
Viscera	5,610	5.85E-17
Shell	1,234.5	2.10E-09
100-109		
Meat	4,946.5	2.82E-14
Gonad	4,455.5	6.81E-09
Viscera	5,354	8.32E-20
Shell	748	3.45E-15

Table A3. Coefficient of determination (R^2), slope (β_1), and how likely a difference in slopes is due to chance (p) for linear models fit to scallop component mass (g) and total mass (g) for three size classes (shell height: 80-89, 90-99, 100-109 mm) of farmed (green; n=167) and wild (orange; n=489) Atlantic sea scallops collected during weeks 32-38 in the Penobscot Bay, Maine, 2020−2022 (from Fig. 9). We found that all slopes were all significantly different at α=0.05.

Mass (g)	R^2		β_1		p
	Wild	Farmed	Wild	Farmed	
Meat	0.6705	0.7153	0.0919	0.1163	8.08e-05
Gonad	0.3824	0.5705	0.0591	0.1157	8.05e-12
Viscera	0.8671	0.9152	0.2935	0.4293	< 2e-16
Shell	0.9743	0.9501	0.7066	0.5707	< 2e-16

APPENDIX B: RESULTS: MODEL OUTPUTS FOR GENERATION AND DEGRADATION EXPERIMENTS

Table B1: Generation biexponential 5p model output for each biomass.

Biomass = 0g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		230.29033	230.55746	30203.365	1887.7103	43.447788	0.1374795
Parameter	**Estimate**	**Std Error**	**Wald ChiSquare**	**Prob > ChiSquare**	**Lower 95%**	**Upper 95%**	
Asymptote	42.934618	.	0	1.0000	.	.	
Scale 1	-2.69e-30	.	0	1.0000	.	.	
Decay Rate 1	-2.214555	.	0	1.0000	.	.	
Scale 2	-42.93462	.	0	1.0000	.	.	
Decay Rate 2	371.60189	.	0	1.0000	.	.	

Biomass = 70g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		769.07118	769.33832	4.192e+15	2.62e+14	16186296	0.2698938
Parameter	**Estimate**	**Std Error**	**Wald ChiSquare**	**Prob > ChiSquare**	**Lower 95%**	**Upper 95%**	
Asymptote	23697044	4179283.5	32.150275	<.0001*	15505799	31888289	
Scale 1	-3.3e-125	2.14e-117	2.386e-16	1.0000	-4.2e-117	4.19e-117	
Decay Rate 1	-9.465132	2023286.9	2.188e-11	1.0000	-3965579	3965560.1	
Scale 2	-23697037	10237112	5.3583759	0.0206*	-43761408	-3632666	
Decay Rate 2	5724.4987	0	.	<.0001*	5724.4987	5724.4987	

Biomass = 140g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		520.27316	519.78602	9.231e+10	6.1541e+9	78448.142	0.8321277

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	194347.41	52519.798	13.693404	0.0002*	91410.498	297284.32
Scale 1	1.8815e+9	1.291e+12	2.1246e-6	0.9988	-2.53e+12	2.532e+12
Decay Rate 1	0.1912641	0.0477724	16.029248	<.0001*	0.097632	0.2848963
Scale 2	-1.882e+9	1.291e+12	2.1251e-6	0.9988	-2.53e+12	2.528e+12
Decay Rate 2	0.1913615	0.0477847	16.037297	<.0001*	0.0977052	0.2850178

Biomass = 280g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		643.10506	642.61791	4.291e+13	2.86e+12	1691270.3	0.4423092

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	248366.78	701710.45	0.1252769	0.7234	-1126960	1623694
Scale 1	2.748e+11	1218443	5.085e+10	<.0001*	2.748e+11	2.748e+11
Decay Rate 1	0.4376981	0.1516135	8.3343791	0.0039*	0.140541	0.7348551
Scale 2	-2.75e+11	0	.	<.0001*	-2.75e+11	-2.75e+11
Decay Rate 2	0.4377125	0.1516182	8.3344197	0.0039*	0.1405463	0.7348786

Biomass = 560g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		718.42878	718.69592	3.759e+14	2.349e+13	4847162.7	0.2826204

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	-2194124	6824613.5	0.1033633	0.7478	-15570121	11181872
Scale 1	5.719e+11	7849384.7	5.3093e+9	<.0001*	5.719e+11	5.72e+11
Decay Rate 1	0.1164872	0.0830848	1.9656812	0.1609	-0.046356	0.2793304
Scale 2	-5.72e+11	0	.	<.0001*	-5.72e+11	-5.72e+11
Decay Rate 2	0.1164917	0.083086	1.9657745	0.1609	-0.046354	0.2793373

Biomass = 1120g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		750.25053	750.51766	1.711e+15	1.069e+14	10340354	0.5535198

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	749746.29	4602208.5	0.0265397	0.8706	-8270417	9769909.2
Scale 1	2.023e+12	7630902.3	7.029e+10	<.0001*	2.023e+12	2.023e+12
Decay Rate 1	0.3644276	0.1006093	13.120364	0.0003*	0.1672369	0.5616182
Scale 2	-2.02e+12	0	.	<.0001*	-2.02e+12	-2.02e+12
Decay Rate 2	0.36444	0.1006123	13.120493	0.0003*	0.1672436	0.5616364

Table B2: Degradation biexponential 5p model output for each biomass.

Biomass = 0g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		243.68982	243.95696	57169.727	3573.108	59.77548	0.082606

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	-15.68575	126.50636	0.015374	0.9013	-263.6337	232.26217
Scale 1	1813460.1	137.64843	173569539	<.0001*	1813190.3	1813729.9
Decay Rate 1	0.0739768	0.1073827	0.4745944	0.4909	-0.136489	0.2844431
Scale 2	-1813422	0	.	<.0001*	-1813422	-1813422
Decay Rate 2	0.073983	0.107386	0.4746452	0.4909	-0.13649	0.2844556

Biomass = 70g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		693.03536	693.3025	1.122e+14	7.012e+12	2647956.8	0.6952054

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	2169965	953302.37	5.1813644	0.0228*	301526.74	4038403.4
Scale 1	10060938	1775189.8	32.120874	<.0001*	6581629.7	13540246
Decay Rate 1	0.2441871	0.1018973	5.7427537	0.0166*	0.0444721	0.4439021
Scale 2	-9.41e-82	1.985e-81	0.2247468	0.6354	-4.83e-81	2.95e-81
Decay Rate 2	-4.883597	0	.	<.0001*	-4.883597	-4.883597

Biomass = 140g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		517.69271	517.95985	2.653e+10	1.6581e+9	40719.966	0.8961745

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	37547.402	11778.759	10.161568	0.0014*	14461.459	60633.345
Scale 1	6875525.1	5703027.9	1.4534522	0.2280	-4302204	18053254
Decay Rate 1	0.6376733	0.1634141	15.227103	<.0001*	0.3173875	0.9579591
Scale 2	-6698663	5706362.8	1.3780263	0.2404	-17882928	4485602.5
Decay Rate 2	2762.4963	0	.	<.0001*	2762.4963	2762.4963

Biomass = 280g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		527.57691	527.84404	4.248e+10	2.6548e+9	51524.456	0.8289054

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	42392.11	29309.074	2.0920202	0.1481	-15052.62	99836.84
Scale 1	195703.93	138938.46	1.9840571	0.1590	-76610.44	468018.3
Decay Rate 1	2984.3107	0	.	<.0001*	2984.3107	2984.3107
Scale 2	102024.82	118181.92	0.745263	0.3880	-129607.5	333657.12
Decay Rate 2	0.1202404	0.248542	0.2340458	0.6285	-0.366893	0.6073738

Biomass = 560g

Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		554.79406	555.06119	1.552e+11	9.7028e+9	98502.704	0.8223415

Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	84852.321	120782.96	0.4935329	0.4824	-151877.9	321582.57
Scale 1	302977.92	139070.11	4.7462913	0.0294*	30405.522	575550.33
Decay Rate 1	2763.0299	0	.	<.0001*	2763.0299	2763.0299
Scale 2	297963.43	95753.505	9.6831484	0.0019*	110290.01	485636.86
Decay Rate 2	0.0633385	0.0834367	0.5762642	0.4478	-0.100194	0.2268715

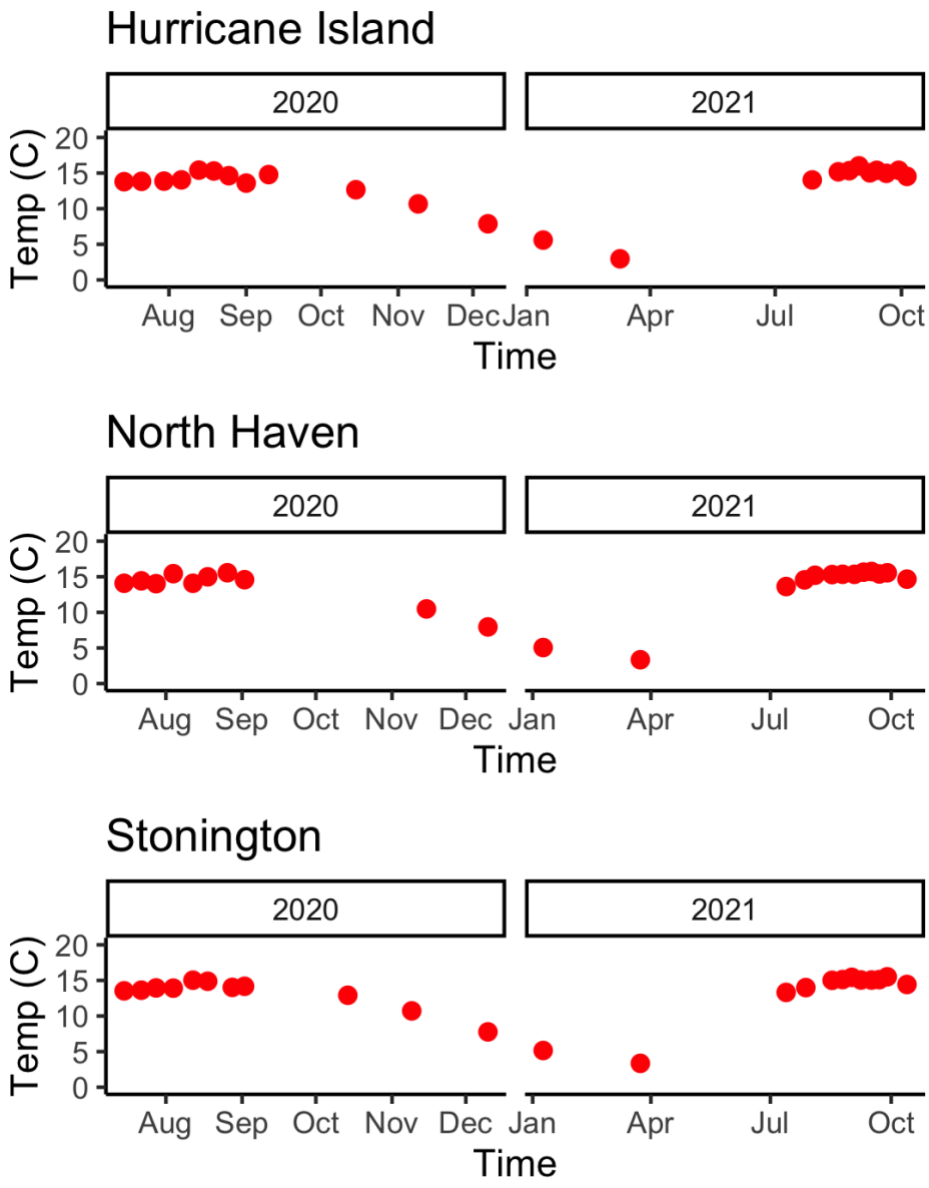
Biomass = 1120g

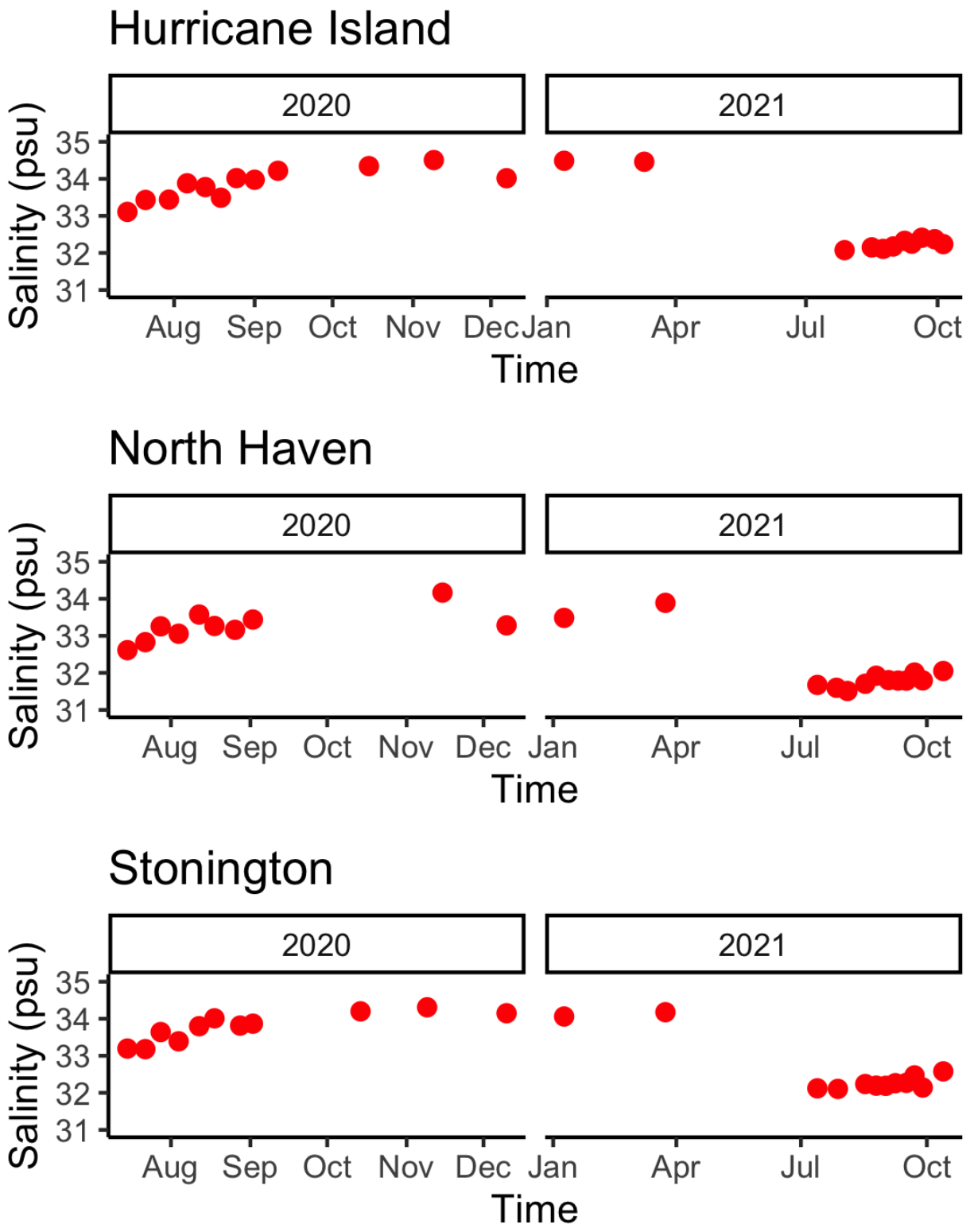
Model		AICc	BIC	SSE	MSE	RMSE	R-Square
Biexponential 5P		599.7093	599.97643	1.318e+12	8.237e+10	287003.52	0.8935212

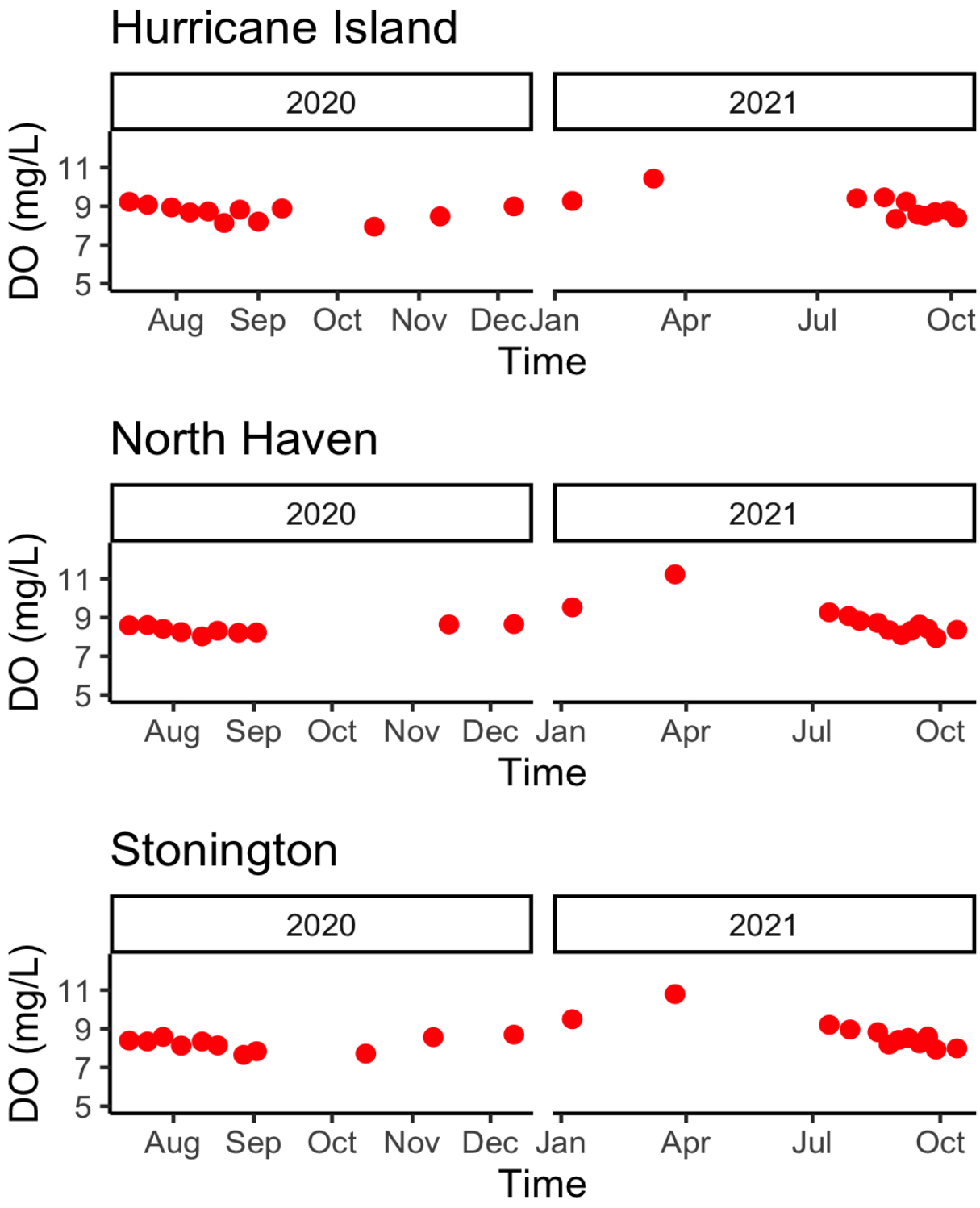
Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Asymptote	435003.94	841748.35	0.267068	0.6053	-1214793	2084800.4
Scale 1	2188173.6	544817.72	16.130993	<.0001*	1120350.5	3255996.7
Decay Rate 1	0.2560681	0.1211184	4.4698292	0.0345*	0.0186805	0.4934558
Scale 2	5936.7482	323423.75	0.0003369	0.9854	-627962.1	639835.65
Decay Rate 2	-0.068866	1.1346772	0.0036835	0.9516	-2.292792	2.1550605

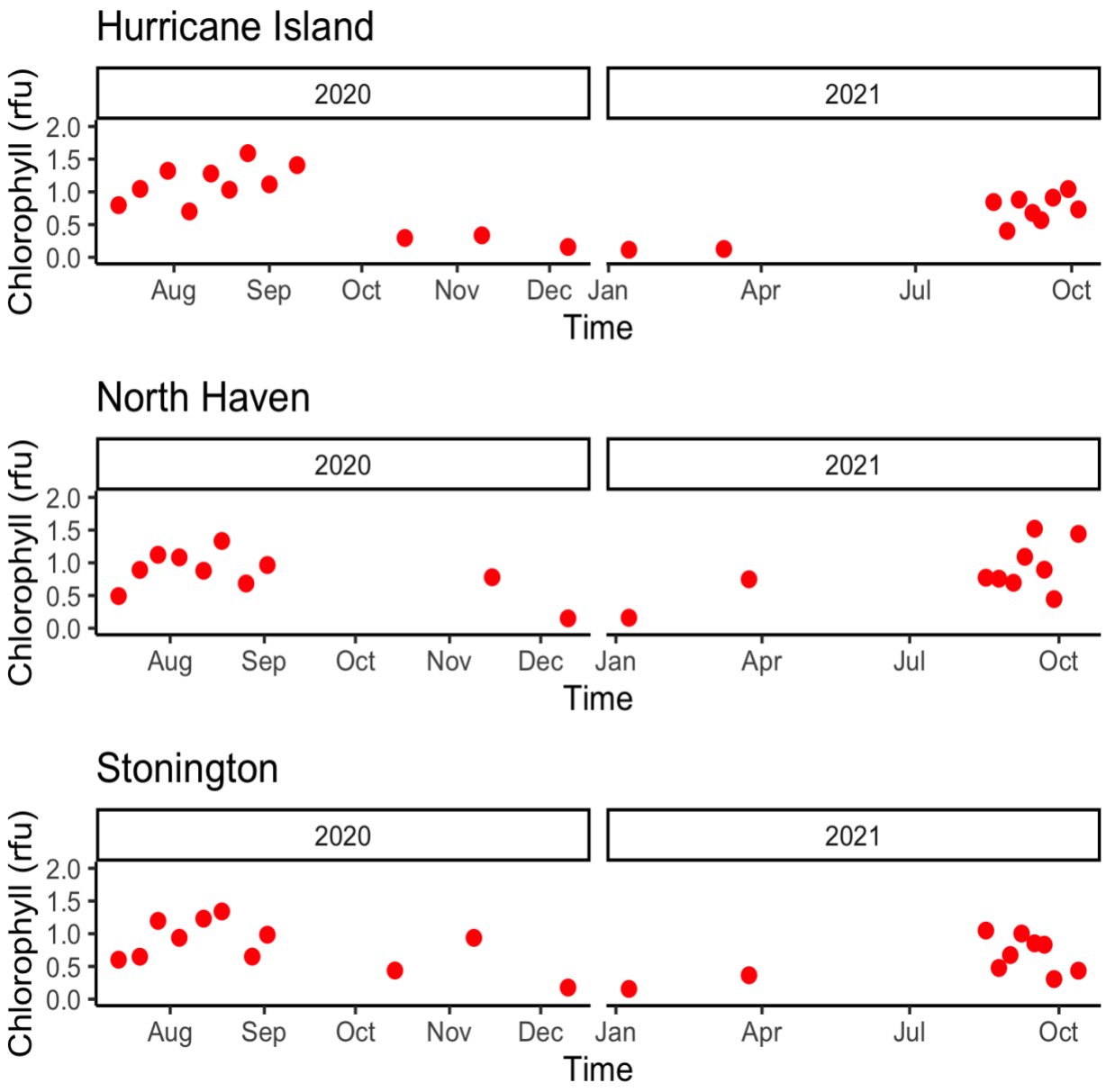
APPENDIX C: RESULTS: ENVIRONMENTAL DATA FROM SEA SCALLOP AQUACULTURE FARMS

Figure C1: Environmental data from sea scallop aquaculture farms. Temperature (°C), salinity (psu), dissolved oxygen (mg/L), and chlorophyll (rfu) readings in 2020 and 2021 at net-level depths on Hurricane Island (5m), North Haven (7m), and Stonington (9m) farm sites. Standard error is included but very small and difficult to visualize.




Hurricane Island
2020
2021
Salinity (psu)
35
34
33
32
31
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
North Haven
2020
2021
Salinity (psu)
35
34
33
32
31
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
Stonington
2020
2021
Salinity (psu)
35
34
33
32
31
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time


Hurricane Island
2020
2021
DO (mg/L)
11
9
7
5
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
North Haven
2020
2021
DO (mg/L)
11
9
7
5
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
Stonington
2020
2021
DO (mg/L)
11
9
7
5
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time


Hurricane Island
2020
2021
Chlorophyll (rfu)
2.0
1.5
1.0
0.5
0.0
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
North Haven
2020
2021
Chlorophyll (rfu)
2.0
1.5
1.0
0.5
0.0
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time
Stonington
2020
2021
Chlorophyll (rfu)
2.0
1.5
1.0
0.5
0.0
Aug
Sep
Oct
Nov
Dec
Jan
Apr
Jul
Oct
Time

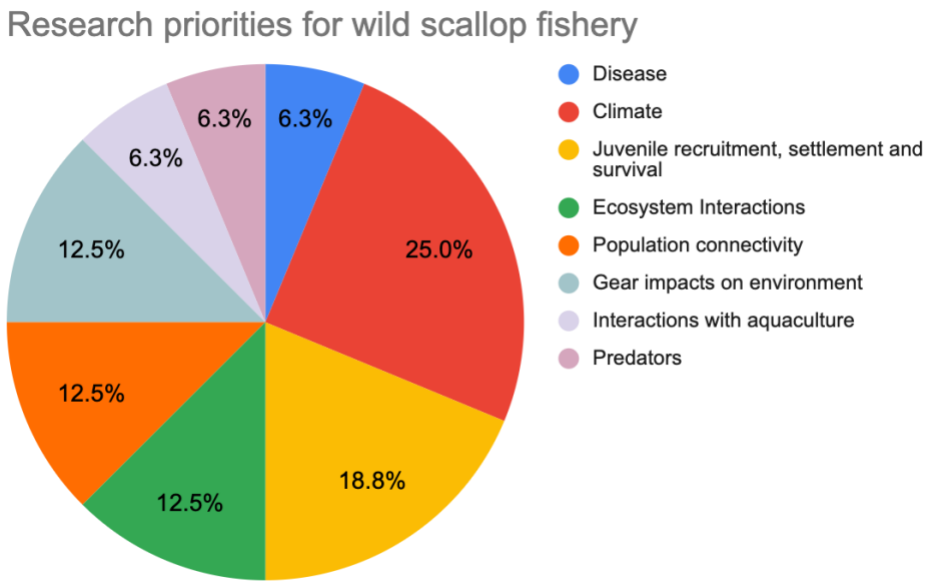
APPENDIX D: OUTCOMES: SCALLOP RESEARCH COLLABORATIVE (SRC) TO IDENTIFY RESEARCH AND MANAGEMENT PRIORITIES

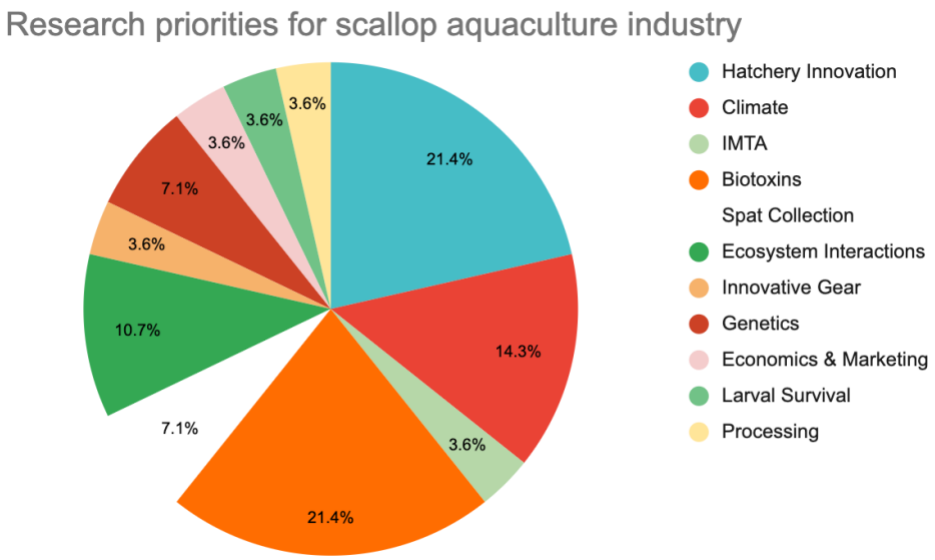
In 2022, with funding from the Northeast Regional Aquaculture Center (NRAC), I partnered with Meggan Dwyer of the UMaine Aquaculture Research Institute to establish the Scallop Research Collaborative (SRC). The purpose of the SRC was to convene aquaculturists, wild harvesters and researchers to work synergistically to (1) determine the current state of scallop research and identify research bottlenecks to industry economic growth; (2) network researchers with industry (aquaculturists, harvesters, processors and dealers) to meet the needs of the industry using existing forums and broader funding initiatives; (3) collaborate on funding proposals that address the research and capacity needs of the scallop industry; (4) propose synergistic research solutions that can be used as models in the NE region; (5) conduct activities that elucidate the mutual benefits of collaboration between wild harvest and aquaculturists.

As part of this effort, we completed a survey to identify wild caught and aquaculture scallop industry research and development needs (Fig. D1) and created a scallop research database highlighting current scallop research occurring in Maine. We hosted a field trip to Hurricane Island in August 2022 where members of the research community presented their work to other researchers, students, aquaculturists and fishers. The full-day event involved hands-on activities such as farm tours and sorting scallop spat with 20 people in attendance representing wild caught, aquaculture, distribution, education and research sectors. In late January 2023, I chaired a scallop session inviting crosscutting researchers to present at Maine Aquaculture R&D Summit, which is Maine's premier event for bringing together researchers and aquaculturists. In March 2023 meeting I ran a scallop session and workshop at the Maine Fishermen's Forum, which is Maine's premier event for bringing together fishermen and

aquaculturists. A feedback session captured crosscutting objectives from regulators, fishers and farmers which resulted in four basic collaborative research topics: Research into species interactions, spat settlement and distribution, source-sink dynamics with federal waters, and genetic distribution.

Figure D1: SRC Survey outcomes identifying research priorities for wild (top) and farmed (bottom) scallop industries.




Research priorities for scallop aquaculture industry
21.4%
14.3%
3.6%
21.4%
7.1%
10.7%
3.6%
7.1%
3.6%
3.6%
3.6%
Hatchery Innovation
Climate
IMTA
Biotoxins
Spat Collection
Ecosystem Interactions
Innovative Gear
Genetics
Economics & Marketing
Larval Survival
Processing

BIOGRAPHY OF THE AUTHOR

Phoebe Jekielek was born in Dubois, PA, on July 12, 1981, because the doctor was out fishing and could not come for the home delivery in Punxsutawney, PA. She was raised in Punxsy, Home of the Groundhog, graduating from Punxsutawney Area High School in 1999. She attended Boston University and graduated in 2003 with a bachelor's degree in biology with a Specialization in Marine Science. Since then, her path has not been linear, thankfully. She spent the next six years as a Fisheries Observer on trawlers and gillnetters out of Portland, ME, and Gloucester, MA, and as a marine science instructor, aquarist, and Assistant Program Director at the Catalina Island Marine Institute where she learned many of her hard and soft skills that she uses on a daily basis in her work and life. In 2009 she returned to Maine to enter the Dual Master's in Marine Biology and Marine Policy program at the University of Maine. After receiving her degrees in 2012, she worked at the NSF-funded UMaine Center for Science Education Excellence (CoSEE) as a Science Communication Specialist and then as the Research Technician in the lab of Dr. Rick Wahle. It was in this lab where she started her love affair with scallops as part of the Midcoast Collaborative Scallop Project working with fishermen to monitor local scallop populations inside and outside of a small-scale closure. In 2014, she took the year to do some traveling and diving and volunteer research with her best friend in the South Pacific, to run a dock on Block Island, RI, and to drive the pump out boat (look it up). In March 2015, she started working for the Hurricane Island Center for Science and Leadership as the Marketing Director, then the Programs and Marketing Director, then the Programs, Marketing and Research Director and many other hats for the next 9 years. She started her PhD in August 2020 at the University of Maine and will start a post-doctorate as soon as she defends. She's excited about next life and work :)