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Detrital Protein Contributes to Oyster Nutrition and Growth in the Damariscotta Estuary, Maine, USA

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DETRITAL PROTEIN CONTRIBUTES TO OYSTER NUTRITION AND GROWTH

IN THE DAMARISCOTTA ESTUARY, MAINE, USA

By

Cheyenne M. Adams

B.S. Southern Illinois University, 2014

A THESIS

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Oyster aquaculture is an expanding industry that relies on identifying and utilizing natural estuarine conditions for the economically viable production of a filter-feeding crop. The eastern oyster, *Crassostrea virginica*, is the principal species currently cultured in Maine. In addition to preferentially consumed phytoplankton, various detrital complexes (non-algal and/or non-living organic matter) may provide some nutrition to *C. virginica* between times of phytoplankton abundance. Here I investigated the importance of detrital proteins in supporting the growth of oysters cultured in the upper Damariscotta Estuary. Oyster aquaculture in this area is highly successful and previous reports indicate that labile detrital protein is seasonally abundant.

I coupled *in vitro* chemical assays of seston quantity and quality (protein lability is a key parameter of quality) with *in vivo* bioassays of feeding and growth of *C. virginica* to test the hypothesis that detrital protein contributes to oyster nutrition in the Damariscotta Estuary. From May to October 2016, enzymatically hydrolyzable amino acids (EHAA, labile protein), extracted chlorophyll-α (CHL), particulate organic matter (POM), and plankton abundance (via FlowCam) analyses were conducted biweekly along with continuous monitoring of temperature, turbidity, and CHL by a Land/Ocean
Biogeochemical Observatory (LOBO) buoy. Oyster feeding and growth were measured biweekly under natural conditions and in a controlled laboratory experiment to assess responses to detrital food.

Oysters readily absorbed phytodetritus (dead and decaying phytoplankton) under laboratory conditions and cleared phytodetritus under natural field conditions. Additionally, estimates of POM absorption rates indicate that oysters absorbed more organic matter than was available from phytoplankton alone, suggesting a role for additional organics such as detritus in oyster nutrition. Bioavailable EHAA was nearly completely absorbed by oysters, consistent with EHAA limitation of dietary demand. Seasonal EHAA concentrations correlate well with growth rates (along with temperature, turbidity, and ciliate abundance), corroborating protein limitation of oyster growth. Finally, not all EHAA can be attributed to phytoplankton throughout the season, implying seasonally abundant labile detrital protein. Considering the strong influence of EHAA abundance on this aspect of biota in the Damariscotta Estuary, EHAA measurements may prove helpful in future studies of both aquaculture site selection and ecological nutrient flows.
DEDICATION

This thesis is dedicated to my grandmother, Martha Evangeline McClanahan Dillman (1921-2016), who had a song, poem or grammar correction for every occasion.
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LIST OF ABBREVIATIONS

gDW – gram Dry Weight of oyster soft tissue

CR – Clearance Rate

AR – Absorption Rate

AE – Absorption Efficiency

CHL – Chlorophyll-α

POM – Particulate Organic Matter

PIM – Particulate Inorganic Matter

TPM – Total Particulate Matter

PON – Particulate Organic Nitrogen

POC – Particulate Organic Carbon

EHAA – Enzymatically Hydrolyzable Amino Acids

SELRG – Selected Organic Matter

REMORG – Remaining Organic Matter

Ciliate-C – Carbon from ciliate biomass

EHAA-N – labile protein in nitrogen equivalents

EHAA-C – labile protein in carbon equivalents

Temp – Temperature

Turb – Turbidity

LOBO – Land/Ocean Biogeochemical Observatory buoy
CHAPTER 1

INTRODUCTION

Worldwide shellfish aquaculture presents a significant opportunity to meet an increased global food demand without relying on diminishing wild fish stocks or limited arable land (Costa-Pierce 2010). In Maine, aquaculture of the Eastern oyster, Crassostrea virginica, has expanded more than 350% from 2011 to 2016, with recent landings of $5.9 million. With Maine’s ~3,500 miles of shoreline, there is high economic and ecological potential for oyster aquaculture, though currently nearly 75% of the statewide harvest takes place in the Damariscotta Estuary (Maine DMR commercial harvest 2016 www.maine.gov/dmr).

Expansion of oyster aquaculture into new estuarine systems depends on better understanding the environmental parameters of a suitable culture habitat. The quantity and quality of natural seston available as nutrition to filter-feeding oysters, over which farmers exert no control, can strongly affect yield. In the natural estuarine habitat of wild and cultured C. virginica, seston concentration and composition are highly variable and subject to numerous physical, chemical, and biological factors (Berg & Newell 1986, Small & Haas 1997, Thompson 2006). Crassostrea virginica exhibits feeding behavioral plasticity in response to this environmental variability (Nelson 1960, Langdon & Newell 1990, Ward & Shumway 2004, Galimany et al. 2017). They can identify, select and take advantage of a range of particulate material including organic matter rich in chlorophyll-α (CHL) from phytoplankton, as well as other potentially nutritious organic particles such as detritus (e.g. Palmer & Williams 1980, Newell & Jordan 1983, McDonald & Ward 1994, Hawkins et al. 2013b).

Particle selection behaviors may also include the ability to choose particles based on chemical composition, as has been demonstrated for nitrogen and CHL content in the scallop Placopecten magellanicus (Brillant & MacDonald 2003). Like P. magellanicus, C. virginica has relatively advanced particle selection capabilities (Ward & Shumway 2004), has demonstrated post-capture particle
selection (Newell & Jordan 1983, Shumway et al. 1985, Ward et al. 1998), and therefore may possess a
similar ability to discriminate based on chemical composition of particles. The ability to select for
nitrogenous material could play an important role in coping with protein limitation. In many marine and
estuarine systems, nitrogen is the limiting element for biological productivity (Roman 1983, Gruber
2008). Inorganic forms of nitrogen are relatively low in surface waters compared to the enriched deep
oceans, which often limits primary production in the photic zone (Gruber 2008). Likewise, secondary
producers such as oysters and other marine heterotrophs often have higher protein (Roman 1983) or
essential amino acid (Brown et al. 1997) content than their autotrophic prey, and this trophic imbalance
in chemical composition can result in protein limitation for these heterotrophs (Kreeger & Langdon

Many studies have characterized food quality based on chloropigments and particulate organic
matter/carbon (POM/C) concentrations to assess generalized bulk nutritional requirements of bivalves
protein have characterized nutritional limitations of bivalves in both laboratory (e.g. Romberger &
field studies (e.g. Gremare et al. 1997, Bayne 2009). For example, Urrutia et al. (1996) estimated that
the cockle *Cerastoderma edula* absorbs nitrogen with a higher efficiency than total organic matter,
possibly due to higher absorption efficiency for dietary proteins than for other biochemical components.
Indeed, Ibarrola et al. (2000) found that proteins are absorbed more efficiently and contribute less to
fecal loss than lipids. Similarly, other marine heterotrophic filter feeders, such as the copepod *Acartia
clausurae*, appear to optimize protein intake by modulating feeding behavior in response to changes in
dietary biochemical composition (Mayzaud et al. 1996). In bivalves, growth rates generally increase with
increasing protein availability up to a maximum ration (Hawkins & Bayne 1991, Kreeger & Landon 1993,
Wikfors et al. 1996, Chi et al. 2010), but the bioavailable fraction of the total protein that provides
nutrients to the animal is largely unknown.

The bioavailability of protein has significant implications for the nutritional quality and
movement of nitrogen through ecosystems (Mayer et al. 1995, Dauwe et al. 1999), especially for key
benthic-pelagic couplers such as *C. virginica* (Newell 2004, Dumbauld et al. 2009). Therefore, it is critical
to assess the fraction of total protein in naturally occurring seston that is labile and therefore available
for absorption by heterotrophs (Hawkins et al. 2013b, Bayne 2017). Methods quantifying enzymatically
hydrolyzable amino acids (EHAA, Mayer et al. 1995) allow estimation of the relatively labile fraction of
protein and show encouraging relationships with biological responses in benthic ecosystems, illustrating
how food webs respond to bioavailable proteins. For example, Gremare et al. (1997) and Bonifacio et al.
(2014) have shown that sediment EHAA concentrations positively correlate with bivalve growth rate and
macroinvertebrate species diversity at the ecosystem level, respectively.

A previous study of EHAA within seston of the Damariscotta Estuary found levels that could be
attributed to varying combinations of live phytoplankton and phytoplankton-derived detritus (Laursen
1995). Detritus is dead and decomposing organic matter that represents either nonpredatory losses
from a trophic level or exogenous (i.e. terrigenous) inputs to the ecosystem (Roman 1983). Detritus is
loosely defined due to the varied source material, size, and biochemical nature, making a clear
quantification metric elusive (Cebrian & Lartigue 2004). Several studies have attempted to characterize
detritus as amorphous and morphous particles (D’Avanzo et al. 1991, Alber & Valiela 1994, Alber &
Valiela 1995) while others have quantified it as all non-chlorophyll associated, or non-phytoplanktonic,
POM (Hager 1984, Hawkins et al 2013a). The latter approach estimates detritus as all particulates other
than algal biomass, and therefore ‘detritus’ can include algal necromass (i.e. phytodetritus),
heterotrophic biomass, heterotrophic necromass, and aggregates containing complexes among the
three. Detrital food sources, which often comprise the majority of POM in marine and estuarine systems
(Steinberg and Saba 2008), could provide significant nutrition to oysters, especially considering nitrogenous enrichment of certain types of detritus during degradation (Newell 1982, Paerl 1984, Biddanda 1988, Rice & Hanson 1988, Hansen et al. 1992, Albert & Valiela 1996).

Detritus can contribute to meeting oyster nutritional requirements for growth (Levinton et al. 2002, Byron et al. 2011), even if phytoplankton are preferentially selected (Ward et al. 1998, Ward & Shumway 2004). *Crassostrea virginica* can obtain as much as 40% of their carbon requirements via saltmarsh detritus (Lucas & Newell 1984). However, some detrital components are refractory and cannot contribute significantly to oysters nutritional demands (Newell & Langdon 1986) unless bacterial colonization of particles converts detritus into more bioavailable compounds (Crosby et al. 1990). For example, oysters absorb only 3% of the total nitrogen present in salt-marsh detritus but have a higher absorption efficiency of 57% for nitrogen from cellulolytic bacteria colonizing that detritus, suggesting an important role for bacteria in cycling nutrients through the ecosystem (Crosby et al. 1990). Attached (i.e. detrital) and free bacteria together can provide up to 27% of total nitrogen requirements of *C. virginica* (Langdon & Newell 1990). Troost et al. (2010) confirmed that detrital contributions can be significant to bivalve diets but is both site- and species-specific. Phytodetritus is particularly nutritious relative to other detrital particles and may be able to provide an ephemerally significant amount of nutrition to filter feeders, especially following and between phytoplankton blooms (Lopez & Levinton 2011). Hawkins et al. (2013a) demonstrated that live phytoplankton alone can provide only a small fraction of the total diet of *C. gigas* and the remainder (up to 90% of all energy absorbed) is derived from detritus, protozoa, bacteria, and/or complexes among them.

Bivalve growth models have variously included detritus, primarily with estimates of POM-derivatives, but generally lack resolution of detrital characteristics or quality (Pouvreau et al. 2000, Ren & Ross 2001, Bourlès et al. 2009, Hawkins et al. 2013a). Only a small subset of detrital POM may be assimilable by filter-feeders, yet detritus is often an important fraction of bivalve diet. Therefore,
distinguishing detrital quality may improve traditional growth models. Growth models for mussels across diverse sites are sensitive to experimental manipulation of POC content of POM (Grant & Bacher 1998) and C:N ratio of detritus (Campbell & Newell 1998). Other models have found that POM (Ren & Ross 2001) and CHL (Bourlès et al. 2009) fail to predict oyster growth rates, which could be attributed to a lack of food quality information in these studies. Even the ‘biopolymeric’ estimate of the labile fraction of POM, calculated as carbon equivalents of various biochemical components of seston dissolved in strong solvents, is not entirely bioavailable via enzymatic hydrolysis (Hawkins et al. 2013b). Although the foregoing has focused on protein from detritus, we also need to estimate the biologically hydrolyzable fraction of carbohydrates and lipids in natural seston (Hawkins et al. 2013a).

Considering the success of oyster aquaculture in the upper Damariscotta Estuary, the environmental factors affecting oyster growth in this system are potentially important parameters to inform site selection decisions in other estuaries. Therefore, I characterized the quantity and quality of food available to oysters in the Damariscotta Estuary, including seasonal variations in labile detrital protein concentrations, to improve our understanding of parameters affecting oyster aquaculture site success. I coupled measures of algal and detrital EHAA to corresponding bioassays of animal response, which has not been done previously by Laursen (1995) or other studies to the author’s knowledge. I tested the hypothesis that detrital protein contributes to oyster nutrition in the Damariscotta Estuary, by coupling in vitro chemical assays of seston quality (protein lability is a key parameter of quality) with in vivo bioassays of feeding and growth of C. virginica. While it is beyond the scope of this study, future assessments of filter-feeder nutritional quality should include estimates of hydrolyzable carbohydrates (Puscéddu et al. 2003) and lipids.
CHAPTER 2

MATERIALS AND METHODS

2.1. Research Strategy

Tests of hypothesis-derived questions included an artificial laboratory experiment, a field experiment conducted twice, and a seasonal field study with extensive environmental monitoring combined with measures of oyster physiological responses. I asked the following questions: Is phytodetritus nutritious and can oysters absorb it for respiration and growth? Do oysters in the field clear detrital material from the water column? Do in situ variations in oyster growth rates correlate with digestible protein, especially detrital forms?

2.2. Laboratory Algal Rot

The laboratory experiment subjected a dense diatom culture to decay by a naturally occurring microbial community to mimic one pathway of detritus formation and monitored the feeding response of oysters to this detritus. A culture of *Thalassiosira weissflogii* was grown to approximately 1 × 10^6 cells mL^-1 and gently centrifuged into a paste using an Evodos 10 Dynamic Settler (Evodos, B.V., Netherlands). The concentrate was homogenized, separated into 50 mL aliquots, and frozen at -80⁰ C to render the algal culture non-viable. Aliquots were thawed at room temperature and resuspended to 2 g-dry weight L^-1 in 15 L natural estuarine seawater filtered at 1 µm and UV sterilized. Microscopic examination confirmed algal cells were intact. A 1 mL unsterilized sample of seawater from the Damariscotta Estuary was filtered to 1 µm and used as an inoculum to induce bacterial decomposition of algal material. The suspension was covered in black aluminum foil, left undisturbed at 19.5⁰ C in a dark room to inhibit algal growth, and monitored daily. At days 0, 2, 5, and 12, subsamples of 433, 554, 607, and 658 mL, respectively, of the suspension were removed and diluted into 900 L of filtered, sterilized seawater to an average concentration of 2.7 ± 0.1 mg-POM L^-1. Powdered kaolinite was added to a concentration of 5.8 ± 0.4 mg L^-1. Varying volumes of algal rot suspension were diluted to maintain constant POM in the
dilution after accounting for carbon losses from bacterial respiration in the rot suspension. The concentrations of POM and particulate inorganic matter (PIM) in the dilution were similar to natural field conditions observed in the Damariscotta Estuary throughout the seasonal study.

Particulates were filtered from triplicate subsamples from these dilutions onto Whatman GF/F filters. POM was measured gravimetrically from 1 L subsamples as in Hawkins (2013b). CHL and pheopigments were measured on a Turner 10-AU fluorometer from 300 mL subsamples using standard acetone extraction procedure (Holm-Hansen & Riemann 1978). Particulate organic carbon and nitrogen (POC/N) were measured from 250 mL subsamples on a Perkin-Elmer CHNS/O 2400B analyzer. Bacterial and algal cell enumerations were conducted by flow cytometry at the J.J. Maclsaac Facility for Aquatic Cytometry, Bigelow Laboratory for Ocean Sciences (JJMFAC/BLOS). The nucleic acid stain SYBR Green was used to identify bacterial cells and CHL fluorescence to identify algal cells. Enzymatically hydrolyzable amino acids (EHAA) were measured from 1 L subsamples using the protocol of Mayer et al. (1995) as adapted for seston filters (Laursen et al. 1996); this biomimetic approach uses a nonspecific proteinase to digest samples and quantifies the resulting production of amino acids and oligopeptides. To assess food quality via elemental ratios, EHAA concentrations were converted to carbon (EHAA-C = EHAA ÷ 2) and nitrogen (EHAA-N = EHAA ÷ 6) equivalents based on average elemental composition (½ carbon and ¼ nitrogen by weight) reported by Mayer et al. (1995) for EHAA. All equipment was pre-cleaned with either RBS-35 or 10% hydrochloric acid.

The feeding response of oysters (C. virginica) to changes in chemical composition of algal rot were assessed by the biodeposition method. Hawkins et al. (2013b) and Iglesias et al. (1998) provide a complete discussion of the biodeposition method and calculations; a brief description of equations is given in the Appendix (Table 2). Individual oysters were placed in flow-through feeding chambers and provided with 200 mL min⁻¹ of diluted algal rot suspension. Two empty control chambers were monitored simultaneously in each run of the experiment to correct for settlement of particulates.
Oysters fed for 6 h on each experimental day, after which all true feces and pseudofeces were collected separately for analysis. POM and PIM concentrations from water samples were compared to POM and PIM in biodeposits to calculate absorption rate (AR), clearance rate (CR), and absorption efficiency (AE). Insufficient biodeposits were recovered for EHAA or CHL analyses. Feeding rates were standardized per gram dry weight (gDW) of soft tissue. Following each experiment, soft tissue was dissected from each oyster, dried at 60° C for 2 days, and weighed on an MT5 analytical microbalance (Mettler Toledo LLC, Ohio, USA). Oysters were maintained at 19.5° C, fed live T. weissflogii culture between experiments, and depurated via starvation for 48 h prior to each experiment. Oysters that were not actively feeding were removed from analysis, leaving final sample sizes of 6-9 oysters for each experimental day.

2.3. Field Feeding Experiment

The field feeding experiment tested the feasibility of detrital consumption by oysters under natural conditions. FlowCam (Fluid Imaging Technologies, Scarborough, ME) analysis was conducted by the JJMFAC/BLOS to identify and quantify seston particles, including phytodetritus, with a 4X objective and 300 µm flow cell on fluorescence trigger mode for particles >20 µm. Images were analyzed in Visual Spreadsheet Software (Fluid Imaging Technologies) and biovolumes were estimated by combining the method in Sieracki et al. (1989) with an algorithm described in Burger & Burge (2008) and Chang et al. (2004). The biomasses of diatoms and ciliates were estimated from biovolume (Menden-Deuer & Lessard 2000) but given the heterogeneous nature of phytodetritus, estimation of phytodetritus biomass from biovolume is impossible.

The same flow-through feeding chambers that were used for the laboratory algal rot were deployed in situ at the Pemaquid Oyster Company lease site in the upper Damariscotta Estuary (site description below). Market-size oysters (61-114 mm) from surface holding cages (held May to October 2016; acclimated to natural field conditions) were placed in feeding chambers and supplied with water from 1 m depth at 150 mL min⁻¹. Relatively large oysters were selected, and water flow reduced for use
in this experiment to ensure sufficient drawdown of particles for accurate FlowCam water sample analysis. Biodeposits were collected to confirm that oysters were actively feeding (unpubl. data).

Comparisons of particle concentration between outflow and control chambers were used to estimate particle removal by the oysters. The experiment was conducted twice, July 11\textsuperscript{th} and August 23\textsuperscript{rd}, and 7 actively feeding oysters were selected for analysis on both experimental days. A full description of methods will be available in Lubelczyk et al. (in progress).

2.4. Seasonal Field Study

The seasonal field study examined how the feeding and growth responses of oysters varied in relation to environmental conditions on an operating oyster farm in the upper Damariscotta Estuary. The upper Damariscotta Estuary is a drowned-river valley with very low freshwater input (1-3 m\textsuperscript{3} s\textsuperscript{-1}) resulting in typical summer salinities of 25-32 PSU (McAlice 1977, Mayer et al. 1996). Being shallow (4-10 m), it has high seasonal temperatures and primary productivity (McAlice 1977, Mayer et al. 1996) that support the production of eastern oysters (Ingersoll 1880, Maine DMR commercial harvest 2016 www.maine.gov/dmr).

Environmental monitoring consisted of hourly data from a Land/Ocean Biogeochemistry Observatory (LOBO) buoy moored in the upper Damariscotta Estuary east of Perkins Point. On board the LOBO, a WQM (Sea-Bird Scientific) at 1.5 m measured temperature (-5 to 35\textdegree{} C), turbidity (backscattering, 0 to 25 NTU), and chlorophyll-\(\alpha\) (fluorescence, 0 to 50 \(\mu\)g L\textsuperscript{-1}). The continuous monitoring was supplemented with biweekly water samples at the LOBO mooring. Triplicate water samples were taken from 1 m depth, filtered onto GF/F Whatman filters, and analyzed for EHAA and pigments as above (section 2.2). POM was sampled similarly but without replication. Water samples were also sent to JJM/BLOS for FlowCam analysis of plankton, as above (section 2.3). Seasonal variation in EHAA:CHL ratios were used to assess the mix of live algal and detrital proteins in the seston by
determining if EHAA abundance is in excess of what can be attributed to average phytoplankton. An EHAA:CHL ratio of 40 is estimated to represent pure phytoplankton (Laursen et al. 1996).

The physiological response of oysters to environmental changes was measured via biweekly feeding and growth rate estimates from May 31st to October 11th, 2016 at Pemaquid Oyster Company lease site. Feeding activity was measured on the same day that water samples were collected near the LOBO buoy and growth rates were measured on alternate weeks. Growth and feeding rates were sampled on alternating weeks so that: 1) growth rates integrate the physiological effects of the environment over the preceding ~8 days, and 2) feeding rates provide a single discrete analysis of biological response to the environment at the time of sampling.

For feeding behavior assessment, the flow-through chambers were deployed in situ as in the field feeding experiment (section 2.3), but the deployment was extended to cover a 12.5-hour, nighttime tidal cycle and used 50-98 mm oysters. Smaller oysters were used in this experiment to prevent reduction of seston in chambers of >50% that might affect animal feeding behavior. These differences in experimental design between the field feeding experiment and seasonal field study do not allow for direct comparisons between feeding results. Ambient water was sampled every 30 minutes during deployment by an ISCO 3700 Sampler (ISCO Inc., Nebraska, USA) to obtain a composite water sample representative of the 12.5 h tidal cycle. This composite water was analyzed for POM, PIM, and EHAA for direct comparison with the same measurements from biodeposits from each chamber to calculate feeding behavior as in the algal rot experiment (section 2.2). For feeding rate calculations that include CHL (SELORG/REMORG fraction of diet), we used the LOBO buoy CHL measurements averaged over each 12.5 h deployment period. Buoy fluorometer data were adjusted with a regression against extracted CHL values obtained from biweekly water samples, excluding times of photoquenching from the buoy data, with a correction factor of 1.40. Insufficient biodeposits were recovered for CHL analysis, but EHAA analysis was possible on two occasions allowing for estimation of EHAA absorption efficiency by
substituting EHAA for POM in the AE equation (Appendix Table 2). Feeding rates were standardized per gDW of soft tissue with DW data obtained as above (section 2.2). Oysters that were not actively feeding were removed from analysis and final sample sizes for each sampling date were 6-10.

The organic matter diet of oysters in the field feeding experiment were partitioned into various nutritional pools to assess the relative potential contribution of each, as done by Hawkins et al. (2013a and 2013b). The potential contribution of algal biomass to oyster diet was calculated as the chlorophyll-rich organic matter that is preferentially selected by oysters (SELRG); the potential non-living and/or non-algal organic matter contribution to oyster diet was calculated as all remaining organic matter (REMORG), which includes, but is not limited to, detrital material. The fraction of the diet from SELORG is calculated as the maximum potential fraction assuming 100% absorption efficiency, and the fraction of the diet from REMORG is calculated as the remaining organic absorption requirements. Similarly, the potential contributions of algal-nitrogen (Algal-N; C:CHL ratio of 50, Redfield C:N ratio of 6.625) and all remaining EHAA-nitrogen (REMEHAA-N; EHAA-N minus algal-N) to oyster diets were calculated with similar results. See Appendix Table 2 for equations.

Specific growth rates of 63 individual oysters were calculated from biweekly shell height measurements. Specific growth rate is a log ratio that accounts for initial oyster size at each interval and has conventionally been used to assess environmental impacts on bivalve success (e.g. Clausen & Riisgård 1996, Mills 2000, Karayücel 2010, Chi et al. 2010, Riisgård et al. 2012, Malkin et al. 2012). Spat-sized oysters were individually marked by gluing numbered tags (Bee Works, Queen Marking Kit) to the shell. They were deployed in May 2016 in two ADPI oyster culture bags on surface nursery lines at the Pemaquid Oyster Company lease site. Oysters were acclimated to field conditions for two weeks before initial shell height measurements were taken to reduce artifacts from handling. Specific growth rates based on changes in oyster shell height were calculated to normalize to animal size and thus provide
more insight into the physiological response of the animal to environmental changes than linear growth rates.

2.5. Statistical Analyses

Analyses of differences in means of water quality and feeding behaviors (laboratory algal rot and seasonal field study) were conducted with one-way ANOVA and, if significant at an experiment-wise $P < 0.05$, followed by Tukey HSD pairwise comparisons. For direct comparisons between two mean values (AE of EHAA and POM), a two-way student’s $t$-test was performed, and significance determined at $P \geq 0.05$. Specific growth rates over a given time-period were correlated with water quality data measured during that same time-period. There was a lag of 7-10 days between water quality and growth rate sampling, and growth rates were compared to averaged water quality data from the previous two sampling dates (May 31st and June 14th) on one occasion (June 16th). Varying lag time between water quality and growth sampling was assessed with residual plots (correlation residuals vs. lag time), which had slope = 0 in all cases ($P > 0.05$).

All single parameter explanations of oyster growth were analyzed by non-parametric rank correlation coefficients (Spearman’s rho, $\rho$, ranging from -1 to +1) because sample size was small ($n = 8-10$, FlowCam and POM analyses were each impossible on one sampling date). Residual plots (predicted values vs. residuals) from all correlations are randomly distributed (slope = 0, $P > 0.05$). To assess the combined effects of multiple measured parameters on variability in growth rates, a stepwise multiple linear regression model was conducted in MATLAB. Moving averages of water quality ranging from 1 to 7 days prior to growth sampling were included in model runs, which were constrained to $\leq 3$ independent variables. All possible combinations of independent variables were generated and the best fit models from each model run are presented here. In each model, positive and negative correlations are indicated by the sign of the regression coefficient for each term (+ and -, respectively) and residual plots (predicted values vs. residuals) from all model runs are randomly distributed (slope = 0, $P > 0.05$).
CHAPTER 3

RESULTS

3.1. Laboratory Algal Rot

As the algal rot progressed, CHL concentrations declined from 3.3±0.1 to 0.7±0.1 µg L\(^{-1}\) and pheopigments increased from 0.0±0.0 to 3.9±0.3 µg L\(^{-1}\) (Fig. 1). The decline in algal cell numbers from 4.0 x 10\(^9\) to 1.7 x 10\(^9\) L\(^{-1}\) (Table 1) was less pronounced than CHL, suggesting intracellular pigment decay. Bacterial cell numbers increased from 2.9 x 10\(^{10}\) to 1.3 x 10\(^{11}\) L\(^{-1}\) (Table 1), indicating their role in algal decomposition. The 58% loss in algal cell number was less than seen in previous algal rot experiments (Mayer, unpubl. data), and the bacterial abundance increased an order of magnitude less than in those experiments. It therefore appears that, as algal biomass decayed, it was converted partially into bacterial biomass and partially into algal necromass to form a product that I call bacteriogenic phytodetritus. The organic carbon concentration in this bacteriogenic phytodetritus decreased with bacterial respiration but the food quality - estimated as EHAA-C:POC - remained relatively constant (Fig. 1). Interestingly, the fraction of total nitrogen in EHAA form (EHAA-N:PON) declined due to stable PON coupled with decreasing EHAA concentrations. These results suggest conservation of total nitrogen relative to total carbon and EHAA as microbial respiration proceeded, but conversion from EHAA-N to other non-proteinaceous nitrogenous compounds such as bacterial peptidoglycan cell walls (see Appendix Fig. 22 for individual POC/N and EHAA concentrations).

The oysters fed on materials from all stages of decay. There were statistically significant increases in AR (from 1.1±0.4 to 3.7±0.5 mg hr\(^{-1}\) gDW\(^{-1}\)) and AE (from 0.41±0.05 to 0.66±0.02) of POM as the algal rot progressed (Fig. 2). Clearance rate likewise increased (Fig. 2), but the change in CR as the rot progressed was not statistically significant. The low AE at Day 0, prior to decay, is at odds with high AE values (0.72-0.90) of phytoplankton cultures typically reported (Romberger & Epifanio 1981; Alber & Valiela 1996). Instead, AE on the day 0 is similar to AE of total POM in the seasonal field study (see Fig.
Table 1. Laboratory algal rot cell enumerations. Variation in algal and bacterial cell numbers on each day the algal rot was sampled, as assessed by flow cytometry.

<table>
<thead>
<tr>
<th>Rot Day</th>
<th>Algal Cells/L</th>
<th>Bacterial Cells/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$4.0 \times 10^9$</td>
<td>$3.0 \times 10^{10}$</td>
</tr>
<tr>
<td>2</td>
<td>$3.1 \times 10^9$</td>
<td>$6.4 \times 10^{10}$</td>
</tr>
<tr>
<td>5</td>
<td>$3.2 \times 10^9$</td>
<td>$1.2 \times 10^{11}$</td>
</tr>
<tr>
<td>12</td>
<td>$1.7 \times 10^9$</td>
<td>$1.3 \times 10^{11}$</td>
</tr>
</tbody>
</table>

Figure 1. Biochemical changes of phytodetritus during laboratory algal rot. In addition to pigments, nitrogen (EHAA-N) and carbon (EHAA-C) equivalents of labile protein were compared to total particulate nitrogen and carbon (PON/C) as the rot progressed. One-way ANOVA analyses were significant for all variables ($P < 0.05$) and letters indicate results of Tukey HSD pairwise comparisons. Bars represent the mean and error bars are ± 1 standard error.
Figure 2. Oyster feeding behavior during laboratory algal rot. Absorption and clearance rates are standardized to gDW of oyster soft tissue. One-way ANOVA analyses were significant for AR and AE (P < 0.05) and letters indicate results of Tukey HSD pairwise comparisons. One-way ANOVA was not significant for CR (P > 0.05). Bars represent the mean and error bars are ± 1 standard error.
11). This discrepancy could be partially attributed to the processing of live algal culture into a non-viable, highly concentrated suspension.

Because biodeposits were collected immediately after the 6-hour feeding period, calculated increases in AR and AE increases over time may reflect increases in gut retention times (GRT). This explanation is unlikely, however, because ingestion rate increased as the rot progressed (Appendix Table 3), implying shorter GRT (Navarro et al. 1992, Navarro et al. 2009). Ingestion rates were calculated with measurements of pseudofeces (unaffected by digestive processes) and PIM of feces. While feces are affected by changes in GRT, increasing GRT would decrease PIM of feces and underestimate ingestion rates. The calculated ingestion rates, therefore, represent the minimum possible ingestion rates and increases over time suggest that GRT is more likely to have decreased than increased.

3.2. Field Feeding Experiment

Oysters in the field experiment also fed on live and dead material. Phytodetritus in field seston was identified by FlowCam and Visual Spreadsheet Software (Fluid Imaging Technologies) as autofluorescing, amorphous, and otherwise unidentifiable particles in the natural seston. Phytodetritus comprised a varying proportion of total seston in the control chamber outflow but was substantially reduced in the outflow of chambers holding oysters on both dates (Fig. 3). Oysters removed 3.5 x 10^7 and 1.3 x 10^8 µm^3 L^{-1} of phytodetritus the July 11th and August 23rd, respectively, as well as various live particles such as ciliates and diatoms (Fig. 4). Among the particle types identified and quantified (diatoms, ciliates, phytodetritus), oysters cleared primarily ciliates by biovolume (1.2 x 10^8 µm^3 L^{-1}) on July 11th and primarily diatoms by biovolume (1.5 x 10^8 µm^3 L^{-1}) on August 23rd.

The variable density and composition of phytodetritus makes accurate volumetric quantification impossible and, therefore, direct comparisons to live particles unreliable. Nevertheless, volumetric concentration of phytodetritus removed from the oyster chamber is compared to the initial concentration (assuming similar nature of inflow and outflow phytodetrital particles) to calculate
Figure 3. Biovolume concentration of particles in field feeding experiment. FlowCam-based analysis identified and quantified biovolume of particles in outflow water from empty (Con) and oyster (Oys) feeding chambers on two occasions.

Figure 4. Oyster clearance of particles from the water in field feeding experiment. Seston particles were identified with a FlowCam and concentrations compared between oyster feeding chamber and control chambers to estimate removal of particles by the oysters.
percent reduction (Fig. 5). Oysters cleared phytodetritus with greater efficiency than ciliates and diatoms on both experimental days (Fig. 5).

3.3. Seasonal Field Study

Environmental conditions from May to October 2016 resembled previous studies of the upper Damariscotta Estuary (McAlice 1977, Laursen 1995, Mayer et al. 1996, Thompson et al. 2008). Temperatures ranged from 10.8°C to a seasonal maximum of 24.3°C in July, and turbidity showed spring/neap tidal periodicity, likely due to sediment resuspension on spring tides in the shallow upper estuary (Fig. 6, see Appendix Fig. 23 for tidal currents). The largest spring tide of the sampling season (July 5th) coincided with the highest PIM to total particulate matter (TPM) ratio (see Appendix Table 5 for PIM:TPM), consistent with tidal sediment resuspension in a shallow estuary (Hawkins et al. 1996, Galimany et al. 2011). Extracted CHL measurements varied seasonally from 2.5±0.0 µg L⁻¹ in late spring to 9.8±0.3 µg L⁻¹ during the fall diatom bloom (Fig. 7; see Appendix Table 5 for diatom enumeration).

Seasonal variations in EHAA concentrations were somewhat similar to changes in CHL, but the EHAA:CHL ratio varied over the course of the season from 174 in the spring to 37 during the diatom bloom on September 13th (Fig. 7). Based on a EHAA:CHL of 40 for pure phytoplankton (Laursen et al. 1996, Fig. 7), only 23% of EHAA can be attributed to algae in the spring, suggesting that the majority of labile protein at that time was non-algal and/or non-living (i.e. detrital). Virtually all the labile protein was phytoplanktonic during the fall diatom bloom. While REMORG generally scales with POM, EHAA concentrations are more variable in relation to POM (Fig. 8). This highlights the importance of measuring the bioavailable fraction of food, which provides greater resolution of the variation of food quality than measurements of total organics.

The rate that particles were cleared from the water (CR) generally scaled with seasonal temperatures, ranging from 0.6±0.1 to 3.0±0.4 L hr⁻¹ gDW⁻¹, with one exception described below (Fig. 9). Clearance rate in C. virginica is known to be strongly influenced by temperature, especially at the
Figure 5. Percent reduction of particles by oysters in field feeding experiment. Percent reduction was calculated for each particles type to indicate preferential clearance by the oyster. Particles were quantified with a FlowCam.

Figure 6. Seasonal variations in sea surface temperature and turbidity in the Damariscotta Estuary. Measurements were collected hourly by a LOBO buoy moored in an active oyster aquaculture area of the estuary.
Figure 7. Seasonal variations in EHAA and CHL concentrations in the Damariscotta Estuary. Measurements from the LOBO buoy show hourly variation in chlorophyll (CHL) and extracted CHL values were compared to labile protein (EHAA:CHL). All measurements were taken at the buoy mooring in an active oyster aquaculture area of the estuary.
Figure 8. REMORG and EHAA relationship to POM concentrations in the Damariscotta Estuary.
REMORG is calculated as all non-algal/non-living organic matter, which generally scales with POM.
EHAA is more variable and does not have a clear relationship with POM. All measurements were taken at the buoy mooring in an active oyster aquaculture area of the estuary.
Figure 9. Seasonal variation in oyster feeding behavior in the Damariscotta Estuary. Clearance rate and absorption rate are standardized to gDW of oyster soft tissue. Clearance rate increases in response to a tidal sediment resuspension event on July 5th while absorption rate decreases to negative values. Bars represent the mean and error bars are ± 1 standard error.
northern end of its range where temperature is often limiting (Loosanoff 1958, Carriker & Gaffney 1996, Comeau 2014). A notable exception occurred on July 5th when CR was unseasonably high (4.7±0.6 L hr\(^{-1}\) gDW\(^{-1}\)) coincident with the largest spring tides and sediment resuspension (PIM:TPM). The CR responses to environmental variation in bivalves are complex, but CR can increase with moderately higher seston loads in mussels (Hawkins et al. 1996), cockles (Iglesias et al. 1996) and oysters (Bayne 2017). *Crassostrea virginica* can increase CR in response to decreasing fraction of organic matter (POM:TPM) in the seston (Galimany et al. 2017), as seen here.

The absorption rate of total organics (AR of POM) ranged seasonally from 0.3±0.1 to 3.0±0.4 mg hr\(^{-1}\) gDW\(^{-1}\) (Fig. 9). Absorption rate correlates negatively with PIM:POM ratio (Fig. 10, p < 0.05) and even became negative during the sediment resuspension event on July 5th (-1.8±1.1 mg hr\(^{-1}\) gDW\(^{-1}\)). Negative estimates of AR might be attributed to metabolic fecal loss (described by Hawkins et al. (2013b) as the sloughing of organic compounds into fecal material during metabolism) due to the high cost of processing inorganic material (Urban & Kirchman 1992) that dilutes organic food particles (Hawkins et al. 1998, Strychar & MacDonald 1999). High PIM concentrations can also inhibit bivalve particle selection capabilities, exacerbating the food dilution effect (Kiørboe et al. 1981).

The efficiency of absorption of total organics (AE of POM) ranged seasonally from 0.35±0.09 to 0.71±0.06, consistent with AE of algae and phytodetritus from the algal rot experiment (Fig. 2). On two occasions, AE of POM (0.67±0.01 and 0.64±0.01) can be compared to AE of EHAA (0.91±0.03 and 0.94±0.02) (Fig. 11). On both occasions, AE of EHAA is significantly higher than AE of POM (two-way t-test, P < 0.001) and higher than AE previously reported for CHL (0.82±0.03, Soletchnik et al. 1996). This notably high AE of EHAA (ave. = 0.93) indicates relatively good agreement between the biomimetic chemical assay and the protein absorption by oysters under natural conditions; essentially all EHAAs are absorbed by the animal and therefore assumed to be bioavailable. However, some bioavailable proteins absorbed by the animal may not be detectable by the EHAA assay. Time constraints limited AE of EHAA
Figure 10. Linear regression between absorption rate of POM by oysters and seston PIM:POM. The ratio between inorganic (PIM) and organic (POM) matter indicates dilution of organic food particles with inorganic material, such as sediment from the tidal resuspension event. Observed variation in PIM:POM negatively affects oyster absorption rate ($R^2 = 0.37$). Circles represent the mean and error bars are ± 1 standard error.
Figure 11. Seasonal variations in absorption efficiency of POM and EHAA by oysters. Absorption efficiency calculations were impossible on July 5th due to negative absorption rates. Oyster biodeposits were analyzed for organic matter (POM) throughout the season and labile protein (EHAA) on two dates. Absorption efficiency was higher for EHAA than POM on both dates (P < 0.001, 2 sample two-way t-test). Bars represent the mean and error bars are ± 1 standard error.
analysis to the 4 most actively feeding oysters on each date, which were compared to AE of POM for the same oysters.

The total organic oyster diet (AR of POM) was subdivided into the potential contributions from algal (SELORG) and other (REMORG) organic material (Fig. 12). For this calculation, I used a C:CHL ratio (wt/wt) of 50, which is a typical value for phytoplankton (Welschmeyer & Lorenzen 1984, Hawkins et al. 2013a); Hawkins et al. (2013b) used a C:CHL of 12 to represent the microalgal cultures that were used in their study. A comparison of diet calculations using both C:CHL ratios is included here (Appendix Table 4), but a ratio of 50 leads to a more conservative estimate of detrital contribution to oyster diet. Calculations assume complete absorption efficiency of SELORG, which is therefore presented as the potential SELORG fraction of the oyster diet, and results in an estimate of the minimum detrital contributions. The potential SELORG fractional contribution to oyster diet ranged from 0.44±0.01 early in the season to 1.00±0.00 during the fall diatom bloom. The minimum REMORG fractional contribution to oyster diet was higher than SELORG on two occasions, June 21st (0.56±0.01) and October 11th (0.53±0.03). Because separation of diet into fractions requires a positive value for AR, the negative estimate for AR on July 5th made dietary fraction calculations impossible on this date. Fraction of diet estimations were also calculated based on nitrogen equivalents (algal-N and REMEHAA-N) and showed similar seasonal patterns (Appendix Fig. 24).

From May to October 2016, average oyster length doubled, from 36.6±0.6 to 73.8±0.8 mm. Specific growth rates were generally higher in the spring with a peak of 1.2±0.06 x 10^{-2} d^{-1} on June 30th and slowly declined to 0.04±0.01 x 10^{-2} d^{-1} on October 26th as water temperature dropped (Fig. 13). Linear growth rates exhibit this same trend (Appendix Fig. 25). An unseasonably low growth rate of 0.36±0.04 x 10^{-2} d^{-1} on July 14th corresponds with the tidal sediment resuspension event on July 5th that also resulted in negative AR likely from the high cost of food processing (Fig. 9 & 10).
Figure 12. Seasonal variations in the SELORG/REMORG fractions of oyster diets. Fraction of diet calculations were impossible on July 5th due to negative absorption rates. Fraction of diet calculations assume complete absorption efficiency of CHL-rich particles (SELORG) and remaining organics (REMORG) meet the rest of oysters’ organic absorption requirements. Calculations are based on C:CHL ratio of 50. One-way ANOVA analysis was significant for REMORG:SELORG ratio (P <0.05) and letters indicate results of Tukey HSD pairwise comparisons. Bars represent the mean and error bars are ± 1 standard error.
Figure 13. Seasonal variations in oyster specific growth rates and sea surface temperature. Oysters roughly doubled in size over the entire sampling season, from average 36.6±0.6 to 73.8±0.8 mm. One-way ANOVA analysis was significant for specific growth rate (P < 0.05) and letters indicate results of Tukey HSD pairwise comparisons.
3.3.1 Significant Single Parameter Growth Correlations

Specific growth rates correlated positively with EHAA concentrations, except for one notable outlier on June 30th - the highest growth rate of the season (EHAA of 0.26 mg L⁻¹, Fig. 14). Using data available for this study, this single, very fast estimated growth rate is only explained well by correlation with ciliate-C as described below. EHAA concentrations correlate significantly (P = 0.006, ρ = 0.83) with specific growth rates when this outlier (which is outside 95% confidence density ellipse for bivariate outlier analysis) is removed from analysis.

Temperature and carbon from ciliate biomass (ciliate-C) are also significant predictors of oyster growth rates. Temperature has a positive relationship with specific growth rates (P = 0.0384, ρ = 0.66), as previously demonstrated for growth and various metabolic rates of *C. virginica* (Loosanoff & Nomejko 1949, Ingle & Dawson 1952, Dame 1972, Loosanoff 1958, Pernet et al. 2008, Lord & Whitlatch 2014). This relationship appears to be exponential (R² = 0.49), although it may, in fact, be a two-phase linear relationship with a growth threshold near 18° C; we lack sufficient data to test the latter hypothesis (Fig. 15). Seasonal ciliate-C is the best single predictor (P = 0.0006), which correlates positively (ρ = 0.91) with specific growth rates (Fig. 16). This unexpected result corroborates the field feeding experiment in which more ciliate biovolume was cleared than any other analyzed particles on July 11th (Fig. 4).

It is worth noting that growth correlations with TPM, POM, and CHL (extracted and buoy data 2-day average as per multiple linear regression below), which are commonly used food parameters in filter-feeding bivalve growth models, were all insignificant in the present study (R² = 0.08, 0.17, and 0.15, respectively; P > 0.05).

3.3.2 Multiple Linear Regression Growth Models

First, oyster growth was predicted with traditional estimates of food quantity (TPM, POM, and CHL) in a simple multiple linear regression. This was followed by a stepwise multiple linear regression in
Figure 14. Correlation between oyster specific growth rate and EHAA concentrations. Specific growth rates correlate positively with seasonal EHAA concentrations, but there is one statistical outlier present. The outlier is outside a 95% confidence density ellipse for bivariate analysis. Spearman’s non-parametric correlation coefficient ($\rho = 0.83$) is significant ($P = 0.006$) when the outlier is removed from analysis.
Figure 15. Correlation between oyster specific growth rate and temperature. Specific growth rates correlate positively with temperature and Spearman’s non-parametric correlation coefficient ($\rho = 0.66$) is significant ($P = 0.0384$). This relationship appears exponential (exp. regression $R^2 = 0.49$).
Figure 16. Correlation between oyster specific growth rate and ciliate-C concentration. Carbon from ciliates (ciliate-C) is the best single-parameter predictor of oyster growth and Spearman’s non-parametric correlation coefficient ($\rho = 0.91$) is highly significant ($P = 0.0006$).
which a baseline model was built on LOBO buoy data and additional parameters were added in subsequent model runs. This growth model also incorporates TPM, POM, and CHL in the final run.

The simple multiple linear regression included both extracted CHL and LOBO buoy CHL, as well as TPM and POM. The best fit model terms were LOBO buoy CHL averaged over 2 days (+), POM (+), and TPM (-) with \( P = 0.0097 \) and \( R^2 = 0.51 \) (Fig. 17). CHL and POM are food estimates and thus are expected to have a positive effect on growth. TPM is likely an indicator of sediment resuspension which negatively affects feeding of oysters in the Damariscotta Estuary (Fig. 9 & 10) by dilution of organic particles with inorganic material, as seen with the sediment resuspension event.

To build the stepwise multiple regression model, a baseline environmental model was established first with temperature, CHL, turbidity, and a ratio of CHL to turbidity (CHL:Turb) using measurements from the LOBO buoy. These variables were chosen to provide an initial assessment of oyster growth because they are traditionally regarded as key parameters for predicting oyster growth and are available in high temporal resolution (hourly). The baseline best fit model includes temperature (+) and CHL:Turb (+) with \( R^2 = 0.21 \) and \( P = 0.0209 \) (Fig. 18). A positive effect of temperature on growth is expected because feeding behavior increases with temperature as discussed above (e.g. Loosanoff 1958). High turbidity, similar to TPM, likely indicates sediment resuspension that negatively affects feeding. The CHL:Turb term thus is affected by both food quantity (CHL) and dilution (Turb), and the positive influence is expected.

Next, parameters measured on biweekly water samples (EHAA, FlowCam plankton enumerations, TPM, POM, PIM, and extracted CHL) were added to re-runs of the baseline model to test for improvements in predictive ability. EHAA (+) addition to the model improves the fit (\( P < 0.0001 \) and \( R^2 = 0.91 \)) when the outlier on June 30th is removed from analysis (Fig. 19). This rejection is justified because the Mahalanobis distance, based on variance of the datum from multivariate mean, is beyond
Figure 17. Multiple regression with traditional estimates of food (TPM, POM, CHL). A simple multiple linear regression best fit growth model with traditional estimates of food, including both extracted CHL and LOBO buoy CHL to assess phytoplankton abundance. The model was constrained to 3 terms. In the model equation, SGR indicates specific growth rate and numbers following term parameter indicates the averaging period (range 1-7 days).
Figure 18. Multiple regression with LOBO buoy variables. A baseline environmental model to provide initial assessment of oyster growth using traditional predictors of oyster growth (temperature, turbidity, chlorophyll) at high temporal resolution (LOBO buoy hourly data). The model was constrained to 3 terms. In the model equation, SGR indicates specific growth rate and numbers following term parameter indicates the averaging period (range 1-7 days).

SGR = -0.009 + (1.0 \times 10^{-3} \text{Temp1}) + (3.0 \times 10^{-3} \text{CHL.Turb1})

Adjusted $R^2 = 0.21$
Figure 19. Multiple regression with LOBO buoy variables and EHAA concentration. The addition of EHAA concentrations to the baseline multiple regression of oyster specific growth rates substantially increases the predictive power of the model ($R^2 = 0.91$). One outlier was removed from analysis (Mahalanobis distance > upper control limit). The model was constrained to 3 terms. In the model equation, SGR indicates specific growth rate and numbers following term parameter indicates the averaging period (range 1-7 days).
the upper control limit (Sikder et al. 2014). In this model, temperature (+) and CHL:Turb (+) remain important terms that contribute significantly to the regression relationship.

Because of its success in explaining specific growth rate in a single parameter correlation, ciliate-C was the next variable added to the model. The addition of Ciliate-C (+) improves model fit over the baseline model (Fig. 20) but has slightly less predictive power ($P = 0.0001$ and $R^2 = 0.90$) than the model with baseline variables and EHAA concentrations. Mahalanobis distances were recalculated for this set of variables and the outlier on June 30th was removed. In this model run, CHL:Turb (+), EHAA (+), and ciliate-C (+) are the 3 terms that result in the best fit model.

Finally, the model was run with all measured environmental parameters including diatoms, phytodetritus, total FlowCam phytoplankton >20µm, extracted CHL, TPM, POM, PIM, and selected ratios between them (Fig. 21). No outliers are present in this larger multivariate dataset (Mahalanobis distances all below upper control limit). The best fit model ($P < 0.0001$ and $R^2 = 0.93$) includes temperature (+), EHAA:POM ratio (-), and ciliate-C (+). The EHAA:POM ratio normalized protein concentrations to total food abundance and provides one indication of food quality. Interestingly, this ratio emerges as an important term, despite the negative coefficient due to the anomalous correlation between growth rate and EHAA on June 30th that was not excluded from this model.
Figure 20. Multiple regression with LOBO buoy variables, EHAA concentration, and Ciliate-C concentration. Since oyster cleared ciliates in the field and oyster growth correlates well with ciliate carbon (ciliate-C), ciliate-C was added to the next model run. The addition of ciliates slightly reduces the predictive power of the previous model from $R^2 = 0.91$ to $R^2 = 0.90$. One outlier was removed from analysis (Mahalanobis distance > upper control limit). The model was constrained to 3 terms. In the model equation, SGR indicates specific growth rate and numbers following term parameter indicates the averaging period (range 1-7 days).
Figure 21. Multiple regression with all measured water quality parameters. The final growth model includes all measured environmental parameters and selected ratios between them. The predictive power is slightly higher than previous models ($R^2 = 0.93$). The ratio between labile protein and organic matter (EHAA:POM) has a negative coefficient due to the inclusion of the anomalous value which is no longer an outlier in this dataset (Mahalanobis distance < upper control limit). The model was constrained to 3 terms. In the model equation, SGR indicates specific growth rate and numbers following term parameter indicates the averaging period (range 1-7 days).
CHAPTER 4

DISCUSSION

The importance of detritus to oyster productivity in the Damariscotta Estuary was assessed through both mechanistic (feeding) and correlative (growth) lenses. While evidence for the contribution of detritus to oyster feeding and growth in this study is indirect, the three approaches employed in this study consistently suggest that oyster utilize detritus as a food source.

4.1. Nutritional Utilization of Detritus: Can Detrital Proteins Fill the Hole in Oyster Diets?

Efficient absorption of phytodetritus by oysters under laboratory conditions strongly indicates the potential physiological significance of detritus. Despite conversion of EHAA-N to other nitrogenous compounds, total particulate nitrogen was conserved relative to particulate carbon and phytoplankton cells in phytodetritus suspension. Bacteria and other heterotrophs can enrich nutritive value of decayed material relative to fresh particles via processes such as bacterial assimilation of dissolved and inorganic nitrogen (Paerl 1984, Rice & Hanson 1984, Biddanda 1988, Sanzone et al. 2001) into particles of an accessible size for oysters (Ward & Shumway 2004). If this detrital nitrogen is bioavailable to oysters, as evidenced by increasing AR and AE as the rot progressed, then similar detrital complexes under natural conditions are highly likely to be a valuable nutritional resource for oysters.

Indeed, particulate phytodetrital complexes observed by FlowCam under natural conditions were cleared from the water column by oysters. Average phytodetritus reduction of 83% implies a significant supply of potentially bioavailable phytodetritus to the oyster digestive system. Clearance rates (rate of removal of particles from the water column) measure only the initial feeding behavior. While clearance of a particle can lead to the assimilation of nutrients, it cannot establish the nutritional importance of cleared particles as cleared particles could also be rejected in pseudofeces. However, clearance of phytodetritus provides evidence of an in-situ oyster feeding capability for detrital particles.
Observation of high AE of EHAA in the seasonal field study could imply utilization of detritus under natural field conditions. However, I was only able to measure AE of EHAA on two dates and the EHAA:CHL ratios were low on both occasions (52 and 37, respectively; Fig. 7) which indicates a largely or entirely phytoplanktonic source of EHAA, as would be expected during the fall diatom bloom on September 13th. Therefore, analysis of AE of labile proteins does not provide direct confirmation of detrital protein absorption on these dates despite the essentially complete absorption of EHAA by oysters. Unfortunately, AE of EHAA calculations were not possible early in the season, particularly on May 31st when a high EHAA:CHL ratio of 174 indicated a larger pool of detrital EHAA. It is unknown if the high AE of EHAA observed around the diatom bloom would hold true for more detrital seston. A better test of absorption of detrital protein would require a period of more detritus-dominated seston in addition to a phytoplankton bloom.

Field-based analysis of SELORG-REMORG contributions to oyster diets indicates that oysters need a source of bioavailable organic material beyond phytoplankton throughout most of the growing season, to which labile detrital protein could contribute. Even assuming complete absorption of all SELORG, oysters require some amount of REMORG to complete their diets. SELORG could supply all measured POM uptake by oysters on only one date (September 27th), when temperature and AR were both low and food was abundant. The necessary inclusion of REMORG in oyster diets on all other sampling days in this study is consistent with observations by Hawkins et al. (2013b) who reported a large role for REMORG in bivalve diets based on a C:CHL ratio of 12. The present study assumed a C:CHL ratio of 50, which is a more conservative assessment of the potential importance of detritus in oyster nutrition than previous studies (Hawkins et al. 2013a & 2013b). Despite using a more conservative estimate, my calculations indicate that REMORG is required in oyster diets and seasonal field measurements indicate an abundance of labile detrital protein that could satisfy this need.
The magnitude of EHAA:CHL-derived estimates of detrital protein observed here are similar to earlier measurements in the Damariscotta Estuary (Laursen 1995). However, the EHAA:CHL variability exhibits reversed seasonal timing. For example, spring detrital protein abundances were relatively high in this study, similar to values recorded July-September by Laursen (1995). The spring detrital EHAA in this study was steadily supplanted by algal EHAA culminating in a fall diatom bloom when algal protein dominated, similar to values observed during the spring bloom by Laursen (1995). Despite changes in the phenology, annual oscillations between predominantly algal and detrital states observed in both studies may be typical for this system. Laursen (1995) hypothesized a phytodetrital source of detrital proteins based on the presence of pigment degradation products. Similarly, in the present study, phytodetritus (µm³ L⁻¹) is a significant component of seston that is on the same order of magnitude as diatoms (µm³ L⁻¹), as assessed by FlowCam (Appendix Table 5).

The abundance of detrital protein and phytodetrital particles in the estuary, the need to absorb additional organic matter beyond phytoplankton, and the ability of oysters to feed on (field) and readily absorb (lab) phytodetritus strongly implicates phytodetrital proteins as a dietary component of C. virginica. Under protein-limiting food regimes, the possible significance of phytodetrital proteins is enhanced. We observed somewhat low POM concentration and organic content of seston in the Damariscotta Estuary (Appendix Table 5) relative to similar field studies of bivalve filter-feeding (Rheault & Rice 1996, Gardner & Thompson 2001, Penney et al. 2001). Additionally, labile protein levels coupled with measured clearance rates indicate that protein intake by oysters is sometimes near the minimum ration reported for mussels (Hawkins & Bayne 1991). Insufficient food supply can limit bivalve growth via insufficient nutrients to meet metabolic demand (Rheault & Rice 1996, Gardner & Thompson 2001, Penney et al. 2001). Observation that oysters absorb essentially all available EHAA implies that proteins are a limiting factor in growth. If Damariscotta Estuary oysters are indeed protein limited, they would be
expected to readily assimilate any bioavailable proteins in the seston, including proteins from the labile
detrital pool observed here.

4.2. What Correlates with Oyster Growth?

Seasonal EHAA concentrations are significant in a variety of correlative explanations of oyster
growth, along with other measured environmental parameters. If EHAA concentrations measure total
labile protein available to the oysters and growth rates generally scale with EHAA concentration, then
nutritional supply of labile protein may limit growth.

As expected, other environmental measurements can also explain some oyster growth
variability. Growth rates increased much more than 3-fold over a roughly 15°C temperature range, as
would be predicted by Q_{10} temperature coefficient of growth determined for mussels (Sanchez-Lazo &
Martinez-Pita 2012), suggesting a role for factors other than temperature to positively affect growth.
Ciliates are an unexpected predictor of oyster growth, but not an entirely unlikely food source. Ciliates
can act as trophic linkages between disparate food webs (David et al. 2006) and oysters can feed on
ciliates when they are abundant and/or during times of phytoplankton scarcity (Paulmier 1972, Le Gall
et al. 1997, Dupuy et al. 1999, Kreeger & Newell 2001). Although ciliate-C in this study can meet only a
small fraction of oysters’ bulk carbon requirements (ave. fraction of diet = 0.03) ciliates may stimulate
oyster growth by providing trace nutrients such as DHA or cholesterol, which can limit growth (Wikfors
et al. 1996). Some ciliate genera can synthesis DHA de novo or accumulate sterols from their prey
(Boëchat et al. 2006, Martinez-Creuzburg et al. 2006, Yang et al. 2015). However, the ciliates observed in
this study were largely unidentified and thus we have no details of their potential for providing trace
nutrients.

Parametric analysis via simple and stepwise linear multiple regressions generally confirm the
non-parametric correlations, with the exception that the simple regression of traditional food estimates
(TPM, POM, and CHL) produced an improved prediction of growth (R^2 = 0.51) over the 3 insignificant
individual correlations. The baseline stepwise growth model was not very powerful in predicting growth \((R^2 = 0.21)\), but the final 3 versions of the growth model \((R^2 = 0.91, R^2 = 0.90, \text{ and } R^2 = 0.94, \text{ respectively})\) all provide considerable improvement.

EHAA values are notable in two ways when considering the multiple regressions. First, an EHAA term is included in all best fit models that were run with EHAA as a possible variable, meaning EHAA concentration provides explanatory power that is unavailable in other environmental data. Second, the baseline and EHAA model provides more explanatory power than the traditional model with TPM, POM, and CHL, meaning that the addition of a single parameter to remote sensing capabilities can greatly improve oyster growth predictions over traditional models. Labile protein (EHAA) is likely a critical factor in overall oyster growth and estimates provide greater resolution of oyster nutritional requirements than total organics (POM). The strong influence of EHAA abundance on growth in the Damariscotta Estuary substantiates the potential that protein, partially detrital in form, enables sufficient oyster diets and growth throughout the season. Therefore, the nearly complete absorption of all available EHAA observed on two occasions may be inferred to hold true for the majority of the season under protein-limiting conditions, and some fraction of EHAA is suspected to be detrital.
CHAPTER 5

CONCLUSIONS

Assessment of seasonal variation in abundance and contribution to oyster diets of ‘detritus’ remains difficult. What has been termed detritus in the Damariscotta Estuary may be a wide variety of products, including algal necromass, heterotrophnic biomass, and heterotrophic necromass. The laboratory algal rot demonstrates that nutritious algal necromass complexes are readily absorbed by oysters, which was corroborated by field observations of oysters clearing phytodetritus. These analyses have shown that non-living and/or non-algal organic matter, as estimated by EHAA:CHL above 40 and REMORG abundance, supports the nutrition and growth of oysters. Labile protein is absorbed very efficiently by oysters, which may include detrital proteins during the spring when detritus dominates. Estimates of EHAA concentrations function well to explain variations in oyster growth in multiple model variations, suggesting that Damariscotta Estuary oysters may be, at least partially, growth-limited by protein availability. Abundance of ciliates also explain growth variations and may provide limited trace nutrients or other trophic linkages.

The EHAA extraction procedure is a biomimetic chemical assay that aims to represent biological processes and, indeed, seems to have predictive power at the ecosystem level. Additionally, EHAA concentrations had an unpredictable relationship with POM, highlighting the importance of measuring the bioavailable fraction of food. Use of the EHAA assay approach should be considered in future studies of bivalve aquaculture site selection, as well as ecosystem nutrient flows and trophic structures. The development of EHAA proxies may be the most realistic application because the biomimetic assay is currently time-consuming, expensive, and requires the use of regulated hazardous materials. Likewise, the biologically hydrolyzable fraction of other biochemical components of seston, such as carbohydrates and lipids, may be equally important in understanding what makes an oyster aquaculture site successful and should be considered in future studies.
The assessment of various forms of ‘detritus’ in supplying vital food resources to filter-feeding bivalves here is encouraging but not conclusive. More conclusive studies should be conducted to measure seasonal EHAA:CHL ratios in tandem with seasonal EHAA absorption efficiency to clearly demonstrate detrital protein absorption by the animal.
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Newell RIE, Langdon CJ (1986) Digestion and absorption of refractory carbon from *Spartina alterniflora* (Loisel) and by the oyster *Crassostrea virginica* (Gmelin). Mar Ecol Prog Ser 34:105-115


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## Appendix:

**Supplementary Material**

<table>
<thead>
<tr>
<th>Description</th>
<th>Abbreviation</th>
<th>Calculation</th>
</tr>
</thead>
</table>
| Clearance Rate         | CR           | \[
\text{PIM egestion rate (mg hr}^{-1}) \div \text{[sestonic PIM (mg L}^{-1})]\]
| Ingestion Rate         | IR           | \[
\text{[PIM egestion rate (mg hr}^{-1}) \times \text{[sestonic POM:PIM]]} \div \text{[pseudofecal POM egestion rate (mg hr}^{-1})]\]
| Absorption Rate        | AR           | \[\text{[IR]} \div \text{[fecal POM egestion rate (mg hr}^{-1})]\]
| Absorption Efficiency  | AE           | \[\text{[AR]} \div \text{[AR]} \div \text{[IR]}][AE]
| Selected Organic Matter| SELORG       | \[\text{[CHL (µg L}^{-1}) \times \text{[C:CHL]} \div [0.38] \div 1,000}\]
| Remaining Organic Matter| REMORG     | \[\text{[sestonic POM (mg L}^{-1}) \div \text{[SELORG (mg L}^{-1})]\]
| Algal Nitrogen         | Algal-N      | \[\text{[CHL (µg L}^{-1}) \times \text{[C:CHL]} \div \text{[Redfield C:N ratio of 6.625]}\]
| Remaining EHAA Nitrogen| REMEHAA-N    | \[\text{[EHAA (µg L}^{-1}) \div \text{[EHAA:N ratio of 6]]} \div \text{[Algal-N (µg L}^{-1})]\]
| Filtration Rate of Y   | FR of Y      | \[\text{[CR]} \times \text{[Y]}\]
|                        |              | Where Y = SELORG, REMORG, Algal-N, REMEHAA-N, ciliate-C                                                                                     |
| Fraction of Diet       | FD           | \[\text{[FR of Y]} \div \text{[AR]}]\]

**Table 2.** Oyster feeding behavior and associated seston fraction calculations.
Figure 22. Particulate biochemical components of phytodetritus in laboratory algal rot. Particulate carbon (POC) and labile protein (EHAA) decreased while total particulate nitrogen (PON) remained constant. One-way ANOVA analyses were significant for POC and EHAA (P < 0.05) and letters indicate results of Tukey HSD pairwise comparisons. Bars represent the mean and error bars are ± 1 standard error.

<table>
<thead>
<tr>
<th>Day of Rot</th>
<th>NIRTOTORG (mg h⁻¹ gDW⁻¹)</th>
<th>Standard Error</th>
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<tr>
<td>0</td>
<td>2.4</td>
<td>0.6</td>
</tr>
<tr>
<td>2</td>
<td>5.0</td>
<td>0.8</td>
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<tr>
<td>5</td>
<td>5.3</td>
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<tr>
<td>12</td>
<td>5.7</td>
<td>0.7</td>
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Table 3. Net ingestion rate of total organics (NIRTOTORG) by oysters during the laboratory algal rot. Error is ± 1 standard error.
Figure 23. Turbidity oscillations with spring/neap tidal cycles. Hourly data from the LOBO buoy in the upper Damariscotta Estuary.

<table>
<thead>
<tr>
<th>Date</th>
<th>C:CHL 12</th>
<th></th>
<th>C:CHL 50</th>
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<tr>
<td></td>
<td>SELORG</td>
<td>REMORG</td>
<td>SELORG</td>
<td>REMORG</td>
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<tr>
<td></td>
<td>Fraction of Diet</td>
<td>Fraction of Diet</td>
<td>Fraction of Diet</td>
<td>Fraction of Diet</td>
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<tr>
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<td>0.15</td>
<td>0.85</td>
<td>0.63</td>
<td>0.37</td>
</tr>
<tr>
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<td>0.80</td>
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<td>0.19</td>
</tr>
<tr>
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<td>0.89</td>
<td>0.44</td>
<td>0.56</td>
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<tr>
<td>5-Jul</td>
<td>0.21</td>
<td>0.79</td>
<td>0.84</td>
<td>0.16</td>
</tr>
<tr>
<td>19-Jul</td>
<td>0.25</td>
<td>0.75</td>
<td>0.43</td>
<td>0.07</td>
</tr>
<tr>
<td>2-Aug</td>
<td>0.37</td>
<td>0.63</td>
<td>0.85</td>
<td>0.15</td>
</tr>
<tr>
<td>16-Aug</td>
<td>0.25</td>
<td>0.75</td>
<td>0.91</td>
<td>0.09</td>
</tr>
<tr>
<td>30-Aug</td>
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<td>0.74</td>
<td>0.86</td>
<td>0.14</td>
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<tr>
<td>13-Sep</td>
<td>0.49</td>
<td>0.51</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>27-Sep</td>
<td>0.11</td>
<td>0.89</td>
<td>0.48</td>
<td>0.53</td>
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Table 4. Comparison between two C:CHL ratios used in Fraction of Diet calculations. Fraction of diet calculations were impossible on July 5th due to negative absorption rates.
Figure 24. Seasonal variations in the algal-N/REMEHAA-N fractions of oyster diets. Fraction of diet calculations were impossible on July 5th due to negative absorption rates. Fraction of diet calculations assume complete absorption efficiency of algal-N and remaining EHAA-N (REMEHAA-N) meet the rest of oysters’ organic absorption requirements. Calculations are based on C:CHL ratio of 50 and assume complete absorption of SELORG. One-way ANOVA analysis was significant for REMEHAA-N:algal-N ratio (P <0.05) and letters indicate results of Tukey HSD pairwise comparisons. Bars represent the mean and error bars are ± 1 standard error.
Table 5. Seasonal gravimetric seston and FlowCam plankton analyses in the Damariscotta Estuary. TPM, POM, and PIM samples on August 2nd and FlowCam samples on September 27th were each impossible due to logistic complications.

<table>
<thead>
<tr>
<th>Date</th>
<th>TPM (mg L⁻¹)</th>
<th>POM (mg L⁻¹)</th>
<th>PIM (mg L⁻¹)</th>
<th>Phytodetritus (µm³ L⁻¹)</th>
<th>Diatom (µm³ L⁻¹)</th>
<th>Diatom (µg C L⁻¹)</th>
<th>Ciliate (µg C L⁻¹)</th>
<th>Dinoflagellate (µg C L⁻¹)</th>
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</thead>
<tbody>
<tr>
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<td>3.8</td>
<td>13.3</td>
<td>1.67 x 10⁸</td>
<td>3.29 x 10⁸</td>
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<td>1.3</td>
<td>5.3</td>
<td>3.66 x 10⁸</td>
<td>5.30 x 10⁸</td>
<td>6.01</td>
<td>12.1</td>
<td>1.37</td>
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<tr>
<td>21-Jun</td>
<td>7.5</td>
<td>1.9</td>
<td>5.6</td>
<td>2.46 x 10⁷</td>
<td>2.45 x 10⁸</td>
<td>3.2</td>
<td>15.8</td>
<td>0.28</td>
</tr>
<tr>
<td>5-Jul</td>
<td>10.3</td>
<td>1.8</td>
<td>8.5</td>
<td>5.81 x 10⁸</td>
<td>8.20 x 10⁷</td>
<td>1.32</td>
<td>8.52</td>
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<tr>
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<td>8.4</td>
<td>1.7</td>
<td>6.7</td>
<td>4.31 x 10⁸</td>
<td>1.31 x 10⁷</td>
<td>12.5</td>
<td>15.9</td>
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<tr>
<td>2-Aug</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>7.46 x 10⁷</td>
<td>1.05 x 10⁸</td>
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<td>10.3</td>
<td>0.32</td>
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<tr>
<td>16-Aug</td>
<td>4.6</td>
<td>1.2</td>
<td>3.4</td>
<td>1.67 x 10⁸</td>
<td>4.32 x 10⁸</td>
<td>5.17</td>
<td>14.0</td>
<td>2.94</td>
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<tr>
<td>30-Aug</td>
<td>8.3</td>
<td>1.8</td>
<td>6.5</td>
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<td>3.12 x 10⁸</td>
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<td>3.25</td>
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<tr>
<td>13-Sep</td>
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<td>1.9</td>
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<td>1.34 x 10⁹</td>
<td>6.36 x 10⁸</td>
<td>45.1</td>
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<tr>
<td>27-Sept</td>
<td>6.2</td>
<td>1.1</td>
<td>5.0</td>
<td>-</td>
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<td>2.50 x 10⁷</td>
<td>1.48 x 10¹⁰</td>
<td>89.3</td>
<td>1.09</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Figure 25. Seasonal variations in oyster linear growth rates and sea surface temperature. The seasonal trend in oyster linear growth rate is similar to that of specific growth rate.
BIOGRAPHY OF THE AUTHOR

Cheyenne Adams was born in Whitefish, Montana on January 1, 1992. She was raised in Normal, Illinois and graduated from El Paso-Gridley High School in 2010. She attended Southern Illinois University at Carbondale and graduated in 2014 with a Bachelor of Science degree in Environmental Biology and minors in Chemistry and Peace Studies. She worked in South Carolina at a marine science summer camp and then in Maine at the National Cold Water Marine Aquaculture Center before entering the Marine Biology graduate program at the University of Maine in 2015. Cheyenne recently accepted a position as the Field Research Technician for the Sustainable Ecological Aquaculture Network at the Darling Marine Center, Walpole, Maine and will continue this work after receiving her degree. The responsibilities of this position primarily include maintaining and validating an array of oceanographic monitoring buoys and sensors along the Maine coast. Cheyenne is a candidate for the Master of Science degree in Marine Biology from the University of Maine in May 2018.