FEEDING PHYSIOLOGY OF SUSPENSION-FEEDING BIVALVES: INTER- AND INTRASPECIFIC PLASTICITY

by

Laura Elizabeth Steeves

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
July 2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Suspension-feeding bivalves play important ecological roles in many marine environments, functioning as links between pelagic and benthic ecosystems, and providing habitat and food for invertebrates and juvenile fish. Through fisheries and aquaculture, bivalves also play important socio-economic roles in many coastal communities, providing jobs and a source of sustainable protein. One of the most well-studied characteristics of bivalves is their suspension-feeding mechanism, which allows them to filter plankton from water with high efficiencies and is one of the primary ways that they interact with their ecosystems. In light of the socio-ecological importance of suspension-feeding marine bivalves, the goal of this thesis is to contribute to the mechanistic understanding of how bivalves acquire energy through suspension feeding. First, an overview of processes that mediate energy acquisition and expenditure, and the extent to which they are subject to plasticity and adaptation is examined in suspension-feeding bivalves. Next, plasticity in feeding physiology is examined both interspecifically and intraspecifically, using a combination of field and laboratory experiments. The results of these experiments show both inter- and intraspecific variability in the feeding physiology of bivalves. Interspecifically, relationships between particle capture efficiency and pumping rate were observed to vary between species of bivalves from different families. Intraspecifically, in the blue mussel, *Mytilus edulis*, plasticity in feeding physiology was observed as mussels were transplanted along a fjord gradient, and high levels of variability in feeing physiology were observed both between and within individuals during 4-day experiments. Finally, recommendations are made for future experiments to observe suspension-feeding mechanisms in marine bivalves. Understanding the mechanisms of suspension-feeding in bivalves is a primary step in predicting how the ecological role of bivalves changes between species and environments.

LIST OF ABBREVIATIONS AND SYMBOLS

µg	Micrograms
µm	Micrometers
CE	Capture efficiency
Chl α	Chlorophyll a
CR	Clearance rate
ESD	Estimated spherical diameter
H	Hour
IR	Ingestion Rate
L	Litres
POC	Particulate organic carbon
PR	Pumping rate
RE	Retention efficiency
SD	Standard deviation
SPM	Suspended particulate matter

ACKNOWLEDGEMENTS

This graduate work was funded by a graduate Natural Sciences and Engineering Researching Council of Canada (NSERC) award, a Killam Predoctoral Scholarship, a MITACS Globalink award, the NSERC Discovery Grant of Dr. Ramón Filgueira, and the project 'Sustainable Low-Trophic Aquaculture' at the Institute of Marine Research (Bergen, Norway).

Laboratory experiments at the Aquatron facility at Dalhousie University would not have been possible without the technical support of John Batt, James Eddington, Stephen Fowler, Lee Jarrett, and Gillian Tobin-Huxley. Experimental animals were provided by Sober Island Oysters (Trevor and Michelle Munroe), Peter Darnell, and Duncan Bates. Scanning electron microscopy expertise and sample preparation was provided by Patricia Scallion and Ping Li. Laboratory and field experiments were assisted by many people including Cécile Vimond, Keryn Winterburn, Cathinka Krogness, Dr. Samuel Rastrick, Justin Trueman, Tom Koppenol, Dr. Antonio Agüera, Anne McKee, Dr. Peter Cranford, and Dr. Sandra Casas. Thank you for your help in all weather conditions and at all hours of the day. Dr. Kristina Arranz and Renato Pavone, your visits to Dalhousie were highlights during my PhD, and I learned much from both of you. Becky Field, Carolyn Young, and Aileen Patterson all provided substantial assistance in navigating graduate studies at Dalhousie.

This thesis has been guided by the expertise and feedback of the advisory committee, Dr. Glenn Crossin, and Dr. Jon Grant. Outside of the advisory committee, the work included in this thesis benefitted substantially from the guidance of Dr. Tore Strohmeier, Dr. Øivind Strand, and Dr. Luc Comeau. I am extremely grateful for the opportunity to have learned from each of you, despite your collective status as a bunch of old men. Ramón, you were extremely fortunate to have me as a student and I wish you the best of luck without me.

I would not have started (or finished) this degree without my parents, my brother, and Eric.

CHAPTER 1 INTRODUCTION

Bivalves are a diverse group of organisms distributed globally in both fresh and marine environments, with over 9000 known living species (Gosling, 2021). Bivalves are the second largest class (Bivalvia) within the phylum Mollusca, which also has eight surviving classes including Cephalopoda (e.g., cuttlefish, octopus), Gastropoda (e.g., snails, slugs), and Polyplacophora (e.g., chitons). Characterized by their bilateral symmetry, and soft tissues enclosed by two hard shells, bivalves include mussels, oysters, scallops, and clams. Bivalves often grow in dense populations of reefs or beds on the sea-floor of both coastal and deep-sea environments. As a result of bed or reef formation, many species of bivalves are described as ecosystem engineers, indicating that they change the physical structure of their environment, and often support increased species biodiversity (Jones et al. 1994, Gutiérrez et al. 2003). Some bivalves are also classified as keystone species, as they affect the broader marine environment through nutrient cycling, water clarification, and habitat provisioning (Gosling 2003, Nizzoli et al. 2005, Coen et al. 2007). Many of the fundamental ecological roles that bivalves play are related to their physiology and behaviour as suspension-feeders. Suspension-feeding organism are those that feed by removing food particles from the water column (Hentschel & Shimeta 2008).

Bivalves are valued socially and economically through the provisioning of food and jobs to coastal communities. In 2019 in Canada over 43 000 and 110 000 tonnes of bivalves were produced in aquaculture farms and caught in wild fisheries, respectively (Department of Fisheries and Oceans Canada 2021a, b). The size and distribution of wild and cultivated bivalve populations are important to understand both for their food provisioning services, and for the fundamental roles that bivalves play in aquatic ecosystems. Survival and distribution of many marine bivalve populations are threatened by both climate change and deteriorating water quality (Beck 2009, Soon & Zheng 2019, Stewart et al. 2021). The growth of some bivalve populations has been supported through restoration efforts for the protection of threatened species (La Peyre et al. 2014, Fitzsimons et al. 2020), the range expansion of some species as a result of climate change (Ouellette-Plante et al., 2017; Russell et al., 2012; Thomas et al., 2016; Timbs et al., 2019), and through their commercial

production in aquaculture farms. In acknowledgment of the important ecological and economic roles of marine bivalves, this thesis focuses on the examination of the variability of suspension-feeding physiology both between and within marine bivalve species (inter- and intraspecifically, respectively). The ecological role and suspension-feeding mechanisms of bivalves are outlined in the following sections.

1.1 THE BIOLOGY AND ECOLOGY OF SUSPENSION-FEEDING BIVALVES

Life cycle

As a result of the diversity in species and environments that bivalves inhabit, the biology of bivalves also varies greatly. However, in this thesis a variety of species were worked with that share a common life-history organization. Bivalves usually reproduce through broadcast spawning of separate gametes (although hermaphrodism is present in the class), resulting in external fertilization. Several hours or days after fertilization, the free-swimming (planktonic) trochophore larvae will form, which mature into veliger larvae that settle onto the ocean floor. Settled veliger larvae subsequently metamorphose into juvenile bivalves. The duration of the planktonic larvae life stage varies substantially between species and contributes to the ability of bivalves to migrate to new environments (Luttikhuizen et al. 2003a, Levin 2006). As adults, bivalves are often described as being sessile, however many species display various forms of mobility. For example, scallops are free living and will use valve movements to propel themselves through water in a swimming motion. Contrastingly, mussels and oysters often attach themselves to hard substrate, including conspecifics using byssal threads and cementation, respectively.

Anatomy & morphology

The body plan of bivalves is characterized by two shells connected by a ligament hinge on the dorsal plane (Figure 1.1). Interiorly, the shells are usually held together by either a single (e.g., scallops), or two (e.g., mussels) adductor muscles which are attached to both shells. Shell length refers to the distance between anterior and posterior ends, whereas shell height refers to the distance between dorsal (hinge) and ventral sides. Inside the shells, the most apparent soft tissue is the mantle, which lines the shells and encloses most internal organs (inside the mantel cavity). Mantle tissue contains haemolymph and

stores reserve energy for reproduction primarily in the form of glycogen (Darriba et al., 2005; Fearman et al., 2009; Honkoop, 2003). Each shell contains two paired gills that are fused dorsally and perform the functions of gas exchange and filter-feeding. Along with the mantle tissue, the gills also contain haemolymph vessels for gas exchange. The gills of bivalves are complexly ciliated to create an inhalant current of water and facilitate particle capture for feeding and ingestion (discussed in detail in section 1.2). The inhalant current of water enters the mantle cavity posteriorly through an inhalant siphon, is moved across the gills, and exhaled posteriorly through an exhalent siphon.

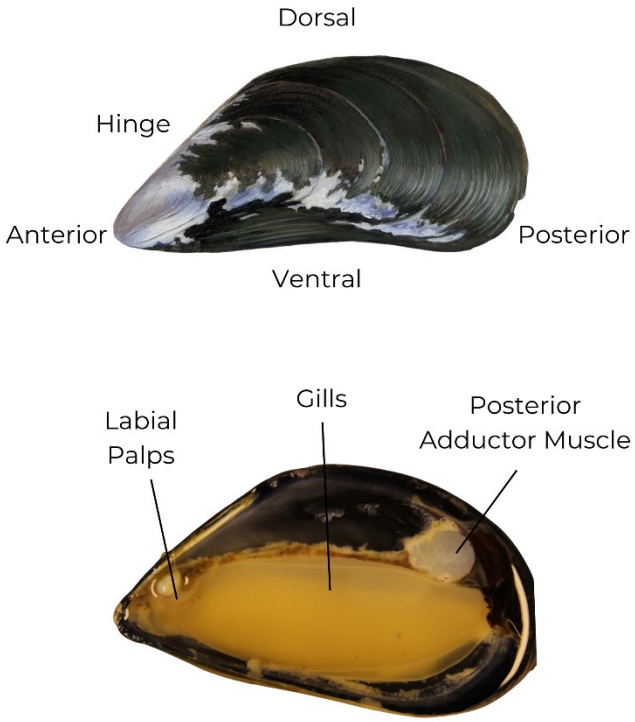


Figure 1.1 Generalized organization and feeding anatomy of a bivalve (blue mussel, *Mytilus edulis)* from the exterior (top) and interior (below). The internal visceral mass has been dissected and removed to reveal the gill, and labial palps.

Ingestion in many bivalve species is facilitated by paired labial palps, which surround a ciliated mouth, and assist in the rejection of pseudofaeces (suspended material (seston) that is captured, but not ingested). (Figure 1.1). Digestion occurs in the stomach

and associated digestive gland, and is facilitated both chemically and physically by digestive enzymes and with the movement of a rod-like crystalline style, which is composed of digestive enzymes, that projects into the stomach from the digestive gland (Kristensen 1972). Digested material is absorbed both in the stomach and intestine, and undigested material is egested as faecal pellets. Absorbed material is taken directly into the haemolymph, which moved through soft tissues by a heart, enclosed in the pericardial cavity and composed of a single ventricle and paired auricles. Finally, the excretion of nitrogenous waste occurs by both kidneys and pericardial glands, which expel nitrogenous waste (primarily ammonia) directly into the exhalent siphon (Gosling 2021).

Ecology

In marine environments, bivalves may inhabit both intertidal and subtidal regions. Bivalves are also characterized as either epifaunal species (those that live on the ocean floor) primarily mussels, oysters, and scallops, and infaunal species (those that burrow into sediments) including clams. Further, bivalves are characterized as those that feed on material suspended in the water column (suspension-feeders) and those that feed from sediments (deposit-feeders), although some bivalve species may be facultatively suspension- and deposit-feed (e.g., the clam *Macoma balthica*) (Ward and Shumway, 2004). Bivalves may exert both top-down (influencing lower trophic level communities) and bottom-up (influencing higher trophic level communities) controls on marine environments though feeding, excretion, and egestion. Top-down controls occur as bivalves filter plankton out of suspension in the water column and subsequently redirect matter to the ocean floor in the form of faeces and pseudofaeces Bottom-up controls occur as bivalves influence nutrient cycling; bivalves can both remove nitrogen and phosphorus from the marine environment as they are assimilated into bivalve tissue, but also add nitrogen to the environment as bivalves excrete ammonia as a nitrogenous waste (Newell 2004, Dame 2012). It is primarily through these top-down and bottom-up processes that suspension-feeding bivalves function as crucial links between pelagic and benthic ecosystems.

Often, the ecological role of bivalves is not studied solely from a biological perspective, but also in the context of both direct and indirect human welfare. This link between natural

ecosystems and human wellbeing can be described as ecosystem services (Fisher et al. 2009). Marine bivalves provide a wealth of ecosystem services including shoreline protection, habitat provisioning for economically important fish, top-down control on phytoplankton blooms, regulating water clarity and depth of light penetration, and both carbon and nitrogen sequestration (Shumway et al. 2003, Coen et al. 2007, Smaal et al. 2019). In terms of food production, bivalves have been identified as a sustainable low-trophic source of protein, as non-fed aquaculture species (Shumway et al. 2003).

Although the ecological functions of bivalves may be described in terms of supporting services, these functions operate in dynamic ecosystems and may result in perceived detrimental environmental effects (Weitzman et al. 2019). Bivalves, particularly when grown in dense populations may increase organic loading and sedimentation rates on benthic environments, which can subsequently reduce oxygen availability in benthic sediments (McKindsey et al. 2009, Gallardi 2014). Further, bivalves can exert significant grazing pressure on plankton communities, contributing to both plankton depletion, and shifts composition of plankton communities, which can have downstream impacts on the food availability for other marine organisms (Bacher, 2003; Jiang et al., 2016; Newell, 2004). In part as a result of linkages between bivalve ecology and societal and economic benefits, bivalve ecology and physiology has been extensively studied. One of the most well studied aspects of bivalve ecology is how they interact with their primary food source: plankton. Despite the important role that bivalves play in ecosystems, there remain unknowns about the feeding physiology of marine bivalves.

1.2 FEEDING PHYSIOLOGY OF SUSPENSION-FEEDING BIVALVES

The feeding physiology of bivalves has been a subject of research for the past century (Jørgensen, 1955; Kellogg, 1903, 1915; Owen and McCrae, 1976; For Review: Ward & Shumway, 2004). Bivalves are active suspension-feeders, they expend energy to create inhalant currents into their mantle cavity. The movement of water and capture of particles on bivalve gills is facilitated by rows of lateral, laterofrontal, and frontal cilia on the gills. The inhalant current of water is pulled into the pallial cavity and over the gills by the movement of the lateral cilia. As inhalant water is passed over the gill, suspended particles

are captured on frontal cilia, which may be facilitated by the laterofrontal or pro-laterofrontal cilia. Most bivalve species have compound (or eu-) laterofrontal cirri, and effectively capture particles greater than ~4 µm, although some species have pro-laterofrontal cilia and effectively capture particles > ~ 6 µm (Riisgård 1988, Riisgård & Larsen 2010). The exact roles that each cilia type play in particle capture are not fully understood, however the ability of bivalves to capture particles of decreasing size (~ <5 µm) is thought to be dependent on both the length and spacing of the laterofrontal cirri (Møhlenberg & Riisgård 1978, Riisgård 1988).

Particle size is often used as a primary predictor of whether a particle will be captured on the cilia of the gills, where small particles are less effectively captured than large particles. However, beyond particle size other characteristics are known to influence the likelihood of capture including wettability, surface charge, lectin-carbohydrate interactions, and fluorescence (Yahel et al. 2009, Pales Espinosa et al. 2009, Rosa et al. 2015, 2017b). Particles that are retained on the cilia are moved by ciliary movement to the margins of the gills on either the dorsal or ventral side. In these margins, particles, facilitated by mucous (in either strings or a slurry), are moved anteriorly towards the mouth (Ward et al. 1993, 1994, Ward 1996). Near the mouth, labial palps assist in either moving captured particles (and mucous) to the mouth for ingestion, or in rejecting captured particles as pseudofaeces.

As primarily sessile organisms, bivalves are generally unable to forage for food in the same manner as motile species. Instead, bivalves are reliant on their ability to selectively ingest or reject particles from the seston to sort material in the available diet of nutritional and non-nutritional quality, respectively. To do this, bivalves have been observed to selectively capture and retain particles of higher nutritional value (organic content) than the composition of seston that they are presented with (Iglesias et al. 1992, Hawkins et al. 1996, Bayne & Svensson 2006). This selection process occurs both on the gills, and the labial palps. At high food concentrations, particle rejection may occur at the gills and labial palps to prevent the gill from being overloaded with material (Jørgenson 1996), or to sort edible material from inorganic silt (Beninger et al. 1999, Riisgård et al. 2011). However, at low food concentrations, the threshold of which varies between species and environments, the rejection of captured material as pseudofaeces is generally not observed.

Although often the mechanisms of pre-ingestive particle sorting remain unknown, they have been hypothesized to be facilitated by both active and passive processes (Rosa et al. 2018). Passive particle sorting implies no physiological or behavioural response from the bivalve, but instead occurs as a response between the gill and particle surface characteristics (e.g., Rosa et al., 2017). Active particle sorting implies a physiological or behavioural response elicited by the bivalve, and mechanisms have not been extensively observed. To describe and compare the processes of particle capture, and feeding rates, this thesis relies on several fundamental metrics of bivalve feeding physiology, which are described in the following section.

Metrics of bivalve feeding physiology

Pumping rate (PR) is the volume of water moved over the gills per unit time (Lh^{-1}). Pumping rate scales with gill area, and as such, values of pumping rate are standardized to gill area in this thesis, to make comparisons between individuals of different sizes (Jones et al. 1992). Capture efficiency (CE) is the proportion of a specific kind of particle captured on the gill filaments compared to those in the inhalant water (Shimeta & Jumars 1991, Rosa et al. 2018). Retention efficiency (RE) is also commonly used to describe this process (e.g., Riisgård 1988, Cranford et al. 2016). However, as suggested by Rosa et al. (2018), the term retention efficiency implies the use of *in vivo* techniques to differentiate between particles that are captured on the gill compared to those retained (i.e., not rejected as pseudofaeces). In this thesis, *in vivo* techniques are not used to examine particle capture mechanisms, and the term capture efficiency will be used. Capture efficiency in this thesis is measured relative to particle size (equivalent spherical diameter, ESD, μm). Generally, capture efficiency of particles increases with increasing particle size, to some maximum, beyond which all particles all completely captured (Evan Ward & Shumway 2004a).

Clearance rate (CR) (Lh^{-1}) is the volume of water cleared of particles by a bivalve per unit time and is reliant on the accurate characterization of capture efficiency. Clearance rate is equivalent to pumping rate for particles that are completely captured on the gills. Ingestion rate (IR) describes the amount of food entering the mouth per unit time, and here is described as $\mu g h^{-1}$. With these defined processes of bivalve suspension-feeding, this thesis aims to describe both the inter- (Chapter 2 & 3) and intraspecific (Chapter 4 & 5)

plasticity of these processes (pumping rate and capture efficiency) as outlined in the following section.

1.3 THESIS OBJECTIVES AND STRUCTURE

The goal of this thesis is to contribute to the mechanistic understanding of how bivalves acquire energy. Understanding bivalve feeding mechanisms is a primary step in predicting both how the ecological role of bivalves changes both between species, and environments. To do this, a suite of methodologies have been employed including field and laboratory experiments, using both natural and cultured diets. This thesis aims to address unkowns about individual bivalve feeding physiology, to better predict both bivalve energy acquisition, and consequently growth, as well as bivalve-ecosystem interactions.

In recognition of the significant research that has already been conducted on the ecophysiology of marine bivalves, **Chapter 2** of this thesis is a review and synthesis of plasticity and adaptation in the processes that determine energy acquisition and expenditure of suspension-feeding marine bivalves. The information in this chapter provides a foundation for the three subsequent data chapters. The focus of **Chapter 3** is on interspecific comparisons of filter-feeding mechanisms in three species of commonly cultured bivalves from different families (the blue mussel, *Mytilus edulis,* the eastern oyster, *Crassostrea virginica,* and the sea scallop *Placopecten magellanicus*). Specifically, in this chapter the relationship between pumping rate and capture efficiency is explored from a hydromechanical perspective. Results from Chapter 3 indicated that the relationship between pumping rate and capture efficiency is dependent upon both particle size and species, where pumping rate was only observed to increase capture efficiency in *C. virginica* for small particles (~2-8 μm ESD). Continuing to examine differences in feeding physiology, **Chapter 4** explores the potential contribution of plasticity and adaptation in feeding physiology of the blue mussel, *M. edulis*, across a geographic gradient in Norway. In this study, using a fully-crossed transplant experiment, we observed differences in key metrics of bivalve feeding rates (pumping rate, and capture efficiency) between and within *M. edulis* from geographically distinct areas. Results from Chapter 4 suggest that capture efficiency may be a plastic trait, driven by environmental conditions, and changes in capture efficiency may be observed more quickly in response to changes in the

environment that in pumping and ingestion rates. **Chapter 5** is focused on the relationships between pumping rate, ingestion rate, and food concentration, and on inter- and intra-individual variability in feeding and ingestion rates in *M. edulis* acclimated to the same conditions. Findings from chapter 5 suggest that for *M. edulis* in low-seston environments, pumping rate may not be closely related to food concentration, and ingestion rates may continue to increase with increasing food concentration. Further, both inter- and intra-individual variability was observed in the feeding physiology of *M. edulis*, where inter-individual variability increased with increasing food concentrations. Information from each of these chapters contributes to the understanding of plasticity in bivalve ecophysiology, which is cornerstone to understanding the growth of both individuals and populations of bivalves.

CHAPTER 2 PLASTICITY AND ADAPTATION IN THE ECOPHYSIOLOGY OF SUSPENSION FEEDING MARINE BIVALVES

2.1 Abstract

As ecologically and economically important species, the limitations of suspension feeding marine bivalves to acclimate and adapt to changing marine environments are important to understand. This review outlines the primary physiological processes of suspension-feeding marine bivalves and examines how these processes may be affected by plasticity and adaptation. These primary physiological processes (feeding, digestion, absorption, and metabolic rate) determine how bivalves acquire and use energy, ultimately determining their overall growth. Generally, marine bivalve physiology is understood to be highly plastic, and therefore designing experiments to assess plasticity and adaption requires careful consideration. Experimental designs that use reciprocal transplants or common garden experiments, in combination with genetic analyses are often best suited assess these processes. Determining the different roles of plasticity and adaptation in physiological traits of suspension feeding marine bivalves is crucial to predict their growth, survival, and distribution in changing marine environments.

2.2 INTRODUCTION

Suspension-feeding marine bivalves are a widely distributed group of species, recognized for their ecological importance (Shumway et al., 2003; Schatte Olivier et al., 2020). In many marine ecosystems, bivalves are keystone species, enhancing nutrient cycling by exerting both top-down and bottom-up ecological controls (Newell, 2004; Gallardi, 2014). Top-down controls are driven by filtration as bivalves suspension feed, removing seston from the water column (Prins et al. 1998). Bottom-up controls are regulated by the excretion of nutrients (Jansen et al. 2012) and the production of faeces and pseudofaeces (Cranford et al., 2007; Zúñiga et al., 2014). Many marine bivalves are ecosystem engineers, forming reefs which provide refuge and substrate for the settlement of other species, supporting increased biodiversity (Snover & Commito 1998, Waser et al. 2016, Herbert et al. 2016).

As primarily sessile organisms, bivalves are exposed to environmental changes, on both short- and long-term scales. Bivalves may experience abiotic environmental changes in the form of temperature, salinity, pH, oxygen availability, desiccation, and energy (wave action and currents) both within the range of natural variation and more substantially as a result of climate change (Gazeau et al. 2010, Thomas et al. 2016). Biotic changes are often related to changes in food quantity and quality, as well as changes in pressure from pathogens, disease, predation, and inter/intraspecific competition (Turner et al. 2016, Hernroth & Baden 2018, Chapman 2020). All of these changes have direct effects on the physiology of marine bivalves and may affect their growth, survival, and distribution. Assisted by plastic and adaptive physiological processes, marine bivalves tolerate a wide variety of environmental conditions. As ecologically and economically important species, the limitations of bivalve physiology to acclimate and adapt to changing marine environments are important to understand.

Predicting the survival and distribution of bivalves in a changing environment is dependent upon understanding growth and its physiological components. Growth potential in terms of energetics may be estimated as the amount of consumed energy, minus the energy expended and lost on metabolism and excretion, respectively (Figure 2.1). The consumption, expenditure, and loss of energy are dependent upon the interactions between

the environment and bivalve physiology, or bivalve ecophysiology. For this review, the degree to which marine bivalve physiology changes in response to environmental conditions are examined through the primary processes that contribute to overall growth: Feeding, digestion, absorption, and metabolic rate (Widdows & Johnson, 1988). Changes in these primary processes of bivalve physiology are investigated to explore the extent to which they are phenotypically plastic and adaptive.

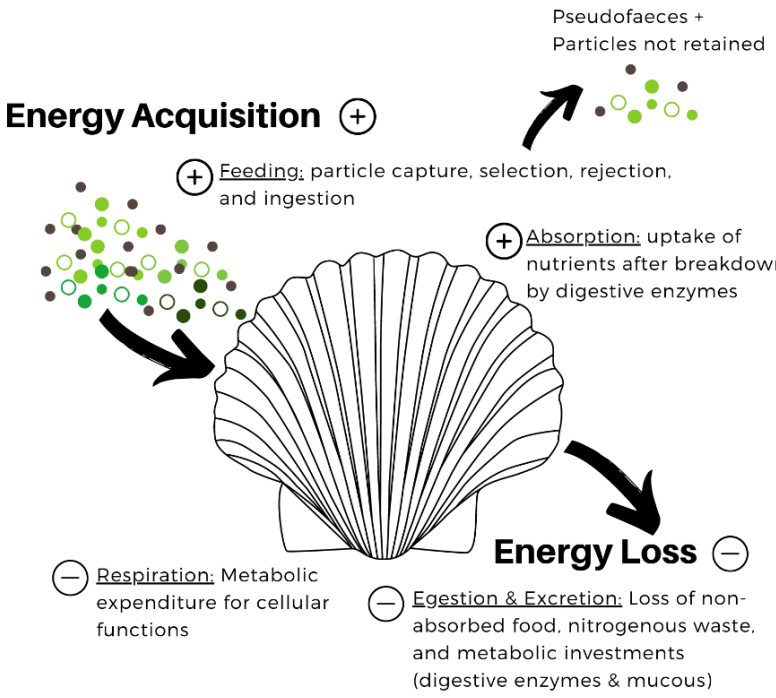


Figure 2.1 Conceptual diagram of the physiological processes that contribute to bivalve growth potential in terms of energetics. Adapted from Cranford (1998).

Phenotypic plasticity is the ability of one genotype to produce multiple phenotypes in different environments (West-Eberhard, 1989) (Figure 2.2). Phenotypic plasticity has been studied as a mechanism by which organisms can survive in changing environments through fast and reversible processes. Marine organisms have phenotypically plastic traits that are behavioural, morphological, physiological, biochemical, chemical, and related to life history stages (Padilla & Savedo 2013). Although plasticity in morphological traits may occur over weeks or months, plasticity in physiology can be observed over just days to

weeks (Paul & Van Alstyne 1992, Padilla 2001). Phenotypic plasticity has been recognized as an important trait in individuals that are subjected to environmental change on small temporal and spatial scales (Miner et al. 2005). Acclimation is often used as a measurement of physiological phenotypic plasticity, wherein a biological trait changes or is regulated to reduce stress in response to environmental variation (Kingsolver & Huey 1998). In this review, plasticity refers to all environmentally induced types of changes in phenotype expression (*sensu* Stearns 1989). As primarily sessile organisms, often living in environments affected by tidal and seasonal cycles, it is understood that bivalves have highly plastic traits for feeding, metabolism, and subsequently overall growth (Levins 1968, Bayne 2004). However, the upper limits of plastic responses for many bivalve traits are not well understood (Padilla & Savedo 2013).

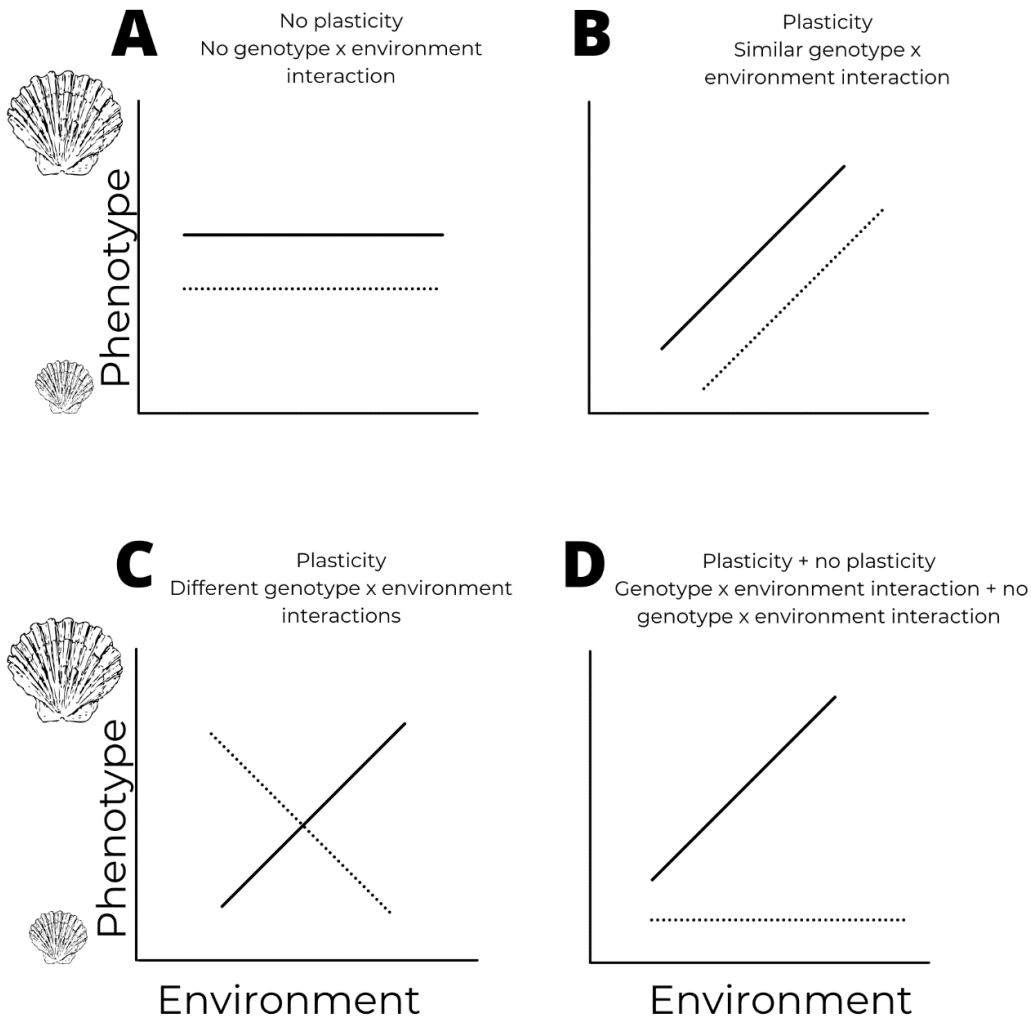


Figure 2.2 Possible responses of two different genotypes (e.g., two genetically distinct populations), solid line and dashed line, to changes in environmental conditions in terms of phenotype expression. A. In both genotypes, a change in environment does not illicit a change in phenotype. B. In both genotypes, a change in environment causes similar changes in phenotypes. C. In both genotypes, a change in environment causes different changes in phenotypes. D. In only one genotype, a change in environment causes a change in phenotype. Adapted from Kusmec et al. (2018).

Adaptation, driven by natural selection, is the change in allele frequencies that results in increased success or fitness. For adaptation to occur, there must be interaction between genotype and the environment, leading to change in allele frequencies that are either the

result of increased fitness of individuals who possess successful alleles, or the result of new genetic mutations (Kawecki & Ebert 2004, Hermisson & Pennings 2005, King et al. 2018). Epigenetics are the processes that produce heritable changes in organisms, without changing DNA sequences (Jablonka & Lamb 2006). Epigenetic changes result from changes in DNA methylation, histone post translational modifications, and non-coding RNA activity, which can modify chromatin structure and change expression patterns (Jablonka & Lamb 2002, Gavery & Roberts 2017, Fallet et al. 2020). These changes can result from environmental cues that contribute to plasticity of phenotypes and can be passed on to later generations, which aids in adaptation processes (Gavery and Roberts 2017, Fallet et al. 2020). Local adaptation is observed in primarily sessile organisms where natural selection is not homogenized by migration between populations (Kirkpatrick & Barton 1997, King et al. 2018). Although marine bivalves have a planktonic larval stage, subjected to drift by ocean currents, the distribution of planktonic bivalve larvae remains not well understood in many locations, limiting knowledge on gene flow between populations (Levin 2006, Ross et al. 2012, McClain et al. 2012). Adaptation has also been subject to debate surrounding definitions at different levels of study including adaptive evolution (Olson-Manning et al. 2012), local adaptation (Kawecki & Ebert 2004), and adaptive plasticity (Ernande et al., 2003; West-Eberhard, 2005). In this review, adaptation refers to changes of allele frequencies of individuals or populations (*sensu* Williams 1966).

There are several experimental designs used to explore both plasticity and adaptation, many of which have benefitted from the improvement of genetic analyses (King et al. 2018): 1. Common garden experiments: exposing individuals from different locations (e.g., geographically separate populations) to a common location, new to both groups of individuals. In common garden experiments, measurements of physiology are often taken on first generation offspring, reared in the new, common environment (de Villemeuil et al. 2016); 2. Transplants: the cross-movement of individuals between two separate environments (e.g., geographically separate populations); or 3: Multigenerational analyses: raising offspring from different populations over several generations and then conducting common garden or transplant studies. In these experiments, measurements of physiology, gene expression, and genetic composition provide information about the relative roles of plasticity and adaption.

The ecological and physiological characteristics of marine bivalves make them model organisms to explore plasticity and adaptation. Many marine bivalves inhabit and tolerate broad environmental conditions; however, their survival and distribution are also threatened by changing environments (Matzelle et al. 2015, 2016). The physiology of bivalves has been extensively studied for over a century, and in recognition of the significant amount of work in this field, the aim of this review is to describe the main physiological processes of marine bivalves and examine how they are affected by plastic and adaptative responses. Further, the benefits and risks of different methodologies used to assess plasticity and adaption will be discussed. Understanding how plasticity and adaptation contribute to changes in their physiology, and ultimately growth, is cornerstone to being able to predict the effects of changing marine environments on bivalves.

2.3 FEEDING

Most bivalves are active suspension-feeders, that is, they expend energy to pump water into their mantle cavity where particles are captured, selected for, and ingested (see Riisgård and Larsen 2001, Ward and Shumway 2004, Cranford et al. 2011 for reviews). Particles are captured on the gills by ciliated ctenidium and moved anteriorly towards the paired labial palps which may reject particles as pseudofaeces or move particles to the mouth for ingestion. Feeding rate is measured in bivalves using several related metrics (Supplemental upplemental Table 2.2), including clearance rate (CR), the volume of water cleared of particles of a given size per unit time (Coughlan 1969). The rate and efficiency with which bivalves pump water and capture food particles varies in response to exogenous environmental conditions. Although it has been proposed that there is no physiological control of CR and that bivalves pump at a maximum rate unless they are subjected to suboptimal conditions (Jørgensen 1990, Jøsrgensen 1996, Riisgård 2001), the majority of research suggests that bivalves do have physiological control over CR and may respond to changes in food quantity and quality (Bayne et al. 1999, Cranford & Hill 1999, Babarro 2000).

Beyond pumping and CR, the other primary mechanisms in feeding regulation are particle selection and rejection (Supplementalupplemental Table 2.2). Marine bivalves

have mechanisms by which food particles can be sorted, and material of low nutritional value (e.g., inorganic material) can be removed from the mantle cavity prior to ingestion. Pre-ingestive selection of filtered particles may occur either at the gill, by differential capture efficiency, or at the labial palps, as rejection as pseudofaeces. Capture efficiency describes the proportion of particles captured at the gill, compared to those in water (Rosa et al. 2018). Often, capture efficiency is related to particle size, where capture efficiency increases with particle size until some maximum is reached, beyond which all particles are completely captured. However, other particle characteristics have been shown to influence capture efficiency including wettability and surface charge (Rosa et al. 2017b, a), lectin-carbohydrate interactions (Pales Espinosa et al. 2009), and fluorescence (Yahel et al. 2009). Particle selection may be either an active or passive process where active selection implies a physiological response to changes in the food environment, and passive selection implies that particle selection occurs as a result particle characteristics and its interaction with the pallial organs (Jøsrgensen 1996; Ward and Shumway 2004; Rosa et al. 2018).

Captured particles are moved via the frontal cilia on the ctenidium to dorsal and ventral margins, where, facilitated by mucous on the gill they are moved towards the anterior paired labial palps (Beninger et al. 1993, Ward & MacDonald 1996, Beninger & St-Jean 1997). Labial palps may either reject captured particles as pseudofaeces, or guide captured particles to the mouth for ingestion (Widdows et al. 1979, Kiørboe & Møhlenberg 1981). The initiation of pseudofaeces production is often triggered at seston loads of ~2.5-5 mg l^{-1} (Widdows et al. 1979),

Plasticity and adaptation in feeding

The feeding physiology of bivalves in terms of pumping, capturing, and rejecting particles is highly plastic (Bayne, 2004). Gills and palps are plastic in size, and in many species may change in response to changes in both seston quantity and quality (Payne et al. 1995, Barillé et al. 2000, Honkoop et al. 2003, Dutertre et al. 2017, Capelle 2021). In areas of low seston quantity, gill area is increased, and palp area is decreased, leading to an overall increased gill-to-palp ratio (Barillé et al. 2000, Dutertre et al. 2009). This relationship is postulated to be a result of the need for increased CR at low food availabilities, and the absence of pseudofaeces production, or pre-ingestive sorting, at low

seston loads (Widdows, Fieth & Worrall, 1979; Bayne, 2004; Dutertre et al., 2007). Conversely, in areas of high seston quantity, marine bivalves often have smaller gills and enlarged palps (Barillé et al. 2000, Dutertre et al. 2007). In high seston environments, though pumping capacity is reduced, pre-ingestive selection to sort particles based on nutritional content is increased, to maximize the ingested fraction of organic material. A transplant experiment with *Crassostrea gigas* measuring temporal variations in gill and palp sizes for one year observed convergence between transplanted and native individuals (Dutertre et al. 2017). This finding highlights that short-term morphological changes in the pallial organs appear to be the result of reversible plasticity (see also Drent et al. 2004). Pallial organ plasticity may not be observed in individuals inhabiting environments with high levels of short-term variability (e.g., tidal/diurnal). This may be due to the time required for morphological plasticity to occur, or the energetic cost of morphological plasticity (Honkoop et al. 2003, Bayne 2004, Dutertre et al. 2017).

Clearance rate responds to changes in environmental conditions, primarily temperature food quantity and quality, and salinity as observed in reciprocal transplants (Worrall and Widdows 1983, Okumus and Stirling 1994, Wong and Cheung 2003, Osores et al. 2017), common garden experiments (Labarta, Fernández-Reiríz & Babarro 1997, Babarro 2000), and laboratory experiments (Bohle 1972). Clearance rate may display a variety of functional responses to food concentration, but generally initiation of feeding is triggered when food concentration surpasses a minimum threshold level. As food levels continue to increase, CR may remain at a constant maximum (e.g., on/off response), or continue to increase with food concentration (Foster-Smith 1975, Riisgard 1991, Clausen & Riisgård 1996, Hawkins et al. 1996). At very high food concentrations, CR may decline to avoid overloading the gills (Navarro, Iglesias & Ortega, 1992; Velasco & Navarro, 2005). Acclimation in CR is observed when the CR of transplanted individuals matches that of native individuals, which may not be observed in the short-term (~<10 days, Navarro et al. 2003; Tang et al. 2020), compared to longer acclimation times (~15 days-4.5 months, Okumus and Stirling 1994, Wong and Cheung 2003). The plastic response of CR may be related to morphological changes in the pallial organs (Capelle et al. 2021), and feedbacks between feeding and digestive activity, where CR responds to the internal state

of an individual (e.g., gut-fullness), as discussed in section 3 (Widdows, Fieth & Worrall 1979, Fréchette 2012).

Both capture and selection efficiency (Supplementary Table S_1) change in response to food quantity and quality (Rosa et al. 2018). Capture efficiency may vary with seston composition and concentration (Barillé et al. 1993), and temporally over the course of a season (Strohmeier et al. 2012, Rosa et al. 2015). Plasticity in capture efficiency has been observed in a transplant experiment with the mussel *Mytilus edulis*, where capture efficiency differed between initial location, and transplant destination in two groups of mussels (Steeves et al. 2020). It was not possible to attribute the change in capture efficiency to passive or active processes; however, the change in response to environment indicates plasticity in feeding (Steeves et al. 2020). Increased mucous production, via the upregulation of genes that control mucosal lectins on the gill may be a mechanism that would increase particle capture efficiency (Palmer & Williams 1980, Pales Espinosa et al. 2009, 2010, Pales Espinosa & Allam 2013, 2018). Selection efficiency varies with diet (Foster-Smith, 1975, Kiørboe, Mølenberg & Nøhr 1980, Iglesias et al. 1992, Hawkins et al. 1996, Beninger, Veniot & Poussart 1999) over the course of a season in response to changes natural diets (Bayne & Svensson 2006), or over the course of several days in laboratory studies using cultured diets (Pales Espinosa & Allam 2013). Plasticity in selection efficiency occurs to maintain high organic ingestion rates, or for the selection of biochemical compounds to meet nutritional requirements (Bayne & Svensson 2006, Pales Espinosa & Allam 2013).

Epigenetics of bivalve gills may facilitate plasticity particularly when exposed to stressors such as toxic algae and parasites. A study on *C. virginica,* found that when exposed to toxic red tide algae (*Karenia brevis*), epigenetic changes occurred in the gills through expression of histone variants (H2A.X, H2A.Z and macroH2A), and reduction of DNA methylation, which likely have a role in protection mechanisms against DNA damage associated with toxins (Gonzalez-Romero et al. 2017). Exposure to *Perkinsus* spp. has also been found to result in epigenetic changes (DNA methylation) in the gills of *Crassostrea gasar* and was hypothesized to be associated with the inhibition of expression of defence genes, which can aid in the progression of *Perkinsus* spp. lifecycle making the oysters more susceptible to infection (Farias et al. 2017).

Much less is known about the role of adaptation in bivalve feeding physiology. Local adaptation may play a role in the absence of morphological plasticity in some bivalve species, as there has been observed to be a heritable component of gill-to-palp ratios (Drent et al. 2004). A greater degree of morphological plasticity in pallial organs have been proposed to contribute to the success of invasive species, when native species display less plasticity in gill and palp size (Dutertre et al. 2009, Ouellette-Plante et al. 2017). Feeding physiology may also adapt to other environmental conditions, for example, a genetically distinct lineage of *Crassostrea virginica* with resistance to the parasite *Perkinsus marinus,* has been found to have significantly lower clearance rates in the presence of *P. marinus* compared to conspecifics without resistance (Ben-Horin et al. 2018). A recent study on *C. virginica* has also found that aquaculture lines selected for parasite resistance (*P. marinus* and *Haplosporidium nelsoni*) were less tolerant to starvation compared to wild conspecifics (McFarland et al. 2020). Further, for populations of bivalves living in low-seston environments, CR has been observed to be relatively higher at very low food concentrations, compared to studies on bivalves living in higher seston concentrations (Strohmeier et al. 2009); however, genetics studies are required to confirm the role of adaptation.

2.4 DIGESTION AND ABSORPTION

Following ingestion, bivalves pass food from the mouth through the esophagus to the stomach (Ward et al. 1994). The stomach of bivalves is composed of grooves, ducts, ridges, and ciliary currents for further particle selection (Reid 1965, Owen 1970, Purchon 1987). Ingested food is broken down by extracellular and intracellular digestion. Extracellular digestion occurs in the stomach by enzymes produced both by the stomach lining and the crystalline style (Kristensen 1972). In intracellular digestion, particles are transported via primary ducts to the digestive tubules of the digestive gland, where food is digested and nutrients are absorbed (Ibarrola et al. 2000). Outside of the digestive gland, absorption also occurs in the stomach and intestine (Kristensen 1972). As a result of the two types of digestion, two types of faeces are produced: intestinal and glandular faeces, produced by extracellular and intracellular digestion, respectively (Widdows, Fieth &

Worrall 1979, Ibarrola et al. 2000). Intracellular digestion is a longer process than extracellular digestion, leading to more efficient absorption of ingested food (Wang 1995, Decho and Luoma 1996)

Digestive enzymes are secreted to aid in the breakdown of carbohydrates, proteins, and fats into smaller units for absorption in both extra- and intracellular digestion (Morton et al. 1983, Karasov & Douglas 2013). The primary digestive enzymes used by marine bivalves are the carbohydrase enzymes amylase, cellulase, laminarinase, as well as proteases and lipases (Brock & Kennedy 1992). Digestive enzymes, in combination with the amount of time food spends in the digestive system determines both the amount of, and rate at which nutrients are absorbed, for a particular diet. Absorption efficiency is a metric used to estimate the fraction of ingested dietary organic matter that is absorbed within the digestive system (Conover 1966), and absorption rate is the organic material absorbed per time (Urrutia et al. 1996) (Supplemental Table 2.2).

Recently there has been interest in the microbiome of bivalves (see (Pierce & Ward 2018) for review). While functional understanding of the microbiome in bivalves is still unknown, in other organisms the microbiome has important roles in digestion, nutrient absorption, and immune function (Crosby, Newell & Langdon 1990, Harris 1993, Wold & Adlerberth 2000, Guarner & Malagelada 2003, Ley et al. 2005, Turnbaugh et al. 2007, Mazmanian, Round & Kasper 2008, Kau et al. 2011, Forberg et al. 2012, Dishaw et al. 2014, Pierce & Ward 2018). Bivalves contain an extensive microbiome within their digestive tract including the stomach, gastric juices, crystalline style, and digestive diverticula (Kueh & Chan 1985). Antibiotic treatments have been used to investigate the role of microbes in host digestive enzyme production, and most studies have found that antibiotic treatments have no impacts on enzymes (Newell & Langdon 1986, Mayasich & Smucker 1987, Crosby, Langdon & Newell 1989); however, Pierce (2016) found that exposure of antibiotics reduced the diversity and number of microbes in the microbiome of oysters, and the enzymes xylanase was impacted, but did not impact cellulase, protease, and amylase, nor the absorption efficiency. However, more research is needed to better understand other contributions of the microbiome to digestion in bivalves, such as understanding microbial-host interactions including horizontal gene transfer of digestive enzymes and other genes associated with metabolic processes (Pierce & Ward 2018).

Plasticity and adaptation in digestion and absorption

Gut passage time, gut volume, and absorption efficiency are interlinked metrics which are highly plastic (Bayne & Newell 1983, Ibarrola, Iglesias & Navarro 1996, Navarro et al. 2003). It is assumed that feeding and digestive processes respond to changes in the food environment to maximize energy uptake (Widdows, Fieth & Worrall 1979, Willows 1992, Hawkins et al. 1999, Hawkins et al. 2001). However, as acclimation in feeding and digestive processes occur on different timescales, the relationships between food quantity and quality and digestion and absorption are complex. Generally, with high quantities of high quality (high organic fraction) diets, ingestion rates increase asymptotically over the course of hours or days (Navarro et al. 1994). To accommodate an increase in newly ingested material, gut volume may increase while gut passage time decreases (Bayne, Klumpp & Clarke, 1984; Navarro, Iglesias & Ortega, 1992; Navarro et al., 2009). Absorption efficiency generally increases with increasing diet quality, despite fast gut passage times (Navarro et al. 1994, Babarro, Fernandez-Reiriz & Labarta 2003, Irisarri et al. 2013), which may be facilitated by increased gut volume, and high digestibility of an organic diet (Navarro et al. 1994). In the short-term acclimation to a diet (2-days), absorption efficiency may increase by increasing gut passage time, as a result of longer contact time between ingested material and the digestive tract, as well as higher ratios of intracellular: extracellular digestion (Bayne, Hawkins & Navarro, 1987). With longer acclimation times to a diet (2-weeks), absorption efficiency may return to baseline levels while reducing gut passage time (Bayne et al. 1989). This is likely facilitated by acclimation in digestive enzyme activity (Fernandez-Reiriz et al. 2001).

Plasticity in absorption efficiency may be dependent upon species, degree of diet change, and acclimation time (Iglesias et al. 1996, Labarta et al. 1997, Wong and Cheung 2003, Galimany *et al*. 2015). In transplant experiments, when acclimation in absorption efficiency occurs (i.e., absorption efficiency of transplanted individuals become similar to native individuals), it usually occurs within 1-8 weeks (Iglesias 1996, Labarta, Fernández-Reiríz & Babarro 1997, Babarro, Fernandez-Reiriz & Labarta 2003, Wong & Cheung 2003, Galimany et al. 2015). Changes in digestive enzyme production and activity are key mechanisms by which bivalves can alter absorption efficiency (Ibarrola et al. 1996, Ibarrola

et al. 1998a, b, Wong and Cheung 2001). Digestive enzyme activity changes in response to diet (Ibarrola et al. 1998a b, Trestrail et al. 2021), temperature (Seiderer & Newell 1979), and salinity (Nie et al. 2020). Change in digestive enzyme production likely aims to maximize absorption and minimize energy loss through the production of excess or unnecessary enzymes (Bayne, Hawkins & Navarro 1988, Willows 1992).

Although digestion and absorption are highly plastic in marine bivalves which encounter high levels of variability in diet, in some cases only partial acclimation in response to new diets is observed (Labarta, Fernández-Reiríz & Babarro 1997, Hawkins et al. 1998). Additionally, morphological constraints (e.g., maximum gut volume) may impose a limit on plasticity, and resultingly suggest adaptive differences between individuals (Hawkins, Navarro & Iglesias 1990, Labarta, Fernández-Reiríz & Babarro 1997, Hawkins et al. 1998). The ability of bivalves to change digestive enzyme production in transplant experiments may vary between species adapted to stable vs. fluctuating food environments (Labarta, & Velasco 2002). The absence of acclimation in absorption efficiency may also be the result of the limit of plasticity in digestive enzyme activity being reached, where change can no longer be induced (Iglesias 1996, Fernandez-Reiriz et al. 2001). Although genetic responses are not as commonly studied in digestion and absorption, genetic polymorphism genes that code for in amylase mRNA have been correlated with higher amylase activity, and growth rates in *C. gigas* (Prudence et al. 2006), indicating a genetic basis for differences in digestion. Additionally, single nucleotide polymorphisms (SNPs) were correlated to high absorption efficiency phenotypes in *C. virginica* (Hall 2017). Further, reference transcriptomes for the digestive gland of commercially produced species have been recently recorded (Gerdol et al. 2014), which also provide a basis for exploring local adaptation in digestion.

Interspecific variability in microbiomes has been observed in bivalves (Zurel et al. 2011, Roterman et al. 2015, Vezzulli et al. 2018, Pierce & Ward 2019, Offret et al. 2020), further, bivalve microbiomes show a great level of plasticity (Hernández-Zárate & Olmos-Soto 2006, King et al. 2012, Vezzulli et al. 2018, Pierce & Ward, 2019). Seston and marine aggregates contribute some of the operational taxonomic units found within microbiomes of bivalves (Pierce 2016, Pierce & Ward 2019); however, this contribution may be as small as 10% of the total microbiome diversity (Pierce & Ward 2019). Environmental conditions

(i.e. temperature, salinity, seasons, etc.) and differences in geographical locations appear to play a role in plasticity of bivalve microbiomes and may be related to both intrinsic factors of the bivalve and extrinsic factors such as microbial community variation (Motes et al. 1998, Pujalte et al. 1999, Cavallo, Acquaviva & Stabili 2009, Zurel et al. 2011, King et al. 2012, Trabal et al. 2012, Trabal Fernández et al. 2014, Lokmer et al. 2016, Pierce, 2016, Wang, He & Wang 2016, Pierce & Ward 2019, Offret et al. 2020). For example, a study by Pierce and Ward (2019) found that the mussel *M. edulis* had a much more stable microbiome seasonally compared to the oyster *C. virginica.* This difference was speculated to be related to bivalve behaviour, as mussels are more physiologically active in the winter, compared to oysters (Pierce & Ward 2019). While functional roles are still not understood, plasticity of microbiome might be associated with acclimation to environment and maintaining metabolic function (Offret et al. 2020).

2.5 METABOLIC RATE

Metabolism is the sum of chemical reactions that occur in organisms, including both the creation (anabolism) and breakdown (catabolism) of chemical components. Metabolism drives maintenance, development, and growth in organisms by turning absorbed nutrients into energy, in the form of adenosine triphosphate (ATP), to fuel cellular processes. Metabolic rate is the rate at which ATP is produced and broken down; however, for aerobic metabolism, metabolic rate is often measured using oxygen consumption rate as a proxy. Endogenous processes that contribute to metabolic rate include movement, growth, protein synthesis, and reproduction (Widdows & Hawkins, 1989), and to a lesser extent, feeding, digestion, absorption, and excretion (Widdows et al. 1984, Widdows & Johnson 1988, Bayne et al. 1989).

As ectotherms, bivalve's oxygen consumption typically increases with increasing temperature (Dame 1972, Newell, Johson & Kofoed 1977, Shumway & Koehn 1982, Navarro et al. 2020), to a critical temperature, beyond which a transfer to anaerobic metabolism may be observed (Peck et al. 2002, Anestis et al. 2007, Eymann et al. 2020). Bivalves are osmoconformers, and when seawater is not at equilibrium with the osmolarity of their tissues, water moves between bivalve cells and the surrounding water until

equilibrium is reached. To avoid the collapse or rupture of cells, bivalves may either protect cells through the production and breakdown of intracellular osmolytes, requiring metabolic energy (Pierce & Greenberg 1973) or by closing their valves, stopping feeding and subsequently reducing metabolic energy supply (Shumway, Gabbott & Youngson, 1977; Shumway & Koehn, 1982; Lavaud et al., 2017; Pourmozaffar et al., 2019). Decreased dissolved oxygen concentration, including hypoxic conditions, also usually cause decreases in metabolic rate (Brand & Morris 1984, Baojun & Riisgård 2018), which in extreme cases drives a transfer to anaerobic metabolism (de Zwaan & Wijsman 1976, Ortmann 2003). As calcifying species, bivalves have complex responses to changes in pH levels (Vargas et al. 2017, Jiang et al. 2019); however, low pH (~PCO_2=650-1200 µatm) usually cause elevated metabolic rates as a result of cellular stress (Lannig et al. 2010, Navarro et al. 2016, 2020, Benítez et al. 2018, Jiang et al. 2021).

Plasticity & Adaptation in Metabolic Rate

Plasticity in metabolic rate has been extensively researched in response to temperature using the Q_{10} temperature coefficient (e.g. Dame 1972 (*C. virginica*); Widdows 1973 (*M. edulis*); Smaal and Zurburg 1997 (*C. edule*), Shumway et al. 1988 (*P. magellanicus*)). Initial change in temperature driven increase or decreases in metabolic rate is a passive process driven by thermodynamics (Arrhenius 1915, Newell 1969; Ghalambor et al. 2007) that does not involve any physiological or behavioural plasticity (Havird et al. 2020). However, acclimation in metabolic rate in response to temperature may be facilitated by changes in gene expression, membrane lipid composition, heat shock protein production, and upregulation of enzyme isoforms that perform well at different temperatures (Hulbert & Else 1999, Pernet et al. 2007, 2008, Seebacher et al. 2010, Havird et al. 2020). Acclimation in metabolic rate may be quantified by the change in Q_{10} values over time (Smaal, Vonck & Bakker, 1997). In *in situ* experiments, thermal acclimation in bivalves may occur over the course of a season (~3 months); however, shorter periods are often observed in laboratory conditions (~2 weeks) (Newell 1969, Shumway 1982, Smaal, Vonck & Bakker 1997, Wilson & Elkaim 1997, Le Luyer et al. 2022). Similar patterns in acclimation of metabolic rate are observed in response to salinity (Shumway & Koehn 1983, Casas et al. 2018) and pH (Parker et al. 2017b). Transplant experiments measuring

rate of oxygen consumption of populations of bivalves across sites varying in temperature, salinity, food availability, or tidal cycle generally observe partial or complete acclimation within two months (Worrall and Widdows 1983; Widdows et al. 1984; Tedengren et al. 1990; Okumus and Stirling 1994; Hummel et al. 2000; Altieri 2006; Jimenez et al. 2015; Osores et al. 2017). Differences in metabolic rates may be attributed to reproductive stage (Widdows et al., 1984), gill area (Tedengren et al. 1990), or adaption (Ramajo et al., 2016; Osores et al. 2017, Ramajo et al. 2021).

Heterosis, or the success of heterozygous individuals, is observed in bivalves where heterozygous individuals have lower basal metabolic rates compared to homozygous individuals (Shumway & Koehn 1982, Koehn & Gaffney 1984, Hawkins, Bayne & Day 1986, Tremblay et al. 1998, Bayne et al. 1999, Tamayo, Ibarrola & Navarro 2013). Metabolic efficiency is facilitated in heterozygous individuals by slower protein turnover rates, as protein synthesis can compromise up to 26% of resting metabolic expenditure in bivalves (Hawkins 1991). The budgeting of available metabolic energy after maintenance costs between growth and reproduction, and the metabolic efficiency of these processes have been proposed to be an adaptive trait in animals (Guderley & Pörtner 2010). Further, metabolic depression (e.g., decreased heart rate or oxygen consumption rate) (Lesser 2016, Liao et al. 2021), or metabolic cold adaptation (Thyrring et al. 2015) may be adaptive responses which promote the growth and reproduction of marine bivalves in otherwise adverse environmental conditions.

Differences in metabolic rate of genetically distinct sub-populations have been observed (Nie et al. 2017, Bernatchez et al. 2019, Li et al. 2020). In a common garden and transplant experiment with two populations of the oyster *Crassostrea ariakensis*, metabolic rate remained different between the populations, despite using first generation progeny, acclimated for three months in the transplant destination (Li et al. 2020). It is possible that the populations displayed adaptive divergence, which may occur when individuals colonize a new environment, and natural selection acts upon phenotypes that are more successful in the new environment, compared to the old, resulting in a new sub-population, or species (Hendry 2001). Physiological phenotypes that may minimize metabolic stress in new high temperature environments maybe related to membrane unsaturation (Pernet *et al*. 2008), protein metabolism efficiency (Hawkins et al. 1986, Meyer and Manahan 2010),

and threshold for induction of heat shock proteins (Li et al. 2017). Population divergence in mussels *Mytilus chilensis* has been observed using transcriptomics, and it has been postulated that many of the functional genes being selected for were related to metabolism (Yévenes et al. 2021). In support of this, despite being raised in a laboratory common garden experiment for two generations, oysters (*Ostrea lurida*) from different environments displayed different responses to salinity stress in terms of transcript expression that regulated functions including ciliary activity and programmed cell death (Maynard et al. 2018). These responses may be related to the adaptive ability of oysters from estuaries to maintain aerobic metabolism when exposed to low salinity water. Evidence for adaptive divergence in metabolism has also been observed in geographically distinct populations of bivalves from stable versus fluctuating environments (Widdows 1976, Le Luyer et al. 2022, Ramajo et al. 2021), and high versus low temperatures (Pante et al. 2019).

2.6 GROWTH

The energy available to bivalves for growth may be estimated from the difference between acquired energy and the sum of energy expended or lost (Brett, 1976; Bayne et al., 1999) (Figure 2.1). Accordingly, plasticity in growth is a function of the plasticity of the processes that mediate energy acquisition and expenditure. Bivalve growth is impacted by previously discussed exogenous variables including temperature (Bayne & Worrall, 1980; Carroll et al., 2011), food availability (Tamayo et al. 2011, Telesca et al. 2019, Gonzalez Giorgis et al. 2020), salinity (Riisgård et al. 2012), pH (Fitzer et al. 2015), hydrodynamics (Lee et al., 2017), and predation pressure (Sherker et al. 2017). However, there is also an endogenous genetic (Jiao et al. 2014) and heritable component (Wang et al. 2013, Kong et al. 2015) to growth.

Interindividual variability in bivalve growth rates is often very high, and determining the endogenous drivers of growth rates has been extensively studied (Bayne 1999, Goff 2011, Prieto et al. 2019). Bayne et al. (1999) outlined three models that contribute to variability in growth rate for bivalves grown in the same conditions: 1. *Increased Acquisition*: fast-growing individuals can feed faster than slow growing

individuals (Bayne 1999, Zhang et al. 2018, Prieto et al. 2018, 2020a,b, Arranz et al. 2020). 2. *Modified Allocation*: fast-growing individuals allocate proportionally more energy to growth than maintenance and reproduction compared to slow-growing individuals (Bayne 2004). 3. *Metabolic Efficiency*: fast-growing individuals grow more for the equivalent amount of energy expended, compared to slow-growing individuals (Bayne & Hawkins 1997, Tamayo et al. 2011, 2015, Fuentes-Santos, Labarta & Fernández-Reiriz, 2018). Intrinsically, high variability in growth rates between bivalves reared in the same conditions has raised questions about the mechanisms of fast- and slow-growing individuals, in particular, if these mechanisms are the result of plasticity or adaptation (Tamayo et al. 2011, Fuentes-Santos et al. 2018, Hulot et al. 2019, Prieto et al. 2020).

Plasticity and adaptation in growth

High levels of plasticity in growth in bivalves has been observed in common garden experiments with populations or sub-populations of bivalves, where acclimation in growth rates is may be observed in as little as three weeks, or up to one year (Rawson & Feindel 2012, Lesser 2016, Hulot et al. 2019). Similarly, fully crossed transplant experiments have found partial to complete acclimation within one year (Tedengren et al. 1990, Montaudouin 1996, Petes et al. 2007). Incomplete acclimation in growth rates may be related to the differential acclimation times for physiological rates (i.e., ingestion, absorption, metabolic rate) or the effect of local adaptation (Worrall & Widdows 1983, Labarta et al. 1997, Babarro et al. 2000, Koch et al. 2015, Osores et al. 2017, Purce et al. 2020). Despite the high level of plasticity in growth rates observed in common garden and transplant experiments, differences in growth rates are also observed between fast- and slow-growing individuals reared in a common location (e.g., Prieto et al. 2018, 2019, 2020…etc.). The physiological differences observed between fast- and slow-growing individuals may be influenced by underlying genetic or epigenetic differences as discussed in the following sections.

Epigenetics has also been found to have a role in regulating gene expression associated with growth and development. Gene expression, including those in the gills and digestive gland, varies widely for several species with fast- and slow-growing individuals (Pernet et al. 2008, Meyer & Manahan 2010, Saavedra et al. 2017, Prieto et al. 2019).

Differential methylation has been observed along the genome of bivalves at different stages of development, which likely has important roles in growth of bivalves (Riviere et al. 2013, 2017, Li et al. 2015). Epigenetics may also have a role in intergenerational success associated with improved growth when parental generation have been exposed to environmental stimulus (Parker et al. 2012, 2015, 2017a, Zhao et al. 2017, 2018, Diaz et al. 2018, Kong et al. 2019). Several studies in bivalves have found that when parental generation is exposed to low pH and spawned, the subsequent generation shows improved growth, and lower metabolic rate (Parker et al. 2012, 2015, 2017a, Zhao et al. 2017, 2018, Diaz et al. 2018). However, the larvae may be more susceptible to mortality when exposed to multiple stressors including high temperature, low feed availability, toxic algae and low salinity (Parker et al. 2017a, Griffith & Gobler 2017). These examples showcase the interplay between parental environment and the impacts to offspring, likely through heritable epigenetic changes.

Heterosis facilitates increased feeding rates and metabolic efficiency in bivalves, and subsequently higher growth rates (Hedgecock et al. 1996; Bayne and Hawkins 1997; Tremblay et al. 1998; Bayne 1999; Pace et al. 2006). Body size itself in bivalves may be a heritable trait, with enough genetic variability to be acted upon by natural selection (Griffiths et al. 2021). Life-history traits, including size at maturity and life stage specific growth rates, influence energy allocation between somatic and reproductive growth and may be adaptive, in that they are acted upon by natural selection (Beverton & Holt 1957; Bayne 2017; Perrin and Sibly). Local adaptation in growth rates may be facilitated by fast-evolving genes responsible for metabolism and reproduction, which have been identified in two scallop species (Wang et al. 2013). Recent research has examined the genetic basis of variation of growth in bivalves using quantitative trait locus analysis, a procedure that links genotypic and phenotypic data to explain trait variation (Jiao et al. 2014, Niu et al. 2017). This technique provides baseline genetic information about species, a resource which is often missing for marine bivalves, and required for further genetic study of growth adaptation (Niu et al. 2017).

2.7 DISCUSSION AND CONCLUSIONS

The body of research on the ecophysiology of bivalves highlights that bivalve physiology is highly plastic. Determining the limits of plasticity, and subsequently the role of adaptation is experimentally difficult (Table 2.1 Examples of plasticity and adaptation in marine bivalve ecophysiology in terms of energy acquisition (feeding, digestion, and absorption) and energy expenditure (metabolic rate) processes, as well as overall growth.). Reciprocal transplants, and common garden experiments are valuable in disentangling the contributions of plasticity and adaptation to physiological traits. However, drawing definition conclusions about the contributions is difficult without further genetic analyses. Different groups of bivalves may perform similarly in transplants or common garden experiments; however, plasticity may mask underlying adaptations. Contrastingly, if groups of bivalves perform differently in transplants or common garden experiments, it may be that the acclimation time was not sufficient to observe a plastic response. To address this, future studies may prioritize experiments that incorporate aspects of physiology, ecology, and genetics, with experimental designs that permit differentiation between plasticity and adaptation.

Table 2.1 Examples of plasticity and adaptation in marine bivalve ecophysiology in terms of energy acquisition (feeding, digestion, and absorption) and energy expenditure (metabolic rate) processes, as well as overall growth.

	Plasticity	**Adaptation**
Feeding	Change in gill and palp size in response to changes in seston load	Differential capture of a parasitic protist between genetically distinct oyster (*Crassostrea virginica*) lineages
	Payne et al. 1995; Barillé et al. 2000, Honkoop et al. 2003, Dutertre et al . 2017, Capelle 2021	*Ben-Horin et al. 2018*

Digestion & Absorption	Change absorption efficiency in response to diet changes (quantity and quality) in reciprocal transplant experiments	Genetic polymorphism genes that code for in amylase mRNA have been correlated with higher amylase activity, and growth rates in the oyster, *Crassostrea gigas* indicating a genetic basis for differences in digestion
	Iglesias 1996, Labarta et al.1997, Babarro et al.2003, Wong and Cheung 2003, Galimany et al.2015	*Prudence et al.2006*
Metabolic Rate	Acclimation in metabolic rate in response to changes in temperature and salinity in reciprocal transplant experiments	Consistently different metabolic rates of genetically distinct populations of the oyster *Crassostrea ariakensis*, in multi-generational common garden experiment
	Worrall and Widdows 1983, Widdows et al.1984, Tedengren et al.1990, Okumus, & Stirling 1994, Hummel et al. 2000, Altieri 2006, Jimenez et al.2015, Osores et al.2017	*Li et al.2020*
Growth	Acclimation in growth rates in common garden and reciprocal transplant experiments	Differences in genetic variation between two geographically separate populations of *Crassostrea ariakensis*, and in a reciprocal transplant experiment, higher growth rates were observed in individuals in their original habitat

	Rawson and Feindel 2012, Lesser 2016, Hulot et al. 2019 Tedengren et al.1990, Montaudouin 1996, Petes et al. 2007	*Li et al. 2020*

There is a need to conduct both controlled laboratory experiments as well as *in situ* field experiments to examine the plastic and adaptive nature of bivalve ecophysiology (Ernande et al. 2003, Bergström & Lindegarth 2016). Laboratory experiments provide control over exogenous variables; however, they are limited in their ability to replicate natural conditions, and produce ecologically relevant results (e.g., artificial diet, static temperatures) (Hewitt & Norkko 2007, Morash et al. 2018). *In situ* experiments are often better designed to assess the additive effects of multiple variables on the physiology of bivalves (Carrier-Belleau et al. 2021). For both laboratory and *in situ* experiments, timescale is important to consider for the acclimation process, and short-term experiments may underestimate the plasticity of individuals (Le Luyer et al., 2022). Experiments that are designed for the purpose of addressing plasticity and adaptation should aim to remove maternal and environmental effects by using first or second generation offspring, in combination with reciprocal transplants (Sanford & Kelly 2011, Thomsen et al. 2017). Often, environmental stress will expose plasticity or adaptation by pushing individuals beyond their physiological limits, and therefore observation of plasticity or adaptation may be missed if individuals are transplanted to a common location, and no differences between individuals are observed. Although physiological differences between individuals may indicate adaptation, genetic techniques provide a mechanistic understanding of adaptive processes.

Bivalves have generally been underrepresented in genomic studies; however, declining costs of genomic sequencing has contributed to the increased application of gene sequencing to evolutionarily important molluscs (Gomes-dos-Santos et al. 2020). At least 17 bivalve genomes are now published (Yang et al. 2020). These publications highlight the high level of genome heterozygosity that is present in bivalves (Zhang et al., 2012; Yan et al., 2019; Peñaloza et al., 2021). High levels of genome heterozygosity, and related genetic

diversity likely play a role in the ability of bivalves to adapt to new and changing environments (Li et al., 2018; Yang et al., 2020). Using sequenced genomes, it is possible to identify local adaptation (e.g., Lal et al. 2018, Xu et al. 2018, Pante et al. 2019), by observing genetic differences between populations, and to attribute that genomic heterogeneity to either natural selection (adaptation) or genetic drift. To do so, analyses such as Q_{ST}-F_{ST} can be applied: Q_{ST}-F_{ST} compares the degree of population differentiation that is measurable in a trait locus (F_{ST}) to the total amount of genetic variance in the trait (Q_{ST}) (Leinonen et al. 2013, Cruz et al. 2020). F_{ST} analyses in populations of the clam *Ruditapes decussatus* have found evidence for a genetic basis of parasite resistance (Cruz et al. 2020). A similar technique, genome scans, have been applied to other molluscs to differentiate between ecotypes along an environmental gradient (Galindo et al. 2010). By continuing to sequence the genomes of marine bivalves these genetics analyses can be further applied to determine the genetic basis of local adaptation in marine bivalves.

As local adaptation occurs when selective forces are stronger than homogenizing (e.g., gene flow) forces, it is important to understand the extent of dispersal in marine bivalve populations, a requisite to defining separate populations (Sanford & Kelly 2011). For many marine bivalves, gene flow between populations is not well understood, and future work should consider exploring the role of hydrodynamics and environment on the extent of drift, and survival of planktonic larvae, and how this mobile life-stage contributes to gene mixing (Luttikhuizen et al. 2003b). Although small numbers of planktonic larvae may be enough to homogenize genetic composition of geographically separate populations for traits not under strong selection pressure, it may not be sufficient to act against traits which are locally selected for. Selection forces are likely particularly relevant in variable and patchy marine environments (Sanford & Kelly 2011). Further, for widely distributed species with long periods of larval drift (e.g., *Pecten maximus*, 6 weeks), although genetic differences may not be observable between populations, differences in proteomic signatures may explain differences in physiology and overall growth (Artigaud et al. 2014)

Understanding the natural levels of environmental variability is useful for determining the limits of plasticity, and role of local adaptation (Vargas et al. 2017). This is relevant in light of climate change which drives the change in ocean conditions, both over the course of decades, and in the short-term (Trenberth 2011, Vargas et al. 2015,

Cubillo et al. 2021, Tangherlini et al. 2021). The climatic variability hypothesis supports the notion that individuals exposed to environments with high levels of natural variability have greater potential for plastic responses (Bozinovic, Calosi & Spicer, 2011; Vargas et al., 2017; Navarro et al., 2020). For example, invasive bivalve species often have higher levels of plasticity in their physiology, and subsequently may be more tolerant to the effects of climate change that non-invasive species (Sarà et al. 2008, Davidson et al. 2011, Pack et al. 2021). In addition, previous exposure to environmental stress may increase the ability of an individual to respond plastically in the future, a trait which may be conferred to future generations (Gibbs et al. 2021). Having baseline information about the natural levels of environmental variability that bivalves are exposed to should also be considered in experimental design (Ventura et al. 2016, Osores et al. 2017, Monaco et al. 2021, Donelan et al. 2021). Finally, bivalves have plastic physiology, which may constrain processes of adaptation by preventing natural selection (Sanford & Kelly 2011), and therefore if adaptation is observed it indicates possibly highly selective environmental pressures.

Although the primary physiological processes that contribute to bivalve growth have been outlined here (feeding, digestion, absorption, respiration), other processes may play a role in determining growth. Energetic losses from nitrogen excretion are often excluded from energetic growth estimates; however, the amount and rate of nitrogenous waste products varies with diet (Widdows & Hawkins 1989, Widdows & Staff 2006). The production and quality of byssal thread in marine bivalves is energetically costly and varies with environmental conditions (Babarro & Carrington 2011, Padin et al. 2021, Roberts et al. 2021). The reproductive effort of bivalves is energetically intensive, seasonal, and impacts many aspects of bivalve physiology, including metabolic rate (Gourault et al. 2018). Finally, the immune response of bivalves (Gerdol et al. 2015, Ben-Horin et al. 2018, Rey-Campos et al. 2021) may represent a significant interaction between the physiology of bivalves and their surrounding ecosystem (e.g., ocean acidification (Schwaner et al. 2020, Zhu et al. 2020)).

The physiology of marine bivalves is highly plastic and determining the relative contributions of plasticity and adaptation in bivalve ecophysiology is experimentally difficult. Epigenetic changes contribute to plastic responses in bivalve ecophysiology by modulating gene expression, and examination at the genetic and molecular levels may

provide insights into adaptive processes. As the energetic components of growth, in terms of both energy acquisition and expenditure have unique plastic and adaptive traits, understanding the cumulative effects of environmental change on bivalve growth is complex. To disentangle the separate contributions of plasticity and adaptation, future studies may consider combining transplant and common garden experiments with genetic analyses. Differentiating between the limits of plasticity and adaptation in physiological traits of important marine bivalves is crucial to predict their growth, survival, and subsequently distribution in changing marine environments.

2.8 SUPPLEMENTAL MATERIAL

upplemental Table 2.2 Glossary of bivalve physiological regulations and rates associated with feeding, digestion, and absorption

Feeding	Pumping Rate	Volume of water flowing through ctenidia per unit time (l h^{-1})	Drinnan 1964
	Clearance Rate	Volume of water cleared of particles, by the bivalve, per unit time (l h^{-1})	Coughlan 1969
	Filtration Rate	Mass of particle cleared by the bivalve per unit time (mg h^{-1})	Winter 1973
	Capture Efficiency	The proportion of particles captured on the gills, compared to those not captured.	Vahl 1972, Shimeta & Jumars 1991, Rosa et al. 2018
	Selection Efficiency	Ratio of a food metric (e.g., organic content) in the inhaled water, compared to the pseudofaeces	Kiørboe et al. 1980
	Ingestion Rate	Rate at which captured and retained food is moved into the mouth (mgh^{-1})	Bayne, 2017
	Feeding rate	Term may be used to describe pumping rate, clearance rate, capture efficiency, and ingestion rate	Wildish and Kristmanson 1997

Digestion & Absorption	Gut residence time	Amount of time ingested food spends in the digestive system before non-absorbed material is egested (h)	Bayne et al. 1988
	Gut passage time	The average gut residence time (h)	Penry & Jumars 1987
	Absorption efficiency	The fraction of ingested dietary organic matter that is absorbed within the digestive system, often estimated with the Conover (1966) method, which compares the amount of organic content of the food and faeces (intestinal or glandular faeces, not pseudofaeces)	Conover 1966, Navarro & Thompson 1994
	Absorption rate	Uptake of nutrients across gut surface per time, often measured as organic ingestion rate minus organic egestion rate (mg h^{-1})	Urrutia et al. 1996
	Digestive enzyme activity	Measured as the amount of product produced by an enzyme (often, µmol product released min^{-1} mg $protein^{-1}$). The size of the digestive gland changes in response to diet, and as a result, enzyme activity is usually standardized to gland size	Ibarrola et al. 1996; 1998
	Endogenous faecal loss	Endogenous fecal losses are digestive investments, mucous and digestive enzymes, which are not reabsorbed by the bivalve	Hawkins and Bayne 1985, Bayne et al. 1987

CHAPTER 3 RELATIONSHIP BETWEEN PUMPING RATE AND PARTICLE CAPTURE EFFICIENCY IN THREE SPECIES OF BIVALVES

This work has been accepted for publication as: Steeves L, Vimond C, Strohmeier T, Casas S, Comeau L, Strand Ø, Filgueira R. (2022). *Marine Ecology Progress Series*.

3.1 ABSTRACT

Capture efficiency (CE) is the proportion of a given type of particle that is cleared from the water by gill filaments compared to other particles that are inhaled. The majority of research on CE variability in suspension-feeding bivalves has focused on particle characteristics (e.g., size, surface properties). This study was designed to explore CE as a function of particle size (of natural seston) and pumping rate (PR), as a proxy for fluid velocity. Bivalve species from different families were chosen for their differences in gill structure: Mytilidae (*Mytilus edulis*), Ostreidae (*Crassostrea virginica*), and Pectinidae (*Placopecten magellanicus*). Structural variation in the gills were hypothesized to influence any relationship between PR and CE. Experiments estimating PR and CE were replicated in both laboratory and field conditions. Results demonstrated that PR may influence CE in bivalves, and that this relationship is dependent upon particle size, and bivalve species (i.e., gill structure). For *C. virginica*, CE increased with PR (range = 0.4-6.9 L h^{-1}) for particles between 2.25 and 7.25, and 4.75 and 8.25 µm, in laboratory and field experiments, respectively. However, for M. edulis and P. magellanicus, no relationship was observed between PR and CE. Among the mechanisms by which particles can be removed from a fluid by a filter, these findings agree qualitatively with the capture

mechanism of direct interception applying to all species where CE depends on particle size, and inertial impaction additionally applying to C. virginica where CE depends on fluid velocity and particle size, in the 2.25 and 8.25 μm range.

3.2 INTRODUCTION

Despite a century of research, there remain many unknowns about the mechanisms of bivalve suspension feeding. Bivalves are active suspension feeders generating currents to pass water over their gills, where particles suspended in the water are either captured on the gills or exhaled (Jørgensen 1955). Capture efficiency (CE) describes the proportion of a given type of particle that is cleared from the water by gill filaments, compared to other particles that are inhaled (Shimeta & Jumars 1991, Rosa et al. 2018). The term retention efficiency (RE) has been commonly used to describe this process instead of CE (e.g., Riisgård 1988, Cranford et al. 2016); however, Rosa et al. 2018 suggested RE could only be applied when *in vivo* measurements allow for differentiation between particles captured and retained, if these measurements are not possible, capture efficiency should be used. CE is often measured relative to particle size, although other particle characteristics (e.g., cell surface properties) have been found to influence it (Strohmeier et al. 2012, Rosa et al. 2017). Generally, CE increases with particle size, until an asymptote is reached, beyond which all particles are captured with equal efficiency (Ward and Shumway 2004). Although CE has been observed to vary in response to changes in particle characteristics or seasons, the mechanisms that govern this variability are still being explored (Strohmeier et al. 2012, Rosa et al. 2018). It has previously been proposed that relationships between particle size and fluid velocity may affect the efficiency of particle capture for suspension-feeding animals (Rubenstein & Koehl 1977). However, despite considerable theoretical research on hydrodynamics and the bivalve pump (Riisgård & Larsen 2001, Newell et al. 2001), the influence of fluid velocity on CE has yet to be experimentally explored in bivalves.

The removal of a particle suspended in fluid by a filter (e.g., a suspension-feeding bivalve) depends on three components: the particle, the filter, and the fluid. Further, the likelihood of particle capture varies with the characteristics of each of these components

(Rubenstein & Koehl 1977). Particles (e.g., natural seston) may vary in size, shape, mass, composition, and surface properties. The filter, which in the case of bivalves is the gill, may vary in morphology. Different species of bivalves have gills with different types of filaments and cilia (Supplemental Supplemental Table 3.3, Supplemental Supplemental Figure 3.8). Cilia are able to move, creating and redirecting flows of water, affecting the likelihood of particle capture. Finally, the fluid, which is either fresh or salt water for bivalves, may vary in density, viscosity, and velocity. Fluid passing over the gills of suspension-feeding bivalves is understood to flow at very low Reynolds numbers (on the order of 10^{-4}), where viscous forces dominate over inertial forces, and with highly laminar flow (Jørgensen 1983, Labarbera 1984).

Rubenstein and Koehl (1997) outlined five mechanisms by which particles can be removed from a fluid and captured by a given filter: (1) Direct interception, (2) Inertial impaction, (3) Gravitational deposition, (4) Diffusional deposition, and (5) Electrostatic attraction. For a given filter, these mechanisms are dependent on particle size and density, and fluid velocity (Figure 3.1). *Direct interception* describes the capture of a particle that encounters a filter filament, or in the context of bivalves, the gills. This capture mechanism is dependent only upon particle size. *Inertial impaction* describes the capture of a particle that, due to its mass, departs from the trajectory of a fluid as it is diverted around a gill filament and captured. The likelihood of capture by inertial impaction increases with particle size and density, and fluid velocity. *Gravitational deposition* describes the capture of a particle denser than the fluid, as it settles onto a gill filament. Likelihood of capture by gravitational deposition increases with particle size and density but decreases with fluid velocity. *Diffusional deposition* describes the capture of particles not following a streamline but moving with random or Brownian forces as a result of being very small or propelled by locomotory motion (e.g., flagellates). Likelihood of capture increases with decreased fluid velocity, as a result of a particle spending more time near a gill filament. Finally, *electrostatic attraction* describes the capture of a charged particle by the gills with an opposite electrical charge. Likelihood of capture is dependent upon the intensity of attraction between the particle and the filter (Rubenstein & Koehl 1977). These mechanisms of particle capture apply to systems with a low Reynolds number (<1), including suspension-feeding (Rubenstein & Koehl 1977, Jørgensen 1981). To estimate

the relative contribution of each of these mechanisms to particle capture, characteristics of the particle, fluid and filter must be considered (Ranz & Wang 1952, Pich 1966, Rubenstein & Koehl 1977).

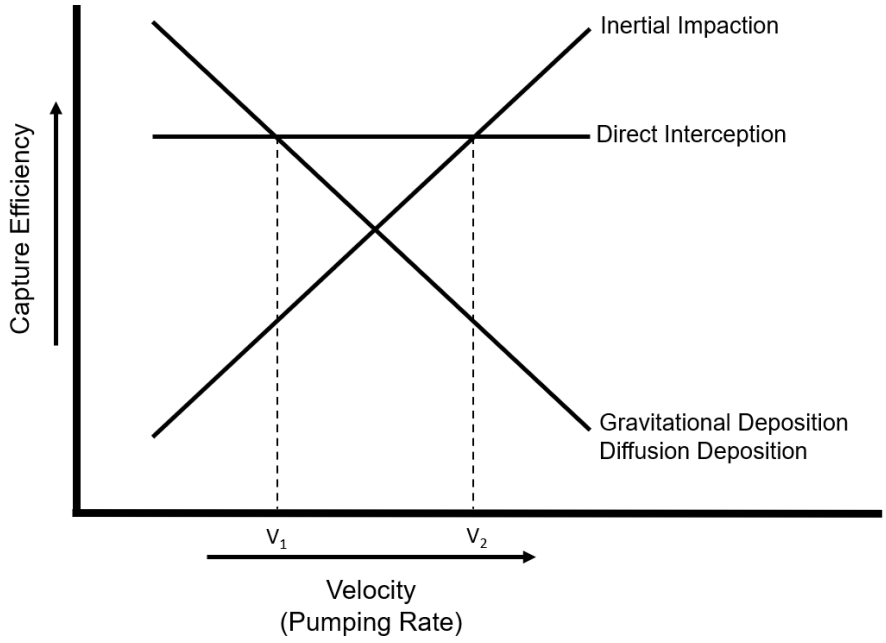


Figure 3.1 Theoretical relationship between capture efficiency (CE) and velocity (using pumping rate as a proxy, PR) for a given particle size and gill structure, highlighting changes in the contribution of the four capture mechanisms: direct interception, inertial impaction, gravitational deposition, and diffusional deposition. (Adapted from Rubenstein & Koehl 1977).

Bivalve gills have rows of lateral, laterofrontal and frontal cilia, the size and density of which vary between families (Supplemental Supplemental Table 3.3, Supplemental Supplemental Figure 3.8). Differences in CE between families of bivalves is generally understood to be a result of the spacing and length of different cilia, primarily the laterofrontal cilia (Møhlenberg & Riisgård 1978, Riisgård 1988). Although the exact role that these cilia play in particle capture has been debated (e.g. Ward et al. 1998b, Riisgård & Larsen 2001), there is evidence that bivalves with higher densities of cilia, compound cirri, and longer laterofrontal cilia/cirri have a higher capacity to capture small particles (ca. 1-5 μm) (Møhlenberg and Riisgård 1978, Riisgård 1988). Video endoscopic work

exploring the mechanisms of particle capture has provided an in-depth understanding of the role of the ctenidial filaments and associated cilia of bivalve gills in particle capture (Ward et al. 1993, 1994). Particle capture is facilitated by both the direct encounter with frontal cilia and currents created by the movement of laterofrontal cirri that redirect flow and suspended particles from the interfilamentar space towards the frontal cilia (Ward 1996, Ward et al. 1998b). The majority of bivalve species, including mussels and oysters, have compound (or eu-) laterofrontal cirri, and effectively capture particles greater than ~4 µm (Riisgård 1988, Riisgård & Larsen 2010). However, for bivalves that have only pro-laterofrontal cilia, including scallops, effective particle capture is greater than ~6 µm (Riisgård 1988, Riisgård and Larsen 2010). The variability in gill complexity of bivalves makes them an ideal model for testing the particle capture theory outlined by Rubenstein and Koehl (1977), where differences in gill structure are reflective of differences in filter efficiency.

Our study was designed to explore relationships between pumping rate (PR) and particle CE in species of bivalves from different families: Mytilidae (*Mytilus edulis*), Ostreidae (*Crassostrea virginica*), and Pectinidae (*Placopecten magellanicus*). PR is defined as the volume of water that passes through the gills per unit time and was used a proxy for fluid velocity. We hypothesized that particle capture would vary in relation to species, fluid velocity (PR), and particle size. For each species, experiments were conducted in both a laboratory setting, and in dockside experiments using natural seawater. By replicating these experiments, we aimed to observe if the relationship between PR and CE for a single species remained consistent in different temporal and spatial environmental conditions. Although surface charge has been found to play a role in particle capture (Rosa et al. 2017), this study does not examine the role of electrostatic attraction. The goal of this research is to contribute to our understanding of the mechanisms that influence CE in suspension-feeding bivalves.

3.3 METHODS

3.3.1 Experimental Design

Six independent experiments were conducted to measure the PR and CE of three species of bivalves: the blue mussel (*M. edulis*), the eastern oyster (*C. virginica*), and the giant scallop (*P. magellanicus*) (Table 3.1). These species were selected as representation from three families of bivalves, with differing gill structures (Supplemental Table 3.3, Supplemental Figure 3.8). For each species, laboratory experiments were conducted at Dalhousie University (Halifax, NS, Canada) between January 2018 and January 2019. Similarly, three field experiments were conducted between May 2018 and July 2020 in Flødevigen, Norway (*M. edulis*), Louisiana, USA (*C. virginica*), and Nova Scotia, Canada (*P. magellanicus*). Capture efficiency was measured as a function of both particle size (using the size distribution of the natural seston) and PR (due to natural variability in CEs and PRs of different individuals for a particle of a given size).

Table 3.1 Summary of conditions in laboratory and field experiments. n-experimental indicates the initial number of individuals measured in each experiment, and n-analyzed indicates the final number of individuals included in analyses. Criterion for individuals included in analyses is described in section 2.2

Experiment	Species	Date	Temperature (°C)	Salinity (ppt) (± standard deviation)	Average shell length or height (mm) (± standard deviation)
Laboratory	*M. edulis*	December 2017	20	30 ± 1	57 ± 5 n-experimental: 47 n-analyzed: 23
	C. virginica	December 2017	20	30 ± 1	64 ± 10 n-experimental: 47 n-analyzed: 25
	P. magellanicus	January 2019	20	30 ± 1	64 ± 2.3 n-experimental: 32 n-analyzed: 31
Field	*M. edulis*	May 2018	9.5 ± 0.7	31.5 ± 0.05	53 ± 4.2 n-experimental: 39 n-analyzed: 33
	C. virginica	February 2018	16.4 ± 1.8	12.4 ± 2.7	89 ± 11 n-experimental: 18 n-analyzed: 12
	P. magellanicus	July 2020	18.9 ± 0.6	30.0 ± 1	59 ± 5.5 n-experimental: 40 n-analyzed: 21

3.3.2 Laboratory Experiments

Laboratory experiments were conducted at the Aquatron Laboratory at Dalhousie University. Wild *C. virginica* and *M. edulis* were collected in the shallow subtidal zone of Sober Island Pond, Nova Scotia, and *P. magellanicus* were collected from a farm in Chester Basin, Nova Scotia. The ambient temperature at time of collection for all species was ~4°C. All specimens were maintained in two aerated 80 L holding tanks (maximum 25 bivalves per tank) on a flow-through design using sand-filtered (50 µm) ambient seawater (~4°C) pumped from 9 to 12 m depth. To acclimate the bivalves to the experimental temperature while avoiding physiological stress (Bricelj et al. 2006), the inflow of seawater was increased by 2°C per day until reaching 20°C. Individuals were acclimated to 20°C for a minimum of two weeks prior to conducting experiments. 20°C was selected as an acclimation temperature for all laboratory experiments as to ensure that the bivalves were physiologically active and would produce PRs above the detectable limit of our methodology (> 0.35 Lh^{-1}). Bivalves were fed cultured *Isochrysis galbana* (~ 4-6 µm, ESD) *ad libitum* with an automatic pump supplying the inflow with ≥ 25 000 cells mL^{-1}. The inflow was set at 680 ± 80 mL min^{-1} for a complete renewal of the holding tanks every 2 hours. The algal stock was kept in an aerated tank to generate a homogenous mixture and prevent sedimentation. Faeces were abundant in the maintenance tanks and cleaned regularly. Raw seawater (from the same source used during the maintenance period) was filtered through a 50 µm mesh screen and used in all laboratory experiments measuring PR and CE.

3.3.3 Field Experiments

For each field experiment, bivalves were collected locally and suspended from wharves in bags at 1 to 3 m depth to acclimate at least one week prior to each experiment. Wild *M. edulis* were collected from natural populations in Flødevigen, Norway, and experiments were conducted at the Flødevigen Research Station in Hisøy, Norway. Wild *C. virginica* were collected from Caillou Lake (Terrebonne Parish, Louisiana, USA) and

moved to the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, Barataria Bay, Louisiana. Cultured *P. magellanicus* were collected from Indian Point Maine Farm LTD, Nova Scotia, Canada, and experiments were conducted in Mahone Bay, Nova Scotia. Water temperature and salinity were monitored throughout field experiments, except for salinity data for the *P. magellanicus* field experiment which was provided from a nearby CTD (Saddle Island, Nova Scotia) (Table 3.1). To measure PR and CE experimental chambers were set up on the wharves adjacent to where the bivalves were suspended, and a submersible pump supplied unfiltered seawater directly from the spot the specimens had been held.

3.3.4 Measurement of Pumping Rate and Capture Efficiency

Measurements of PR and CE were conducted similarly for all species in both laboratory and field conditions. The static method was employed to simultaneously collect PR and CE measurements (Cranford et al. 2016). Individual bivalves were placed in a cylindrical feeding chamber, the dimensions of which were selected based on shell size, ranging in volume from 0.6 to 1 L. The feeding chamber was situated within a flow-through water bath. During the experiments, water in the feeding chambers was constantly maintained at acclimation temperature by continuously flowing water through the water bath (20°C for laboratory experiments, and ambient sea temperature for field experiments) (Table 3.1). Water within each chamber was constantly mixed using magnetic stirring plates to prevent particle sedimentation. Individuals were placed on a semi-rigid mesh shelf at the bottom of the chamber to avoid disturbance from the magnetic stir bar. For all experiments, an identical chamber without a bivalve served as a control, with a minimum of two controls run per sampling day. These controls were also used to characterize the seston in each experiment by measuring particle size distributions.

To begin each experiment, inflow and outflow sampling tubes of a PAMAS S4031GO (PAMAS GmbH) particle counter were carefully set into the chamber containing an individual bivalve. The PAMAS uses light scattering to count particles and estimates particle sizes as equivalent spherical diameter (ESD, µm). The PAMAS sampled 4.5 mL of water from the feeding chambers every 30 s. Particle size distribution was measured for particles between 2.25 to 13.25 µm ESD in 0.5 µm increments, resulting in 18 particle size

classes where the particle size 2.25 µm includes particles in the 2 to 2.5 µm range. Water sampled by the PAMAS was continuously returned to the feeding chamber, maintaining constant volume over time. After beginning particle counting, the experiment was run for 1 h, or until counts of particles size 8 µm ESD within the chambers had declined below 50%.

The determination of CE followed the method described by Cranford et al. (2016). In a static chamber containing a suspension feeder pumping at a constant rate and with no water renewal, the rate of particle removal follows an exponential decline (Coughlan 1969). The PAMAS measures this progressive particle reduction, permitting the measurement of the slope of the natural logarithm of particle concentration over time (λ). To ensure PR was constant, only time periods where the average λ was linear ($r^2 \geq 0.9$) were used (Cranford et al. 2016). For each particle size measured, the slopes of the exponential decay in particle concentration over time were estimated (λ_{size}) to calculate CE for each particle size (CE_{size}) following:

$$CE_{size} = \frac{\lambda sample, size \ - \lambda control, \ size}{\lambda sample, \ average} \qquad 3.1$$

where $\lambda_{sample,\ size}$ is the slope of the exponential decay in particle concentration for a given particle size class in the sample; $\lambda_{control,\ size}$ is the slope of the exponential decay in particle concentration for the same particle size class in the control (all controls were averaged for each sampling date); $\lambda_{sample,\ average}$ is the average $\lambda_{sample,\ size}$ of particles with sizes assumed to be fully captured (CE = 1). For *M. edulis*, *C. virginica,* and field experiment *P. magellanicus*, $\lambda_{sample,\ average}$ was calculated using particles from classes 8.25 to 10.25 µm. For laboratory experiment *P. magellanicus*, particles below 10.25 µm ESD did not appear to be fully captured (asymptote not yet reached), so particles from classes 10.25 to 13.25 µm ESD were used to estimate $\lambda_{sample,\ average}$. An average value across several particle size classes was selected to avoid incorporating potential measurement errors from a single particle size class count. The relative value of each CE_{size} was standardized between 0 and 1, describing particles that were either not captured or fully captured, respectively. Despite this adjustment, values of CE over 1 may be reported as a result of standardizing to an average of particles across several size classes, and not to the maximum λ.

PR, the volume of water passing across the gill per unit of time, was calculated as follows:

$$PR = \lambda_{\text{sample,average}} \times V \times T \quad 3.2$$

where PR is in Lh^{-1}, $\lambda_{\text{sample, average}}$ is the average $\lambda_{\text{sample, size}}$ of particles with sizes assumed to be fully captured (CE = 1), V is the water volume in the static chamber (0.6-1 L), and T is time (3,600 s h^{-1}). The number of particles in the feeding chamber, PR and r^2 values associated with λ_{size} were used to account for analytical error. Accordingly, samples were excluded from further analysis if *(i)* constant PR was only observed when the seawater was significantly depleted in particles (below the precision threshold of <200 particles mL^{-1}), *(ii)* bivalves exhibited a PR lower than 0.35 Lh^{-1}, which was undistinguishable from PRs of 0 Lh^{-1} by the PAMAS/precision of the methodology, and *(iii)* regressions with r^2 below 0.9 (indicating non-constant PR). The numbers of bivalves measured and included in each species analysis are reported in Table 3.1.

To make interspecific comparisons of PR, PRs were standardized by length (mussels) or height (oysters and scallops) to a bivalve of 60 mm, calculated as:

$$PR_{std} = PR_{exp} \times \left(\frac{L_{std}}{L_{exp}}\right)^{b} \quad 3.3$$

where PR_{std} is the standardized PR ($l\ h^{-1}$); PR_{exp} is the unstandardized PR of an individual; L_{std} is the standardized length or height of 60 mm; L_{exp} is the length or height of the experimental bivalve (mm); b is the species specific allometric exponent for length (*M. edulis* = 2.092 (Jones et al. 1992); *C. virginica* = 1.78 (Cranford et al. 2011); *P. magellanicus* = 2 (theoretical value assuming isometric growth)).

3.3.5 Statistical Analyses

All statistical analyses were performed in R version 3.6.2 (Rstudio version 1.4.1717). To describe the relationship between unstandardized PR and CE, linear regressions were fit. Significant regression ($p \leq 0.05$) curves were plotted with a solid line while insignificant relationships were represented by a dashed line (Figures 4-9). To compare standardized PR between laboratory and field experiments within each species, *t*-tests were used. Data were tested for normality and homogeneity of variance using Shapiro-Wilk's and Levene's tests, respectively. If data violated these assumptions, a log10 transformation was applied. Reported values represent means ± 1 standard deviation of the mean.

3.4 RESULTS

3.4.1 Water Quality Parameters

Temperature was lower for field experiments using ambient seawater than for laboratory experiment. Salinity was generally similar across experiments (~30 ppt), excluding the *C. virginica* field experiment, where salinity was below 15 ppt (Table 3.1). For all laboratory and field experiments, particle concentration in the seston generally decreased with particle size (Figure 3.2 A-C). *C. virginica* field experiment exhibited the highest particle concentration for all particle sizes as well as an increase in particle concentration at ~4 to 5 µm ESD (Figure 3.2 B). For both *M. edulis* and *P. magellanicus* experiments, particle concentration was generally higher in the laboratory than field experiments (Figure 3.2 A-C). Particle volume (μm^3 mL^{-1}) generally increased with increasing particle size (Figure 3.2 D-F), and similarly to particle count, was highest for the *C. virginica* field experiment (Figure 3.2 E). For each experiment, no significant relationships were observed between initial particle concentration and individual PR.

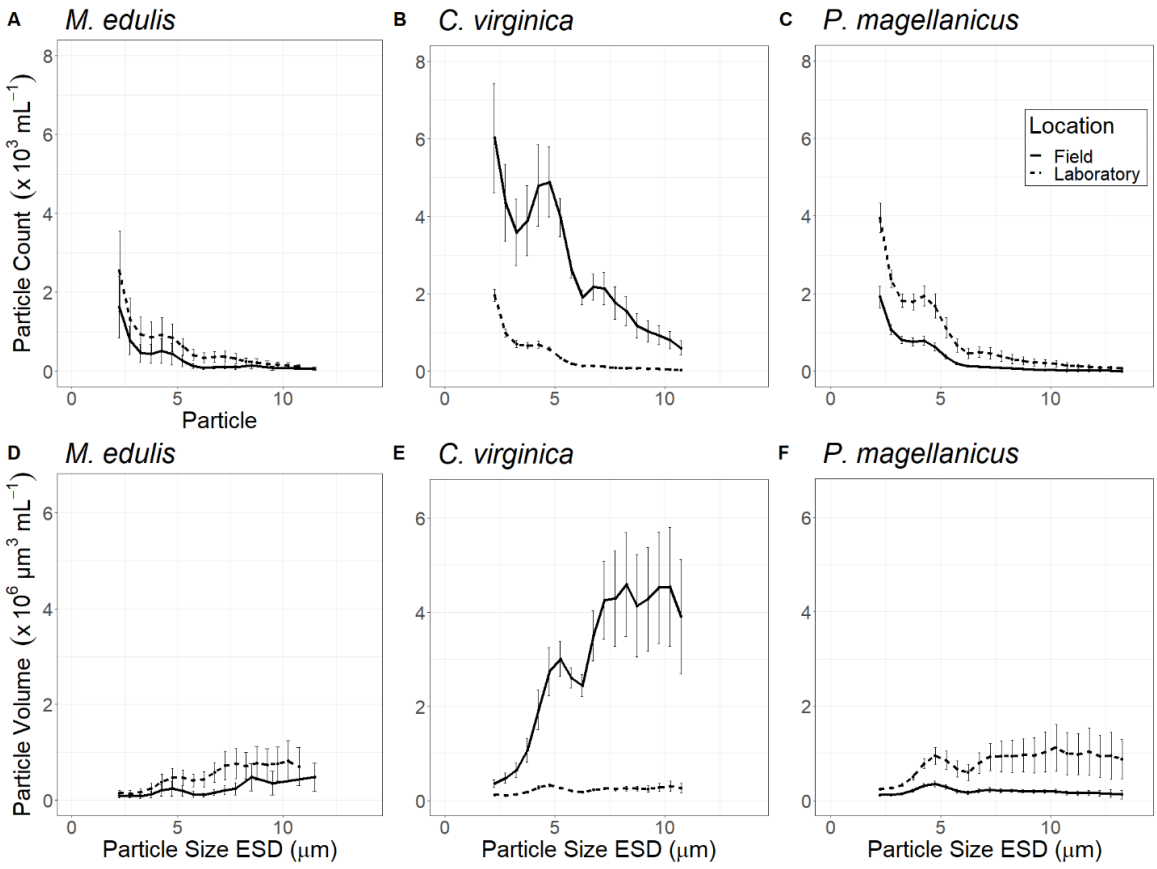


Figure 3.2 A-F. Descriptions of particle count (count mL-1) and volume (um3 mL-1) for each particle size (equivalent spherical diameter, ESD) for laboratory and field experiments of three species of bivalves. Particle count (count mL-1) is shown for (A) *M. edulis*, (B) *C. virginica*, and (C) *P. magellanicus*. Particle volume ($um^3\ mL^{-1}$) is shown for (D) *M. edulis*, (E) *C. virginica*, and (F) *P. magellanicus*.

3.4.2 Capture efficiency in *Mytilus edulis*

No significant differences were observed between PR_{std} in *M. edulis* sampled in the laboratory and field (Figure 3.3, $p = 0.7$). Capture efficiency in both experimental settings increased with particle size until particles were completely captured (Figure 3.4A–B). No statistically significant relationships were observed between CE of each particle size measured and PR measured in the laboratory (Figure 3.5, Supplemental Table 3.2). In the field experiment, one significant relationship was observed between CE and PR for

particles sized 4.25 µm ESD, where CE decreased with increasing PR, even though the explained variance of this relationship was only 12% (Supplemental Table 3.2, $p < 0.05$, $r^2 = 0.12$).

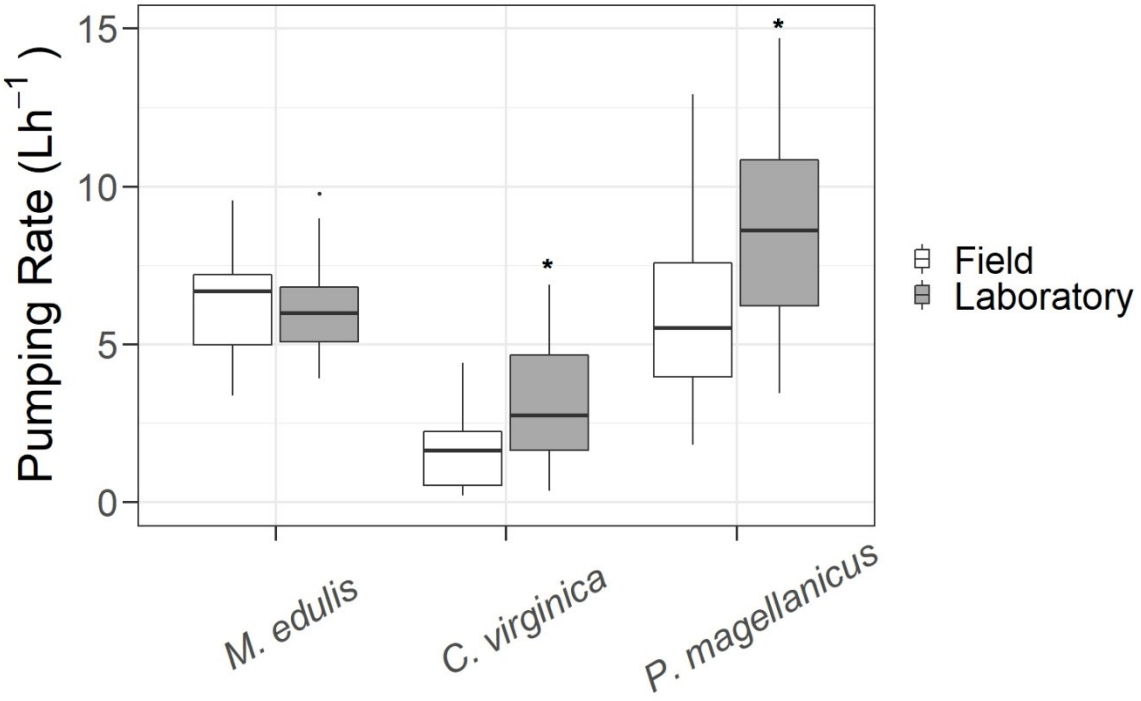


Figure 3.3 Standardized pumping rates (PR_{std}, Lh-1) of *M. edulis, C. virginica*, and *P. magellanicus* from both field (white) and laboratory (grey) experiments. Asterix (*) denotes statistical significance at $p = 0.05$ for within species comparisons. Pumping rates are standardized to shell length (*M. edulis*) or height (*C. virginica* and *P. magellanicus*) of a 60mm bivalve.

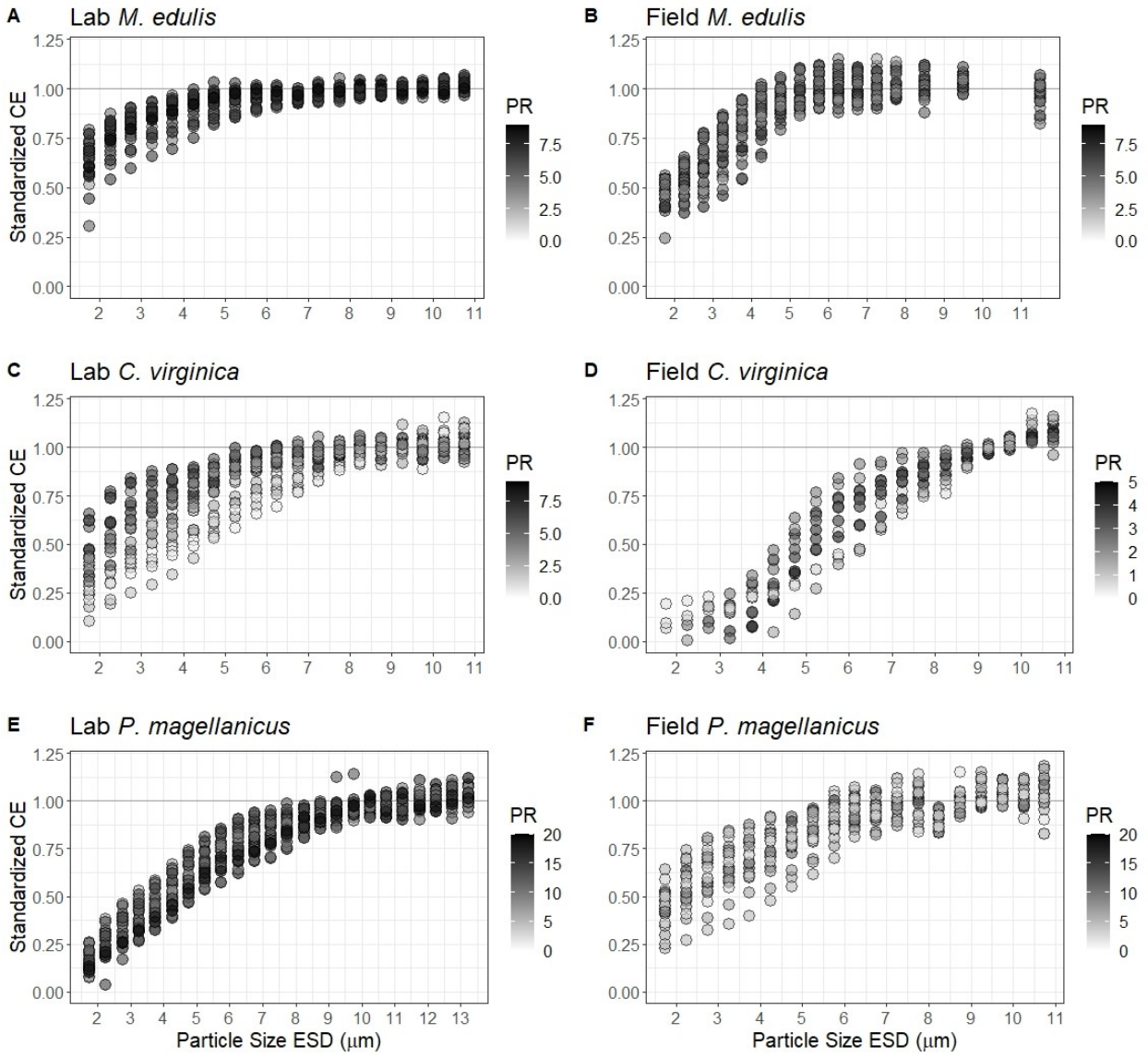


Figure 3.4 A-F. Standardized capture efficiency (CE) of *M. edulis, C. virginica*, and *P. magellanicus* across particle size (µm equivalent spherical diameter (ESD)) in laboratory (A, C, E) and field (B, D, F) experiments: (A) laboratory *M. edulis*, (B) field *M. edulis,* (C) laboratory *C. virginica*, (D) field *C. virginica*, (E) laboratory *P. magellanicus*, and (F) field *P. magellanicus*. Particle sizes expressed as equivalent spherical diameter (µm ESD). Gray-scale colour of data points represents the associated pumping rate (PR_{exp}, Lh^{-1}) with each CE measurement. Line at CE = 1 represents complete particle capture. CE standardized to values between 0–1 using particles from 8.25–10.25 µm ESD for all *M. edulis* and *C. virginica* experiments, and field *P. magellanicus*, and from 10.25–13.25 µm ESD for laboratory *P. magellanicus*.

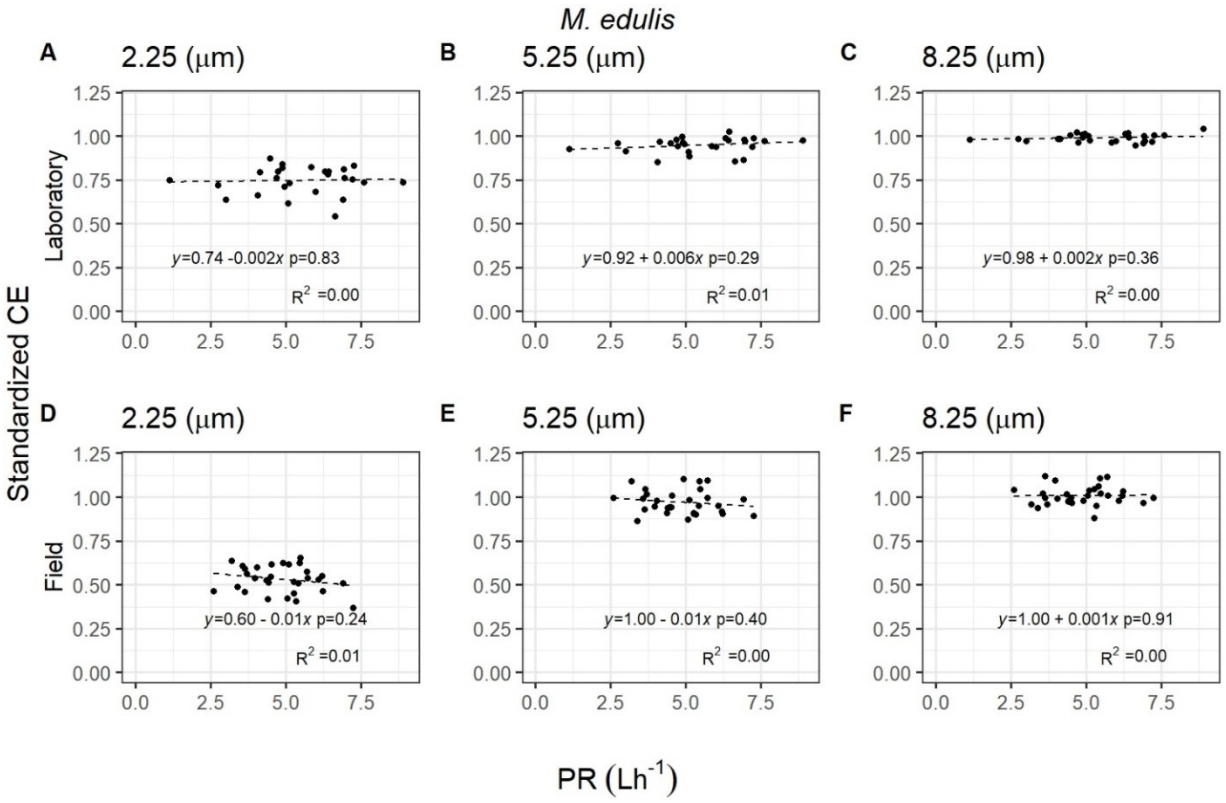


Figure 3.5 *M. edulis* capture efficiency (CE, standardized to 8.25–10.25 µm equivalent spherical diameter (ESD)) measured in relation to pumping rate (PR_{exp}, Lh-1) in laboratory (A-C) and field (D-F) experiments for three particle sizes: (A, D) 2.25 µm ESD, (B, E) 5.25 µm ESD, and (C, F) 8.25 µm ESD. Fitted curves are linear regressions, where dotted lines represent non-significant fits ($p > 0.05$).

3.4.3 Capture efficiency in *Crassostrea virginica*

C. virginica sampled in the laboratory had significantly higher PR_{std} than those in the field (Figure 3.3, $p < 0.05$). Capture efficiency in both laboratory and field experiments increased with particle size to an asymptote beyond which particles were completely captured (Figure 3.4C–D). In the field experiment, despite correcting CE measurements with controls, negative CE values were observed for some individuals at particle sizes smaller than 3.25 µm ESD. Negative values were excluded from figures and further analyses. The laboratory experiment produced positive relationships between PR and CE

of particle sizes between 2.25 and 8.25 µm ESD (Figure 3.6, Supplemental Table 3.1). As particle size increased, the slope of the linear relationship between PR and CE became smaller, and the statistical significance of the regression decreased (Figure 3.6, Supplemental Table 3.2). Significant relationships were not observed between PR and CE for particles sized 7.75 µm ESD, as well as any particles 8.75 µm ESD and larger (Figure 3.6, Supplemental Supplemental Table 3.3). A similar trend was observed for the field experiment, however CE for small (< 4.75 µm ESD) and large (> 8.75 µm ESD) particle sizes did not produce significant relationships with PR (Figure 3.6, Supplemental Table 3.2). Similar to relationships observed in the laboratory samples, as particle size increased, the slope of the linear relationship between PR and CE became smaller (Figure 3.6, Supplemental Table 3.2).

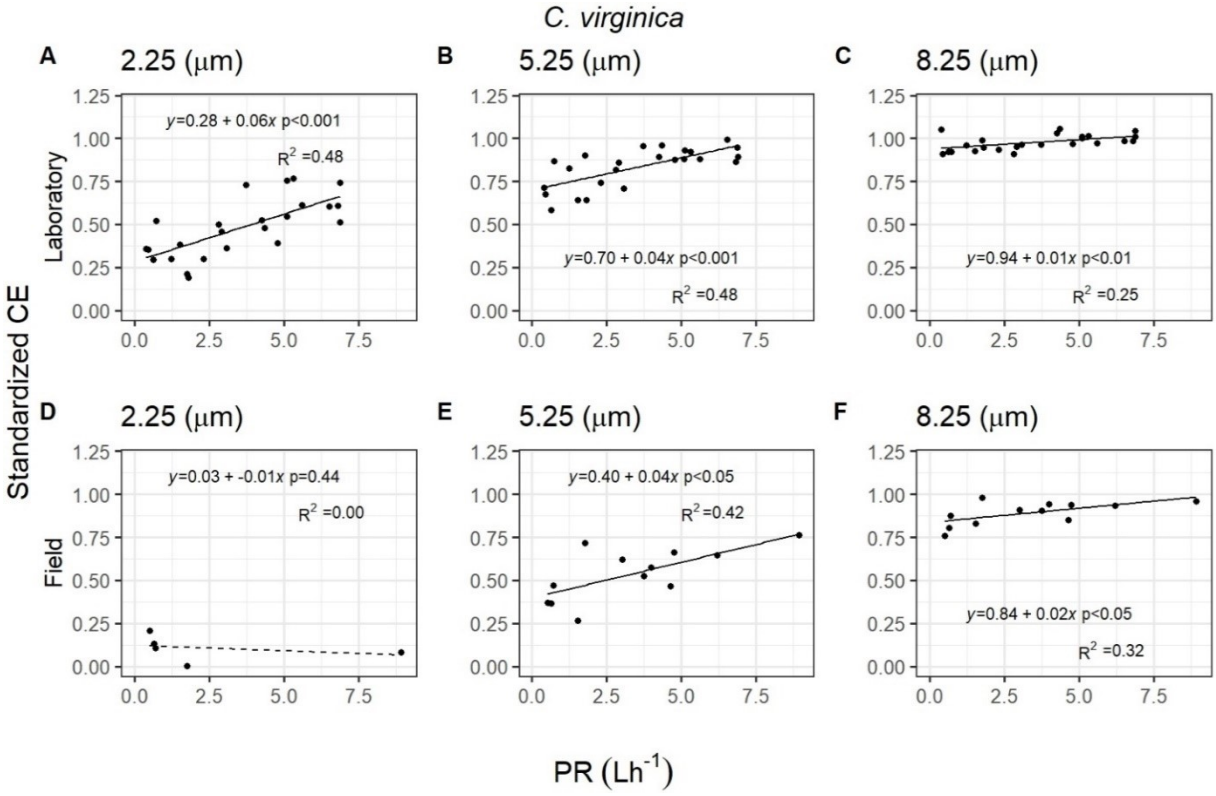


Figure 3.6 *C. virginica* capture efficiency (CE, standardized to 8.25–10.25 µm equivalent spherical diameter (ESD)) measured in relation to pumping rate (PRexp, Lh-1) in laboratory (A-C) and field (D-F) experiments for three particle sizes: (A, D) 2.25 µm ESD,

(B, E) 5.25 µm ESD, (C, F) 8.25 µm ESD. Fitted curves are linear regressions, where dotted lines represent non-significant fits ($p > 0.05$).

3.4.4 Capture efficiency in *Placopecten magellanicus*

PR_{std} were significantly higher for *P. magellanicus* sampled in the laboratory than in the field (Figure 3.3, $p < 0.01$). In both experiments, CE increased with particle size to an asymptote above which all particles were fully captured (Figure 3.4). The laboratory sampling produced no significant relationships between PR and CE for each particle size sampled from 2.25 to 10.75 µm ESD (Figure 3.7, Supplemental Table 3.2). A similar trend was observed for field samples with only one significant relationship detected between PR and CE for particles sized 8.25 µm ESD (Figure 3.7F, $p < 0.05$), although only 27% of variance was explained by this relationship.

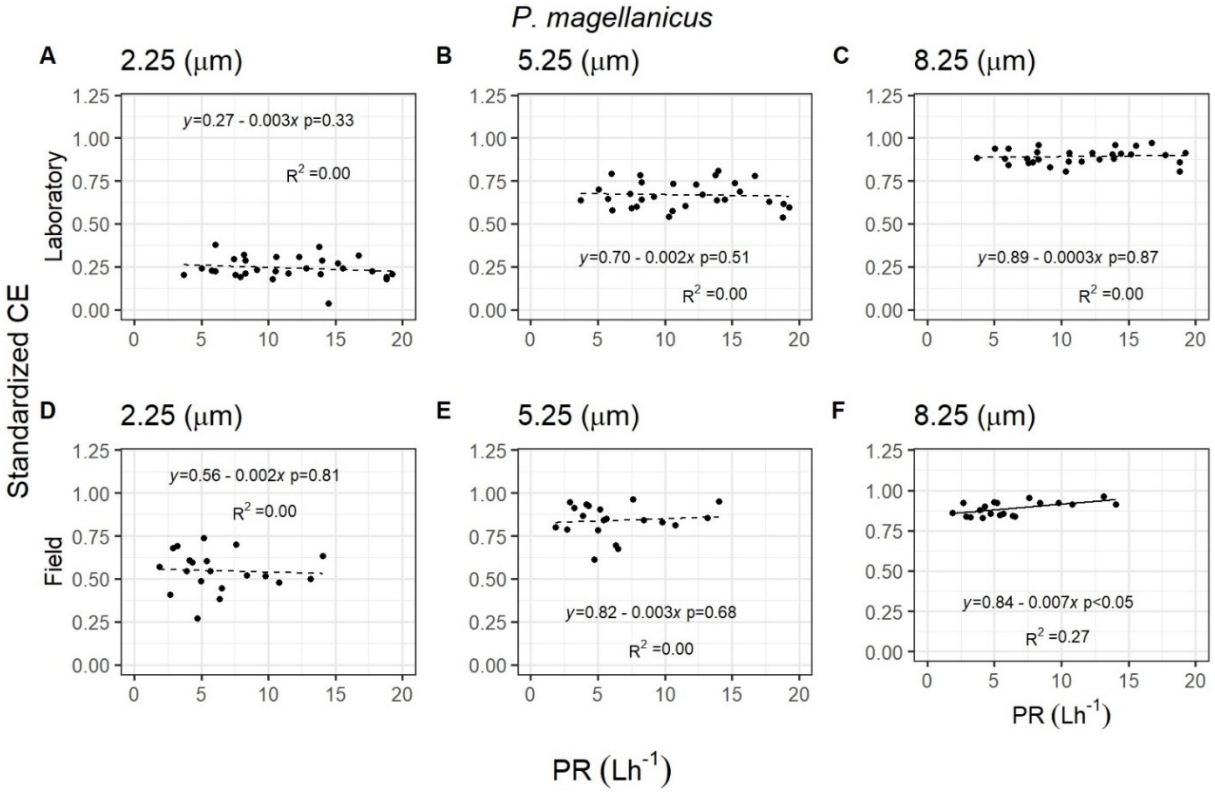


Figure 3.7 *P. magellanicus* capture efficiency (CE) measured in relation to pumping rate (PRexp, Lh-1) in laboratory (A-C) and field (D-F) experiments for three particle sizes: (A, D) 2.25 µm ESD, (B, E) 5.25 µm ESD, and (C, F) 8.25 µm ESD. Fitted curves are linear regressions, where dotted lines represent non-significant fits ($p > 0.05$). Capture efficiency was standardized to 10.25–13.25 µm ESD for laboratory experiments, and 8.25–10.25 µm ESD for field experiments.

3.5 Discussion

This study suggests that pumping rate (PR) influences particle capture efficiency (CE) in some species of bivalves, and that this relationship is dependent upon particle size. For *C. virginica*, CE increased with PR for particles between 2.25 and 8.25, and 5.75 and 8.25 µm ESD, for laboratory and field studies, respectively. Further, for *C. virginica* as particle size increased, the slope of the relationship between CE and PR became less steep, indicating that as particle size increases, PR plays less of a role in CE. For *M. edulis* and

P. magellanicus, there was no indication of a relationship between PR and CE for particles between 2.25 and 10.75 µm ESD. Results presented here qualitatively suggest that the capture mechanism of direct interception applies to all species. Moreover, inertial impaction may additionally apply to C. virginica for particles in the 2.25 and 8.25 µm ESD range, where CE depends on both fluid velocity and particle size. This interpretation is based on relationships between characteristics of the particles, the fluid, and the filter, which is detailed in the following sections.

3.5.1 Particle: Seston Characteristics

Particle size influences every hydrodynamic mechanism by which particles may be captured (Rubenstein & Koehl 1977). For direct interception, inertial impaction, and gravitational deposition, the larger the particle is, the more likely it is to be captured. Of these mechanisms, direct interception is the only one not affected by fluid velocity (Rubenstein & Koehl 1977) (Figure 3.1). For both *M. edulis* and *P. magellanicus*, CE increased with particle size, but was not affected by PR. These results may indicate that the primary mechanism of particle capture for these two species is direct interception, which has previously been suggested for *M. edulis* (Ward 1996). For *C. virginica*, CE increased with both particle size and PR for particles between ~2.25 and 8.75 µm ESD (below 100% CE). These results may indicate that inertial impaction contributes to particle capture for particles in this size range, where the likelihood of particle capture increases with both particle size and fluid velocity. For *C. virginica* capturing particles larger than 8.75 µm ESD (100% CE), there was no relationship between CE and PR, suggesting the primary mechanism of particle capture for these particles may be direct interception.

Beyond size, other particle characteristics not measured in this study may influence CE. Here, particle shape is estimated as a sphere, and mass is assumed to scale with size. This assumption does not account for differentially shaped seston, and seston with similar sizes but different mass, contributing to variations in particle density. (Cranford et al. 2011). For a particle of a given size, increased particle density would increase the likelihood of capture by gravitational deposition and inertial impaction. Particle density may vary based on the composition of the seston, as inorganic material is expected to be more dense than organic matter. Particle density was not measured in this study, as the

diets were characterized by the number and volume of particles for specific size classes. Although seston composition and particle density were not controlled for, we aimed to highlight the consistency in the relationships between PR and CE, despite changes in environmental conditions, by replicating these experiments in field and laboratory conditions. Furthermore, laboratory experiments with *M. edulis* and *C. virginica* were conducted consecutively with the same seawater, minimizing variability in seston composition. As particle density influences the hydromechanical mechanisms that dictate particle capture, future studies may consider separating the effects of particle size and particle density on the relationship between PR and CE.

Particle concentration may also influence CE. Similar to the results observed for the *C. virginica* field experiment, Barillé et al. (1993) observed that *C. gigas* CE for small particles (< 4 μm) was lower at very high seston loads (12.25 mg L^{-1}), compared to moderate seston loads (6.15 mg L^{-1}). Conversely, Ward and Macdonald (1994) observed that when *P. magellanicus* was exposed to seawater supplemented with algae and silt, CE increased for particles below 7 μm. Seston concentration did vary between the experiments in this study, particularly between laboratory and field experiments. Despite this variability in seston concentration, particularly relevant in the case of *C. virginica*, similar relationships between PR and CE were observed in both field and laboratory experiments, strengthening the outcomes of the present study. Although the functional response of PR (and similarly clearance rate) as well as seston concentration has been extensively studied (Riisgård 1991, Hawkins et al. 1996, Navarro et al. 1992), the relationship between CE and seston concentration has not been widely studied. Moreover, to the best of our knowledge, this relationship has not been studied while also considering PR. Future studies may consider examining the relationship between CE and seston concentration, while controlling for PR.

Motile cells (e.g., flagellates) may not follow a streamline motion predicted by non-motile cells in a fluid (Rubenstein & Koehl 1977). Motile cells may move in a pattern akin to random Brownian motion, and therefore the likelihood of particle capture would increase with decreased velocity by diffusional deposition. As only one negative relationship was observed between CE and PR in this study, it is unlikely that diffusional deposition dominated any particle capture process for particle sizes measured here. Finally, particle

charge can influence particle capture by means of electrostatic attraction. Particle charge has previously been shown to differentially influence CE in both *M. edulis* and *C. virginica* (Rosa et al. 2017).

3.5.2 Fluid: Seawater Characteristics

Most mechanisms of particle capture described by Rubenstein and Koehl (1977) are influenced by fluid velocity. As velocity decreases, gravitational deposition and diffusional deposition are enhanced, and as velocity increases, inertial impaction of particles is enhanced (Figure 3.1). For all species measured in this study, PR fell within the expected values for the temperatures at which they were measured (*M. edulis*: Winter 1973, Foster-Smith 1975; *C. virginica*: Riisgård 1988, Pernet et al. 2008; *P. magellanicus*: Cranford & Grant 1990, Cranford & Gordon 1992, MacDonald & Ward 2009, Cranford et al. 2011).

Although flow/fluid velocity at the gill filaments was estimated here using PR as a proxy, it should be noted that it may not represent absolute velocity at the cilia/cirri level. Approach velocity of particles moving towards the gill surface can be estimated as pumping rate divided by gill area (following Riisgård & Larsen 2000). PR has also been found to scale both with shell length or height and gill area in bivalves (Jones et al. 1992, Filgueira et al. 2008). In fact, Ward (1996) observed that approach velocities were higher in actively feeding bivalves, a result that indicates that PR is related to approach velocity. In this study, we aimed to minimize differences in fluid velocity through the gills attributed to variables other than PR by selecting individuals of similar sizes, given that gill area scales isometrically with shell length or height of bivalves. Due to differences in morphology, the length-to-gill area relationship may vary interspecifically, which limits the possibility of making direct interspecific comparisons of PR values. To make direct interspecific comparisons of PR, flow velocity should be measured at the gill for each species pumping at different rates.

Fluid temperature and salinity, and subsequently viscosity and density, may also influence mechanisms of particle capture. These effects were controlled for in laboratory experiments, using constant temperature (20°C), and the collection of deep water of stable salinity (29 to 31 ppt) (Table 3.1). The differential effects of temperature and viscosity on

bivalve feeding have been extensively explored with debate around the extent of physiological control over feeding in response to temperature (Riisgård & Larsen 2007, 2018, Fuchs & Specht 2018). The effects of viscosity are often discussed in terms of ciliary beat frequency of bivalves, relating to overall pumping rates (Specht & Fuchs 2018), where lower viscosity permits higher pumping rates (Riisgård & Larsen 2007), potentially leading to higher fluid velocity at the gills. Changes in flow velocity and fluid viscosity both affect the Reynolds number of the fluid. Although the flow of water at the gill surface is generally understood to have low Reynolds numbers (on the order of 10^{-4}) (Jørgensen 1981, Labarbera 1984), values as large as 0.35 have been estimated (Ward 1996). If Reynolds numbers are greater than 0.1, inertial forces may play a more significant role in particle capture (Shimeta & Jumars 1991). Further, the capture mechanisms outlined by Rubenstein and Koehl (1977) can only be applied to systems with low Reynolds numbers. Despite potential differences in viscosity in this study between laboratory and field conditions, relationships between CE and PR were constant. The explanations presented here are hypothetical. To further explain the relationships between PR and CE, water velocity at the gill should be directly measured (Nielsen et al. 1993, Ward 1996, Riisgård & Larsen 2005, Frank et al. 2008).

3.5.3 Filter: Gill Characteristics

Capture efficiency, particularly the size at which complete particle capture occurs, is understood to vary between families of bivalves (Riisgård 1988). CE generally decreases below 4 µm for *M. edulis,* although Strohmeier et al. (2012) found that *M. edulis* captured particles of 1 µm ESD with a CE between 14% to 64%. *C. virginica* have been found to capture particles below 5 µm with decreasing efficiency, with a CE of 50% for particles of 2 µm (Riisgård et al. 1988). *P. magellanicus* have also been found to sharply reduce CE for particles below 5 to 10 µm (Cranford & Grant 1990). Differences in CE between these species are understood to be a result of differing gill structures (Supplemental Table 3.2, Supplemental Figure 3.1), primarily the composition and length of laterofrontal or pro-laterofrontal cilia/cirri, and interfilamentar space (i.e., distance between ordinary filaments) (Riisgård 1988). For example, in *M. edulis*, previous work has demonstrated that

when the laterofrontal cirri are inactivated, CE is significantly reduced, likely as a result of their inability to redirect particles onto the frontal cilia (Ward et al. 1998a).

The gill filaments of the bivalve species used in this study vary by type and organization (Supplemental Table 3.2, Supplemental Figure 3.1). The heterorhabdic gills of both *C. virginica* and *P. magellanicus* form highly plicated folds, with ordinary filaments forming arches and primary filaments forming troughs (Ward et al. 1994, Beninger et al. 1988), whereas the homorhabdic gills of *M. edulis* lack this plicated structure (Supplemental Table 3.2, Supplemental Figure 3.1). It has previously been suggested that direct interception on the ordinary filaments of *M. edulis* play a primary role in particle capture (Ward 1996, Ward et al. 1998b). Furthermore, it has been suggested that in plicate gills, like those of *C. virginica* and *P. magellanicus*, particles may be captured by entrainment within the troughs formed by ordinary and principal filaments (Ward 1996). Although the more general organization of the gills may not explain differences in the relationship between CE and PR, the finer organization of cilia/cirri on gill filaments may contribute to species-specific relationships in CE and PR.

The mechanisms of particle capture described by Rubenstein and Koehl (1977) are dependent on the thickness and orientation of the filter. Since gill characteristics are specific to each species used in this study (Supplemental Table 3.2, Supplemental Figure 3.1), the processes that dictate the relationships between particle size, PR and CE are expected to be species-specific. Although the interfilamentar space is largest in *M. edulis,* followed by *C. virginica,* and smallest in *P. magellanicus,* the non-occluded portion of the interfilamentar space follows an opposite pattern, where it is smallest in *M. edulis* and largest in *P. magellanicus.* The occlusion of the interfilamentar space in *M. edulis* is caused by the large and complex laterofrontal cirri. If direct interception is the primary mechanism for particle capture in *M. edulis*, this outcome may be a result of the laterofrontal cirri on the gills and the interfilamentar space that they cover. It is possible that velocity has no effect on CE as the spacing is small enough that the gill filaments capture the majority of particles by direct interception. For *C. virginica*, with generally less densely packed and shorter laterofrontal cirri (Owen & McCrae 1976, Ribelin & Collier 1977), the spacing of the cirri may be large enough that in addition to direct interception, increasing velocity increases particle capture by inertial impaction. For *P. magellanicus*, with the largest spacing between filaments and only pro-laterofrontal cirri (Beninger et al. 1988), it is

possible that we did not observe an impact of velocity on CE because low velocities (PR < 4 Lh^{-1}) were not observed. Further, it is possible that there is a threshold of particle size beyond which it is not possible to observe the effects of inertial impaction for this specific type of gill. Finally, it is possible that there were relationships between CE and PR that fell outside the detection limit of the methodology employed in this study.

3.5.4 Future Directions and Conclusions

To date, the majority of research on CE variability in bivalves has been primarily explored in relation to particle characteristics, such as size (Strohmeier et al. 2012) and surface properties (Rosa et al. 2017). Future studies may consider combining particle characteristics, other than size, that are known to influence CE with the effects of flow velocity. Further research may also consider exploring the effect of diet concentration and particle density on the relationship between PR and CE. Although raw seawater was used in the present study to characterize the relationship between PR and CE with a natural diet, this can make accurate observations of CE challenging. For example, the water used in the *C. virginica* field experiment was turbid and contained high concentrations of suspended sediment. In the static chambers used for measuring CE and PR, flocs of sediment may have broken apart, thus increasing the counts of small particles, and resulting in negative estimates of CE.

This study is the first to experimentally explore the relationship between PR and CE in bivalves. Our findings qualitatively indicate that direct interception contributes to particle capture in *M. edulis*, *C. virginica* and *P. magellanicus*. They also suggest that inertial impaction contributes to particle capture in *C. virginica* for *particles* 2.25 and 8.25 µm ESD, where CE is potentially enhanced by increased fluid velocity. By replicating these findings in laboratory and field experiments, and obtaining similar results, we aimed to highlight the robustness of the trends observed. Results presented here indicate that the relationship between PR and CE in bivalves is dependent upon particle size as well as fluid velocity for *C. virginica*. These findings contribute to the understanding of particle capture in suspension-feeding bivalves, although additional experiments are needed to mechanistically explain these observations.

3.6 SUPPLEMENTAL MATERIAL

Supplemental Table 3.2 Results of the linear regressions between capture efficiency and pumping rate (l h-1), for each particle size measured (2.25–10.75/11.5 µm, ESD) *for M. edulis, C. virginica, and P. magellanicus* measured in both laboratory and field experiments. Bold values indicate linear regressions which were significant at $p < 0.05$.

Family	Particle Size (µm, ESD)	Laboratory Experiment				Field Experiment			
Mussels		Slope	Intercept	R^2	p-value	Slope	Intercept	R^2	p-value
M. edulis	2.25	-0.002	0.74	0.00	0.83	-0.01	0.60	0.01	0.24
	2.75	0.005	0.78	0.00	0.58	-0.02	0.73	0.03	0.19
	3.25	0.006	0.81	0.00	0.48	-0.03	0.85	0.065	0.09
	3.75	0.005	0.85	0.00	0.56	-0.04	0.98	0.12	0.03
	4.25	0.006	0.87	0.00	0.39	**-0.03**	**1.02**	**0.12**	**<0.05**
	4.75	0.007	0.89	0.004	0.30	-0.02	1.03	0.10	0.05
	5.25	0.006	0.92	0.01	0.29	-0.01	1.00	0.00	0.40
	5.75	0.005	0.93	0.03	0.20	-0.009	1.04	0.00	0.40
	6.25	0.003	0.95	0.00	0.41	-0.01	1.05	0.00	0.39

	6.75	0.004	0.94	0.04	0.18	-0.008	1.05	0.00	0.47
	7.25	0.006	0.94	0.11	0.06	-0.01	1.07	0.03	0.18
	7.75	0.0008	0.98	0.00	0.80	-0.006	1.04	0.00	0.49
	8.25	0.002	0.98	0.00	0.36				
	8.5					0.001	1.00	0.00	0.91
	8.75	0.0003	0.99	0.00	0.92				
	9.25	-0.0003	1.00	0.00	0.89				
	9.5					-0.0005	1.00	0.00	0.40
	9.75	-0.001	1.01	0.00	0.61				
	10.25	-0.002	1.01	0.00	0.62				
	10.75	0.0004	1.01	0.00	0.90				
	11.5					0.94	0.006	0.00	0.54
Oysters	2.25	**0.06**	**0.28**	**0.48**	**<0.001**	-0.01	0.03	0.00	0.44
C. virginica	2.75	**0.06**	**0.36**	**0.53**	**<0.001**	-0.0005	0.07	0.00	0.75
	3.25	**0.05**	**0.42**	**0.52**	**<0.001**	0.008	0.07	0.00	0.55
	3.75	**0.05**	**0.49**	**0.55**	**<0.001**	0.01	0.16	0.00	0.40
	4.25	**0.05**	**0.55**	**0.52**	**<0.001**	0.02	0.20	0.00	0.06
	4.75	**0.04**	**0.62**	**0.46**	**<0.001**	**0.04**	**0.29**	**0.39**	**<0.05**
	5.25	**0.04**	**0.70**	**0.48**	**<0.001**	**0.04**	**0.40**	**0.42**	**<0.05**
	5.75	**0.03**	**0.77**	**0.50**	**<0.001**	**0.04**	**0.49**	**0.42**	**<0.05**

	6.25	**0.03**	**0.80**	**0.46**	**<0.001**	**0.04**	**0.52**	**0.51**	**<0.01**
	6.75	**0.02**	**0.83**	**038**	**<0.001**	**0.03**	**0.63**	**0.37**	**<0.05**
	7.25	**0.01**	**0.89**	**0.21**	**<0.05**	**0.03**	**0.72**	**0.32**	**<0.05**
	7.75	0.007	0.95	0.10	0.08	**0.02**	**0.79**	**0.41**	**<0.05**
	8.25	**0.01**	**0.94**	**0.25**	**<0.01**	**0.02**	**0.84**	**0.32**	**<0.05**
	8.75	0.003	0.97	0.00	0.44	0.008	0.93	0.21	0.07
	9.25	0.001	1.00	0.00	0.76	-0.003	1.00	0.10	0.16
	9.75	0.0007	1.00	0.00	0.88	-0.0006	1.02	0.00	0.83
	10.25	-0.01	1.06	0.23	0.01	-0.01	1.11	0.40	0.02
	10.75	-0.005	1.03	0.00	0.34	-0.009	1.10	0.05	0.25
Scallops	2.25	-0.003	0.27	0.00	0.33	-0.002	0.56	0.00	0.81
P.	2.75	-0.003	0.35	0.01	0.26	-0.002	0.62	0.00	0.81
magellanicus	3.25	-0.003	0.41	0.007	0.28	-0.003	0.68	0.00	0.71
	3.75	-0.003	0.46	0.00	0.37	-0.003	0.72	0.00	0.72
	4.25	-0.003	0.54	0.00	0.34	-0.005	0.78	0.00	0.58
	4.75	-0.002	0.61	0.00	0.49	0.0007	0.78	0.00	0.92
	5.25	-0.002	0.70	0.00	0.51	0.003	0.82	0.00	0.68
	5.75	-0.003	0.75	0.00	0.40	0.0004	0.89	0.00	0.95
	6.25	-0.002	0.79	0.00	0.47	-0.004	0.98	0.00	0.42
	6.75	-0.003	0.84	0.00	0.33	-0.0008	0.95	0.00	0.87

	7.25	-0.002	0.85	0.00	0.42	-0.006	1.03	0.04	0.19
	7.75	-0.001	0.87	0.00	0.65	0.001	0.99	0.00	0.83
	8.25	-0.0003	0.89	0.00	0.87	**0.007**	**0.84**	**0.27**	**<0.05**
	8.75	-0.001	0.94	0.00	0.48	-0.003	1.02	0.00	0.34
	9.25	-0.0008	0.96	0.00	0.68	-0.006	1.07	0.09	0.11
	9.75	0.0005	0.96	0.00	0.77	-0.003	1.06	0.00	0.33
	10.25	0.002	0.96	0.03	0.18	-0.003	1.04	0.00	0.44
	10.75	-0.001	1.00	0.00	0.37	0.007	0.98	0.009	0.29

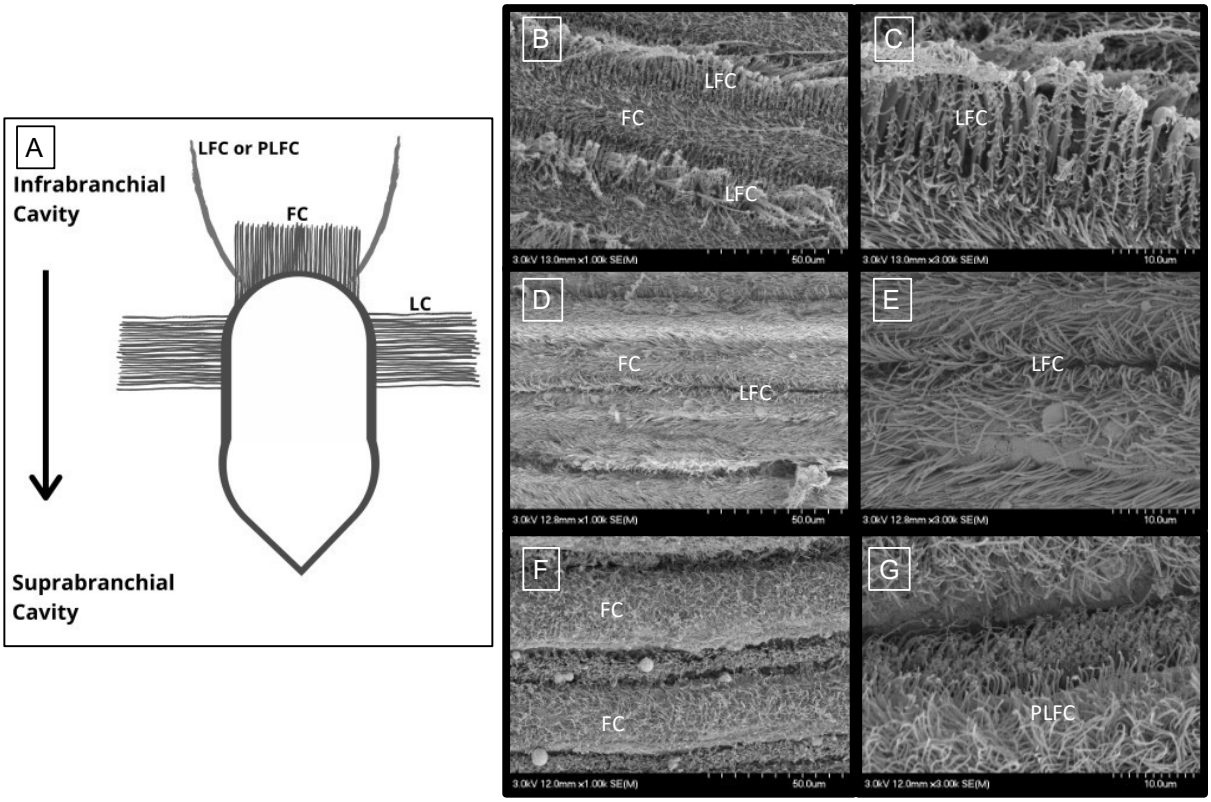
3.6.1 Characterization of bivalve gills: *M. edulis, C. virginica, P. magellanicus*

To explore differences in gill structure and gill filament types between the bivalve species used in laboratory and field experiments, a review of previously existing observations and measurements was conducted (Supplemental Supplemental Table 3.3). Further, to observe gill morphology and filaments in a similar orientation, gill tissues were prepared for scanning electron microscopy (SEM) for *M. edulis*, *C. virginica*, and *P. magellanicus*. Bivalves were collected from the same locations used in the laboratory experiment and held in the Aquatron facility for 5 days in filtered ambient seawater prior to preparing gill tissue for SEM observation. Individuals were shucked and whole gill tissue was immediately fixed in 2.5% glutaraldehyde solution with sea water. Time between shucking and fixation was kept below 30 seconds to minimize mucous production on the gill tissue and preserve the structure of the tissue (Prasetiya et al. 2017). Gill tissues were refrigerated for 20 hours in the glutaraldehyde solution. Glutaraldehyde was then removed, and tissues were rinsed three times in distilled water, soaking tissues for 20 minutes each time. Whole gill tissues were then dissected under a dissection scope into 5mm squares of gill tissue. Tissues were then fixed again in 2% osmium tetroxide solution and held in the dark at room temperature for 2 hours. Osmium tetroxide was then removed, and tissues were again rinsed three times in distilled water, each time for 20 minutes. Tissues were then dehydrated using increasingly concentrated ethanol (25%, 50%, 75%, 95%, 100%). Each time, tissues were soaked for 10 minutes, and the 100% ethanol was applied three times. Tissues were then mounted on aluminum stubs and dried with CO_2 with a critical point dry apparatus (CPD 300 Critical Point Dry Leica EM). Mounted tissue samples were sputter coated with 20nm of gold palladium (Leica EM Coater ACE200) and observed using a Hitachi S-4700 scanning electron microscope at 3.0 kV (Supplemental Supplemental Figure 3.8).

Supplemental Table 3.3 Descriptions of the gill structure of *M. edulis, C. virginica and P. magellanicus*. N/A indicates values were not found in a review of the literature.

	Mytilus edulis	*Crassostrea virginica*	*Placopecten magellanicus*
Family	Mytilidae (Mussels)	Ostreidae (Oysters)	Pectinidae (Scallops)
Inter-Filament Association	Filibranch	Pseudolamellibranch	Filibranch
Filament Type	Homorhabdic (Beninger et al. 1993)	Heterorhabdic (Beninger & Dufour 1996)	Heterorhabdic (Beninger et al. 1993)
Laterofrontal cirri	13-18.3 µm in length (Cannuel et al. 2009) Diameter: 0.06 µm (Owen & McCrae 1976)	13.5 µm in length (Ribelin & Collier 1977)	Pro-laterofrontal cirri N/A

	Spacing= 1.3 µm (Jorgensen 1975)		
Interfilamentar distance	25-30 µm (Owen and Mcrae 1976)	20 µm (Owen and McCrae 1976, *O. edulis*)	N/A
Lateral cilia	2.7 µm length 0.6 µm apart (Moore 1971) 11.9-15.6 µm length (Cannuel et al. 2009)	N/A	8 µm (Beninger et al. 1988)
Frontal Cilia	7.3-9.4 µm (Cannuel et al. 2009)	N/A	N/A



Supplemental Figure 3.8 (A) Transverse view of a generalized ordinary filament from a bivalve gill indicating the orientation and location of different types of cilia. (B-G) Scanning electron micrographs of the frontal view of ordinary gill filaments of: (B-C) *M. edulis* (B. 1000x C. 3000x) (D-E): *C. virginica* (D. 1000x E. 3000x) (F-G) *P. magellanicus* (F. 1000x G. 3000x), indicating different observed cilia or cirri types. FC = frontal cilia or cirri, LFC = laterofrontal cilia or cirri, PLFC = pro-laterofrontal cilia or cirri, LC = lateral cilia or cirri.

Reference

Prasetiya F. S, Decottignies P, Barillé L, Gastineau R, Jacquette B, Figiel A, Morançais M, Tremblay R, Mouget J, and Cognie B 2017. Cell size-based, passive selection of the blue diatom *Haslea ostrearia* by the oyster *Crassostrea gigas*. J. Mollus. Stud. **83**: 145–152. doi:10.1093/mollus/eyx012

CHAPTER 4 EXPLORING FEEDING PHYSIOLOGY OF *MYTILUS EDULIS* ACROSS GEOGRAPHIC AND FJORD GRADIENTS

This work has been published as: Steeves L, Strohmeier T, Filgueira R, Strand Ø. (2020). *Marine Ecology Progress Series*. 651: 71-84.

4.1 ABSTRACT

It is important to be able to predict the growth of filter-feeding bivalves, as they grow in dense populations both naturally and for commercial production. To understand the growth of bivalves it is necessary to have a mechanistic understanding of how they acquire energy through ingestion. This study was designed to understand if capture efficiency (CE), a primary step in ingestion for filter-feeders, is variable in the blue mussel *Mytilus edulis*. CE was measured using natural seston in 3 populations of naturally occurring M. edulis and within 2 populations along a fjord gradient. Differences in CE were found within a single population as well as along the fjord gradient. To determine if these differences were driven by short- or long-term changes, a single population of mussels was reciprocally transplanted between 2 locations along a fjord. This study is the first time CE has been measured within a population of *M. edulis* using a regional transplant experiment. Results showed that CE may vary between populations and change within populations, indicating that CE seems primarily driven by environmental cues. Pumping and overall ingestion rates differed between populations and varied within populations. For widely distributed species in changing environments, it is increasingly relevant to understand the limits of plasticity of specific traits to be able to predict their growth, survival, and distribution. Here, we

aimed to provide a more mechanistic description of CE, pumping rate, and overall ingestion in *M. edulis*.

4.2 INTRODUCTION

As ecosystem engineers in coastal environments, bivalves often grow in dense populations, modifying their habitat naturally and also when farmed for commercial production (Shumway et al. 2003, Borthagaray & Carranza 2007). Modelling bivalve growth is an important tool for exploring these ecological aspects (Beadman et al. 2002, Thomas et al. 2011), but also for potential economic implications (Ferreira et al. 2007). Crucial to estimating growth of these species is understanding how they acquire energy through feeding. Despite a century of research on feeding in bivalves (see Cranford et al. 2011, Rosa et al. 2018 for reviews), there remain many unknowns about the mechanistic underpinnings of this process. Dynamic Energy Budget modelling (Kooijman, 2010) exemplifies this; despite being a state-of-the-art modelling technique widely applied to bivalves, it still requires local calibration for ingestion rates (e.g., Rosland et al. 2009, Picoche et al. 2014). Being able to mechanistically predict ingestion between and within populations of bivalves is a crucial bottleneck in estimating overall growth and ecosystem-interactions of widely distributed species.

Ingestion rate in bivalves is a function of four components: food concentration, pumping rate, capture efficiency (CE), and rejection rate. Pumping rate is defined as the volume of water moved across the gill per unit time, and in combination with food concentration, represents the amount of food that is available at the gills per unit time (Wildish & Kristmanson 1997). Following Rosa et al. (2015), CE according to size describes the proportion of a given type of particles that could be cleared from the water column by gill filaments compared to other particles. Some particles which are captured are not ingested but rejected as pseudofaeces. In the absence of pseudofaeces production (low seston environments, usually below 2.5-5 mg l^{-1}, Widdows et al. 1979) ingestion in bivalves is a function of the food concentration, pumping rate, and CE.

CE had been assumed to increase non-linearly with particle size until an asymptote is reached, beyond which all particles are completely captured (Coughlan 1969, Vahl 1972,

Møhlenberg & Riisgård 1978). Recent research has challenged several aspects of CE of *Mytilus edulis*, including this asymptote (4 µm, Møhlenberg & Riisgård 1978), CE of small particles (1-4 µm) (Rosa et al. 2017a), and the notion that CE is a static trait (Strohmeier et al. 2012). Although variable CE is accepted in the literature, the mechanisms by which changes occur are not well understood (Rosa et al. 2018 for review). Most variability in CE occurs at small particle size (~1-4µm); however, this variability is cornerstone to understanding *M. edulis* energy acquisition as these particles may dominate the seston composition by number (Strohmeier et al. 2012, Rosa et al. 2015, Cranford et al. 2016).

M. edulis are a widely distributed species on a global scale (Sukhotin et al. 2007), making them a model species for exploring the effects of localized conditions on the response of CE and pumping rate. These responses may be plastic, e.g., operate in the short-term and be reversible, or adaptive long-term irreversible changes. Many feeding and growth traits of bivalves are highly plastic, particularly pumping rates which change in response to food quantity and quality (Bayne et al. 1993, Bayne 2004, Rosa et al. 2018 for review). Contrastingly, traits with genetic underpinnings may be adapted over long periods of time to the environment and may not easily respond to short-term environmental changes (e.g. salinity tolerance, Riginos & Cunningham 2004). Genetic differences in sessile marine bivalves tend to vary widely between populations due to the limited gene flow on a broad geographic scale, despite having planktonic larval stages (Levin 2006). Although differences in a trait may be observed between populations, these differences cannot be directly attributed to plastic or adaptive responses without further investigation (e.g., transplants, or genetic research). *In situ* transplant experiments permit the exploration of plastic versus adaptive traits (Worrall & Widdows 1983, Widdows et al. 1984). Although variations in CE have been observed in *M. edulis* (Strohmeier et al. 2012), CE has not been measured in a transplant experiment in this species and it is not well understood if changes in CE are happening on short- or long-term scales. Predicting changes in CE in response to environmental change contributes to a mechanistic understanding of ingestion, important for predicting growth of bivalves without local calibration.

This study was designed to understand the degree of variability in CE of *M. edulis* across a wide latitudinal gradient, and within fjord gradients. To address this, CE was measured between three populations of mussels, and within two populations along two

fjord gradients. Using natural seston, CE, pumping rate, and ingestion rate were measured in all five sampling locations, which covered a broad range of environmental conditions, reflecting the diverse habitats *M. edulis* grow in. Given that differences in CE were observed in *M. edulis* within the same population along a fjord gradient, mussels were reciprocally transplanted between these two locations along the fjord to determine if these differences were driven by short- or long-term changes in the environment. This study aims to provide a clearer understanding of particle capture, pumping rate, and ingestion in filter-feeding bivalves.

4.3 METHODS

4.3.1 Experimental Design

Two sequential experiments were carried out between April and June 2018 in Norway. In experiment 1 feeding trials were conducted at five field sites (Figure 4.1). Field sites (Austevoll, Hardangerfjord, Flødevigen, and Åfjord) covered a geographic range from 58ºN to 63ºN, and two fjord gradients, from inner to outer area (Hardangerfjord-Austevoll, and Åfjord 1-Åfjord 2) (Figure 4.1). Subsequently, in experiment 2, mussels were transplanted between two sites along a fjord gradient, previously sampled in experiment 1, Austevoll and Hardangerfjord. These mussels were acclimated for three weeks and feeding trials were conducted, measuring both native and transplanted mussels at each site.

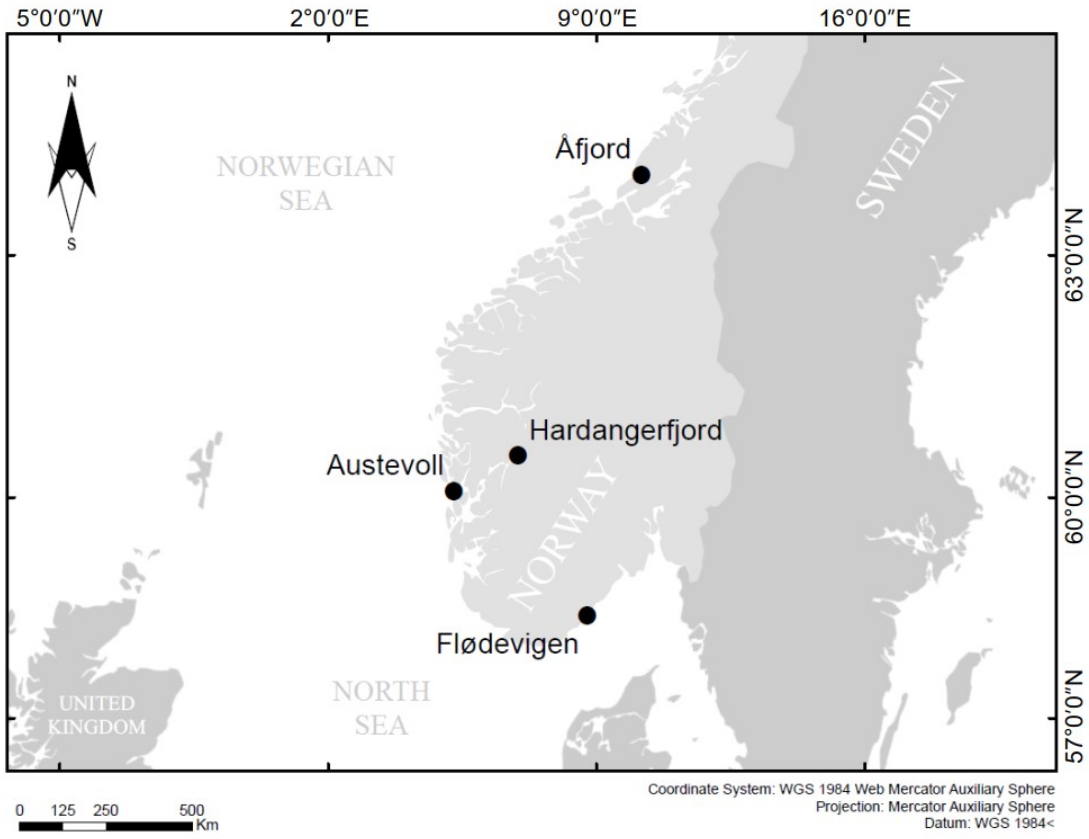


Figure 4.1 Location of the 5 field sites used in this study. For Expt 1, measurements were taken at Austevoll (60° 6' 45.77'' N, 5° 11' 23.95'' E) on 16-19 April; Hardangerfjord (60° 32' 38.86'' N, 6° 56' 47.60'' E) on 24-25 April; Flødevigen (58° 25' 34.42'' N, 8 ° 45' 16.09'' E) on 8-9 May; and Åfjord (63° 56' 22.94'' N, 10° 9' 57.60'' E). Within the Åfjord site, 2 samples were taken: one in the inner fjord (23-24 May) and one in the outer fjord (25-26 May). For Expt 2, mussels were transplanted between the Austevoll (8-10 June) and Hardangerfjord (13-14 June) sites

4.3.2 Water Quality Measurements

At each field site for both experiment 1 and 2, measurements were taken to describe water and seston characteristics. A CTD (SAIV A/S Model 204, Norway) was deployed in the header tank to record temperature and salinity. Each day a feeding trial was run, water

characteristics were determined at the beginning, middle, and end of the feeding trials by collecting water from the pump supplying water to the trial. To measure chlorophyll *a* concentration, 250mL of water was filtered onto a 1.2 μm filter (Whatman GF/C), and the fluorescence method was used (Strickland & Parsons 1968), using a fluorometer (Turner Designs Model 10-AU), previously calibrated as outlined in Strohmeier et al. (2012). Particulate organic carbon was measured by filtering 150mL of water onto rinsed (distilled water) and precombusted 1.2 μm filter (Whatman GF/C). Particle counts by size were determined using a PAMAS S4031 GO (PAMAS), which uses light scattering to count particles between 1 and 200 μm. Particle sizes are estimated as equivalent spherical diameter (ESD, μm). Using these counts (in triplicate) and associated size (ESD, μm), particle volume by size class could be determined.

4.3.3 Feeding Trials

Feeding trials were conducted using the static method to measure mussel CE and pumping rate (Cranford et al. 2016). At each site, the day before sampling began, wild mussels were graded for length (50.1 ± 4.3mm for all populations), cleansed of epibionts, and held at 3m depth. For each experiment, 40 individual mussels were sampled. During trials, mussels were held in a tank provided with flowing water pumped from 3m depth. CE was measured following Cranford et al. (2016). This technique is based on the continuous monitoring at high temporal resolution (30 seconds), of the number of particles of different sizes in a static feeding chamber (following Coughlan 1969). A single mussel was placed in a cylindrical PVC chamber (0.98L volume), where water was continually mixed using a magnetic stirrer to avoid sedimentation during the trial. Three controls were taken over the course of each sampling day by repeating the feeding trial without a mussel in the chamber. The feeding chamber was placed in a flow-through bath of ambient seawater. After a mussel was placed in the chamber, flowing water was pumped through until the mussel had opened. The flow was then stopped, and particle count measurements were carried out every 30 seconds using a PAMAS, as described in section 2.2. The PAMAS sampled 4.5 mL of water and estimated the number of particles between 1.75 and 11.5 μm, at 0.5 μm intervals. The PAMAS uses an internal pump that takes the sample

from the chamber and then returns it to the feeding chamber, providing constant volume over time. During the experiment, mussels were observed for pseudofaeces production. Each trial was run for a maximum duration of one hour.

Estimation of Capture Efficiency, Pumping Rate, and Ingestion Rate

In a static chamber, particle removal by a bivalve pumping at a constant rate follows an exponential decline (Coughlan 1969). To ensure only periods of constant pumping were used to calculate CE, only periods where the slope of the natural logarithm of particle concentration over time, λ, produced a linear line were selected ($r^2 \geq 0.9$) (Cranford et al. 2016). The comparison of the slopes for different particle sizes, λ_{size}, was used to calculate the CE for each particle size, (CE_{size}). CE_{size} is expressed as a relative value between 0 and 1, to describe how effectively particles of certain sizes are captured compared to others, wherein 1 represents particles captured with the highest efficiency, and 0 represents particles that are not captured. The calculation for CE_{size} is as follows:

$$CE_{size} = \frac{\lambda_{sample,size} - \lambda_{control,size}}{\lambda_{average}} \quad 4.1$$

Where $\lambda_{sample,\ size}$ is the slope of the exponential decay in particle concentration of a specific size, for the sample measurement taken with a bivalve present. $\lambda_{control,\ size}$ is the slope of the exponential decay in particle concentration of the same size, in the absence of a bivalve, which accounts for sedimentation in the feeding chamber. $\lambda_{\ average}$ is an average of the control-corrected slope of exponential decay in particle concentration of particle sizes that are known to be fully captured (CE of 1). For this study, $\lambda_{\ average}$ was calculated using particles from 8.5 to 11.5µm. All particle sizes are expressed as equivalent spherical diameter (ESD, µm).

To compare capture efficiencies of *M. edulis* across locations, each data set was modelled using a non-linear least square fit from an exponential growth function with an asymptote set to a value of 1:

$$CE = \frac{1}{1 + e^{(-phi2*(size-phi3))}} \quad 4.2$$

Where CE is capture efficiency, size is particle size (ESD, µm), phi2 is the steepness of the curve (1/(ESD, µm)), and phi3 is the theoretical particle size when CE is 0.5 (ESD, µm). The shape of this curve fits an expected relationship between CE and particle size, where CE increases with particle size until an asymptote is reached at a value of 1, representing the highest capture efficiency, or particles that are always captured (Cranford et al. 2016). To determine if these models were different across locations, the parameters from each model were compared using an Extra Sum of Squares F-test (see Peteiro et al. 2006).
From the assumption that $\lambda_{average}$ accurately describes particles which are captured with complete efficiency, pumping rate (PR, l h^{-1}), the volume of water moved across the gill per unit time can be calculated as:

$$PR = \lambda_{average} \text{ x } V \text{ x } 60 \text{ x } 60 \quad 4.3$$

Where V is the chamber volume (l), and 60 x 60 is used to convert the units of PR to l h^{-1}. Using PR, CE_{size} and particle counts for each size class (1.75-9.5µm) from the PAMAS, a volumetric ingestion rate (VIR, um^3h^{-1}), can be calculated as:

$$VIR = \sum_{size=1.75}^{9.5} (PR * CE_{size}) * (Particle\ Count_{size} * Particle\ Volume_{size}) \quad 4.4$$

Where $Particle\ Count_{size}$ and $Particle\ Volume_{size}$ are the number of particles of a given size, and its respective volume (calculated from its estimated spherical diameter), respectively. VIR is the sum of the total volume of particles cleared for each size class.

Ingestion rate was also calculated using both POC, and chlorophyll *a*, as other measures of food concentration:

$$Ingestion = PR * POC\ or\ Chlorophyll\ \alpha \quad (4.5)$$

Where PR is pumping rate, POC is in units of mg l^{-1}, Chlorophyll *a* is µg l^{-1}, and ingestion rate is in mg or µg h^{-1}.

Standardization of Pumping and Ingestion rate

Pumping rate was standardized to average gill area using the following formula:

$$PR_{std} = PR * (\frac{GA_{std}}{GA_{ind}}) \quad (4.6)$$

Where PR_{std} is the standardized pumping rate, GA_{std} is the average gill area from all individuals used in feeding trials (averaged separately for experiment 1 and 2), and GA_{ind} is the gill area for the individual being standardized. Gill area was measured for each individual directly after each feeding trial. To expose the surface of the gills for analysis, the anterior and posterior adductor muscles were cut with a scalpel. Once the shell was open, the gills were exposed by cutting away inner organs and the mantle, on both sides of the shell, leaving two exposed gills in each half of the shell (Sunde 2013). To avoid gill contraction, seawater was added to the shell halves to float the gills in. Assuming that all four gills were equal in size, a picture was taken of a shell half, containing two stacked gills. A top-down view of a shell half with two gills in it shows half of the surface area of one gill. This area was measured using freehand selections in ImageJ (v. 1.52 f). This area (in mm^2) was then multiplied by 8 (two sides of four gills), to estimate total gill area. Average gill area in experiment 1 was 233mm^2 and is equivalent to a length of 51.4mm. For experiment 2, gill areas were estimated using the relationship between gill area and length of experiment 1 (gill area (mm^2) = 9.335 x length (mm) – 246.5, $r^2 = 0.59$, n = 49). The average estimated gill area for experiment 2 was 204 mm^2, equivalent to a length of 48.3 mm.

4.3.4 Statistics

Parametric tests (analysis of variance, or student's t-tests) were employed to compare environmental parameters, and feeding physiology measurements (pumping rate, volumetric ingestion rate). Normality and homogeneity of variances were tested for, and if they were not found ($\alpha < 0.05$), data were $\log_{10}$ transformed. Statistical analyses were performed in GraphPad Prism v.8.2 and RStudio (R v.3.6.1).

4.4 RESULTS

4.4.1 Experiment 1: Water Quality Parameters

Temperature ranged between 5.5-13.8°C (Austevoll and Åfjord site 2, respectively), and salinity ranged from 20.8-31.6 (Flødevigen and Austevoll, respectively) (Table 4.1). Chlorophyll *a* was highest at Hardangerfjord ($df_{4,21}$, $p < 0.05$, Figure 4.2-A), followed equivalently by Åfjord 2, Åfjord 1, and Flødevigen ($df_{4,21}$, $p > 0.05$, Figure 4.2-A). Austevoll had the lowest chlorophyll *a* levels, significantly lower than both Hardangerfjord and Åfjord 2 ($df_{4,21}$, $p < 0.05$, Figure 4.2-A. POC levels were highest at Åfjord 2, followed equivalently by Åfjord 1, Hardangerfjord, and Flødevigen ($df_{4,21}$, $p < 0.05$, Figure 4.2-B). Austevoll again had the lowest levels of POC, lower than both Åfjord 1 and 2 ($df_{4,21}$, $p < 0.05$, Figure 4.2-B). Volume of particles for each size class (ESD, µm) varied with particle size (Figure 4.2-C), with a notable peak in the Hardangerfjord data between 4-6 µm, and Austevoll at 4 µm (Figure 4.2-C).

Table 4.1 Average (±SD) temperature and salinity measurements from Expt 1 for the 5 sampling locations

	Location				
	Austevoll	Hardangerfjord	Flødevigen	Åfjord 1	Åfjord 2
Temperature (°C)	5.5 ± 0.4	6.6 ± 0.1	9.5 ± 0.7	6.5 ± 1.3	13.8 ± 0.7
Salinity	31.6 ± 0.2	31.5 ± 0.05	20.8 ± 1.3	29.8 ± 3.4	30.2 ± 0.1

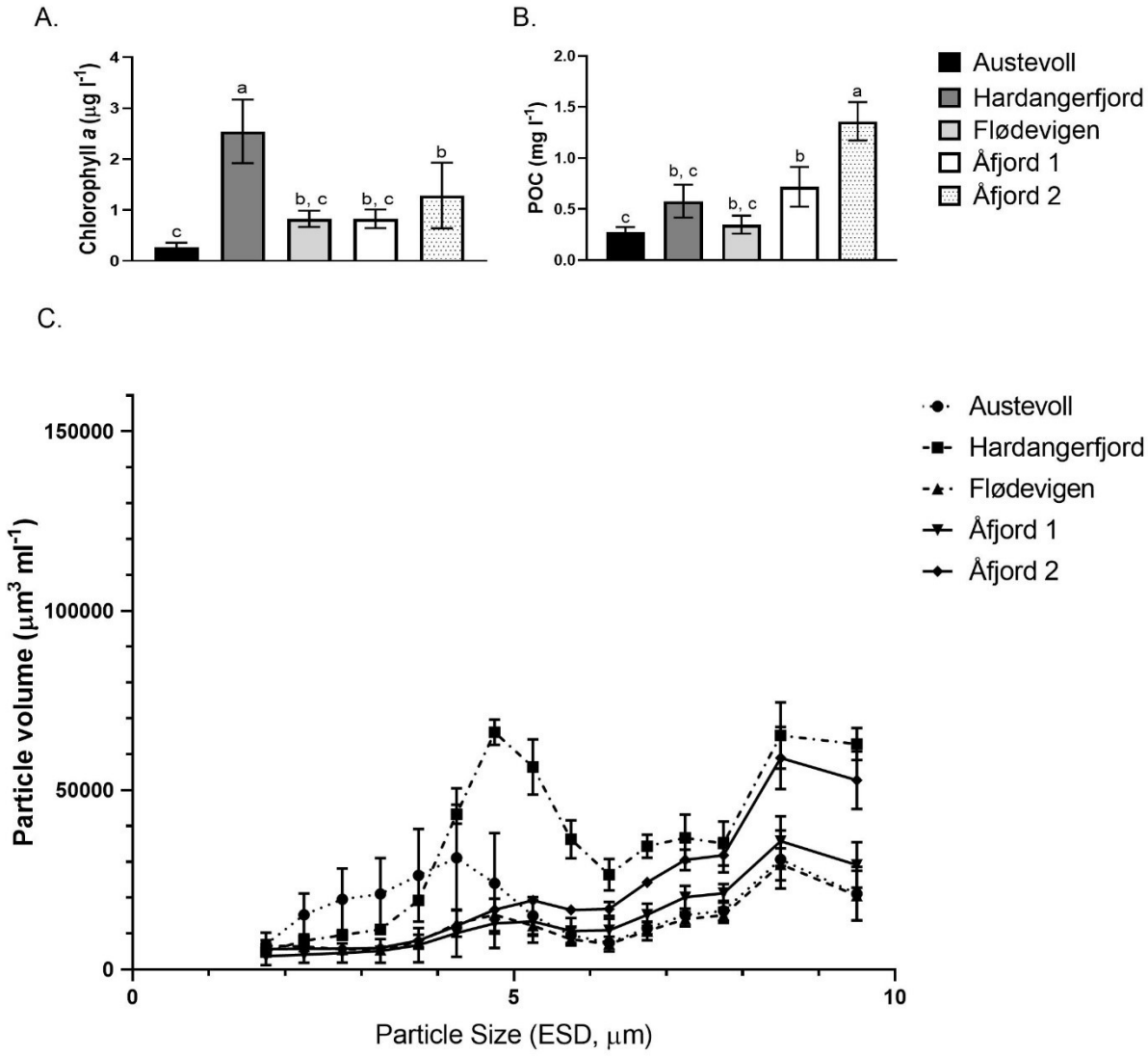


Figure 4.2 Water quality measurements (±SD) from all locations sampled in Exp1 1. (A) chl a (µg l-1), (B) particulate organic carbon (mg C l-1), and (C) total particle volume (um3 ml-1) for each size class measured (equivalent spherical diameter, µm). Particle volume between was estimated using 0.5 µm diameter steps excluding the last two measurements (8.5 and 9.5 µm) which used 1 µm steps due to low particle counts. Letters denote statistical significance at $\alpha = 0.05$.

4.4.2 Experiment 1: Feeding Trials

No pseudofaeces production was observed during any of the feeding trials. The Hardangerfjord population had an uncharacteristic peak of high CE values for particles between 2-3 µm ESD (Figure 4.3-B). The steepness of the curves (phi 2) was different between all populations, Flødevigen and Hardangerfjord being the highest and lowest, respectively ($p < 0.001$, Figure 4.4-A). The particle size when CE is at 0.5 (phi 3) was lowest for Åfjord 1 ($p < 0.05$) followed by Hardangerfjord and Flødevigen, which were statistically similar between them ($p > 0.05$, Figure 4.4-B). For Austevoll, phi 3 was not significantly different from Åfjord 2 (the highest), or Hardangerfjord and Flødevigen ($p > 0.05$, Figure 4.4-B). Hardangerfjord mussels had the lowest CE for particles of 4 µm ESD (0.54±0.13), and Flødevigen had the highest (0.83±0.11) ($p < 0.05$, Figure 3.4-C). There were no significant differences for CE at 4 µm ESD between the other populations (Austevoll, Åfjord 1, Åfjord 2) ($df_{4,120}$, $p > 0.05$, Figure 4.4-C). For all populations CE of 1 was reached at different particle sizes; the highest was Hardangerfjord (9.5 µm ESD), and Flødevigen had the lowest (4.75 µm ESD) (Figure 4.4-D).

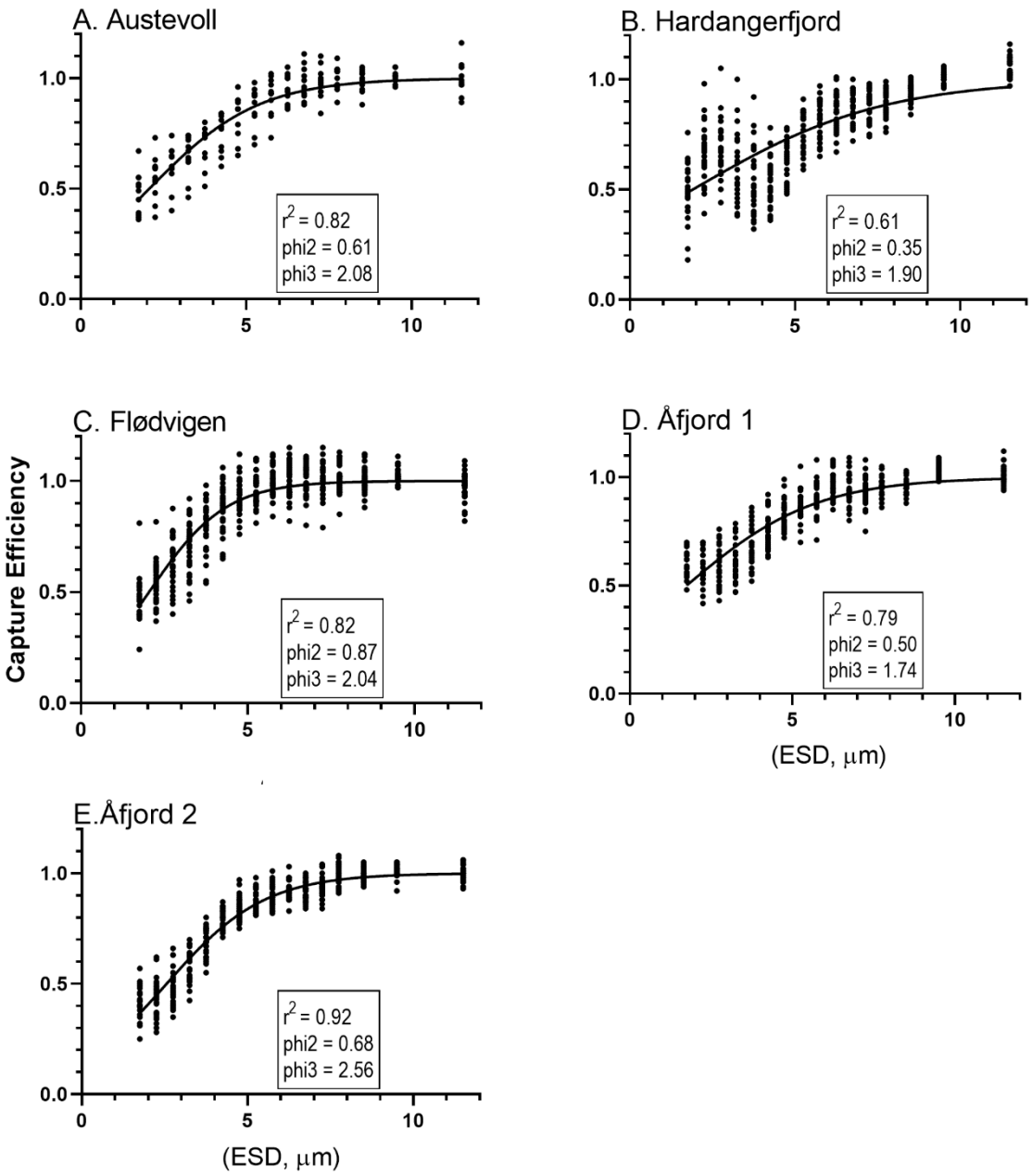


Figure 4.3 Standardized capture efficiency for each population of mussels sampled in Expt 1: (A) Austevoll, (B) Hardangerfjord, (C) Flødevigen, (D) Åfjord site 1, (E) Åfjord site 2. Particle sizes are expressed as equivalent spherical diameter (ESD); fitted curves and parameters shown are calculated using equation 4.2.

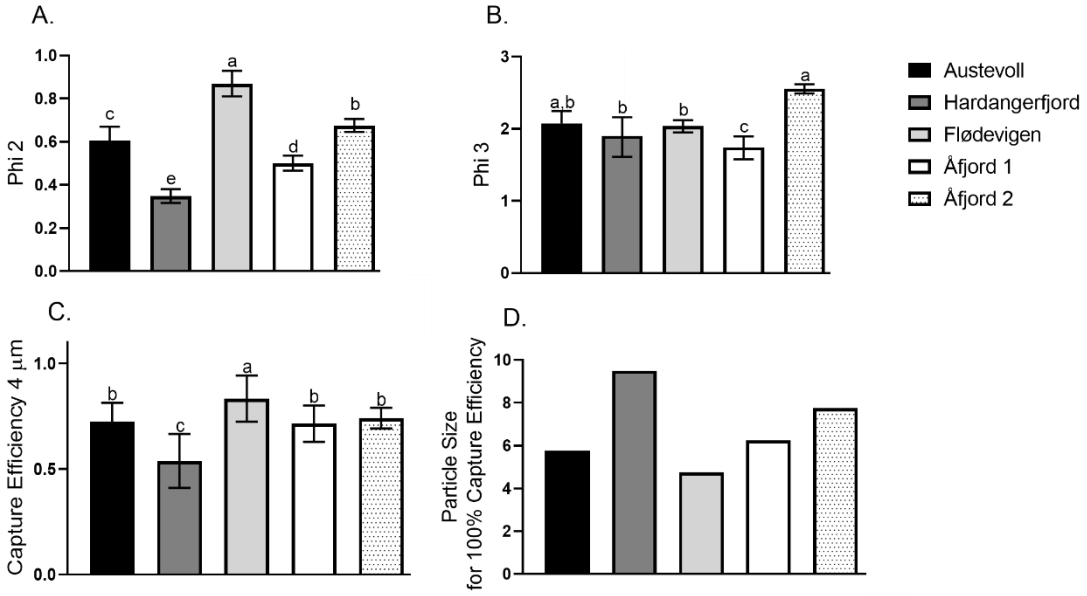


Figure 4.4 Mathematical descriptions of the capture efficiency (CE) curves shown in Fig. 3.3: (A) steepness of the curve (phi2) (1 / [equivalent spherical diameter {ESD}, µm]), (B) particle size when CE = 0.5 (phi3) (ESD, µm), (C) CE values for 4 µm particle size, and (D) particle size when CE first reaches 1 µm. Error bars show ± SD and letters denote statistical significance at $\alpha = 0.001$ (A,B) and 0.05 (C)

Pumping rate (lh^{-1}) was significantly lower for both Austevoll and Hardangerfjord, compared to all other populations ($df_{4,120}$, $p < 0.05$, Figure 4.5-A). Volumetric ingestion rate was highest for Hardangerfjord and Åfjord 2 ($df_{4,120}$, $p>0.05$, Figure 4.5-B), and lowest for all other locations ($df_{4,120}$, $p > 0.05$, Figure 4.5-B). Åfjord 1 had the third highest volumetric ingestion rate (mm^3h^{-1}), followed by Flødevigen and then Austevoll ($df_{4,120}$, $p < 0.05$, Figure 4.5-B). Ingestion rates calculated using POC and Chlorophyll *a* did not provide additional relevant information for both experiment 1 and 2 (Supplemental Figure 4.10).

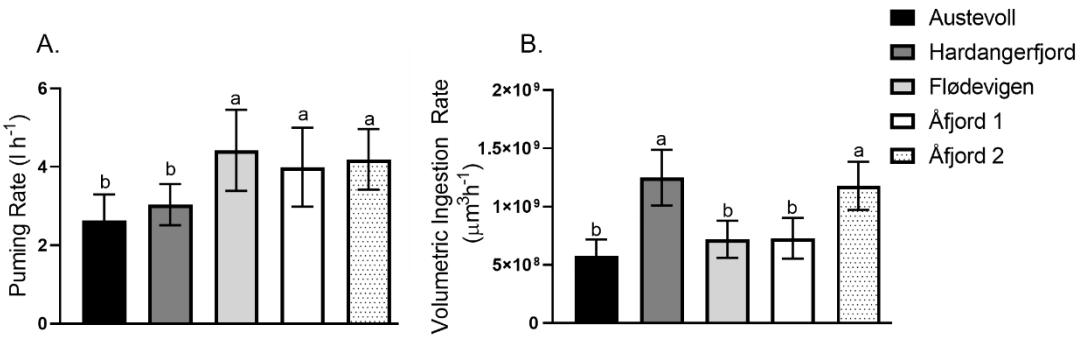


Figure 4.5 (A) Pumping rate and (B) volumetric ingestion rate from populations of mussels from Expt 1. Error bars show ± SD and letters denote statistical significance at $\alpha = 0.05$

4.4.3 Experiment 2: Water Quality Parameters

Temperature was higher in Hardangerfjord (19.1 ± 0.4°C) compared to Austevoll (16.5 ± 0.6°C). Salinity was also higher in Austevoll (29.6 ± 0.1) compared to Hardangerfjord (7.8 ± 0.9). Chlorophyll *a*, and POC were both higher in Hardangerfjord ($df_{3,24,}$ $p < 0.05$, Figure 4.6-A-B). Total particle volume by size class was similar in both locations until 5 µm ESD, beyond which Hardangerfjord particles had greater overall volume by size (Figure 4.6-C).

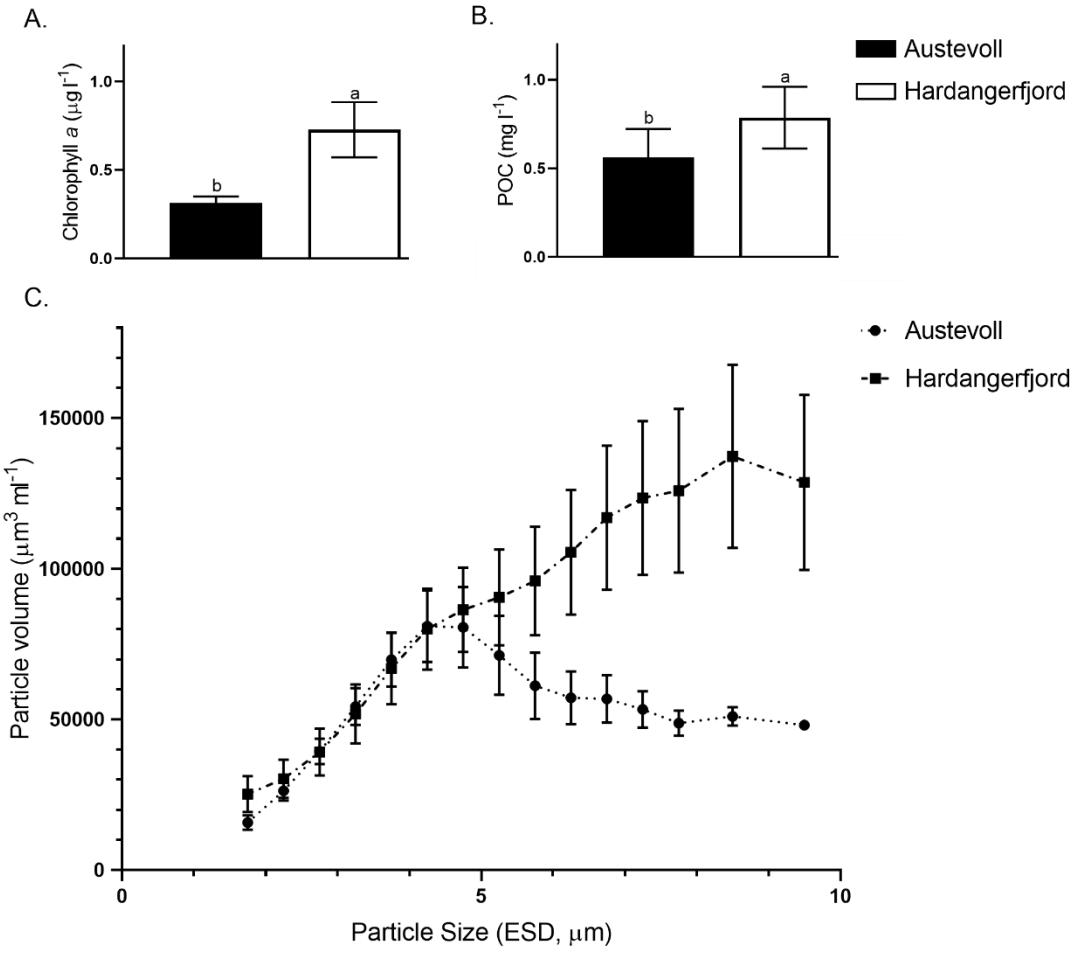


Figure 4.6 Water quality measurements (± SD) from all locations sampled in Expt 2: (A) chl a, (B) particulate organic carbon (POC), and (C) total particle counts for all size classes measured. Letters denote statistical significance at $\alpha = 0.05$

4.4.4 Experiment 2: Feeding Trials

CE values were similar within each sampling location, regardless of the origin of the population of *Mytilus edulis* sampled (Figure 4.7-A-D). The steepness of curves (phi 2) was significantly greater for mussels sampled in Austevoll, compared to those sampled in Hardangerfjord ($p < 0.001$, Figure 4.8-A). In addition, the particle size when CE is equal to 0.5 (phi 3) was higher for mussels sampled in Austevoll (Figure 4.7-A-B, respectively), compared to those sampled in Hardangerfjord (Figure 4.7-C-D, respectively) ($p < 0.001$,

Figure 4.8-B). For both phi 2 and 3, no differences were found based on the effect of population origin. Despite general differences in CE curves, all mussels sampled in experiment 2 has similar values for CE at 4 µm ESD, 0.89 ± 0.08 ($df_{3,44}$, $p > 0.05$, Figure 4.8-C). Particle size at which CE reached 1 was similar for both populations, but differed between sampling location, being 4.75 µm ESD for mussels in Austevoll, and 8.5 and 7.25 for Austevoll and Hardangerfjord mussels in Hardangerfjord, respectively (Figure 4.8-D).

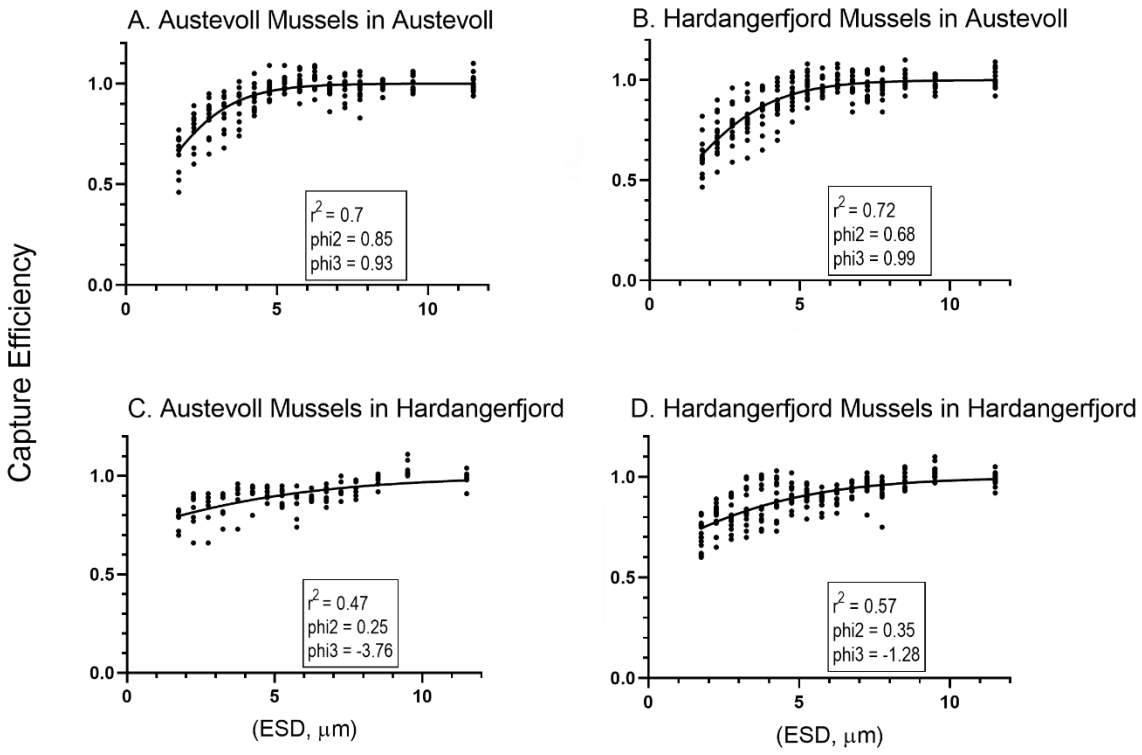


Figure 4.7 Standardized capture efficiency for each population of mussels sampled in Expt 2: (A) Austevoll mussels in Austevoll, (B) Hardangerfjord mussels in Austevoll, (C) Austevoll mussels in Hardangerfjord, and (D) Hardangerfjord mussels in Hardangerfjord. Particle sizes are expressed as equivalent spherical diameter (ESD); fitted curves and parameters shown are calculated using equation 4.2

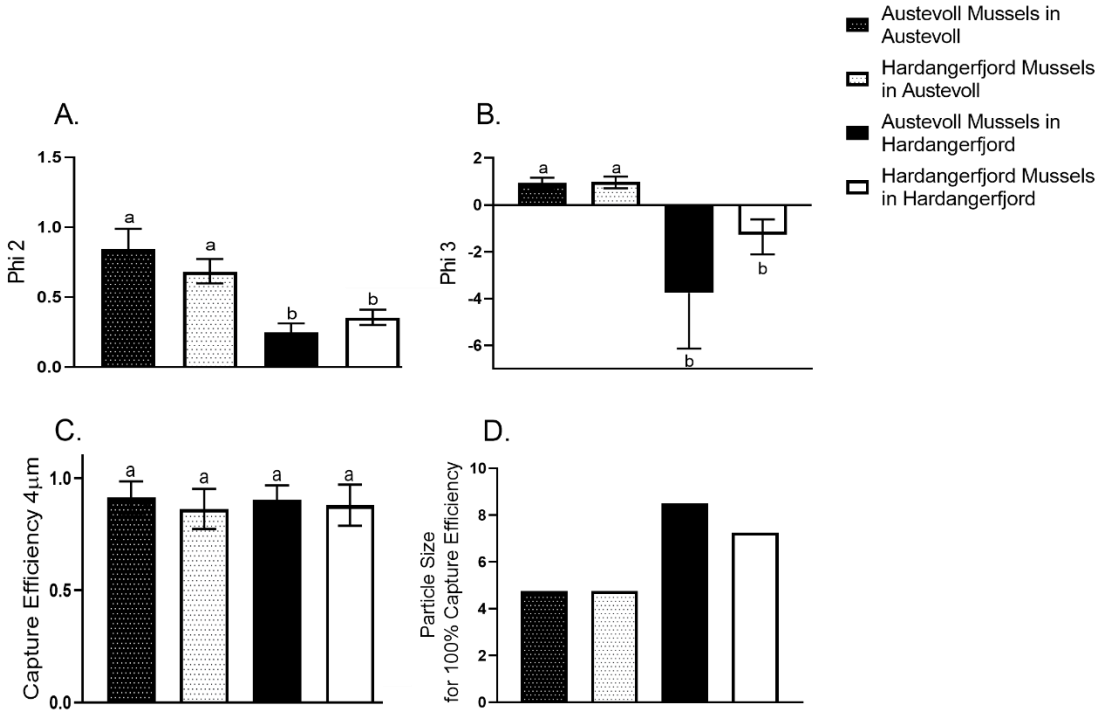


Figure 4.8 Mathematical descriptions of the capture efficiency (CE) curves shown in Fig. 7: (A) steepness of the curve (phi2) (1 / [equivalent spherical diameter {ESD}, µm]), (B) particle size when CE = 0.5 (phi3) (ESD, µm), (C) CE values for 4 µm particle size, and (D) particle size when CE first reaches 1 (µm). Error bars show ± SD and letters denote statistical significance at $\alpha = 0.001$ (A, B) and 0.05 (C)

Pumping rate (lh^{-1}) varied both by sampling location, and population origin; it was highest for Hardangerfjord mussels in Austevoll, and lowest for Austevoll mussels in Hardangerfjord ($df_{3,44}$, $p < 0.05$, Figure 4.9-A). There were no statistical differences in pumping rate between the Austevoll mussels in Austevoll, and the Hardangerfjord mussels in Hardangerfjord ($df_{3,44}$, $p > 0.05$, Figure 4.9-A). Within each location, Austevoll mussels consistently had statistically lower pumping rates than Hardangerfjord mussels.

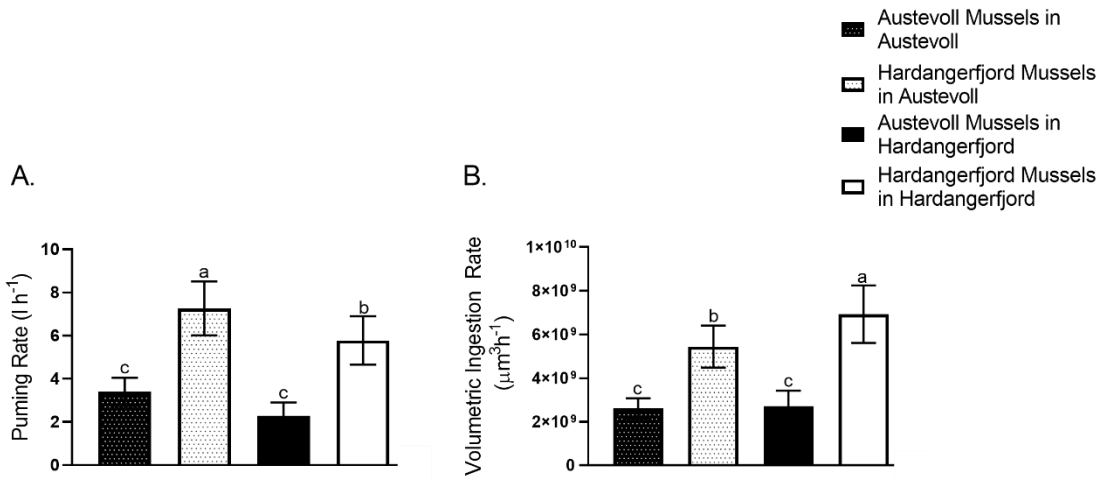


Figure 4.9 (A) Pumping rate and (B) volumetric ingestion rate from populations of mussels from Expt 2. Error bars show ± SD and letters denote statistical significance at $\alpha = 0.05$

Volumetric ingestion rate ($\mu m^3 h^{-1}$) was generally higher for mussels from Hardangerfjord than those from Austevoll. The highest ingestion rate was measured in Hardangerfjord mussels in Hardangerfjord, followed by Hardangerfjord mussels in Austevoll, which was statistically similar to Austevoll mussels in Hardangerfjord ($df_{3,44}$, $p < 0.05$, Figure 4.9-B). The lowest volumetric ingestion rates were measured in Austevoll mussels in both locations ($df_{3,44}$, $p > 0.05$, Figure 4.9-B).

4.5 DISCUSSION

This study demonstrates that CE, pumping rate, and ingestion rate of *M. edulis* all varied both between populations and along fjord gradients. CE was different between three geographically distinct populations of mussels and changed temporally within two populations. Further, when mussels were reciprocally transplanted along a fjord gradient, mussels of different origin had similar CE when placed in the same location, suggesting that CE was primarily driven by environmental cues. Ingestion rates were not similar between and within populations of *M. edulis*. Further, when mussels were transplanted between two locations, both pumping and ingestion rates were driven by both origin and environmental cues.

4.5.1 Capture Efficiency

CE of *M. edulis* generally increased with particle size to an asymptote, beyond which particles were completely captured. However, CE of small particles was different between the three populations and five sampling sites in experiment 1. Additionally, in experiment 1 the Hardangerfjord population had a CE for particles of ~4 µm under 50%, which is unusual for this species (Møhlenberg & Riisgård 1978, Cranford et al. 2016), although has been previously observed (Strohmeier et al. 2012). For this population, CE initially increased with particle size as expected; however, ~4-6 µm particles had lower CE than smaller particles (3.25 µm). This unexpected response in CE coincided with high chlorophyll *a* levels, and also a peak of seston volume at ~4-6 µm, suggesting that CE could be driven by the dominance of a single planktonic species that is not efficiently captured by mussels. Based on previous literature, this experiment was likely conducted after the peak of the spring bloom in the Hardangerfjord, but the physical and biological characteristics of this fjord are subject to high levels of variability due to freshwater inputs, large depths, and coastal advective processes (Braarud 1976, Sakshaug & Olsen 1986, Asplin et al. 2014). Therefore, it is plausible that the end of the bloom, or an input of freshwater from the spring melt, may have driven the bloom of this planktonic species, subsequently triggering the CE response. When the Hardangerfjord mussels were sampled again in experiment 2, both the low CE for ~4-6 µm particles and peak in seston volume (~4-6 µm) were not observed, strengthening the hypothesis that the response of CE during experiment 1 was driven by environmental cues.

CE of mussels sampled in the same location in the Hardangerfjord changed over three months during experiments 1 and 2. Strohmeier et al. (2012) measured CE of one population of *M. edulis* over four months and found that CE for small particles (~1-4 µm) was higher later in the season when the seston had higher concentration of particles that size. Similarly, Rosa et al. (2015) observed that over 9 months, CE for particles ≤ 5 µm significantly increased within a population of *M. edulis*, using natural seston; however, no mechanism was proposed that would facilitate this. These changes in CE may be made in response to changes in seston composition, particularly shifts in particle size distribution (Strohmeier et al. 2012). In this experiment, the increase in CE for small particles in the

Hardangerfjord mussels over three months was not explained by changes in concentration of small particles (following Strohmeier et al. 2012), seston volume, chlorophyll *a* or POC concentration. Alternative hypotheses are required to determine drivers in changes of CE for *M. edulis,* including identifying seston composition by plankton groups.

The accurate characterization of CE is necessary for calculating ingestion rate in bivalves. The results of this study highlight that using a single CE curve for *M. edulis* may not reflect the physiology of local populations or capture temporal shifts in CE. The traditionally accepted notion that complete particle capture is reached for *M. edulis* at 4 µm (Møhlenberg & Riisgård 1978) can create compound errors in calculations of ingestion (Cranford et al. 2016). Further, the majority of research on CE has been interested in the particle size at which CE reaches a maximum; however, the contribution of small particles (e.g., picoplankton) to filter-feeder energetics also warrants a clear understanding (Sonier et al. 2016, Rosa et al. 2018). Understanding why CE changes over time is important to be able to predict differences in particle capture, and overall ingestion.

4.5.2 Ingestion Rates

Ingestion rates, as measured by seston volume, POC, and chlorophyll *a*, were different in several of the sampling locations of experiment 1. Further, ingestion rates in the Hardangerfjord mussels varied between experiment 1 and 2 over a three-month period. No compensatory mechanisms between CE and pumping rate to maintain similar ingestion rates were observed. It has previously been postulated that as the available diet changes, *M. edulis* uses a variety of physiological mechanisms, including ingestion and rejection rates, and digestive processes (e.g., absorption efficiency), to maintain constant energy uptake (Willows 1992, Bayne et al. 1993). Similarly, it has also been suggested that feeding rates respond to maintain stomach fullness (Bayne et al. 1989, Willows 1992). Lack of similarity between ingestion rates observed in this study do not support any of these hypotheses.

In this study, ingestion was measured using proxies for energy content commonly used in the literature (Carver & Mallet 1990, Sarà et al. 2012). Although POC may be good a indicator of energy (Strohmeier et al. *in prep*), measurements of ingestion using energy

would more accurately assess hypotheses of constant energy uptake. Further, different methodologies for calculating ingestion rates may also contribute to inconclusive findings. Here, volumetric ingestion only included particles as large as 9.5µm and is therefore missing the contribution of larger particles. However, particle count declined steeply after 9.5 µm, and ingestion as measured by POC and chlorophyll *a* provided similar results to volumetric ingestion estimations (Supplemental Supplemental Figure 4.10). Differences in the internal states of the naturally occurring populations of *M. edulis* may have also contributed to differences in ingestion rates. Condition index varied significantly between populations in the transplant experiment (Supplemental Supplemental Figure 4.11), indicating physiological states may have been variable. As this experiment was conducted in the spring, it is possible that spawning may have recently occurred, potentially introducing a physiological stress (Worrall & Widdows 1983). Further, although differences in ingestion were observed in this study, constant energy uptake may have been maintained through internal changes in digestion such as gut passage time, and absorption efficiency (Navarro & Winter 1982). Another restraint on the explanation of constant energy uptake is the use of natural seston as a food source. While natural seston allows for the examination of ingestion under natural conditions, it is possible that the gradient of food quantity and quality was not large enough to allow for compensatory mechanisms in feeding. Although different ingestion rates were observed between populations of *M. edulis*, it could not be determined if differences are driven by the internal state, environmental conditions, or local adaptation.

4.5.3 Using Transplant Experiments to Explore Plasticity and Adaptation

To understand if the observed differences in CE and ingestion were driven by short- or long-term responses to environmental conditions, mussels were reciprocally transplanted along the Hardangerfjord in experiment 2. After the three-week acclimation period in experiment 2, CE was determined by transplant location, and contrastingly pumping and ingestion rates seem more closely linked to the origin location. Previous transplant experiments with bivalves suggest a gradient of acclimation by traits, species, and acclimation time. Navarro et al. (2003) conducted a transplant experiment using

Mulinia edulis and *Mytilus chilensis* between intertidal and subtidal zones. After 7 days of being exposed to the new environment, and different diets, total ingestion rates showed a higher degree of acclimation than clearance rates for both species. Longer acclimation periods (63 days, *Mytilus chilensis*) of a transplant experiment found that origin site still had a significant effect on clearance and ingestion rates (Osores et al. 2017). Other transplant experiments have focused on overall energy acquisition. Labarta et al. (1997) transplanted intertidal and raft cultivated *Mytilus galloprovincialis* to a laboratory setting and determined scope for growth of both populations. After 15 days, both populations of mussels had increased clearance and ingestion rates. However, higher scope for growth was maintained in the cultivated mussels through higher absorption efficiencies. Results from these studies highlight the complex relationships between acclimation time and feeding physiology in bivalves. This experiment supports the notion that different components of feeding in bivalves respond to environmental change over different timeframes. Here, CE changed more quickly than pumping or overall ingestion rates.

As primarily sessile organisms that grow in diverse environments, *M. edulis* are a good model species to explore plastic and adaptive traits. Changes in CE within a population of *M. edulis* were observed in this study, suggesting that CE is not an adapted trait in this location. However, as changes in CE in this study cannot be determined to be driven by either physiological control or changes in seston characteristics, it is not clear if this is a plastic response. When mussels were transplanted, pumping and ingestion rates were determined both by origin and transplant destination. This indicates that a longer acclimation time may be required to observe a plastic response, or that these traits may be locally adapted. As not all traits in organisms are plastic, it has been hypothesized that the limitations imposed on plasticity are a trade-off between succeeding in a variable environment, and the cost of phenotypic plasticity (Murren et al. 2015). Limitations of plasticity may be driven by underlying processes, for example, changes in protein induction and metabolic rate (Osores et al. 2017, Byrne et al. 2020). Adaptive responses may more commonly be used in response to slower rates of change, that do not exceed levels natural variability in the environment (Boyd et al. 2016). Understanding plastic and adaptive traits of feeding physiology in bivalves is key to a mechanistic understanding of growth in different environmental conditions.

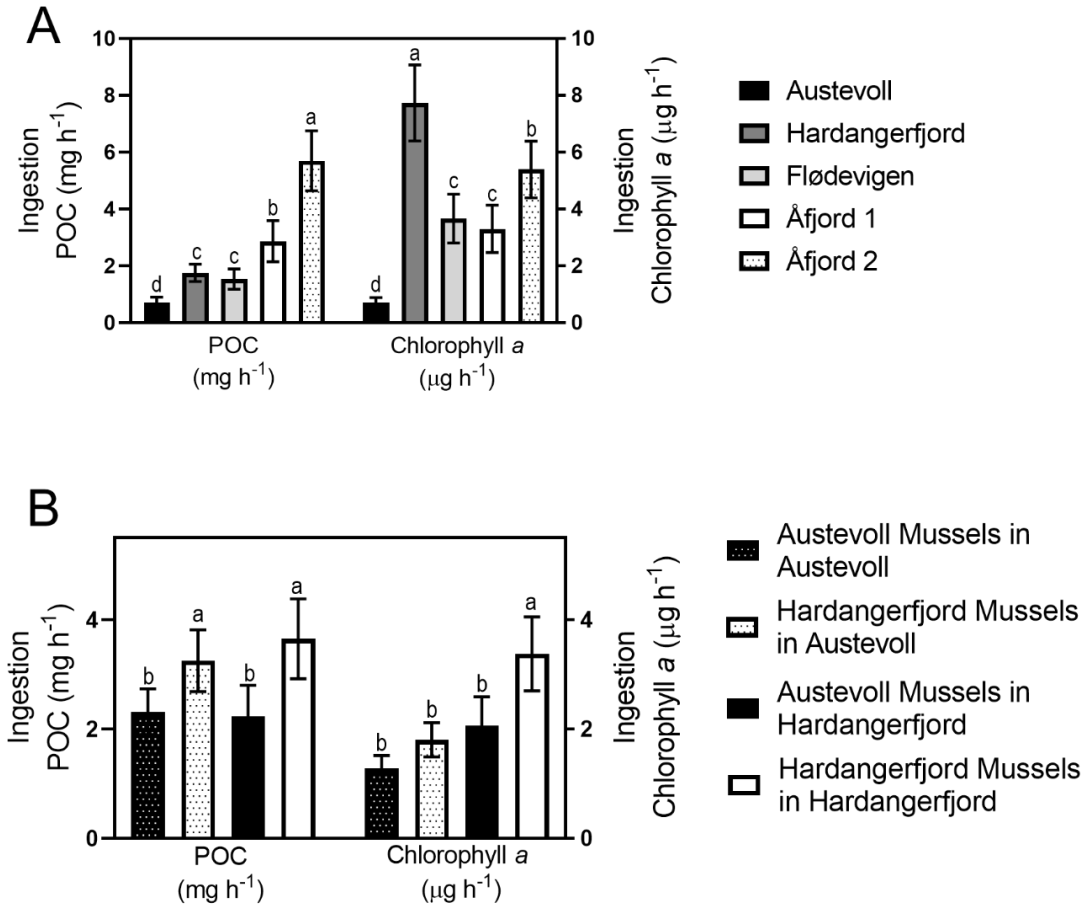
4.5.4 Conclusions and Future Directions

Findings from this study indicate that for *M. edulis* short-term changes are observable in CE; however, limited inferences can be made about what may have been driving these changes. To expand upon these findings, future studies should consider analyses of seston composition and physicochemical properties, transplants across larger environmental gradients, and genetic analysis of bivalve populations. Several aspects of seston composition have been previously shown to influence CE in laboratory experiments (e.g., hydrophobicity (Rosa et al. 2017b), lectin-carbohydrate interactions (Pales Espinosa et al. 2009), fluorescence (Yahel et al. 2009)). Future *in situ* experiments should consider measuring these seston properties to determine drivers of CE change using natural seawater. Beyond analysis of seston composition, transplant experiments across larger environmental gradients would provide further information on plasticity of feeding. Although the use of natural seston is imperative to understanding feeding physiology, it also limits control over differences in environmental conditions. Larger differences in food quality and quantity may be required to observe acclimation in pumping and ingestion rates. Finally, genetic analysis of transplanted mussels would permit exploration of population distribution and levels of genetic mixing in natural populations. Short-term changes in feeding physiology of *M. edulis* were observed in this study, and future research should consider both the drivers and limits of these changes.

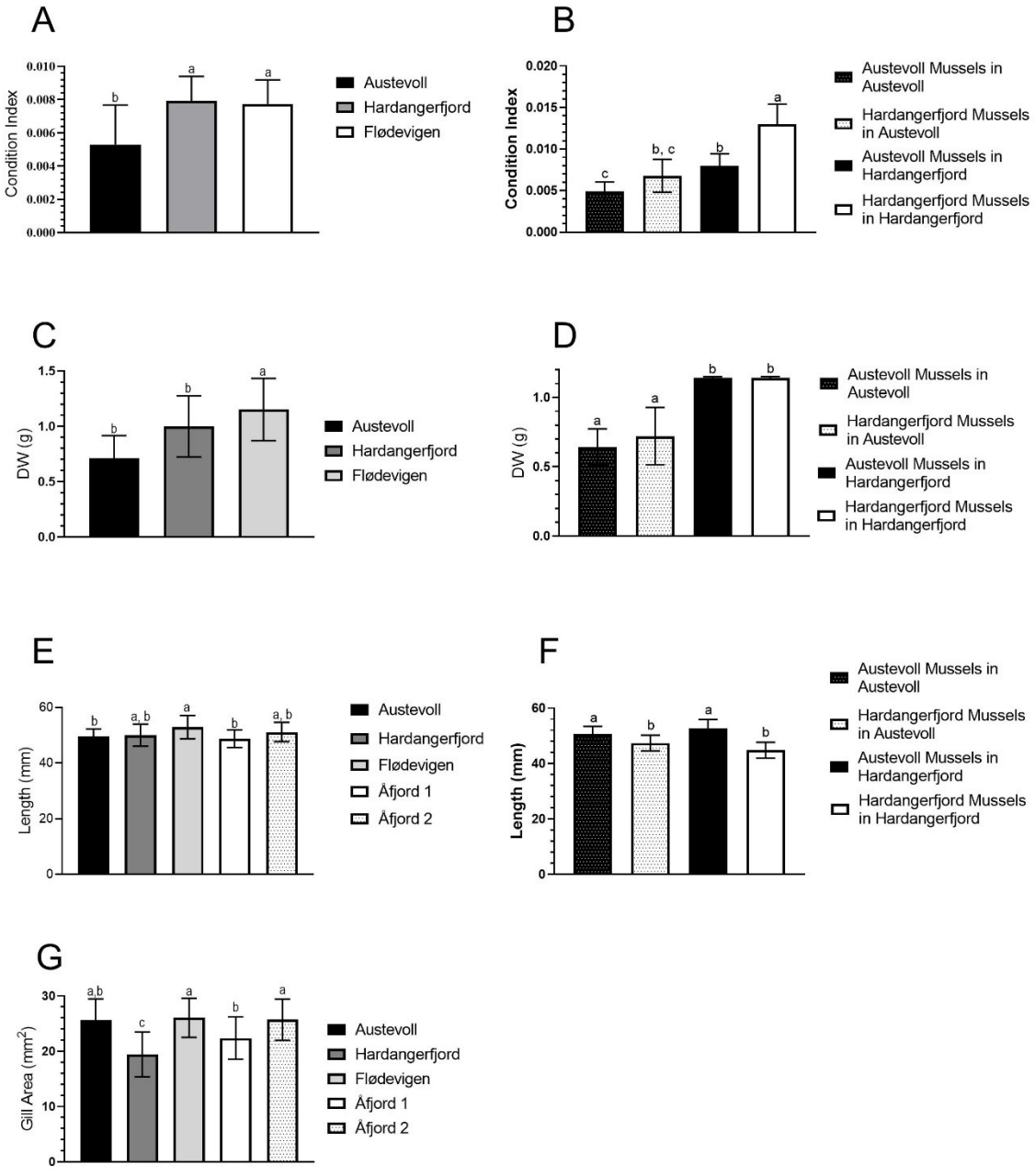
This study demonstrated that feeding physiology, measured as CE, pumping rate, and ingestion rate were variable both between populations of *M. edulis* and within populations along a fjord. This study is the first-time CE has been measured in a transplant experiment with *M. edulis.* These findings further corroborate that CE of small particles can change in *M. edulis,* and that full CE does not occur at 4 μm for all individuals. Overall, ingestion rates differed both between populations, and changed within populations over time. Understanding the limits of acclimation and plasticity of feeding physiology is increasingly relevant for widely distributed species in a changing climate. Although environmental conditions may change quickly, responses may happen slowly, and vary for individual processes. The accurate characterization of CE and pumping rate are necessary

to measure ingestion in bivalve filter feeders. Having a mechanistic understanding of ingestion in filter-feeding bivalves is necessary to fully understand how bivalves acquire energy, and how that information can be used to better predict individual growth and species distribution.

4.6 SUPPLEMENTAL MATERIAL



Supplemental Figure 4.10 Ingestion rates for experiment 1 (A) and 2 (B) calculated using particulate organic carbon ($mgh^{-1}mm^{-2}$) and chlorophyll *a*. Letters denote statistical significance at $p<0.05$.



Supplemental Figure 4.11 Biological measurements of mussels from experiment 1 and 2: (A) condition index experiment 1 (excluding Åfjord sites) (B) condition index experiment 2 (C) dry weight (g) experiment 1 (excluding Åfjord sites) (D) dry weight (g) experiment 2 (E) length (mm) experiment 1 (F) length (mm) experiment 2 (G) gill area (mm^2) experiment 1. Letters denote statistical significance at $p<0.05$.

CHAPTER 5 INTRA- AND INTERINDIVIDUAL RESPONSES OF FEEDING AND INGESTION RATES IN *M. EDULIS* TO NATURAL DIETS

5.1 ABSTRACT

The feeding activity of bivalves is understood to change in response to a suite of environmental conditions including food quantity and quality. It has previously been hypothesized that by varying feeding rates in response to changes in the available diet, bivalves may be able to maintain relatively constant ingestion rates, allowing them to have constant energy uptake despite changes in food availability. The purpose of this study was to use a novel methodology to measure the feeding rate of *M. edulis* with high temporal resolution (every 20-60 minutes) to determine both the levels of inter- and intra-individual variability in feeding rates, and the relationship between feeding rate and food availability. Three four-day experiments were conducted to measure pumping and ingestion rates in response to fluctuations in seston and chlorophyll *a* concentrations using natural seawater. Experiments were conducted in dock-side experiments over the spring season (April-June). Physiological rates were measured using a novel methodology with high temporal resolution, (every 20-60 minutes) while mussels (n=10) were held in a flow-through system. This high temporal resolution of pumping and ingestion rate measurements permitted the observation of both intra- and interindividual variability of feeding rates. Results show both intra- and interindividual variability in feeding rates, with pumping rates varying within individuals over 4-days, and some individuals pumping on average at high rates (~5 Lh^{-1} $individual^{-1}$) and some at low (~1 Lh^{-1} $individual^{-1}$), despite being held in similar conditions. Further, experiment population-level pumping rate was generally not

related to changes in food availability (fluorescence concentration, μgL^{-1}), and instead population-level ingestion rates were driven by food availability. These results suggest that for this population of *M. edulis*, feeding rates may not vary with the available diet to produce constant energy uptake over time.

5.2 INTRODUCTION

Suspension-feeding marine bivalves play important ecological roles by filtering plankton and detritus that are suspended in the water column, and subsequently producing faeces and pseudofaeces that sink to the ocean floor. This top-down control on planktonic communities, as well as bottom-up control from bivalve excretion, can affect planktonic community structure and functioning (Prins et al. 1998, Newell 2004, Trottet et al. 2008). Concomitantly, the quantity and quality of food (seston) available to suspension-feeding bivalves affects their performance in terms of growth and survival (Smaal et al. 1986, 2013). Many coastal marine environments are characterized by large fluctuations in seston composition and concentration, over both long (seasonal) and short (diel) timeframes (Bratbak et al. 2011).Understanding the relationships between food availability and bivalve feeding behaviour is crucial to predicting both bivalve growth, and bivalve-ecosystem interactions.

Suspension-feeding bivalves have several mechanisms by which the quantity and composition of ingested food can be regulated. Pumping rate, the volume of water moved over the gills per unit time (PR) is a metric of feeding activity and may change by several litres/hour in an individual exposed to diets of differing concentration and composition (Foster-Smith 1975, Shumway et al. 1985, Velasco & Navarro 2002). Generally, the initiation of pumping is triggered when food concentration surpasses a minimum threshold level, which may vary both by species and population (Foster-Smith 1975, Bayne et al. 1993, Smaal et al. 1997, Strohmeier et al. 2009) As food levels continue to increase beyond the minimum threshold, PR may remain at a constant maximum, or increase with food concentration (Foster-Smith 1975, Riisgard 1991, Clausen & Riisgård 1996, Hawkins et al. 1996). When food levels become very high, PR may decline or become intermittent to avoid overloading the gills (Navarro et al. 1992, Velasco & Navarro 2005) or the digestive

system (Willows 1992, Rueda & Smaal 2002), suggesting that the maximum ingestion rate has been reached. Bivalves may also regulate ingestion rates through the rejection of pseudofaces, a process which is usually not observed in low seston environments (< ~ 2.5-5 mgL^{-1}) (Widdows et al. 1979). Although bivalve PR in response to diets of varying composition has been extensively studied, a mechanistic understanding of this process is still relatively unknown (Jøsrgensen 1996, Riisgård et al. 2011).

For primarily sessile species exposed to high levels of variation in the available diet, the ability to regulate the amount and quality of ingested food, through the processes described above, is an important mechanism in energy acquisition in bivalves. Although bivalves are exposed to frequently changing diets, these pre-ingestive mechanisms may help to maintain a relatively stable ingestion rate (IR) over time (Winter 1976). In the absence of pseudofaeces production, IR may be estimated as a function of PR and food concentration. For situations when food concentration is increasing and PR is decreasing, a relatively stable IR may be observed (Navarro & Winter 1982, Navarro & Widdows 1997). It has been theorized that this relationship between PR and food availability that can produce stable IRs may also contribute to constant energy uptake by bivalves in a fluctuating food environment (Winter 1976). In bivalves, the relationship between IR and food concentration is often modelled using Holling functional responses, which describe the relationship between prey density and predator consumption rates (Holling 1966, Picoche et al. 2014, Montalto et al. 2017). Holling functional responses may describe a linear increase (Type I) or asymptotic increases (Type II and III) in consumption rate with increasing prey density. The ability to accurately predict bivalve IRs in variable environmental conditions is a foundational step in predicting how bivalves acquire energy for growth.

The goal of this study was to examine the levels of intra- and inter-individual variability in PR and IR, and to explore the relationships between PR and IR in response to fluctuations in natural diets. Often, the relationships between feeding, ingestion, and the food environment are studied using artificial diets (or natural seawater supplemented with artificial diets) in laboratory experiments (Bayne et al. 1993, Strohmeier et al. 2009, Vajedsamiei et al. 2021). However, experiments with natural diets are needed to understand the physiological responses of bivalves to the complexities of naturally occurring

planktonic communities. Further, the current knowledge on the physiological responses in feeding activity to variability in diet comes primarily from environments with high seston concentration (> 4µgL^{-1}), either in laboratory studies, or in sites where bivalves are cultivated (Prins et al. 1998, Figueiras et al. 2002). However, many bivalves reside in environments that usually have lower seston concentrations, which become more commonly used for aquaculture farms due to space limitations in high seston environments. Metrics of feeding and ingestion rates are often reported as an average of a group (e.g., one measurement on each individual), or by taking repeated measurements on the same individuals over the course of several hours (Cranford & Grant 1990, Velasco & Navarro 2005). These studies may overlook the short-term fluctuations in PR that can be captured with methodologies that allow high-frequency physiological measurements (e.g., Vajedsamiei et al. 2021). This study uses a novel methodology to estimate feeding and ingestion rates of *M. edulis* with a high temporal resolution (every 18 minutes, for 4 days), using natural seawater under flow-through conditions. As seston concentration may change over the course of hours and days, this study aims to capture the functional feeding response of *M. edulis* over short timescales. *M. edulis* was selected as a model species as it is widely distributed, commercially important, and its feeding behaviour has been extensively studied. It was hypothesized that as the concentration and composition of the seston varied, *M. edulis* would vary PR to maintain constant IRs, above a minimum threshold of food concentration, following Winter (1976).

5.3 METHODS

5.3.1 Experimental design

Three independent 4-day experiments were conducted to measure *Mytilus edulis* pumping rates (PR), ingestion rates (IR), and environmental conditions (Table 5.1). Dockside experiments were conducted in the spring of 2019 and 2020 in Austevoll, Norway at the Institute of Marine Research station (60°05'12.9"N 5°15'51.5"E) Experiment 1 and 2 (Exp. 1, 2) were conducted in May and June of 2019, respectively. Experiment 3 (Exp. 3) was conducted in April of 2020. Blue mussels (*M. edulis*) (30-60mm) were collected from a local population and held at 3m depth from a dock at the

research station in hanging lantern nets for acclimation prior to all experiments. *M. edulis* were collected in February of 2019 (Exp 1. and 2.), and February 2020 (Exp. 3). All experiments used the same experimental set-up, in the same location. At least 24h prior to each experiment, 10 experimental mussels were removed from the lantern, cleared of epibionts, and measured for shell length. Mussels were then placed in individual flow-through chambers (See Strohmeier et al. 2009 for chamber design). The individual chambers were designed to ensure direct flow of water over the mussels and to avoid recirculation, preventing refiltration (Palmer and Williams 1980). The size of the rectangular chambers (internal measurements) are as follows: width of 3.8 cm, a length of 19.5 cm and a height of 8.1 cm. Two chambers had water flowing through them with no mussels, to serve as controls.

Table 5.1 Summary of environmental and *M. edulis* physiology data from all experiments. Values represent mean for environmental data and median for physiological data. ± indicates standard deviation, and the coefficient of variation (%) is shown in parentheses.

	Exp.1	**Exp. 2**	**Exp. 3**
Dates	May 07 – 11	June 04 – 08	April 06-13
Temperature (°C)	8.31 ± 0.16 (2)	10.51 ± 0.63 (6)	6.85 ± 0.14 (2)
Fluorescence (μg L^{-1})	0.67 ± 0.44 (66)	1.47 ± 0.47 (32)	2.99 ± 0.89 (30)
Suspended particulate matter (mg L^{-1})	1.68 ± 0.31 (18)	2.64 ± 0.52 (20)	1.92 ± 0.57 (30)
Energy (J L^{-1})	5.83 ± 1.74 (30)	11.00 ± 2.83 (26)	9.00 ± 1.87 (21)
Shell length (mm)	55.9 ± 1.6 (3)	59.5 ± 1.4 (2)	35.0 ± 2.5 (7)
Median pumping rate (L h^{-1})	2.0 ± 0.7 (35)	3.2 ± 0.4 (13)	3.1 ± 1.1 (35)
Median ingestion rate (μg h^{-1})	0.8 ± 1.2 (150)	4.4 ± 2.3 (52)	8.9 ± 4.1 (46)

Ambient, unfiltered seawater was pumped using an air pump (PlusAir: PA.15FVT) directly from the dock where mussels were being held to a water reservoir (600 L). From the water reservoir, seawater was gravity fed to a header tank located directly above the individual flow-through chambers. From the header tank, water was flowed through 12 individual chambers. Following (Filgueira et al. 2006) and Strohmeier et al. (2009), flow-rates were regulated to aim for 20-30% particle depletion of particles that are completely captured by mussels. Flow-rate through each chamber was measured a minimum of 4 times per day, and flow-rates were corrected as needed.

5.3.2 Water quality measurements

Water temperature (°C) and fluorescence (μgL^{-1}) measurements were taken every 30 minutes in the experimental water reservoir using a CTD (SAIV A/S Model 204). Water from the header tank was also filtered for suspended particulate matter (SPM; mgL^{-1}) and energy density (JL^{-1}). To do this, water filtered from a pressurized tank through pre-combusted and washed 1.2 µm 90mm filters (Whatman GF/D 2.0 µm pore width). Volumes filtered varied between 30-50L, depending on filtration rate. The timing of SPM and energy density measurements was similar for Exp. 1 and 2 and changed for Exp. 3 due to availability of filters. For Exp. 1 and 2, water from the header tank was filtered for SPM and energy density measurements once every 12h, with six replicates for each measurement. For Exp. 3, SPM and energy density were measured before and after the experiment (April 2 and 20, 2020) in replicates of 10 and 5, respectively. All filters were rinsed twice with 50mL of 0.5M ammonium formate to remove any salts and kept frozen until analyzed. To measure SPM concentration, filters were dried in a 60°C oven until weights were stable. Energy density measurements were estimated from filters as outlined in Strohmeier et al. *in prep*, using a bomb calorimeter (BC, IKA model C6000). Filters were dried at 60°C until stable weights were recorded, after which 500 mg of combustion aid (paraffin oil) was added to the filters to aid with complete combustion. Filters were combusted, and the measurement of temperature change (to the nearest 0.0001 K) was used to estimate energy density (JL^{-1}). Energy produced by the combustion aid and filter itself

were subtracted from overall energy density to report values of energy from the water column only.

5.3.3 Mussel physiology measurements

Feeding activity of *M. edulis* was measured as both PR and IR using the flow-through method (Palmer & Williams 1980, Filgueira et al. 2006, Strohmeier et al. 2009). This method relies on the accurate characterization of particles in the outflow of flow-through chambers (both from those containing a mussel, and empty control chambers). In this experiment, the outflow of each chamber was connected to a normally closed solenoid valve. When a valve was closed, the outflow from that chamber would be directed to a drain. When opened, the outflow from that chamber was directed to an electronic laser particle counter (PAMAS S4031GO, GmbH), through silicone tubing. The solenoid valves from each individual chamber were opened sequentially, to ensure that the outflow from only one chamber at a time was delivered to the PAMAS. Solenoid valves were controlled by an Arduino Nano (3.X) connected to a relay board. The outflow of each chamber was sampled by the PAMAS for 60 seconds (volumetric equivalent of 10mL), and then the particle counter was flushed for 30 seconds with the outflow of the following chamber before the next sample was recorded. This flushing period was employed to clean the PAMAS between samples. For Exp. 1 and 2, PR and IR measurements were taken on each individual and control every 18 minutes, and for Exp. 3 measurements were taken on each individual every hour.

The PAMAS estimates particle size as equivalent spherical diameter (ESD, µm), and uses light scattering to count particles by size class at predefined intervals (0.5 µm in this study). From the estimates of particle counts for distinct size classes, both PR and IR were estimated. Pumping rate was estimated as:

$$PR = \left(\frac{P_c - P_b}{P_c}\right) \times FR \qquad 5.1$$

Where PR is pumping rate (Lh^{-1}), P_c is the count of particles exiting the control chamber, and P_b is the number of particles exiting the experimental chamber containing a bivalve, and FR is flow-rate through the chamber (Lh^{-1}) (Strohmeier et al. 2015). P_c and P_b were calculated using only particles understood to be completely captured on the gills (7.25, 7.75, and 8.25 µm ESD) (Steeves et al. 2022 *in press*). Three size classes were used to minimize the potential error from a single particle size count. Although larger particles (>8.25 µm ESD) are also expected to be completely captured, the abundance of these particles in the natural seston was low and were excluded to avoid introducing error into the calculation of PR. Chambers were monitored for pseudofaeces production during all experiments, and none was observed at any time.

Pumping rates of individual mussels were standardized to gill area following (Steeves et al. 2020):

$$PR_{std} = PR \times (\frac{GA_{std}}{GA_{ind}}) \quad 5.2$$

Where PR_{std} is the standardized PR, GA_{std} is the gill area for the averaged size mussel from all experiments (46 mm, 22.38 cm^2) and GA_{ind} is the gill area for the individual mussel being standardized. Gill area was measured for all mussels in Exp. 1 and 2. Mussels were dissected by severing the anterior and posterior adductor muscles with a scalpel and separating both shell halves. In one half shell, gills were exposed by removing inner organs and mantel (Sunde 2013). The gills were then floated in seawater to avoid contraction, and a photograph was taken from a top-down view. The area of one gill was then measured in ImageJ (v. 1.52 f), and multiplied by 8 (accounting for 4 gills, with 2 sides each), resulting in a total gill area of cm^2. For Exp. 3, no gill area pictures were taken, and gill area estimates were made from shell length following the relationship between length and gill area previously established for the same population of mussels: Gill Area [cm^2] = 0.0004 x length [mm]$^{2.85}$, $r^2 = 0.79$, n = 27 (Steeves et al. 2020).

PR_{std} measurements were subsequently corrected for variations in temperature using an Arrhenius function (Kooijman 2010):

$$PR(T)_{std} = PR_1 \times \exp\left(\frac{T_A}{T_{AL}} - \frac{T_A}{T}\right) \times \frac{s(T)}{s(T_1)} \quad 5.3$$

$$s(T) = (1 + \exp\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right) + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right))^{-1}$$

Where $PR(T)_{std}$ is the PR_{std} corrected to temperature T, T is the absolute temperature (218.15K or 8°C), T_1 is the reference temperature (K), PR_1 is the uncorrected PR at T_1, T_A is the Arrhenius temperature (5800K), and T_{AL} (45430K) and T_{AH} (31376K) are the rates of PR decrease at the lower and upper temperature boundaries, respectively. T_L (275K) and T_H (296K) are the upper and lower temperature tolerance range, respectively. All Arrhenius parameters were obtained from van der Veer et al. (2006).

Ingestion rate was estimated from both PR and F values from the CTD as:

$$IR = PR(T)_{std} \times F \quad 5.4$$

Where IR is ingestion rate ($\mu g h^{-1}$) calculated using PR standardized to both gill area and temperature, and F is fluorescence (proxy for chlorophyll *a*) in $\mu g L^{-1}$. This calculation of IR is valid for conditions in which there is no production of pseudofaeces.

5.3.4 Statistical Analyses

All statistical analyses were performed in R version 3.6.2 (RStudio version 1.4.1717). For periods during experiments where two control measurements were not reliably collected (e.g., if water was not sufficiently sampled from the outflow of the control chamber and air was introduced into the PAMAS, artificially reducing particle counts), all PR data were removed. If PR values for an individual mussel were unreasonably high (e.g., P_b counts ~0) it was assumed that no outflow water was being sampled by the PAMAS and PR data for that individual was removed. For one sampling

period (Exp. 1 and 2: 18 minutes, Exp.3: 1 hour) if fewer than 6 mussels were successfully sampled, all data were removed. Due to limitations in the precision of the particle counter, if $PR(T)_{std}$ was <0.2 Lh^{-1}, values were considered indistinguishable from 0 and the data were replaced with 0 but included in the data set.

Within each experiment, median PR, IR, and chlorophyll *a* concentration (fluorescence) was visualized by fitting a locally estimated scatterplot smoothing (LOESS) regression (Cleveland & Devlin 1988). For this regression, low-degree polynomials are fit to subsets of the data using weighted least squares. The size of the subsets of the data are determined using a smoothing parameter (α), which is a fraction of the number of datapoints. In this study, α =0.1, resulting in low-degree polynomials being fit to the data every ~10h. For the LOESS regression, PR, IR and chlorophyll *a* datasets were interpolated with a linear function.

5.4 RESULTS

5.4.1 Environmental Conditions

Environmental conditions varied between all experiments from April to June following a seasonal trend (Table 5.1). Mean temperature values ranged between 6.9 and 10.5 °C, with values being lowest in April (Exp. 3) and highest in June (Exp. 2). Mean chlorophyll *a* concentrations varied from 0.7 (Exp. 1) to 3.0 μgL^{-1} (Exp. 3; Table 5.1). Suspended particulate matter (mgL^{-1}), and energy density (JL^{-1}) had similar trends with lowest values in Exp. 1 (1.7 and 5.8, respectively) and highest values in Exp. 2 (2.6 and 11.0, respectively; Table 5.1).

5.4.2 Pumping Rate

M. edulis in Exp. 1 had the lowest median population-level PR ($2.0Lh^{-1}$), with values ranging between 0.1 and $3.6Lh^{-1}$ (Table 5.1, Figure 5.1A). Notably, the population median PR was lowest between May 9 and 10 (Figure 5.1A). To further examine the variability in the population PR, examples of mussels with mussels high and low interquartile range (IQR) in PR were analyzed (Figure 5.1B). At the same point in time, the PR between two

mussels varied as much as ~3Lh^{-1}, which was particularly noticeable at the end of the experiment (May 11) (Figure 1B). Although both mussels periodically stopped pumping (PR=0), the timing and frequency of closures varied between individuals (Figure 5.1B). Additionally, some individuals had relatively stable PRs compared to others (Figure 5.1C), with the coefficient of variation in PR ranging from 28 to 162%. Overall, some individuals pumped at higher rates than others, with average PRs ranging from 0.8 to 2.8Lh^{-1} (Figure 5.1C). Further, the degree of variability in PR, as shown by interquartile range in Figure 5.1C, was different between individuals.

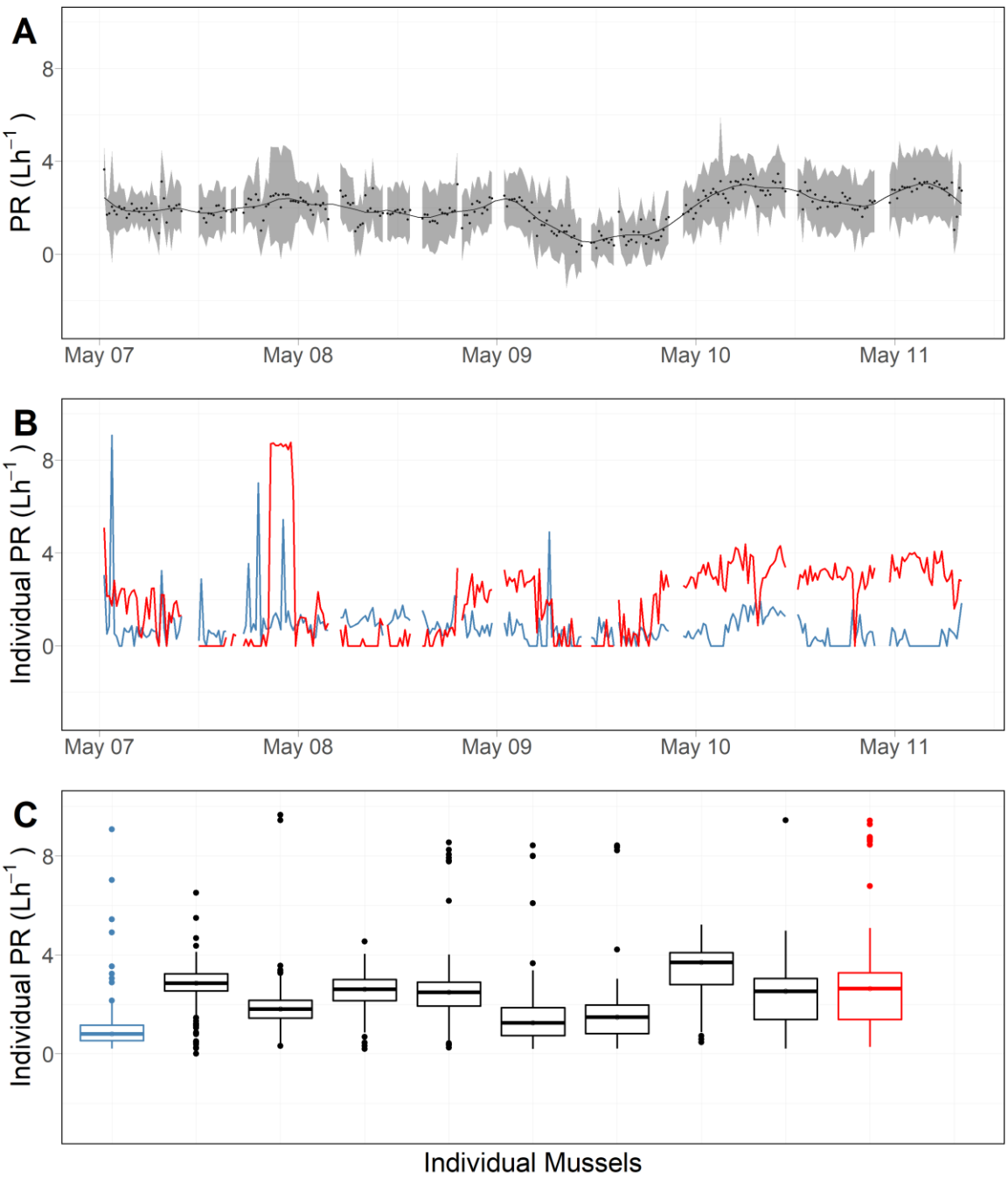


Figure 5.1 Summary of pumping rate (PR) (Lh^{-1}) data from Exp.1: A. Median PR of all individuals ± SD over 4 days; B. Individual PR of two mussels with lowest (blue) and highest (red) interquartile range in PR; C. Boxplots of PR of each individual averaged for the entire duration of Exp. 1.

M. edulis in Exp. 2 had the highest population-level median PR ($3.2 Lh^{-1}$), which was also the most stable of all experiments, ranging between 1.2 and $4.0 Lh^{-1}$ (Table 5.1, Figure 5.2A). In Exp. 2, one individual was excluded from the population median PR calculation, as PR was often not distinguishable from zero (Figure 5.2C, indicated with an asterisk). In general, there was no extended period of time (e.g., days) over which the median population PR was generally higher or lower (Figure 5.2A). In examining the PR of the individuals with high and low IQR in PR (Figure 5.2B), it was observed that the individual with the low IQR had a highly stable PR over 4 days. This mussel pumped consistently at an intermediate rate of ~ $3 Lh^{-1}$, with few interruptions, until the end of the experiment. Contrastingly, the individual with the high IQR showed generally high PRs for the first 3 days of the experiment (~$5 Lh^{-1}$), and low around the 4^{th} day (~$2 Lh^{-1}$). This mussel abruptly stopped pumping several times during the first two days of the experiment for short periods of time, before returning to a relatively high PR (~4 Lh^{-1}) (Figure 5.2). Towards the end of the experiment, this mussel had more gradual changes in PR, occurring over the course of several hours. Similar to Exp. 1, at a single point in time there was at times a ~3 Lh^{-1} difference in PR between two individuals (Figure 5.2B). Variability in PR within individuals was generally lower than Exp. 1, with coefficient of variation in PR ranging from 11 to 91% (Figure 5.2C). Some individuals pumped at higher rates than others, with average PRs for each individual ranging from 2.0 to $3.7 Lh^{-1}$ (Figure 5.2C).

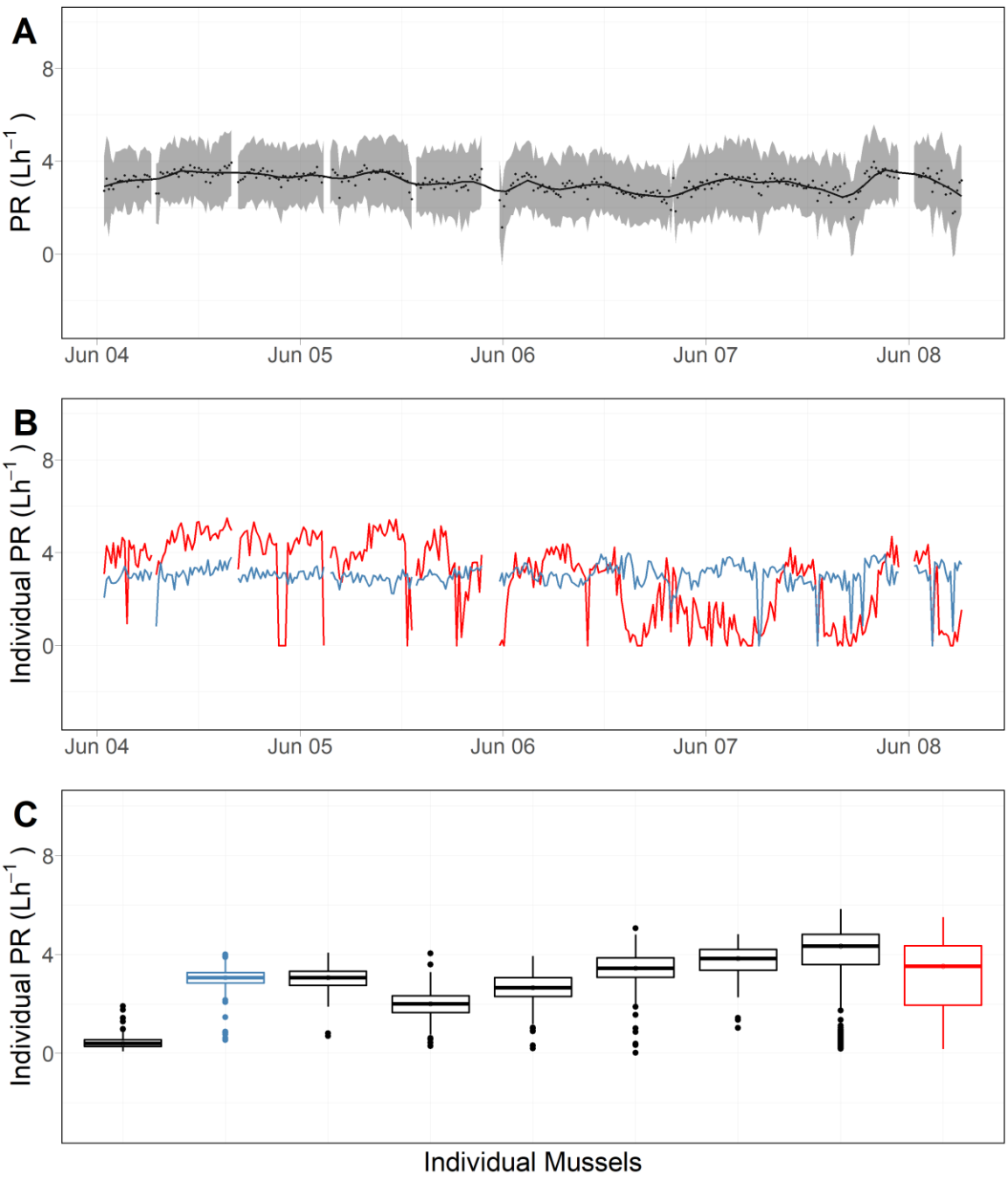


Figure 5.2 Summary of pumping rate (PR) (Lh^{-1}) data from Exp.2: A. Median PR of all individuals ± SD over 4 days; B. Individual PR of two mussels with low (blue) and high (red) interquartile range in PR; C. Boxplots of PR of each individual from the entire duration of Exp. 2.

M. edulis in Exp. 3 had a similar median population-level PR to Exp. 2 ($3.1 Lh^{-1}$); however, the variability in PR was markedly higher than the first two experiments, both between and within individuals (Table 5.1, Figure 5.3A). The median population PR ranged from 1.0 to $7.5 Lh^{-1}$ (Figure 5.3A). Similar to Exp. 2, there was no extended periods of high or low median population PRs, but PRs were generally variable over the 4 days. When examining the individuals with high and low IQR in PR, there was a marked difference between their PRs during the experiment. Although there were three mussels with lower IQR in PR (Figure 5.3C), the fourth lowest individual was selected to highlight in Figure 5.3B as this individual had a more complete PR dataset during the experiment. The mussel with the low IQR in PR pumped at low rates over the course of the experiment ($1.3 \pm 0.9 Lh^{-1}$), compared to the mussel with the highest IQR in PR ($6.1 \pm 2.4 Lh^{-1}$) (Figure 5.3B, C). The high level of variability in the mussel pumping at 6.1 Lh^{-1} was driven by a decrease in PR over the last several days of the experiment (Figure 5.3B). Further, at a single point in time there was a difference ~7 Lh^{-1} in PR between two individuals (Figure 5.3B). Variability in PR within individuals was generally lower than Exp. 1, with coefficient of variation in PR ranging from 31 to 135% (Figure 5.3C). Similar to the first two experiments, some individuals pumped at higher rates than others, with average PRs ranging from 0.5 to $6.1 Lh^{-1}$ (Figure 5.3C).

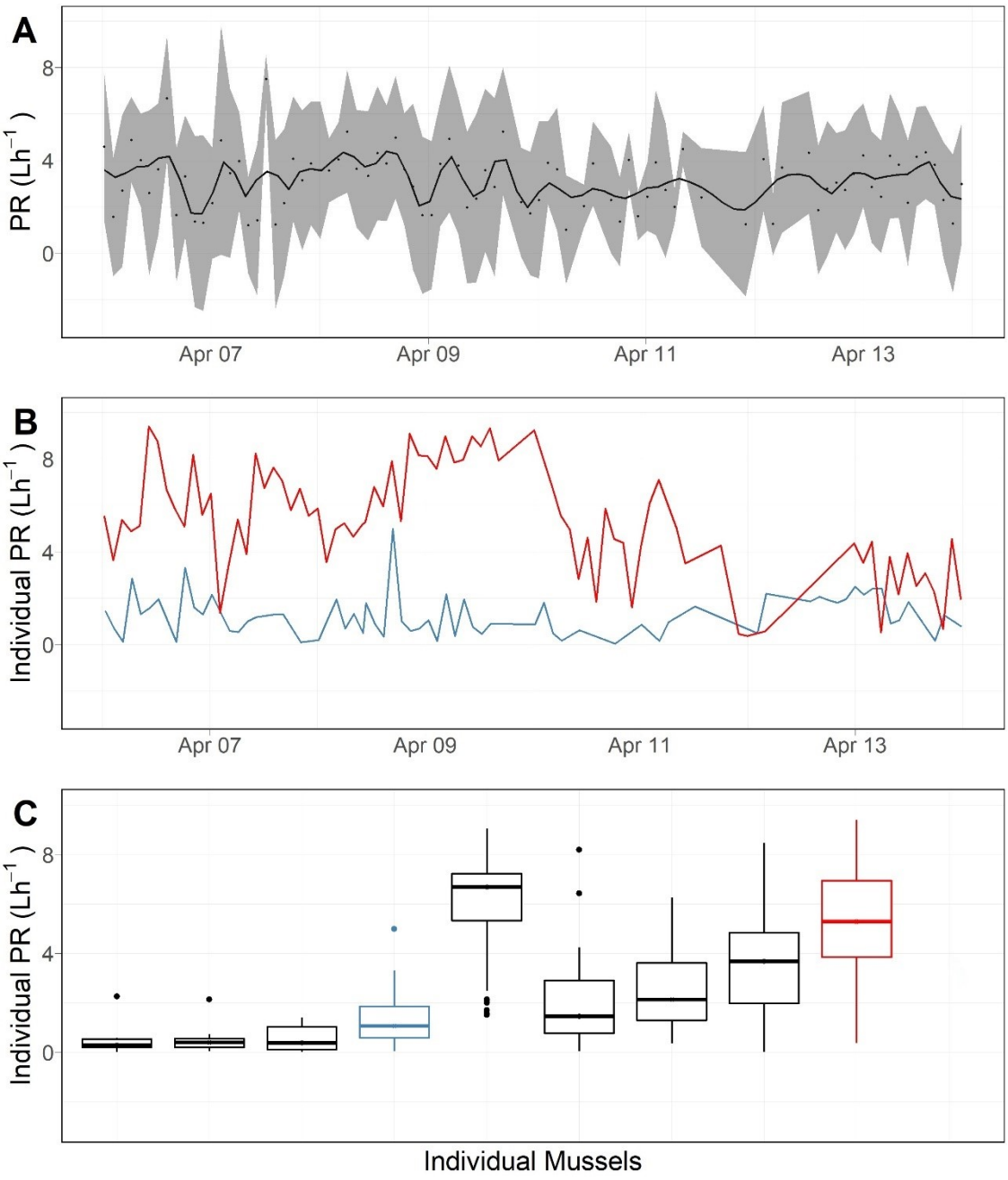


Figure 5.3 Summary of pumping rate (PR) (Lh^{-1}) data from Exp.3: A. Median PR of all individuals ± SD over 4 days; B. Individual PR of two mussels with low (blue) and high (red) interquartile range in PR C. Although the mussel wit the low interquartile range (blue) in PR is not the lowest, it did have a more complete PR dataset and was selected for visualization purposes. Boxplots of PR of each individual from the entire duration of Exp. 3.

5.4.3 Ingestion Rate

In Exp. 1, the population-level median IRs of *M. edulis* were the lowest of all experiments (0.8 $\mu g h^{-1}$) (Table 5.1, Figure 5.4A). Ingestion rate in Exp. 1 closely followed the pattern of median PR over time, with low rates between May 9 and 10, and rising on May 11, matching the increase in PR (Figure 5.4A). The variability in population IR was highest in Exp.1, with a coefficient of variation of 85%; however, the range was lowest (4.3 $\mu g h^{-1}$) (Table 5.1, Figure 5.4A). Exp. 2 had the second highest population level median IR (4.4 $\mu g h^{-1}$), lowest variability (coefficient of variation: 36%), and doubled the range of Exp. 1 (8.8 $\mu g h^{-1}$) (Table 5.1, Figure 5.4A). In Exp. 2, IR more closely followed the trend of fluorescence compared to PR over time, with a marked decrease in IR at the end of June 6, and an increase early on June 7, matching the pattern of fluorescence (Figure 5.4B). In Exp. 3, population level median IR was the highest (8.9 $\mu g h^{-1}$), the second most variable (coefficient of variation: 45%) and had the highest range (17.4 $\mu g h^{-1}$) (Table 5.1, Figure 5.4C). Additionally, IR did not follow the pattern of either PR or fluorescence for the entire duration of the experiment (Figure 5.4C). Between April 8-9, IR closely followed the fluctuating pattern of PR; however, during the beginning and end of the experiment, IR closely followed the patterns in fluorescence (Figure 5.4C).

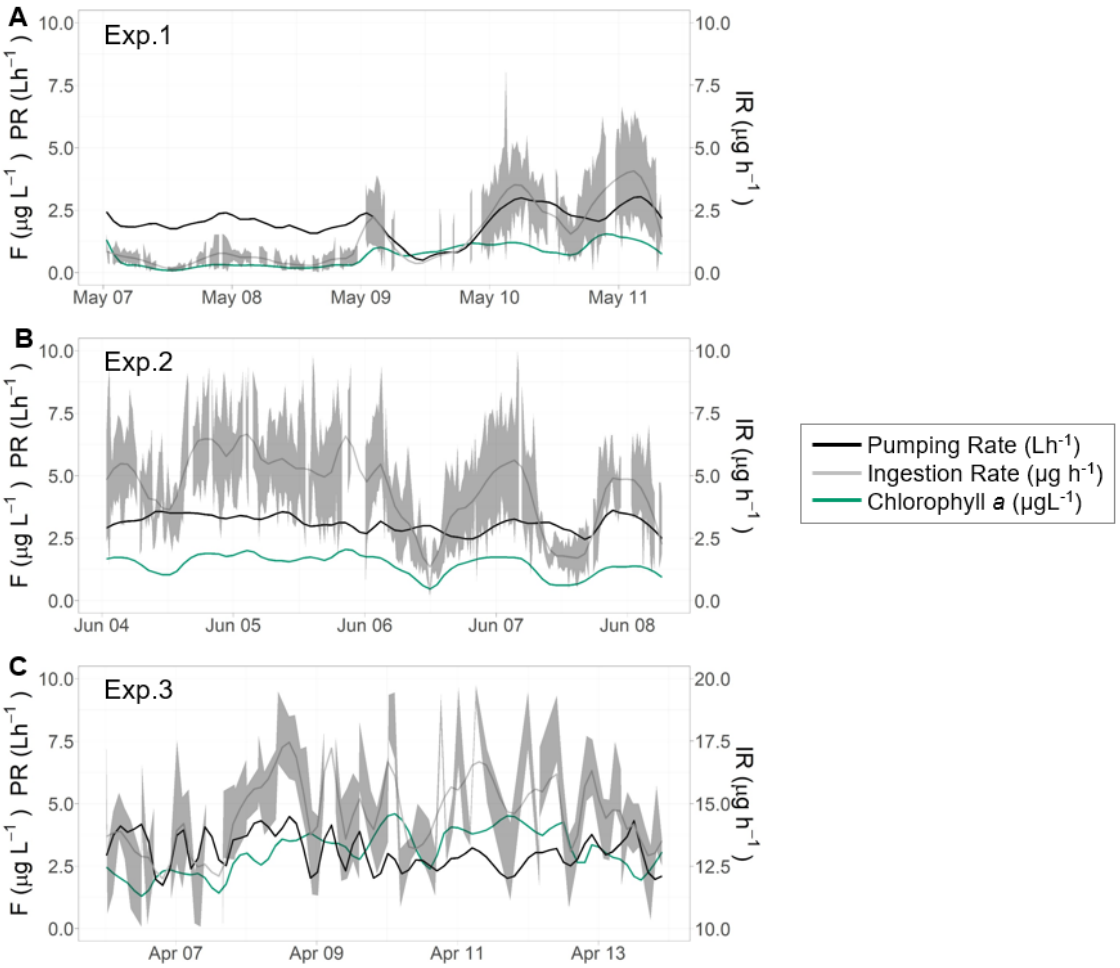


Figure 5.4 Pumping rate (PR) (Lh^{-1}) (black), chlorophyll *a* (F) (μgL^{-1}) (green), and ingestion rate (IR) (μgh^{-1}) (gray) for A. Exp. 1, B. Exp. 2. C. Exp. 3. The gray shaded area is the standard deviation for IR.

5.4.4 Functional responses to food availability

To examine the relationships between PR, IR, and food availability (chlorophyll *a*), the population-level results from all experiments were combined (Figure 5.5). When considering the population level response in PR to chlorophyll *a* in all the experiments, no consistent trends were observed (Figure 5.5A). PR generally did not increase with increasing chlorophyll *a*; however, interindividual variability in PR increased at higher fluorescence levels (>2μgL^{-1}) (Figure 5.5A). For all experiments, population-level IR

generally increased with increasing fluorescence (Figure 5.5B). At low concentrations of chlorophyll *a* (<2 μgL^{-1}), IR increases were highly linear with chlorophyll *a*; however, as chlorophyll *a* concentration increased beyond 2 μgL^{-1}, the increase in IR became less linear (Figure 5.5B). Further, interindividual variability in IR increased in each subsequent experiment with increasing concentrations of chlorophyll *a* (particularly when chlorophyll *a* was >2 μgL^{-1}) (Figure 5.5B). The relationship between IR and increasing chlorophyll *a* was visualized with Holling functional responses (Type I, II, and III) to illustrate the potential response in IR being either linear or asymptotic.

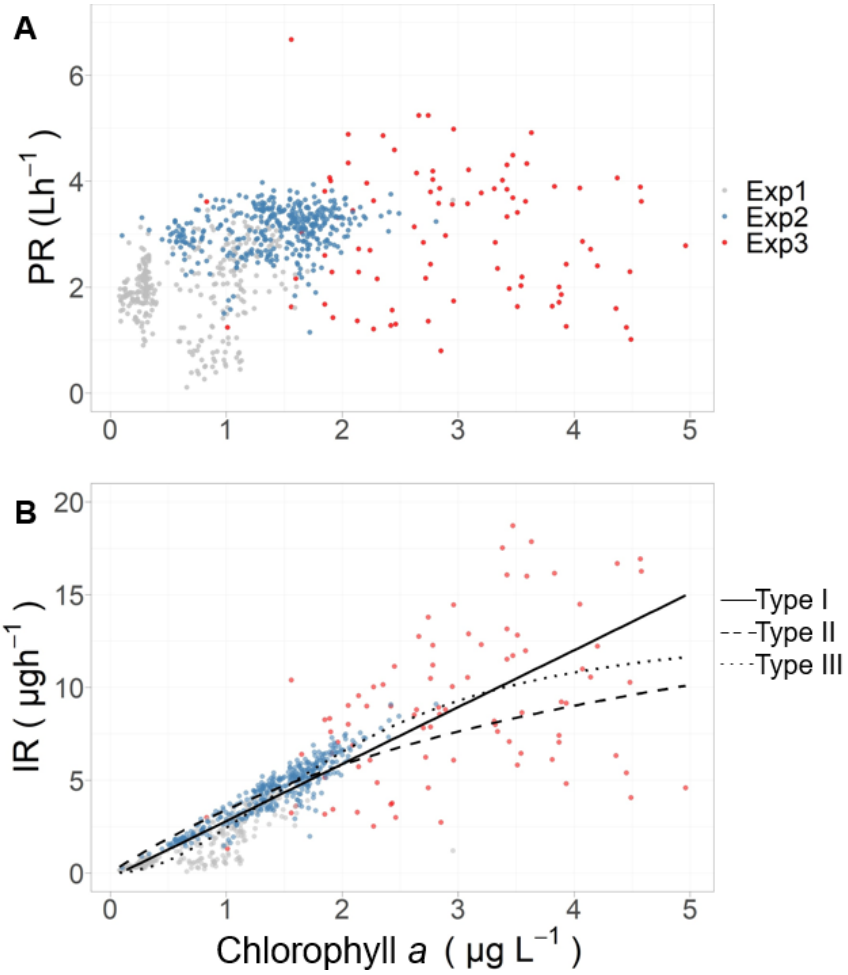


Figure 5.5 Relationships between (A) pumping rate (PR) (Lh^{-1}) and (B) ingestion rate (IR) (μgh^{-1}) and chlorophyll *a* (μgL^{-1}) for all experiments. Drawn lines on (B) represent Holling functional responses (Type I, II, and III, solid, dashed, and dotted, respectively) to indicate potential relationships between chlorophyll *a* and IR.

5.5 Discussion

This study used a novel flow-through methodology to measure feeding (pumping and ingestion rates) in *M. edulis* in response to natural fluctuations in diet. Although it has previously been hypothesized that bivalves alter pumping rates to maintain relatively constant ingestion rates, these compensatory processes were not observed in this study. Pumping rates displayed no consistent response to changes in food availability, as measured by chlorophyll *a*. Subsequently, ingestion rate generally increased with

increasing food availability. The high frequency of pumping and ingestion rate calculation made in this study permitted the exploration of both intra- and interindividual variability on a much finer temporal scale (minutes) compared to previous studies (hours/days/weeks). High levels of variability in pumping and ingestion rates were observed both between and within individuals during these 4-day experiments.

5.5.1 Feeding activity in response to natural fluctuations in diet

The range of PRs recorded in this experiment (mean ± standard deviation: 3.0 ± 1.8 Lh^{-1}) are similar to those reported for *M. edulis* in similar environmental conditions (Strohmeier et al. 2009, 2012, Cranford et al. 2016, Steeves et al. 2020b). Food concentration (or *diet quantity*) was characterized by chlorophyll *a* concentration and increased with each subsequent experiment from ~1 to ~3 μgL^{-1}, which is within the range of values commonly reported during spring in this region (Erga 1989, Frette et al. 2004, Strohmeier et al. 2015). In all experiments, PR generally was not related to changes in food concentration. Food concentration is understood to be a primary determinant of feeding rates in bivalves, where feeding is initiated at a minimum food concentration and continues to increase to a maximum rate as food concentration increases (Bayne et al., 1993); finally, at food levels above a saturation threshold, feeding rates often decline, to avoid overloading the gills, or because maximum ingestion rate may have been reached (Filgueira et al., 2009; Navarro et al., 1992). Although a cessation in PR of mussels has been observed at low food concentrations (<0.5 $\mu g\ L^{-1}$, Pascoe et al., 2009), a previous study on the same population of *M. edulis* used in this study observed PRs between 2.5-4.7 Lh^{-1} at very low chlorophyll *a* concentrations (0.1-0.6 μgL^{-1}) (Strohmeier et al. 2009). Further, a decline in PR was not expected as food concentrations (< $3\mu gL^{-1}$) did not reach the saturation threshold expected to trigger a reduction in feeding rates (Filgueira et al. 2009, Riisgård et al. 2011). Therefore, the lack of relationship between population-level PR and chlorophyll *a* in any of the 4-day experiments is not unexpected for the low levels of fluorescence observed in this study.

In this population of *M. edulis*, relatively stable PRs have also been observed despite changes in a diet of similar quantities (chlorophyll *a* concentration) (Strohmeier et al., 2009). It is possible that the lack of relationship between PR and chlorophyll *a* observed

in this experiment indicates that for individuals adapted to maximize ingestion rates in low seston environments, PR is initiated at very low food concentration, and remains high as food concentration increases (Strohmeier et al. 2009). Bivalves inhabiting low-seston environments have often been observed to have very high feeding rates in field studies (Hawkins et al. 1998, Pouvreau et al. 1999, 2000, Strohmeier et al. 2009). At chlorophyll *a* levels much higher than those observed in this study (> 3 μgL^{-1}) PR of *M. edulis* may decline; however, these conditions are not frequent in this region (Erga 1989, Frette et al. 2004, Strohmeier et al. 2015). It has previously been recognized by Cranford et al. (2011) that the strategy of bivalves to regulate the amount of ingested material may vary by species, wherein *M. edulis* has often been observed to regulate ingestion through pseudofaeces production, while continuing to pump at high rates (Foster-Smith 1975, Smaal et al. 1997, Hawkins et al. 1998). As the range in diet observed in this study remained under the threshold for the production of pseudofaeces, it is likely that the mussels were continuing to pump at high rates. The lack of relationship between PR and chlorophyll *a* levels observed in this experiment may also indicate that for short-term fluctuations in diet quantity, a physiological response in PR may not be elicited. This "time-averaged" behaviour may be an explanation for why PRs do not change in response to diet changes that only last on the scale of minutes to hours (Cranford et al. 2011).

Aspects of diet composition (or *diet quality*) that may affect feeding rates include seston load, and fraction of non-digestible inorganic material (Filgueira et al., 2010; Hawkins et al., 1999; Iglesias et al., 1992; Montalto et al., 2017; Rueda and Smaal, 2002). By characterizing the diet using chlorophyll *a*, some qualitative aspects of the diet known to influence PR may not be captured (Velasco & Navarro 2002, 2005). Although chlorophyll *a* concentration increased from Exp. 1 to Exp. 3, the highest concentrations of suspended particulate matter and energy were observed in Exp. 2, indicating that diet quality was also changing between experiments. Although chlorophyll *a* concentration does not comprehensively describe the available diet, it is easily measured with high temporal frequency, compared to the more time-intensive methods required for the filtration of water for SPM and energy concentration (Vajedsamiei et al. 2021). Resultingly, high temporal resolution measurements of chlorophyll *a* concentration may

provide one of the best available methods to take measurements of diet and feeding physiology on similar temporal scales.

The functional response of ingestion rate (IR) to food concentration in bivalves has been previously described using different Holling functional responses. Most commonly used are the Type II and III functional responses which are characterized by stable IRs at high food concentrations (Picoche et al. 2014, Montalto et al. 2017). The population-level IR in this experiment generally increased with increasing chlorophyll *a* concentration; however, this relationship had the highest slope when food concentration was low (<2 μgL^{-1}). The population-level response in IR to increasing food concentrations in this study suggests that any of the Holling functional responses may statistically represent the observed relationship. However, the data collected in this study is heavily concentrated with observations at low food concentrations (<2 μgL^{-1}), compared to higher concentrations (~2-5 μgL^{-1}), which limits the ability to estimate an asymptotic relationship. Although a stable ingestion rate at high food levels has been previously hypothesized (Holling Type II and III) (Winter 1976, Navarro & Winter 1982, Bayne et al. 1989, Navarro & Widdows 1997), it is likely that food levels in this experiment did not reach high enough concentrations to observe maximum and constant ingestion rates. As previously described, it is possible that the strategy of individuals adapted to low-seston environments may be to continuously pump at a high rate, resulting in increasing ingestion rates with increasing food concentration (Strohmeier et al. 2009).

Despite the lack of clear stabilization of ingestion rates at high food concentrations, the observations revealed increasing levels of inter-individual variability in both ingestion and pumping rates at high chlorophyll *a* concentrations. This variability in feeding physiology at increasing food concentrations may indicate the periodic stopping or slowing of feeding driven by digestive processes (e.g., gut capacity being reached, maximum ingestion rate being reached) (Holling 1966, Hawkins & Bayne 1984, Bayne et al. 1987, Willows 1992). Accordingly, it is possible that an asymptote in ingestion rates reflective of a Holling Type II or III response may emerge at higher food concentrations (e.g., > 3 μgL^{-1}) if periodic slowing or stopping of PR becomes more frequent at the population-level.

5.5.2 Intra- and interindividual variability in feeding activity

The high temporal resolution of the methodology used in this experiment was selected to be able to examine both intra- and interindividual variability in pumping and ingestion rates in response to real-time fluctuations in diet. By observing the range of physiological rates within an individual over the scale of hours and days, it is possible to more accurately observe short-term fluctuations in feeding physiology in response to environmental variability in terms of food quantity and quality (Frechette & Bourget 1987, Cranford et al. 1998). In previous studies, when physiological rates have been measured only one time per individual, or repeatedly on an individual with coarse temporal resolution, it is possible to overlook the full range of intra- and interindividual variability over short timescales (Vajedsamiei et al. 2021).

Inter-individual variability was observed during each 4-day experiment between the PRs of individual mussels. Despite being exposed to the same conditions, the average PR of the mussels ranged ~3Lh^{-1} between individuals. Inter-individual variability in physiological rates, including feeding rates, has been explored as a potential explanation for different growth rates between fast- and slow-growing individuals (Bayne et al. 1999b), and similar inter-individual variability in feeding rates of bivalves exposed to the same conditions has been observed in other studies (Tamayo et al. 2011, Fuentes-Santos et al. 2018). In this experiment, differences between experimental individuals were minimized by selecting *M. edulis* of the same size and age-class from the same location. The goal in selecting similar individuals was to minimize differences in inter-individual variability driven by factors not examined in this study. However, it is possible that there were differences between the *M. edulis* used in this study that were not accounted for, including sex (potentially influencing energetic requirements), genetic differences, and maternal effects (Hawkins et al. 2000, Fernández-Reiriz et al. 2015, Griffith & Gobler 2017, Zhang et al. 2019). Future experiments may consider further minimizing differences between individuals by rearing first generation offspring together in a common conditions (e.g., de Villemereuil et al., 2016), or by increasing the duration of the experiments to observe if average physiological rates between individuals are similar across longer periods of time (e.g., seasonally, or annually).

Intraindividual variability was observed in all experiments, where PR and IR varied within individuals over the 4-day periods. Variability in the feeding physiology of bivalves may be driven by changes in environmental conditions, including those previously discussed (e.g., temperature, diet) (Jørgensen 1990, Clausen & Riisgård 1996, Hawkins et al. 1996). However, the periodic cessation of feeding in *M. edulis* observed in this study was unsynchronized between individuals, suggesting that feeding rates may have been regulated by internal drivers rather than external environmental conditions. For example, if gut capacity is reached, feeding rates may slow down; however, gut capacity may not be reached at the same time for all individuals (Rueda and Smaal, 2002; Willows 1992). The high temporal resolution of the PR data presented here indicates that PR activity varies between individuals in terms of how consistent PR is over time, and how quickly PR may increase or decrease (e.g., on the scale of minutes to hours). These results suggest that there is a broad range of PR activity between individuals exposed to the same conditions, and that for these conditions, may not be driven by environmental factors. Further, these data do not suggest that there is a consistent or synchronized on/off response in PR in all individuals. Observing intraindividual variability in the feeding physiology of *M. edulis*, and characteristics of the natural diet at high temporal resolution provides insights into the drivers of feeding physiology of bivalves.

5.5.3 Energy acquisition

Fluorescence is used in this study to estimate chlorophyll *a* as a proxy for food concentration; however, chlorophyll *a* is limited as a proxy for the amount of food that is captured and ingested from the seston by *M. edulis.* Chlorophyll *a* alone is not able to capture the complexity of the seston in terms of particle sizes and surface properties, which both may affect particle capture efficiency. Capture efficiency describes the proportion of particles captured on the gill, compared to those in the water (Rosa et al. 2018), and is often described according to particle size, where capture efficiency increases with increasing particle size to some maximum, beyond which all particles are captured (Coughlan 1969, Møhlenberg & Riisgård 1978). However, capture efficiency has also been related to other particle characteristics including hydrophobicity (Rosa et al. 2017), lectin-carbohydrate

interactions (Pales Espinosa et al. 2009), and fluorescence (Yahel et al. 2009). Additionally, capture efficiency has been observed to vary in *M. edulis* across seasons in response to natural seston composition (Strohmeier et al. 2012, Steeves et al. 2020). As IR is described in this experiment using chlorophyll *a*, if changes in capture efficiency occurred, it would not be accounted for in estimates of ingestion. Further, estimation of ingestion rate using chlorophyll *a* instead of the total volume of ingested material, may not be used to estimate gut capacity, which may limit maximum ingestion rates (Rueda and Smaal, 2002; Willows 1992).

It has been theorized that as the quality and quantity of their diet changes, bivalves will make use of behavioural and physiological mechanisms to maintain constant energy uptake (Bayne et al., 1993; Widdows et al., 1979; Willows, 1992; Winter, 1976). Although in this study constant ingestion rates were not observed as food concentration changed, it is possible that other mechanisms were employed to maximize energy uptake. Specifically, changes in digestive processes may contribute to constant levels of energy absorption despite variability in the quantity and quality of diet in the digestive system (Bayne et al. 1987, 1988, Navarro et al. 1994, Ibarrola et al. 1998a). For example, changing in digestive enzyme activity may increase absorption efficiency of bivalves acclimated to low quality diets (Ibarrola et al. 1998b). In addition, gut passage time may increase in response to diets of low quality to prolong the time available for digestion and absorption of nutrients (Ibarrola et al., 1998a). The relationships between digestive processes and diet quantity and quality are complex, particularly as natural diets may fluctuate on both short- and long-term timescale; however, they have been empirically modelled (Willows 1992, Scholten & Smaal 1998, 1999). Changes in digestive processes may contribute to stable energy uptake, despite variations in ingestion rate.

5.5.4 Conclusions

This study examined the functional relationships between pumping and ingestion rate in *M. edulis* in response to changes in the diet concentration in a low-seston environment. Results indicated that there were no clear relationships between population-level pumping rate and food concentration, measured as chlorophyll *a*, and resultingly,

ingestion rate increased with increasing food concentration. Using novel methodology that permitted the measurement of feeding activity with high temporal resolution, approximately every 20 minutes, this study highlights the variability in physiological rates both between and within individuals exposed to the same environmental conditions. Both intra- and interindividual variability in pumping and ingestion rates were observed in all experiments. Understanding the range of both intra- and interindividual variability in physiological rates is beneficial when scaling physiological rates from the individual to population level, and for estimating interactions between suspension-feeders and food source.

CHAPTER 6 DISCUSSION

6.1 MAJOR FINDINGS

This thesis examined plasticity in the feeding physiology of suspension-feeding marine bivalves. The focus of interspecific plasticity was examined both broadly in terms of energy acquisition and expenditure processes (**Chapter 2**) and specifically in terms the relationships between pumping rate and capture efficiency (**Chapter 3**). In the examination of intra-specific plasticity, specific focus was given to the blue mussel *Mytilus edulis* in low-seston environments (**Chapter 3 and 4**).

Chapter 2 examined the contributions of plasticity and adaptation to the fundamental physiological processes that determine how suspension feeding marine bivalves acquire (feeding, digestion, absorption) and use (metabolic rate) energy to determine growth potential. This chapter synthesized the limits of plasticity in the physiology of these highly plastic species and made recommendations about how to design experiments to appropriately assess the role of plasticity and adaptation. Experiments that make use of reciprocal transplants, or common garden experiments, in combination with genetic analyses are the best experimental tools available to assess plasticity and adaption in the physiology of marine bivalves. **Chapter 3** more specifically examined inter-specific plasticity in two primary metrics feeding physiology: pumping rate and capture efficiency. Primary findings from this chapter indicate that the relationship between pumping rate and capture efficiency is dependent upon species and particle size. Increases in pumping rate increased particle capture efficiency only in the oyster *Crassostrea virginica*, for small particles between ~2-8 µm ESD. However, no relationship was observed between pumping rate and capture efficiency in either the mussel *Mytilus edulis* or the scallop *Placopecten magellanicus*. These diverse species were selected as they belong to three distinct families of bivalves, with different characteristic gill morphology. This finding implies that both how bivalves acquire food, and how bivalves interact with the seston, may in some cases be dependent on pumping rate and gill structure.

Chapter 4 examined how primary metrics of feeding physiology (pumping rate, capture efficiency, and ingestion rate) varied both between and within populations of *M. edulis*. Between three populations of *M. edulis*, and within two populations along a fjord

gradient, differences in pumping rate, capture efficiency, and ingestion rate were observed. To further determine if these differences were driven by short-term (plastic) or long-term (adaptive processes), a single population of mussels was transplanted in a fully-crossed experiment along a fjord gradient and feeding physiology was re-measured. We observed that capture efficiency of small particles ($\sim < 5$ μm) changed within populations of mussels when moved along a fjord gradient on a short-term time-scale (3 weeks). This finding suggests that capture efficiency may change in the short term, and be driven by environmental conditions, rather than adaptations between populations. On the same time-scale, less clear acclimation in pumping and ingestion rates were observed, suggesting that perhaps these processes require more time for a plastic response to be observed, or are also driven by adaptive processes. **Chapter 5** further examined the feeding physiology of *M. edulis* with a focus on inter- and intra-individual variability of the relationships between food concentration, pumping rate, and ingestion rate. Observations of feeding physiology in this chapter were taken at a very high temporal resolution (every 20 minutes) providing novel observations of individual feeding rates with high frequency. Results indicated that for the population of mussels used in this experiment in low-seston environments, there was no apparent relationship between pumping rate and food concentration, and resultingly, ingestion rate increased with increasing food concentration. Differences in pumping rates were observed both between individuals exposed to the same environmental conditions, and within individuals over the duration of 4-day experiments. Further, the level of inter-individual variability increased with increasing food concentration.

The mechanisms of suspension-feeding examined in this thesis contributes to the understanding of how economically and ecologically important bivalve species acquire energy and interact with their ecosystems. The feeding physiology of bivalves, and the extent to which it varies both between and within species is important to understand to be able to identify the ecological roles that bivalves play, and to avoid detrimental impacts from farming and fishing bivalves. Bivalve fisheries and aquaculture contribute to the economy of coastal communities while providing a source of sustainable low-tropic protein. The feeding physiology of bivalves (pumping rate, capture efficiency, and ingestion rate) is cornerstone to understanding both the condition of wild populations as natural food availability changes, and the carrying capacity of bivalves grown in

aquaculture farms. Ecologically, bivalves may act as fundamental links between the pelagic and benthic environments, a process dependent upon the capture, selection, and ingestion of seston from the water column. The findings from this thesis contribute to our knowledge of how the primary components of feeding physiology, including pumping rate, capture, efficiency, and ingestion rate, may vary between and within bivalve species, and also between and within individuals, in response to different environmental conditions.

6.2 Future Directions

6.2.1 On Laboratory and Field Experiments

Laboratory and field studies provide different conditions in which the feeding physiology of bivalves can be observed. The controlled conditions of laboratory experiments are well suited to isolating single cause- and effect relationships that are required to inform field studies. However, laboratory studies are also more limited in their ability to draw broad ecological conclusions. Cultured diets do not permit observations of feeding physiology in response to the natural diversity of the seston in terms of particle shape, size, concentration, and surface properties. Resultingly, observations of bivalve physiology in these conditions may not reflect true variability, of either diet or physiological rates, in natural settings. Although natural diets and *in situ* studies may provide more ecologically relevant observations of feeding physiology in terms of pumping and ingestion rates and capture efficiency, field studies are subject to uncontrolled changes in environmental conditions. Field experiments may be limited by the confounding effects of multiple environmental variables fluctuating simultaneously. For example, salinity and temperature may both influence feeding physiology of bivalves and are often influenced by weather and diel cycles and may fluctuate on short-term scales. If temperature and salinity vary widely during an experiment, it may be difficult to isolate the feeding response of bivalves to diet characteristics without the confounding effects of temperature and salinity. Although in some cases these confounding effects can be controlled for (e.g., temperature corrected pumping rates in **Chapter 5**), highly variable environmental conditions may make it difficult to observe specific physiological relationships. In addition, if only diet quantity is being measured, confounding effects of changing diet quality may also make it difficult to characterize bivalve feeding relationships. Relying solely on

natural diets also limits the ability to make observations of feeding physiology over a specific range of food concentrations. For example, in **Chapter 5**, field experiments were replicated over three months to capture different concentrations of food both between and within experiments. However, the recorded data is biased towards low food concentrations, and maximum concentrations are still relatively low compared to many lab studies. By making observations of feeding physiology over the course of seasons using natural seston, future studies may aim to record higher variability in natural diets. Similarity, a field experiment in **Chapter 3** used a natural diet with high levels of suspended material, and the breakdown of flocs make feeding physiology difficult to observe and interpret. To isolate specific drivers of feeding physiology, and maintain ecological relevance, future studies should consider the combination of both laboratory and field studies on bivalve feeding physiology, including pumping rate and capture efficiency.

6.2.2 On Assessing Plasticity & Adaptation

There are several ways in which the design of experiments can be improved to better assess plasticity in bivalve feeding physiology. To begin, genetic information, including genome sequencing and mapping, for bivalve species provide a basis on which adaptation may be measured. Despite advances in the fields of genomics, and its application in mapping bivalve genomes, many ecologically and economically important species remain understudied. Baseline genetic information is required to establish genetically distinct populations on which experiments can be conducted to assess plastic and adaptive processes. Relatedly, a better understanding of bivalve larval transport is required to better characterize geographically and genetically distinct bivalve populations. With foundational genetic knowledge, experiments to assess plasticity and adaptation, including reciprocal transplant and common garden experiments become more powerful experimental tools. For example, in **Chapter 4**, differences in feeding physiology were observed between three geographically separate "populations" of *M. edulis*; however, without genetic information about mussels in each location, the extent to which these mussels are distinct populations may only be speculated based on hydrodynamics of larval transport. Further, the transplant experiment in **Chapter 4** was conducted along a fjord

gradient, where larval transport may permit genetic mixing between the two transport locations. With genome sequencing, it is possible to observe both different populations on a genetic level (e.g., local adaptation), and also to relate genetic differences to environmental conditions, genetic drift, and natural selection. Reciprocal transplant studies benefit from the ecological relevance of field experiments, and the use of natural diets; however, they are limited in practicality in terms of moving bivalves over large geographic scales. Beyond field transplant studies, laboratory common garden studies may also be used to assess plasticity and adaptation and are best performed on the first-generation offspring of bivalves reared in common conditions, to remove any maternal effects. Studies assessing plasticity and adaptation should aim to incorporate aspects of physiology, ecology, and genetics to provide a holistic understanding of the changes in feeding physiology of bivalves.

6.2.3 On the mechanisms of filter-feeding

Research on the mechanisms of bivalve suspension-feeding has been advanced by technology including electronic particle counters, scanning electron microscopy, and *in vivo* endoscopy. In this thesis, feeding physiology was investigated using an electronic particle counter designed for portable use in both field and laboratory experiments. The benefits of this methodology are that it is mobile and permits high-throughput measurements using both the static (**Chapter 3** and **4**) or flow-through (**Chapter 5**) methods. Using the static method, high frequency measurements of particle counts (every 30s) permit measuring particle decline over time with high temporal resolution. This methodology is able to ensure that the bivalve was pumping at a constant rate while the measurement was being taken, an assumption required to calculate pumping rate. This portable methodology allows for fast measurements of feeding physiology, without the need to preserve and transport water samples to a laboratory for further analysis.

Particle counting methodologies are limited in their ability to describe the seston beyond particle size. The electronic particle counter used in this thesis described particles as estimated spheres, at 0.5 μm intervals, with no qualitative description of particle characteristics. The size, shape, and surface properties of plankton species may not always

be correctly estimated as a sphere, particularly for species with high aspect ratios. Future studies may combine the use electronic particle counter, and high temporal resolution measurements, with tools that permit more descriptive measurements of the diet. As factors beyond particle size are understood to influence capture efficiency, the diet may be further characterized by filtering water samples to determine the organic fraction of the diet, size-fractioned chlorophyll *a*, and energetic content (**Chapter 5**). Visual analysis of water samples, including microscopy, fluid imaging technologies, or flow cytometry may be used to identify plankton species groups that are present in the diet, and may be captured with higher efficiencies, or preferentially ingested. Finally, as particle surface characteristics are known to influence capture efficiency of particles in some species of bivalves, particles present in the diet may further be characterized by wettability and surface charge.

In addition to describing the available diet, *in vivo* visual techniques including video endoscopy provide a way to observe the mechanisms of particle capture on the gill surfaces. *In vivo* techniques would permit the direct observation of suspension-feeding activity and would allow for the differentiation between capture efficiency and retention efficiency, contributing to the understanding of particle capture, and rejection mechanisms. Video endoscopy has previously been used to record the capture of particles on the gill surfaces, and the movement of particles along marginal grooves for ingestion or rejection. Microscopy can also be used to make observations of gill cilia shape and size, both between and within species, and this information may be related to the characteristics of fluid movement at the gill surface, and particle capture (e.g., scanning electron microscopy in **Chapter 3**). A combination of techniques making use of the best available technologies in particle counting, particle characterization, and in *vivo* observations would provide the most complete observations of particle capture mechanisms in suspension-feeding bivalves.

6.2.4 On variability in feeding physiology

Interspecifically, the variability in the feeding physiology of marine bivalves has important implications in the ecosystem interactions of these diverse species. As observed from the literature in **Chapter 2**, and experimentally in **Chapter 3**, there exist interspecific

differences in the rates at which bivalves feed (pumping rate) and the particles that they capture (capture efficiency). For example, in **Chapter 3,** *M. edulis* is more efficient than *C. virginica* and *P. magellanicus* at capturing small particles (< 4 μm ESD), indicating that these species will interact with plankton communities in different ways. Resultingly, there exist species-specific ecosystem interactions in terms of the rates of biodeposition, water filtration, and plankton community pressures. These ecosystem interactions are important to consider for anthropogenic activities including the establishment of aquaculture farms, the design of "living shorelines" for coastal protection, and the restoration of populations of threatened species. Although in-depth knowledge on commercially important species is often the priority of research programmes, the breadth of knowledge that can be provided by interspecific studies can provide information about the diverse ecological roles of bivalves. Future studies may consider more comparative studies on different species of bivalves in terms of pumping rate and capture efficiency for both ecological and management implications.

Intraspecifically, feeding physiology may vary both between individuals in different environmental conditions (**Chapter 4**) and between individuals held in the same environmental conditions (**Chapter 5**). Variation in feeding physiology between individuals in different environments provides information about the environmental drivers of feeding physiology. These relationships have been explored in this thesis primarily in terms of natural diets (food quantity and quality) but may also be related to the abiotic environment (e.g., temperature, salinity), and has been extensively explored. However, this thesis highlights that the expected functional relationships between physiological rates (pumping and ingestion) may vary within species, depending on the specific environment of a population of individuals. This thesis dealt extensively with the blue mussel, *M. edulis*, which is a widely distributed species, and inhabits a variety of environments. For the population of mussels used in **Chapter 4** and **5**, the environment consistently contains low levels of food, and as such, the relationships between feeding physiology and environmental conditions may reflect local adaptation of this population. **Chapter 5** also observed high levels of variability in physiological rates in *M. edulis*, despite individuals being acclimated to the same conditions. Future studies may consider further examining the drivers of interindividual variability in feeding physiology by using common garden

experiments on first generation offspring to minimize extraneous differences between individuals (e.g., maternal effects, parasite load).

High levels of intra-individual variability were also observed in this thesis in the feeding physiology of *M. edulis* in **Chapter 5.** Over the course of 4 days, despite relatively stable environmental conditions, the pumping rates of individuals varied several litres per hour. This variability suggests that instead of being driven by environmental cues, that rates of feeding physiology are instead being driven by internal feedbacks. For example, pumping rate may slow when maximum ingestion rate, or gut capacity has been reached. Similarly, if diet quality is high lower pumping rates may be needed to maintain high rates of organic ingestion. Future studies may consider incorporating aspects of digestive physiology including gut volume, gut passage time, and absorption efficiency to examine the potential feedbacks between feeding and digestive physiology that may be driving intra-individual variability in feeding rates.

6.3 CONCLUSIONS

Understanding how bivalves acquire energy through feeding is a foundational step in predicting the growth, survival, and distribution of these ecologically and economically important species. This thesis has contributed to our understanding of the variability in bivalve suspension-feeding physiology, as explored through inter- and intraspecific plasticity. Bivalves have highly plastic physiology that allows them to vary their rates of energy acquisition and expenditure. This plasticity varies between species, and in some cases, may be limited by adaptative processes. Specifically, the process that mediate particle capture was observed to vary interspecifically in this thesis, where particle capture efficiency was influenced by pumping rate in *C. virginica*, but not *M. edulis* or *P. magellanicus*. Intraspecifically, it was found that primary components on feeding physiology (pumping rate, capture efficiency, and ingestion rate) may vary between *M. edulis* from different populations, but that the primary driver of this variability may be environmental conditions and not local adaptation. High temporal resolution measurements of pumping and ingestion rates in this species also suggested that despite being held in common conditions, there may be high levels of inter- and intraindividual variability in feeding and ingestion rates in *M. edulis*. The combination of laboratory and field

experiments, and their application to various bivalve species has provided information about the drivers of variability in the feeding physiology of bivalves. In light of rapidly changing marine environments, understanding the mechanisms, and extent of plasticity, in the feeding physiology of bivalves is crucial to predicting their ability to grow and survive.

REFERENCES

Altieri A. H (2006) Inducible variation in hypoxia tolerance across the intertidal-subtidal distribution of the blue mussel *Mytilus edulis*. Mar Ecol Prog Ser 325:295–300.

Anestis A, Lazou A, Pörtner HO, Michaelidis B (2007) Behavioral, metabolic, and molecular stress responses of marine bivalve *Mytilus galloprovincialis* during long-term acclimation at increasing ambient temperature. Am J Physiol-Regul Integr Comp Physiol 293: R911–R921.

Arranz K, Urrutxurtu I, Ibarrola I, Urrutia MB, Saavedra C, Cordero D, Pérez-Larruscain J, Navarro E (2020) Physiological processes modulate acute and chronic responses to dietary protein/energy ratio fluctuations in individuals and families of Manila clam (*Ruditapes philippinarum*) selected for variable growth rates. Aquaculture 521:735056.

Artigaud S, Lavaud R, Thébault J, Jean F, Strand Ø, Strohmeier T, Milan M, Pichereau V (2014) Proteomic-based comparison between populations of the Great Scallop, *Pecten maximus*. J Proteomics 105:164–173.

Asplin L, Johnsen IA, Sandvik AD, Albretsen J, Sundfjord V, Aure J, Boxaspen KK (2014) Dispersion of salmon lice in the Hardangerfjord. Mar Biol Res 10:216–225.

Babarro J, Carrington E (2011) Byssus secretion of *Mytilus galloprovincialis*: effect of site at macro- and micro-geographical scales within Ría de Vigo (NW Spain). Mar Ecol Prog Ser 435:125–140.

Babarro JMF (2000) Metabolism of the mussel *Mytilus galloprovincialis* from two origins in the R|¨ a de Arousa (north-west Spain). J Mar Biol Assoc U K:8.

Babarro MF, Fernandez-Reiriz MJ, Labarta U (2003) In situ absorption efficiency processes for the cultured mussel *Mytilus galloprovincialis* in Ria de Arousa (north-west Spain). J Mar Biol Assoc U K:1059–1064.

Bacher C (2003) Modelling the effect of food depletion on scallop growth in Sungo Bay (China). Aquat Living Resour 16:10–24.

Baojun T, Riisgård HU (2018) Relationship between oxygen concentration, respiration and filtration rate in blue mussel *Mytilus edulis*. J Oceanogr Limnol 36:395–404.

Barillé L, Haure J, Cognie B, Leroy A (2000) Variations in pallial organs and eulatero-frontal cirri in response to high particulate matter concentrations in the oyster *Crassostrea gigas*. Can J Fish Aquat Sci 57:837–843.

Barillé L, Prou J, Héral M, Bourgrier S (1993) No influence of food quality, but ration-dependent retention efficiencies in the Japanese oyster *Crassostrea gigas*. J Exp Mar Biol Ecol 171:91–106.

Bayne B, Hawkins A, Navarro E, Iglesias I (1989) Effects of seston concentration on feeding, digestion and growth in the mussel *Mytilus edulis*. Mar Ecol Prog Ser 55:47–54.

Bayne B, Worrall C (1980) Growth and production of mussels *Mytilus edulis* from two populations. Mar Ecol Prog Ser 3:317–328.

Bayne BL (2017) Biology of Oysters, 1st ed. Academic Press, London.

Bayne BL (2004) Phenotypic flexibility and physiological tradeoffs in the feeding and growth of marine bivalve molluscs. Integr Comp Biol 44:425–432.

Bayne BL (1999) Physiological components of growth differences between individual oysters (*Crassostrea gigas*) and a comparison with *Saccostrea commercialis*. Physiol Biochem Zool 72:705–713.

Bayne BL, Hawkins AJS (1997) Protein metabolism, the costs of growth, and genomic heterozygosity: Experiments with the mussel *Mytilus galloprovincialis* Lmk. Physiol Zool 70:391–402.

Bayne BL, Hawkins AJS, Navarro E (1987) Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. J Exp Mar Biol Ecol 111:1–22.

Bayne BL, Hawkins AJS, Navarro E (1988) Feeding and digestion in suspension-feeding bivalve molluscs: The relevance of physiological compensations. Am Zool 28:147–159.

Bayne BL, Hedgecock D, McGoldrick D, Rees R (1999a) Feeding behaviour and metabolic efficiency contribute to growth heterosis in Pacific oysters [*Crassostrea gigas* (Thunberg)]. J Exp Mar Biol Ecol 233:115–130.

Bayne BL, Iglesias JIP, Hawkins AJS, Navarro E, Heral M, Deslous-Paoli JM (1993) Feeding behaviour of the mussel, *Mytilus edulis* : responses to variations in quantity and organic content of the seston. J Mar Biol Assoc U K 73:813–829.

Bayne BL, Klumpp DW, Clarke KR (1984) Aspects of feeding, including estimates of gut residence time, in three mytilid species (Bivalvia, Mollusca) at two contrasting sites in the Cape Peninsula, South Africa. Oecologia 64:26–33.

Bayne BL, Newell RC (1983) 9 - Physiological Energetics of Marine Molluscs. In: *The Mollusca*. Saleuddin ASM, Wilbur KM (eds) Academic Press, p 407–515

Bayne BL, Svensson S (2006) Seasonal variability in feeding behaviour, metabolic rates and carbon and nitrogen balances in the Sydney oyster, *Saccostrea glomerata* (Gould). J Exp Mar Biol Ecol 332:12–26.

Bayne BL, Svensson S, Nell JA (1999b) The physiological basis for faster growth in the Sydney Rock Oyster, *Saccostrea commercialis*. Biol Bull 197:377–387.

Beadman H, Willows R, Kaiser M (2002) Potential applications of mussel modelling. Helgol Mar Res 56:76–85.

Beck MW (2009) Shellfish reefs at risk: a global analysis of problems and solutions. Nature Conservancy.

Ben-Horin T, Allen S, Small J, Proestou D (2018) Genetic variation in anti-parasite behavior in oysters. Mar Ecol Prog Ser 594:107–117.

Beninger P, Dufour S (1996) Mucocyte distribution and relationship to particle transport on the pseudolamellibranch gill of *Crassostrea virginica* (Bivalvia: Ostreidae). Mar Ecol Prog Ser 137:133–138.

Beninger P, St-Jean S (1997) Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca:Bivalvia). Mar Ecol Prog Ser 147:117–127.

Beninger P, St-Jean S, Poussart Y, Ward J (1993) Gill function and mucooyte distribution in Placopecten magellanicus and *Mytilus edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. Mar Ecol Prog Ser 98:275–282.

Beninger P, Veniot A, Poussart Y (1999) Principles of pseudofeces rejection on the bivalve mantle:integration in particle processing. Mar Ecol Prog Ser 178:259–269.

Beninger PG, Pennec ML, Salaiin M (1988) New observations of the gills of *Placopecten magellanicus* (Mollusca: Bivalvia), and implications for nutrition. 10.

Benítez S, Lagos N, Osores S, Opitz T, Duarte C, Navarro J, Lardies M (2018) High pCO2 levels affect metabolic rate, but not feeding behavior and fitness, of farmed giant mussel *Choromytilus chorus*. Aquac Environ Interact 10:267–278.

Bergström P, Lindegarth M (2016) Environmental influence on mussel (*Mytilus edulis*) growth – A quantile regression approach. Estuar Coast Shelf Sci 171:123–132.

Bernatchez S, Xuereb A, Laporte M, Benestan L, Steeves R, Laflamme M, Bernatchez L, Mallet MA (2019) Seascape genomics of eastern oyster (*Crassostrea virginica*) along the Atlantic coast of Canada. Evol Appl 12:587–609.

Bohle B (1972) Effects of adaptation to reduced salinity on filtration activity and growth of mussels (*Mytilus edulis* L.). J Exp Mar Biol Ecol 10:41–47.

Borthagaray AI, Carranza A (2007) Mussels as ecosystem engineers: Their contribution to species richness in a rocky littoral community. Acta Oecologica 31:243–250.

Boyd PW, Cornwall CE, Davison A, Doney SC, Fourquez M, Hurd CL, Lima ID, McMinn A (2016) Biological responses to environmental heterogeneity under future ocean conditions. Glob Change Biol 22:2633–2650.

Bozinovic F, Calosi P, Spicer JI (2011) Physiological correlates of geographic range in Animals. 42: 155-179.

Braarud T (1976) The natural history of the Hardangerfjord: 13. The ecology of taxonomic groups and species of phytoplankton related to their distribution patterns in a fjord area. Sarsia 60:41–62.

Brand AR, Morris DJ (1984) The respiratory responses of the dog cockle *Glycymeris glycymeris* (L.) to declining environmental oxygen tension. J Exp Mar Biol Ecol 83:89–106.

Bratbak G, Jacquet S, Larsen A, Pettersson LH, Sazhin AF, Thyrhaug R (2011) The plankton community in Norwegian coastal waters—abundance, composition, spatial distribution and diel variation. Cont Shelf Res 31:1500–1514.

Brett JR (1976) Scope for Metabolism and Growth of Sockeye Salmon, *Oncorhynchus nerka* , and Some Related Energetics. J Fish Res Board Can 33:307–313.

Bricelj VM, Ouellette C, Anderson M, Brun NT, Pernet F, Ross N, Landry T (2006) Physiological and biochemical responses of juvenile quahogs, *Mercinaria*

mercinaria, to low temperatures: Potential for mitigation of overwhelming mortalities. Can Tech Rep Fish Aquat Sci:vii + 47.

Brock V, Kennedy VS (1992) Quantitative analysis of crystalline style carbohydrases in five suspension- and deposit-feeing bivalves. J Exp Mar Biol Ecol:51–58.

Byrne M, Foo SA, Ross PM, Putnam HM (2020) Limitations of cross- and multigenerational plasticity for marine invertebrates faced with global climate change. Glob Change Biol 26:80–102.

Cannuel R, Beninger PG, McCombie H, Boudry P (2009) Gill development and its functional and evolutionary implications in the blue mussel *Mytilus edulis* (Bivalvia: Mytilidae). Biol Bull 217:173–188.

Capelle JJ (2021) Adaptation of gill-palp ratio by mussels after transplantation to culture plots with different seston conditions. Aquaculture 541: 736794.

Carrier-Belleau C, Drolet D, McKindsey CW, Archambault P (2021) OPEN Environmental stressors, complex interactions and marine benthic communities' responses. Sci Rep 11.

Carroll ML, Ambrose WG, Levin BS, Locke V WL, Henkes GA, Hop H, Renaud PE (2011) Pan-Svalbard growth rate variability and environmental regulation in the Arctic bivalve *Serripes groenlandicus*. J Mar Syst 88:239–251.

Carver CEA, Mallet AL (1990) Estimating the carrying capacity of a coastal inlet for mussel culture. Aquaculture 88:39–53.

Cavallo RA, Acquaviva MI, Stabili L (2009) Culturable heterotrophic bacteria in seawater and *Mytilus galloprovincialis* from a Mediterranean area (Northern Ionian Sea – Italy). Environ Monit Assess 149:465–475.

Chapman EJ (2020) Effects of climate change on coastal ecosystem food webs: Implications for aquaculture. Mar Environ Res:10.

Clausen I, Riisgård H (1996) Growth, filtration and respiration in the mussel *Mytilus edulis*:no evidence for physiological regulation of the filter-pump to nutritional needs. Mar Ecol Prog Ser 141:37–45.

Cleveland WS, Devlin SJ (1988) Locally weighted regression: An approach to regression analysis by local fitting. J Am Stat Assoc 83:596–610.

Coen L, Brumbaugh R, Bushek D, Grizzle R, Luckenbach M, Posey M, Powers S, Tolley S (2007) Ecosystem services related to oyster restoration. Mar Ecol Prog Ser 341:303–307.

Conover RJ (1966) Assimilation of organic matter by zooplankton. Limnol Oceanogr 11:338–345.

Coughlan J (1969) The estimation of filtering rate from the clearance of suspensions. Mar Biol 2:356–358.

Cranford P, Hill P (1999) Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. Mar Ecol Prog Ser 190:223–239.

Cranford P, Strain P, Dowd M, Hargrave B, Grant J, Archambault M (2007) Influence of mussel aquaculture on nitrogen dynamics in a nutrient enriched coastal embayment. Mar Ecol Prog Ser 347:61–78.

Cranford P, Strohmeier T, Filgueira R, Strand Ø (2016) Potential methodological influences on the determination of particle retention efficiency by suspension feeders: *Mytilus edulis* and *Ciona intestinalis*. Aquat Biol 25:61–73.

Cranford PJ, Emerson CW, Hargrave BT, Milligan TG (1998) In situ feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. J Exp Mar Biol Ecol 219:45–70.

Cranford PJ, Grant J (1990) Particle clearance and absorption of phytoplankton and detritus by the sea scallop *Placopecten magellanicus* (Gmelin). J Exp Mar Biol Ecol 137:105–121.

Cranford PJ, Ward JE, Shumway SE (2011) Bivalve filter feeding: Variability and limits of the aquaculture biofilter. In: *Shellfish Aquaculture and the Environment*. Shumway SE (ed) Wiley-Blackwell, Oxford, UK, p 81–124

Crosby MP, Langdon CJ, Newell RIE (1989) Importance of refractory plant material to the carbon budget of the oyster *Crassostrea virginica*. Mar Biol 100:343–352.

Crosby MP, Newell RIE, Langdon CJ (1990) Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. Limnol Oceanogr 35:625–639.

Cruz A, da Costa F, Fernández-Pérez J, Nantón A, Fernández-Boo S, Insua A, Méndez J (2020) Genetic variability in *Ruditapes decussatus* clam combined with *Perkinsus* infection level to support founder population selection for a breeding program. PeerJ 8:e9728.

Cubillo AM, Ferreira JG, Lencart-Silva J, Taylor NGH, Kennerley A, Guilder J, Kay S, Kamermans P (2021) Direct effects of climate change on productivity of European aquaculture. Aquac Int 29: 1561-1590.

Dame RF, Kenneth MJ (2012) Ecology of marine bivalves: An ecosystem approach. Taylor & Francis: 284.

Dame RF (1972) The ecological energies of growth, respiration and assimilation in the intertidal American oyster *Crassostrea virginica*. Mar Biol 17:243–250.

Darriba S, San Juan F, Guerra A (2005) Energy storage and utilization in relation to the reproductive cycle in the razor clam *Ensis arcuatus* (Jeffreys, 1865). ICES J Mar Sci 62:886–896.

Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis: Invasive species have higher phenotypic plasticity. Ecol Lett 14:419–431.

Department of Fisheries and Oceans Canada (2021a) Seafisheries landed by province, 2020. Accessed online: https://www.dfo-mpo.gc.ca/stats/commercial/land-debarq/sea-maritimes/s2020pv-eng.htm

Department of Fisheries and Oceans Canada (2021b) Aquaculture production and value. Accessed online: https://www.dfo-mpo.gc.ca/stats/aqua/aqua20-eng.htm

Decho AW, Luoma SM (1996) Flexible digestion strategies and trace metal assimilation in marine bivalves. Limnol Oceanogr 41:568–572.

Diaz R, Lardies MA, Tapia FJ, Tarifeño E, Vargas CA (2018) Transgenerational effects of pCO2-driven ocean acidification on adult mussels *Mytilus chilensis* Modulate Physiological Response to Multiple Stressors in Larvae. Front Physiol 9:1349.

Dishaw LJ, Cannon JP, Litman GW, Parker W (2014) Immune-directed support of rich microbial communities in the gut has ancient roots. Dev Comp Immunol 47:36–51.

Donelan SC, Breitburg D, Ogburn MB (2021) Context-dependent carryover effects of hypoxia and warming in a coastal ecosystem engineer. Ecol Appl.

Donelson JM, Munday PL, McCormick MI, Pitcher CR (2012) Rapid transgenerational acclimation of a tropical reef fish to climate change. Nat Clim Change 2:30–32.

Drinnan, R. E (1964) An apparatus for recording the water-pumping behaviour of lamellibranchs. Neth J Sea Res 2:223–232.

Dutertre M, Barillé L, Beninger PG, Rosa P, Gruet Y (2009) Variations in the pallial organ sizes of the invasive oyster, *Crassostrea gigas*, along an extreme turbidity gradient. Estuar Coast Shelf Sci 85:431–436.

Dutertre M, Barillé L, Haure J, Cognie B (2007) Functional responses associated with pallial organ variations in the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). J Exp Mar Biol Ecol 352:139–151.

Dutertre M, Ernande B, Haure J, Barillé L (2017) Spatial and temporal adjustments in gill and palp size in the oyster *Crassostrea gigas*. J Molluscan Stud 83:11–18.

Erga SR (1989) Ecological-studies on the phytoplankton of Boknafjorden, Western Norway. 1. The effect of water exchange processes and environmental-factors on temporal and vertical variability of biomass. Sarsia 74:161-176

Ernande B, Boudry P, Clobert J, Haure J (2003) Plasticity in resource allocation based life history traits in the Pacific oyster, *Crassostrea gigas*. I. Spatial variation in food abundance: Plasticity in resource allocation in a marine bivalve. J Evol Biol 17:342–356.

Evan Ward J, Shumway SE (2004a) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83–130.

Evan Ward J, Shumway SE (2004b) Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J Exp Mar Biol Ecol 300:83–130.

Eymann C, Götze S, Bock C, Guderley H, Knoll AH, Lannig G, Sokolova IM, Aberhan M, Pörtner H-O (2020) Thermal performance of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758)—explaining ecological findings under climate change. Mar Biol 167:17.

Fallet M, Luquet E, David P, Cosseau C (2020) Epigenetic inheritance and intergenerational effects in mollusks. Gene 729:144166.

Farias ND, de Oliveira NFP, da Silva PM (2017) Perkinsus infection is associated with alterations in the level of global DNA methylation of gills and gastrointestinal tract of the oyster *Crassostrea gasar*. J Invertebr Pathol 149:76–81.

Fearman J-A, Bolch CJS, Moltschaniwskyj NA (2009) Energy storage and reproduction in mussels, *Mytilus galloprovincialis* : The influence of diet quality. J Shellfish Res 28:305–312.

Fernández-Reiriz M, Garrido J, Irisarri J (2015) Fatty acid composition in *Mytilus galloprovincialis* organs: trophic interactions, sexual differences and differential anatomical distribution. Mar Ecol Prog Ser 528:221–234.

Fernandez-Reiriz MJ, Labarta U, Navarro JM, Velasco LA (2001) Enzymatic digestive activity in *Mytilus chilensis* (Hupé 1854) in response to food regimes and past feeding history. J Comp Physiol [B] 171:449–456.

Ferreira JG, Hawkins AJS, Bricker SB (2007) Management of productivity, environmental effects and profitability of shellfish aquaculture — the Farm Aquaculture Resource Management (FARM) model. Aquaculture 264:160–174.

Filgueira R (2010) Clearance rate of the mussel *Mytilus galloprovincialis*. II. Response to uncorrelated seston variables (quantity, quality, and chlorophyll content). Cienc Mar 36:15–28.

Filgueira R, Fernandez-Reiriz MJ, Labarta U (2009) Clearance rate of the mussel *Mytilus galloprovincialis*. I. Response to extreme chlorophyll ranges. Cienc Mar 35:405–417.

Filgueira R, Labarta U, Fernández-Reiriz MJ (2008) Effect of condition index on allometric relationships of clearance rate in *Mytilus galloprovincialis* Lamarck, 1819. Rev Biol Mar Oceanogr 43.

Filgueira R, Labarta U, Fernandez-Reiriz MJ (2006) Flow-through chamber method for clearance rate measurements in bivalves: design and validation of individual chambers and mesocosm: CR protocol for validation. Limnol Oceanogr Methods 4:284–292.

Fisher B, Turner RK, Morling P (2009) Defining and classifying ecosystem services for decision making. Ecol Econ 68:643–653.

Fitzer SC, Vittert L, Bowman A, Kamenos NA, Phoenix VR, Cusack M (2015) Ocean acidification and temperature increase impact mussel shell shape and thickness: problematic for protection? Ecol Evol 5:4875–4884.

Fitzsimons JA, Branigan S, Gillies CL, Brumbaugh RD, Cheng J, DeAngelis BM, Geselbracht L, Hancock B, Jeffs A, McDonald T, McLeod IM, Pogoda B, Theuerkauf SJ, Thomas M, Westby S, zu Ermgassen PSE (2020) Restoring shellfish reefs: Global guidelines for practitioners and scientists. Conserv Sci Pract 2.

Forberg T, Vestrum RI, Arukwe A, Vadstein O (2012) Bacterial composition and activity determines host gene-expression responses in gnotobiotic Atlantic cod (Gadus morhua) larvae. Vet Microbiol 157:420–427.

Foster-Smith RL (1975) The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J Exp Mar Biol Ecol 17:1–22.

Frank DM, Ward JE, Shumway SE, Holohan BA, Gray C (2008) Application of particle image velocimetry to the study of suspension feeding in marine invertebrates. Mar Freshw Behav Physiol 41:1–18.

Fréchette M (2012) A model of clearance rate regulation in mussels. J Sea Res 73:32–40.

Frechette M, Bourget E (1987) Significance of small-scale spatio-temporal heterogeneity in phytoplankton abundance for energy flow in *Mytilus edulis*. Mar Biol 94:231–240.

Frette O, Erga SR, Hamre B, Aure J, Stamnes JJ (2004) Seasonal variability in inherent optical properties in a western Norwegian fjord. Sarsia 89:276-291

Fuchs H, Specht J (2018) Evidence for diverse responses to viscosity in suspension-feeding bivalves: Reply to Riisgård & Larsen (2018). Mar Ecol Prog Ser 596:267–270.

Fuentes-Santos I, Labarta U, Fernández-Reiriz MJ (2018) Characterizing individual variability in mussel (*Mytilus galloprovincialis*) growth and testing its physiological drivers using Functional Data Analysis. PLOS ONE 13:e0205981.

Galimany E, Rose JM, Dixon MS, Wikfors GH (2015) Transplant experiment to evaluate the feeding behaviour of the Atlantic ribbed mussel, *Geukensia demissa*, moved to a high inorganic seston area. Mar Freshw Res 66:220.

Galindo J, Grahame JW, Butlin RK (2010) An EST-based genome scan using 454 sequencing in the marine snail *Littorina saxatilis*: EST-genome scan via 454 sequencing. J Evol Biol 23:2004–2016.

Gallardi D (2014) Effects of bivalve aquaculture on the environment and their possible mitigation: a review. Fish Aquac J 2014.

Gavery MR, Roberts SB (2017) Epigenetic considerations in aquaculture. PeerJ 5:e4147.

Gazeau F, Gattuso J-P, Dawber C, Pronker AE, Peene F, Peene J, Heip CHR, Middelburg JJ (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. Biogeosciences 7:2051–2060.

Gerdol M, De Moro G, Manfrin C, Milandri A, Riccardi E, Beran A, Venier P, Pallavicini A (2014) RNA sequencing and de novo assembly of the digestive gland transcriptome in *Mytilus galloprovincialis* fed with toxinogenic and non-toxic strains of Alexandrium minutum. BMC Res Notes 7:722.

Gerdol M, Venier P, Pallavicini A (2015) The genome of the Pacific oyster Crassostrea gigas brings new insights on the massive expansion of the C1q gene family in Bivalvia. Dev Comp Immunol 49:59–71.

Ghalambor CK, McKAY JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Funct Ecol 21:394–407.

Gibbs MC, Parker LM, Scanes E, Byrne M, O'Connor WA, Ross PM (2021) Adult exposure to ocean acidification and warming remains beneficial for oyster larvae following starvation. ICES J Mar Sci:fsab066.

Goff SA (2011) A unifying theory for general multigenic heterosis: energy efficiency, protein metabolism, and implications for molecular breeding: Tansley review. New Phytol 189:923–937.

Gomes-dos-Santos A, Lopes-Lima M, Castro LFC, Froufe E (2020) Molluscan genomics: the road so far and the way forward. Hydrobiologia 847:1705–1726.

Gonzalez Giorgis Y, Cruz Sueiro M, Márquez F (2020) Phenotypic plasticity at fine-grained spatial scales: the scorched mussel *Perumytilus purpuratus* growing on Patagonian rocky salt-marshes. Sci Mar 84:393–401.

Gonzalez-Romero R, Suarez-Ulloa V, Rodriguez-Casariego J, Garcia-Souto D, Diaz G, Smith A, Pasantes JJ, Rand G, Eirin-Lopez JM (2017) Effects of Florida red tides on histone variant expression and DNA methylation in the Eastern oyster *Crassostrea virginica*. Aquat Toxicol 186:196–204.

Gosling EM (2003) Bivalve molluscs: biology, ecology, and culture. Fishing News Books, Oxford ; Malden, MA.

Gourault M, Lavaud R, Leynaert A, Pecquerie L, Paulet Y-M, Pouvreau S (2018) New insights into the reproductive cycle of two Great Scallop populations in Brittany (France) using a DEB modelling approach. J Sea Res 143: 207-221.

Griffith AW, Gobler CJ (2017) Transgenerational exposure of North Atlantic bivalves to ocean acidification renders offspring more vulnerable to low pH and additional stressors. Sci Rep 7:11394.

Griffiths JS, Johnson KM, Sirovy KA, Yeats MS, Pan FTC, La Peyre JF, Kelly MW (2021) Transgenerational plasticity and the capacity to adapt to low salinity in the eastern oyster, *Crassostrea virginica*. Proc R Soc B Biol Sci 288:20203118.

Guarner F, Malagelada J-R (2003) Gut flora in health and disease. Lancet Lond Engl 361:512–519.

Guderley H, Pörtner HO (2010) Metabolic power budgeting and adaptive strategies in zoology: examples from scallops and fish. Can J Zool 88:753–763.

Gutiérrez JL, Jones CG, Strayer DL, Iribarne OO (2003) Mollusks as ecosystem engineers: the role of shell production in aquatic habitats. Oikos 101:79–90.

H. Karasov W, Douglas AE (2013) Comparative digestive physiology. In: *Comprehensive Physiology*. Terjung R (ed) John Wiley & Sons, Inc., Hoboken, NJ, USA, p c110054

Harris JM (1993) The presence, nature, and role of gut microflora in aquatic invertebrates: A synthesis. Microb Ecol 25:195–231.

Havird JC, Neuwald JL, Shah AA, Mauro A, Marshall CA, Ghalambor CK (2020) Distinguishing between active plasticity due to thermal acclimation and passive plasticity due to Q_{10} effects: Why methodology matters. Funct Ecol 34:1015–1028.

Hawkins A, James M, Hickman R, Hatton S, Weatherhead M (1999) Modelling of suspension-feeding and growth in the green-lipped mussel *Perna canaliculus* exposed to natural and experimental variations of seston availability in the Marlborough Sounds, New Zealand. Mar Ecol Prog Ser 191:217–232.

Hawkins A, Smith R, Bayne B, Héral M (1996) Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis*. Mar Ecol Prog Ser 131:179–190.

Hawkins AJS (1991) Protein turnover: A functional appraisal. Funct Ecol 5:222.

Hawkins AJS, Bayne B. L, Day A J (1986) Protein turnover, physiological energetics and heterozygosity in the blue mussel *Mytilus edulis*: the basis of variable age-specfic growth. Proc R Soc B Biol Sci 229:161–176.

Hawkins AJS, Bayne BL (1984) Seasonal variation in the balance between physiological mechanisms of feeding and digestion in *Mytilus edulis* (Bivalvia: Mollusca). Mar Biol 82:233–240.

Hawkins AJS, Bayne BL (1985) Seasonal variation in the relative utilization of carbon and nitrogen by the mussel *Mytilus edulis*: budgets, conversion efficiencies and maintenance requirements. Mar Ecol Prog Ser 25:181–188.

Hawkins AJS, Bayne BL, Bougrier S, Heral M, Iglesias JIP, Navarro E, Smith RFM, Urrutia MB (1998) Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. J Exp Mar Biol Ecol:17.

Hawkins AJS, Fang JG, Pascoe PL, Zhang JH, Zhang XL, Zhu MY (2001) Modelling short-term responsive adjustments in particle clearance rate among bivalve suspension-feeders: separate unimodal effects of seston volume and composition in the scallop *Chlamys farreri*. J Exp Mar Biol Ecol 262:61–73.

Hawkins AJS, Magoulas A, Héral M, Bougrier S, Naciri-Graven Y, Day AJ, Kotoulas G (2000) Separate effects of triploidy, parentage and genomic diversity upon feeding behaviour, metabolic efficiency and net energy balance in the Pacific oyster *Crassostrea gigas*. Genet Res 76:273–284.

Hawkins AJS, Navarro E, Iglesias JIP (1990) Comparative allometries of gut-passage time, gut content and metabolic faecal loss in *Mytilus edulis* and *Cerastoderma edule*. Mar Biol 105:197–204.

Hedgecock D, McGoldrick DJ, Manahan DT, Vavra J, Appelmans N, Bayne BL (1996) Quantitative and molecular genetic analyses of heterosis in bivalve molluscs. J Exp Mar Biol Ecol 203:49–59.

Hendry AP (2001) Adaptive divergence and the evolution of reproductive isolation in the wild: An empirical demonstration using introduced sockeye salmon. In: *Microevolution Rate, Pattern, Process*. Contemporary Issues in Genetics and Evolution, Hendry AP, Kinnison MT (eds) Springer Netherlands, Dordrecht, p 515–534

Hentschel BT, Shimeta J (2008) Suspension Feeders. In: *Encyclopedia of Ecology*. Elsevier, p 3437–3442

Herbert RJH, Humphreys J, Davies ClareJ, Roberts C, Fletcher S, Crowe Tasman P (2016) Ecological impacts of non-native Pacific oysters (*Crassostrea gigas*) and management measures for protected areas in Europe. Biodivers Conserv 25:2835–2865.

Hermisson J, Pennings PS (2005) Soft sweeps: Molecular population genetics of adaptation from standing genetic variation. Genetics 169:2335–2352.

Hernández-Zárate G, Olmos-Soto J (2006) Identification of bacterial diversity in the oyster *Crassostrea gigas* by fluorescent in situ hybridization and polymerase chain reaction. J Appl Microbiol 100:664–672.

Hernroth BE, Baden SP (2018) Alteration of host-pathogen interactions in the wake of climate change – Increasing risk for shellfish associated infections? Environ Res 161:425–438.

Hewitt JE, Norkko J (2007) Incorporating temporal variability of stressors into studies: An example using suspension-feeding bivalves and elevated suspended sediment concentrations. J Exp Mar Biol Ecol 341:131–141.

Holling CS (1966) The functional response of invertebrate predators to prey density. Mem Entomol Soc Can 98:5–86.

Honkoop PJC (2003) Physiological costs of reproduction in the Sydney rock oyster *Saccostrea glomerata*: How expensive is reproduction? Oecologia 135:176–183.

Honkoop PJC, Bayne BL, Drent J (2003) Flexibility of size of gills and palps in the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). J Exp Mar Biol Ecol 282:113–133.

Hulbert AJ, Else PL (1999) Membranes as possible pacemakers of metabolism. J Theor Biol 199:257–274.

Hulot V, Saulnier D, Latchere O, Maihota N, Gaertner-Mazouni N (2019) Phenotype plasticity, local adaptation, and biofouling influence on growth of the pearl oyster *Pinctada margaritifera*: A common garden approach. Aquaculture 512:734309.

Hummel H, Bogaards RH, Bachelet G, Caron F, Sola JC, Amiard-Triquet C (2000) The respiratory performance and survival of the bivalve *Macoma balthica* (L.) at the southern limit of its distribution area: a translocation experiment. J Exp Mar Biol Ecol 251:85–102.

Ibarrola I, Iglesias JIP, Navarro E (1996) Differential absorption of biochemical components in the diet of the cockle *Cerastoderma edule* : enzymatic responses to variations in seston composition. Can J Zool 74:1887–1897.

Ibarrola I, Larretxea X, Iglesias JIP, Urrutia MB, Navarro E (1998a) Seasonal variation of digestive enzyme activities in the digestive gland and the crystalline style of the common cockle *Cerastoderma edule*. Comp Biochem Physiol:25–34.

Ibarrola I, Navarro E, Iglesias JIP (1998b) Short-term adaptation of digestive processes in the cockle *Cerastoderma edule* exposed to different food quantity and quality. J Comp Physiol [B] 168:32–40.

Ibarrola I, Navarro E, Iglesias JIP, Urrutia MB (2000) Temporal changes of feeding and absorption of biochemical components in *Cerastoderma edule* fed an algal diet. J Mar Biol Assoc U K 80:119–125.

Iglesias (1996) Microgeographic variability feeding, absorption, and condition of mussels (*Mytilus galloprovincialias* LMK.): a transplant experiment.

Iglesias JIP, Navarro E, Alvarez Jorna P, Armentina I (1992) Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentration and quality. J Exp Mar Biol Ecol 162:177–198.

Irisarri J, Fernández-Reiriz MJ, Robinson SMC, Cranford PJ, Labarta U (2013) Absorption efficiency of mussels *Mytilus edulis* and *Mytilus galloprovincialis* cultured under Integrated Multi-Trophic Aquaculture conditions in the Bay of Fundy (Canada) and Ría Ares-Betanzos (Spain). Aquaculture 388–391:182–192.

Jablonka E, Lamb MJ (2002) The changing concept of epigenetics. Ann N Y Acad Sci 981:82–96.

Jansen HM, Strand Ø, Verdegem M, Smaal A (2012) Accumulation, release and turnover of nutrients (C-N-P-Si) by the blue mussel *Mytilus edulis* under oligotrophic conditions. J Exp Mar Biol Ecol 416–417:185–195.

Jiang T, Chen F, Yu Z, Lu L, Wang Z (2016) Size-dependent depletion and community disturbance of phytoplankton under intensive oyster mariculture based on HPLC pigment analysis in Daya Bay, South China Sea. Environ Pollut 219:804–814.

Jiang W, Wang X, Rastrick SPS, Wang J, Zhang Y, Strand Ø, Fang J, Jiang Z (2021) Effects of elevated *p* CO2 on the physiological energetics of Pacific oyster, *Crassostrea gigas*. ICES J Mar Sci:fsab139.

Jiang Z, Wang X, Rastrick SPS, Fang J, Du M, Gao Y, Li F, Strand Ø, Fang J (2019) Metabolic responses to elevated pCO2 in the gills of the Pacific oyster (*Crassostrea gigas*) using a GC-TOF-MS-based metabolomics approach. Comp Biochem Physiol Part D Genomics Proteomics 29:330–338.

Jiao W, Fu X, Dou J, Li H, Su H, Mao J, Yu Q, Zhang L, Hu X, Huang X, Wang Y, Wang S, Bao Z (2014) High-resolution linkage and quantitative trait locus mapping aided by genome survey sequencing: Building up an integrative genomic framework for a bivalve mollusc. DNA Res 21:85–101.

Jimenez AG, Jayawardene S, Alves S, Dallmer J, Dowd WW (2015) Micro-scale environmental variation amplifies physiological variation among individual mussels. Proc R Soc B Biol Sci 282:20152273.

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373.

Jones HD, Richards OG, Southern TA (1992) Gill dimensions, water pumping rate and body size in mussel *Mytilus edulis* L. J Exp Mar Biol Ecol:213–237.

Jørgensen (1990) Effects of temperature on the mussel pump. Mar Ecol Prog Ser 28: 89-97.

Jørgensen CB (1981) A hydromechanical principle for particle retention in *Mytilus edulis* and other ciliary suspension feeders. Mar Biol 61:277–282.

Jørgensen CB (1983) Fluid mechanical aspects of suspension feeding. Mar Ecol Prog Ser 11:89–103.

Jørgensen CB (1955) Quantiatative aspects of filter feeding in invertebrates. Biol Rev 30:391–453.

Jøsrgensen CB (1996) Bivalve filter feeding revisited. Mar Ecol Prog Ser 142:297–302.

Kau AL, Ahern PP, Griffin NW, Goodman AL, Gordon JI (2011) Human nutrition, the gut microbiome, and immune system: envisioning the future. Nature 474:327–336.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecol Lett 7:1225–1241.

Kellogg JL (1915) Ciliary mechanisms of lamellibranchs with descriptions of anatomy. J Morphol 26:625–701.

Kellogg JL (1903) Feeding habits and growth of Venus mercenaria. University of the State of New York

King GM, Judd C, Kuske CR, Smith C (2012) Analysis of stomach and gut microbiomes of the Eastern Oyster (*Crassostrea virginica*) from coastal Louisiana, USA. PLOS ONE 7:e51475.

King NG, McKeown NJ, Smale DA, Moore PJ (2018) The importance of phenotypic plasticity and local adaptation in driving intraspecific variability in thermal niches of marine macrophytes. Ecography 41:1469–1484.

Kingsolver JG, Huey RB (1998) Evolutionary analyses of morphological and physiological plasticity in thermally variable environments. Am Zool 38:545–560.

Kiørboe T, Møhlenberg F (1981) Particle selection in suspension-feeding bivalves. Mar Ecol Prog Ser 5:291–296.

Kiørboe T, Mølenberg F, Nøhr O (1980) Feeding, particle selection and carbon absorption in *Mytilus edulis* in different mixtures of algae and resuspended bottom material. Ophelia 19:193–205.

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. Am Nat 150:1–23.

Koch V, Rengstorf A, Taylor M, Mazón-Suástegui JM, Sinsel F, Wolff M (2015) Comparative growth and mortality of cultured Lion's Paw scallops (*Nodipecten subnodosus*) from Gulf of California and Pacific populations and their reciprocal transplants. Aquac Res 46:185–201.

Koehn RK, Gaffney PM (1984) Genetic heterozygosity and growth rate in *Mytilus edulis*. Mar Biol 82:1–7.

Kong H, Jiang X, Clements JC, Wang T, Huang X, Shang Y, Chen J, Hu M, Wang Y (2019) Transgenerational effects of short-term exposure to acidification and hypoxia on early developmental traits of the mussel *Mytilus edulis*. Mar Environ Res 145:73–80.

Kong N, Li Q, Yu H, Kong L-F (2015) Heritability estimates for growth-related traits in the Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. Aquac Res 46:499–508.

Kooijman S (2010) Dynamic energy budget theory for metabolic organisation. Cambridge university press.

Kristensen JH (1972) Structure and function of crystalline styles of bivalves. Ophelia 10:91–108.

Kueh CS, Chan KY (1985) Bacteria in bivalve shellfish with special reference to the oyster. J Appl Bacteriol 59:41–47.

La Peyre M, Furlong J, Brown LA, Piazza BP, Brown K (2014) Oyster reef restoration in the northern Gulf of Mexico: Extent, methods and outcomes. Ocean Coast Manag 89:20–28.

Labarbera M (1984) Feeding currents and particle capture mechanisms in suspension feeding animals. Am Zool 24:71–84.

Labarta F-R, J. N, A. V (2002) Enzymatic digestive activity in epifaunal (*Mytilus chilensis*) and infaunal (*Mulinia edulis*) bivalves in response to changes in food regimes in a natural environment. Mar Biol 140:669–676.

Labarta U, Fernández-Reiríz M, Babarro J (1997) Differences in physiological energetics between intertidal and raft cultivated mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 152:167–173.

Lal MM, Southgate PC, Jerry DR, Zenger KR (2018) Genome-wide comparisons reveal evidence for a species complex in the black-lip pearl oyster *Pinctada margaritifera* (Bivalvia: Pteriidae). Sci Rep 8:191.

Lannig G, Eilers S, Pörtner HO, Sokolova IM, Bock C (2010) Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*—Changes in metabolic pathways and thermal response. Mar Drugs 8:2318–2339.

Lavaud R, La Peyre MK, Casas SM, Bacher C, La Peyre JF (2017) Integrating the effects of salinity on the physiology of the eastern oyster, *Crassostrea virginica*, in the northern Gulf of Mexico through a Dynamic Energy Budget model. Ecol Model 363:221–233.

Le Luyer J, Milhade L, Reisser C, Soyez C, Monaco CJ, Belliard C, Le Moullac G, Ky C-L, Pernet F (2021) Gene expression plasticity, genetic variation and fatty acid remodelling in divergent populations of a tropical bivalve species. Ecology.

Lee KH, Jeong HJ, Lee K, Franks PJS, Seong KA, Lee SY, Lee MJ, Hyeon Jang S, Potvin E, Suk Lim A, Yoon EY, Yoo YD, Kang NS, Kim KY (2019) Effects of warming and eutrophication on coastal phytoplankton production. Harmful Algae 81:106–118.

Lee Y-J, Kang HY, Lee WC, Kang C-K (2017) Hydrodynamic effects on growth performance of the Pacific Oyster *Crassostrea gigas* cultured in suspension in a temperate bay on the coast of Korea. Estuaries Coasts 40:1551–1565.

Leinonen T, McCairns RJS, O'Hara RB, Merilä J (2013) QST–FST comparisons: evolutionary and ecological insights from genomic heterogeneity. Nat Rev Genet 14:179–190.

Lesser MP (2016) Climate change stressors cause metabolic depression in the blue mussel, *Mytilus edulis*, from the Gulf of Maine. Limnol Oceanogr 61:1705–1717.

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integr Comp Biol 46:282–297.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI (2005) Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 102:11070–11075.

Li A, Li L, Song K, Wang W, Zhang G (2017) Temperature, energy metabolism, and adaptive divergence in two oyster subspecies. Ecol Evol 7(16): 6151-6162.

Li A, Wang C, Wang W, Zhang Z, Liu M, She Z, Jia Z, Zhang G, Li L (2020) Molecular and fitness data reveal local adaptation of southern and northern estuarine oysters (*Crassostrea ariakensis*). Front Mar Sci 7:589099.

Li L, Li A, Song K, Meng J, Guo X, Li S, Li C, De Wit P, Que H, Wu F, Wang W, Qi H, Xu F, Cong R, Huang B, Li Y, Wang T, Tang X, Liu S, Li B, Shi R, Liu Y, Bu C, Zhang C, He W, Zhao S, Li H, Zhang S, Zhang L, Zhang G (2018) Divergence and plasticity shape adaptive potential of the Pacific oyster. Nat Ecol Evol 2:1751–1760.

Li Y, Guan Y, Li Q, He M (2015) Analysis of DNA methylation in tissues and development stages of pearl oyster *Pinctada fucata*. Genes Genomics 37:263–270.

Liao M, Li G, Wang J, Marshall DJ, Hui TY, Ma S, Zhang Y, Helmuth B, Dong Y (2021) Physiological determinants of biogeography: the importance of metabolic depression to heat tolerance. Glob Change Biol:gcb.15578.

Liu LL, Stickle WB, Gnaiger E (1990) Normoxic and anoxic energy metabolism of the southern oyster drill Thais haemastoma during salinity acclimation. Mar Biol 104:239–245.

Lokmer A, Kuenzel S, Baines JF, Wegner KM (2016) The role of tissue-specific microbiota in initial establishment success of Pacific oysters. Environ Microbiol 18:970–987.

Luttikhuizen PC, Drent J, Baker AJ (2003a) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol Ecol 12:2215–2229.

Luttikhuizen PC, Drent J, Delden WV, Piersma T (2003b) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. J EVOL BIOL:13.

Matzelle AJ, Sarà G, Montalto V, Zippay M, Trussell GC, Helmuth B (2015) A bioenergetics framework for integrating the effects of multiple stressors: Opening a 'Black Box' in climate change research. Am Malacol Bull 33:150–160.

Mayasich SA, Smucker RA (1987) Role of *Cristispira sp*. and other bacteria in the chitinase and chitobiase activities of the crystalline style of *Crassostrea virginica* (Gmelin). Microb Ecol 14:157–166.

Maynard A, Bible JM, Pespeni MH, Sanford E, Evans TG (2018) Transcriptomic responses to extreme low salinity among locally adapted populations of Olympia oyster (*Ostrea lurida*). Molec Ecol 27(21): 4225-4240.

Mazmanian SK, Round JL, Kasper DL (2008) A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 453:620–625.

McClain CR, Stegen JC, Hurlbert AH (2012) Dispersal, environmental niches and oceanic-scale turnover in deep-sea bivalves. Proc R Soc B Biol Sci 279:1993–2002.

McFarland K, Plough LV, Nguyen M, Hare MP (2020) Are bivalves susceptible to domestication selection? Using starvation tolerance to test for potential trait changes in eastern oyster larvae. PLOS ONE 15:e0230222.

McKindsey CW, Lecuona M, Huot M, Weise AM (2009) Biodeposit production and benthic loading by farmed mussels and associated tunicate epifauna in Prince Edward Island. Aquaculture 295:44–51.

Meyer E, Manahan DT (2010) Gene expression profiling of genetically determined growth variation in bivalve larvae (*Crassostrea gigas*). J Exp Biol 213:749–758.

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. Trends Ecol Evol 20:685–692.

Møhlenberg F, Riisgård HU (1978) Efficiency of particle retention in 13 species of suspension feeding bivalves. Ophelia 17:239–246.

Monaco CJ, Sangare N, Le Moullac G, Basset C, Belliard C, Mizuno K, Smith DL, Lo-Yat A (2021) Dynamic Energy Budget model suggests feeding constraints and physiological stress in black-lip pearl oysters, 5 years post mass mortality event. Mar Pollut Bull 167:112329.

Montalto V, Helmuth B, Ruti PM, Dell'Aquila A, Rinaldi A, Sarà G (2016) A mechanistic approach reveals non linear effects of climate warming on mussels throughout the Mediterranean sea. Clim Change 139:293–306.

Montalto V, Martinez M, Rinaldi A, Sarà G, Mirto S (2017) The effect of the quality of diet on the functional response of *Mytilus galloprovincialis* (Lamarck, 1819): Implications for integrated multitrophic aquaculture (IMTA) and marine spatial planning. Aquaculture 468: 371-377.

Montaudouin XD (1996) Factors involved in growth plasticity of cockles *Cerastoderma edule* (L.) identified by field survey and transplant experiments. J Sea Res 36:251–265.

Moore HJ (1971) The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding. Mar Biol 11:23–27.

Morash AJ, Neufeld C, MacCormack TJ, Currie S (2018) The importance of incorporating natural thermal variation when evaluating physiological performance in wild species. J Exp Biol:8.

Motes ML, DePaola A, Cook DW, Veazey JE, Hunsucker JC, Garthright WE, Blodgett RJ, Chirtel SJ (1998) Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic Coast oysters (*Crassostrea virginica*). Appl Environ Microbiol 64:1459–1465.

Murren CJ, Auld JR, Callahan H, Ghalambor CK, Handelsman CA, Heskel MA, Kingsolver JG, Maclean HJ, Masel J, Maughan H, Pfennig DW, Relyea RA, Seiter S, Snell-Rood E, Steiner UK, Schlichting CD (2015) Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. Heredity 115:293–301.

Navarro E, Iglesias JIP, Ortega MM (1992) Natural sediment as a food source for the cockle *Cerastoderma edule* (L.): effect of variable particle concentration on feeding, digestion and the scope for growth. J Exp Mar Biol Ecol 156:69–87.

Navarro E, Iglesias JIP, Ortega MM, Larretxea X (1994) The basis for a functional response to variable food quantity and quality in cockles *Cerastoderma edule* (Bivalvia, Cardiidae). Physiol Zool 67:468–496.

Navarro E, Méndez S, Ibarrola I, Urrutia M (2009) Comparative utilization of phytoplankton and vascular plant detritus by the cockle *Cerastoderma edule*: digestive responses during diet acclimation. Aquat Biol 6:247–262.

Navarro J, Widdows J (1997) Feeding physiology of *Cerastoderma edule* in response to a wide range of seston concentrations. Mar Ecol Prog Ser 152:175–186.

Navarro JM, Duarte C, Manríquez PH, Lardies MA, Torres R, Acuña K, Vargas CA, Lagos NA (2016) Ocean warming and elevated carbon dioxide: multiple stressor impacts on juvenile mussels from southern Chile. ICES J Mar Sci 73:764–771.

Navarro JM, Labarta U, Fernández-Reiriz MJ, Velasco A (2003) Feeding behavior and differential absorption of biochemical components by the infaunal bivalve *Mulinia edulis* and the epibenthic *Mytilus chilensis* in response to changes in food regimes. J Exp Mar Biol Ecol 287:13–35.

Navarro JM, Thompson RJ (1994) Comparison and evaluation of different techniques for measuring absorption efficiency in suspension feeders. Limnol Oceanogr 39:159–164.

Navarro JM, Villanueva P, Rocha N, Torres R, Chaparro OR, Benítez S, Andrade-Villagrán PV, Alarcón E (2020) Plastic response of the oyster *Ostrea chilensis* to temperature and pCO2 within the present natural range of variability. PLOS ONE 15:e0234994.

Navarro JM, Winter JE (1982) Ingestion rate, assimilation efficiency and energy balance in *Mytilus chilensis* in relation to body size and different algal concentrations. Mar Biol 67:255–266.

Newell CR, Wildish DJ, MacDonald BA (2001) The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. J Exp Mar Biol Ecol 262:91–111.

Newell R (2004) Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: A review. J Shellfish Res 23:51–61.

Newell RC (1969) Effect of fluctuations in temperature on the metabolism of intertidal invertebrates. Am Zool 9:293–307.

Newell RC, Johson LG, Kofoed LH (1977) Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis*. Oecologia 30:97–110.

Newell RIE, Langdon CJ (1986) Digestion and absorption of refractory carbon from the plant *Spartina alterniflora* by the oyster *Crassostrea virginica*. Mar Ecol Prog Ser 34:105–115.

Nie H, Chen P, Huo Z, Chen Y, Hou X, Yang F, Yan X (2017) Effects of temperature and salinity on oxygen consumption and ammonia excretion in different colour strains of the Manila clam, *Ruditapes philippinarum*. Aquac Res 48:2778–2786.

Nie H, Jahan K, Zhang W, Huo Z, Yan X (2020) Physiological and biochemical responses of different shell color strains of Manila clam to low salinity challenges. Aquac Rep 16:100260.

Nielsen NF, Larsen PS, Riisgård HU, Jørgensen CB (1993) Fluid motion and particle retention in the gill of *Mytilus edulis*: Video recordings and numerical modelling. Mar Biol 116:61–71.

Niu D, Du Y, Wang Z, Xie S, Nguyen H, Dong Z, Shen H, Li J (2017) Construction of the first high-density genetic linkage map and analysis of quantitative trait loci for growth-related traits in *Sinonovacula constricta*. Mar Biotechnol 19:488–496.

Nizzoli D, Welsh DT, Bartoli M, Viaroli P (2005) Impacts of mussel (*Mytilus galloprovincialis*) farming on oxygen consumption and nutrient recycling in a eutrophic coastal lagoon. Hydrobiologia 550:183–198.

Offret C, Paulino S, Gauthier O, Château K, Bidault A, Corporeau C, Miner P, Petton B, Pernet F, Fabioux C, Paillard C, Blay GL (2020) The marine intertidal zone shapes oyster and clam digestive bacterial microbiota. FEMS Microbiol Ecol 96.

Okumus, I., Stirling H. P. (1994) Physiological energetics of cultivated mussels (*Mytilus edulis*) populations in two scottish west coast sea lochs. Mar Biol Lett 119:125–131.

Olson-Manning CF, Wagner MR, Mitchell-Olds T (2012) Adaptive evolution: evaluating empirical support for theoretical predictions. Nat Rev Genet 13:867–877.

Ortmann C (2003) Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*. J Exp Biol 206:4167–4178.

Osores SJA, Lagos NA, San Martín V, Manríquez PH, Vargas CA, Torres R, Navarro JM, Poupin MJ, Saldías GS, Lardies MA (2017) Plasticity and inter-population variability in physiological and life-history traits of the mussel *Mytilus chilensis*: A reciprocal transplant experiment. J Exp Mar Biol Ecol 490:1–12.

Ouellette-Plante J, Morden AL, Johnson LE, Martel AL, Ricciardi A (2017) Acclimation by invasive mussels: spatiotemporal variation in phenotypic response to turbidity. Freshw Sci 36:325–337.

Owen, G (1970) The fine structure of the digestive tubules of the marine bivalve *Cardium edule*. Philos Trans R Soc Lond B Biol Sci 258:245–260.

Owen G, McCrae JM (1976) Further studies on the latero-frontal tracts of bivalves. Proc R Soc B Biol Sci 194:527–544.

Pace DA, Marsh AG, Leong PK, Green AJ, Hedgecock D, Manahan DT (2006) Physiological bases of genetically determined variation in growth of marine invertebrate larvae: A study of growth heterosis in the bivalve *Crassostrea gigas*. J Exp Mar Biol Ecol 335:188–209.

Pack KE, Rius M, Mieszkowska N (2021) Long-term environmental tolerance of the non-indigenous Pacific oyster to expected contemporary climate change conditions. Mar Environ Res 164:105226.

Padilla DK, Savedo MM (2013) A systematic review of phenotypic plasticity in marine invertebrate and plant systems. In: *Advances in Marine Biology*. Elsevier, p 67–94

Padin XA, Babarro JMF, Silva E, Longa Portabales MÁ, Calvo S, Nolasco R (2021) Variability in strength of byssus attachment and index condition of subtidal mussels during the maximum growth stage. Aquac Res 52:3485–3497.

Pales Espinosa E, Allam B (2013) Food quality and season affect gene expression of the mucosal lectin MeML and particle sorting in the blue mussel *Mytilus edulis*. Mar Biol 160:1441–1450.

Pales Espinosa E, Allam B (2018) Reverse genetics demonstrate the role of mucosal C-type lectins in food particle selection in the oyster *Crassostrea virginica*. J Exp Biol:jeb.174094.

Pales Espinosa E, Hassan D, Ward JE, Shumway SE, Allam B (2010) Role of epicellular molecules in the selection of particles by the blue mussel, *Mytilus edulis*. Biol Bull 219:50–60.

Pales Espinosa E, Perrigault M, Ward JE, Shumway SE, Allam B (2009) Lectins associated with the feeding organs of the oyster *Crassostrea virginica* can mediate particle selection. Biol Bull 217:130–141.

Palmer RE, Williams LG (1980) Effect of particle concentration on filtration efficiency of the bay scallop *Argopecten irradians* and the oyster *Crassostrea virginica*. Ophelia 19:163–174.

Pante E, Becquet V, Viricel A, Garcia P (2019) Investigation of the molecular signatures of selection on ATP synthase genes in the marine bivalve *Limecola balthica*. Aquat Living Resour 32:3.

Parker LM, O'Connor WA, Byrne M, Coleman RA, Virtue P, Dove M, Gibbs M, Spohr L, Scanes E, Ross PM (2017a) Adult exposure to ocean acidification is maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of multiple stressors. Biol Lett 13:20160798.

Parker LM, O'Connor WA, Raftos DA, Pörtner H-O, Ross PM (2015) Persistence of positive carryover effects in the oyster, *Saccostrea glomerata*, following transgenerational exposure to ocean acidification. PLOS ONE 10:e0132276.

Parker LM, Ross PM, O'Connor WA, Borysko L, Raftos DA, Pörtner H-O (2012) Adult exposure influences offspring response to ocean acidification in oysters. Glob Change Biol 18:82–92.

Parker LM, Scanes E, O'Connor WA, Coleman RA, Byrne M, Pörtner H-O, Ross PM (2017b) Ocean acidification narrows the acute thermal and salinity tolerance of the Sydney rock oyster *Saccostrea glomerata*. Mar Pollut Bull 122:263–271.

Pascoe P, Parry H, Hawkins A (2009) Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish. Aquat Biol 6:181–190.

Payne BS, Miller AC, Hubertz ED, Lei J (1995) Adaptive variation in palp and gill size of the zebra mussel (*Dreissena polymorpha*) and Asian clam (*Corbicula fluminea*). Can J Fish Aquat Sci 52:1130–1134.

Peck LS, Pörtner HO, Hardewig I (2002) Metabolic demand, oxygen supply, and critical temperatures in the Antarctic bivalve *Laternula elliptica*. Physiol Biochem Zool 75:123–133.

Peñaloza C, Gutierrez AP, Eöry L, Wang S, Guo X, Archibald AL, Bean TP, Houston RD (2021) A chromosome-level genome assembly for the Pacific oyster *Crassostrea gigas*. GigaScience 10:giab020.

Penry DL, Jumars PA (1987) Modeling animal guts as chemical reactors. Am Nat 129:69–96.

Pernet F, Tremblay R, Comeau L, Guderley H (2007) Temperature adaptation in two bivalve species from different thermal habitats: energetics and remodelling of membrane lipids. J Exp Biol 210:2999–3014.

Pernet F, Tremblay R, Redjah I, Sevigny J-M, Gionet C (2008) Physiological and biochemical traits correlate with differences in growth rate and temperature adaptation among groups of the eastern oyster *Crassostrea virginica*. J Exp Biol 211:969–977.

Perrin N, Sibly RM (1993) Dynamic models of energy allocation and investment. Annu Rev Ecol Syst 24:379–410.

Petes LE, Menge BA, Murphy GD (2007) Environmental stress decreases survival, growth, and reproduction in New Zealand mussels. J Exp Mar Biol Ecol 351:83–91.

Picoche C, Le Gendre R, Flye-Sainte-Marie J, Françoise S, Maheux F, Simon B, Gangnery A (2014) Towards the determination of *Mytilus edulis* food preferences using the Dynamic Energy Budget (DEB) Theory. PLoS ONE 9:e109796.

Pierce M (2016) The microbiome of the eastern oyster, *Crassostrea virginica* (Gmelin, 1791): Temporal and spatial variation, environmental influences, and its impact on Host Physiology. Dr Diss.

Pierce ML, Ward JE (2019) Gut microbiomes of the eastern oyster (*Crassostrea virginica*) and the blue mussel (*Mytilus edulis*): Temporal variation and the influence of marine aggregate-associated microbial communities. mSphere e00730-19.

Pierce ML, Ward JE (2018) Microbial ecology of the Bivalvia, with an emphasis on the family ostreidae. J Shellfish Res 37:793–806.

Pierce SKP, Greenberg MJ (1973) The initiation and control of free amino acid regulation of cell volume in salinity-stressed marine bivalves. J Exp Biol 59:435–440.

Pourmozaffar S, Tamadoni Jahromi S, Rameshi H, Sadeghi A, Bagheri T, Behzadi S, Gozari M, Zahedi MR, Abrari Lazarjani S (2019) The role of salinity in physiological responses of bivalves. Rev Aquac:raq.12397.

Pouvreau S, Bodoy A, Buestel D (2000) In situ suspension feeding behaviour of the pearl oyster, *Pinctada margaritifera*: combined effects of body size and weather-related seston composition. Aquaculture 181:91–113.

Pouvreau S, Jonquières G, Buestel D (1999) Filtration by the pearl oyster, *Pinctada margaritifera*, under conditions of low seston load and small particle size in a tropical lagoon habitat. Aquaculture 176:295–314.

Prieto D (2020) variable capacity for acute and chronic thermal compensation of physiological rates contributes to inter-individual differences in growth rate in mussels (*Mytilus galloprovincialis*). Front Mar Sci 7:13.

Prieto D, Markaide P, Urrutxurtu I, Navarro E, Artigaud S, Fleury E, Ibarrola I, Urrutia MB (2019) Gill transcriptomic analysis in fast- and slow-growing individuals of *Mytilus galloprovincialis*. Aquaculture 511:734242.

Prieto D, Tamayo D, Urrutxurtu I, Navarro E, Ibarrola I, Urrutia MB (2020) Nature more than nurture affects the growth rate of mussels. Sci Rep 10:3539.

Prieto D, Urrutxurtu I, Navarro E, Urrutia MB, Ibarrola I (2018) *Mytilus galloprovincialis* fast growing phenotypes under different restrictive feeding conditions: Fast feeders and energy savers. Mar Environ Res 140:114–125.

Prins TC, Smaal AC, Dame RF (1998) A review of the feedbacks between bivalve grazing and ecosystem processes. Aquat Ecol 31:349–359.

Prudence M, Moal J, Boudry P, Daniel JY (2006) An amylase gene polymorphism is associated with growth differences in the Pacific cupped oyster *Crassostrea gigas*. Anim Genet:384–351.

Pujalte MJ, Ortigosa M, Macián MC, Garay E (1999) Aerobic and facultative anaerobic heterotrophic bacteria associated to Mediterranean oysters and seawater. Int Microbiol Off J Span Soc Microbiol 2:259–266.

Purce DNS, Donovan DA, Maeda-Martínez AN, Koch V (2020) Scope for growth of cultivated Pacific and Gulf of California populations of lion's paw scallop *Nodipecten subnodosus*, and their reciprocal transplants. Lat Am J Aquat Res 48:538–551.

Purchon, R. D (1987) The stomach in the bivalvia. Philos Trans R Soc B Biol Sci:183–276.

Ramajo L, Osores SJ, Lagos NA, Broitman BR, Navarro JM, Vargas CA, Manríquez PH, Lardies MA (2021) Estuarine conditions more than pH modulate the physiological

flexibility of mussel *Perumytilus purpuratus* populations. Estuar Coast Shelf Sci 249:107098.

Ramajo L, Prado L, Rodriguez-Navarro A, Lardies M, Duarte C, Lagos N (2016) Plasticity and trade-offs in physiological traits of intertidal mussels subjected to freshwater-induced environmental variation. Mar Ecol Prog Ser 553:93–109.

Rawson P, Feindel S (2012) Growth and survival for genetically improved lines of Eastern oysters (*Crassostrea virginica*) and interline hybrids in Maine, USA. Aquaculture 326–329:61–67.

Reid RGB (1965) The structure and function of the stomach in bivalve molluscs. J Zool 147:156–184.

Rey-Campos M, Novoa B, Pallavicini A, Gerdol M, Figueras A (2021) Comparative genomics reveals 13 different isoforms of mytimycins (A-M) in *Mytilus galloprovincialis*. Int J Mol Sci 22:3235.

Ribelin BW, Collier A (1977) Studies on the gill ciliation of the American oyster *Crassostrea virginica* (Gmelin). J Morphol 151:439–449.

Riginos C, Cunningham CW (2004) Invited Review: Local adaptation and species segregation in two mussel (*Mytilus edulis* × *Mytilus trossulus*) hybrid zones. Mol Ecol 14:381–400.

Riisgård (2001) On the measurement of filtration rates in bivalves - the stony road to reliable data: review and interpretation. Mar Ecol Prog Ser 211: 275-291.

Riisgård H (1988) Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Mar Ecol Prog Ser 45:217–223.

Riisgård H, Larsen P (2010) Particle capture mechanisms in suspension-feeding invertebrates. Mar Ecol Prog Ser 418:255–293.

Riisgård H, Larsen P (2018) Thermal and viscous effects on ciliary suspension-feeding bivalves — no need for a new explanation: Comment on Specht & Fuchs (2018). Mar Ecol Prog Ser 596:263–265.

Riisgård H, Larsen P (2007) Viscosity of seawater controls beat frequency of water-pumping cilia and filtration rate of mussels *Mytilus edulis*. Mar Ecol Prog Ser 343:141–150.

Riisgård H, Larsen P (2005) Water flow analysis and particle capture in ciliary suspension-feeding scallops (Pectinidae). Mar Ecol Prog Ser 303:177–193.

Riisgard HU (1991) Filtration rate and growth in the blue mussel, *Mytilus edulis* Linneaus,1758: Dependence on algal concentration. J Shellfish Res 10:29–35.

Riisgård HU, Bøttiger L, Pleissner D (2012) Effect of salinity on growth of mussels *Mytilus edulis*, with special reference to Great Belt (Denmark). Open J Mar Sci 02:167–176.

Riisgård HU, Egede PP, Barreiro Saavedra I (2011) Feeding behaviour of the mussel, *Mytilus edulis* : New observations, with a minireview of current knowledge. J Mar Biol 2011:1–13.

Riisgård HU, Larsen PoulS (2000) A comment on experimental techniques for studying particle capture in filter-feeding bivalves. Limnol Oceanogr 45:1192–1195.

Riisgård HU, Larsen PS (2001) Minireview: Ciliary filter feeding and bio-fluid mechanics-present understanding and unsolved problems. Limnol Oceanogr 46:882–891.

Riviere G, He Y, Tecchio S, Crowell E, Gras M, Sourdaine P, Guo X, Favrel P (2017) Dynamics of DNA methylomes underlie oyster development. PLOS Genet 13:e1006807.

Riviere G, Wu G-C, Fellous A, Goux D, Sourdaine P, Favrel P (2013) DNA Methylation Is Crucial for the Early Development in the Oyster *C. gigas*. Mar Biotechnol 15:739–753.

Roberts EA, Newcomb LA, McCartha MM, Harrington KJ, LaFramboise SA, Carrington E, Sebens KP (2021) Resource allocation to a structural biomaterial: induced production of byssal threads decreases growth of a marine mussel. Funct Ecol:1365-2435.13788.

Rosa M, Ward JE, Frink A, Shumway SE (2017a) Effects of surface properties on particle capture by two species of suspension-feeding bivalve molluscs. Am Malacol Bull 35:181–188.

Rosa M, Ward JE, Holohan BA, Shumway SE, Wikfors GH (2017b) Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs. J Exp Mar Biol Ecol 486:59–68.

Rosa M, Ward JE, Ouvrard M, Holohan BA, Pales Espinosa E, Shumway SE, Allam B (2015) Examining the physiological plasticity of particle capture by the blue mussel, *Mytilus edulis* (L.): Confounding factors and potential artifacts with studies utilizing natural seston. J Exp Mar Biol Ecol 473:207–217.

Rosa M, Ward JE, Shumway SE (2018) Selective capture and ingestion of particles by suspension-feeding bivalve molluscs: A Review. J Shellfish Res 37:727–746.

Rosland R, Strand ø., Alunno-Bruscia M, Bacher C, Strohmeier T (2009) Applying Dynamic Energy Budget (DEB) theory to simulate growth and bio-energetics of blue mussels under low seston conditions. J Sea Res 62:49–61.

Ross PM, Hogg ID, Pilditch CA, Lundquist CJ, Wilkins RJ (2012) Population genetic structure of the New Zealand estuarine clam *Austrovenus stutchburyi* (Bivalvia: Veneridae) reveals population subdivision and partial congruence with biogeographic boundaries. Estuaries Coasts 35:143–154.

Rubenstein DI, Koehl MAR (1977) The mechanisms of filter feeding: Some theoretical considerations. Am Nat 111:981–994.

Rueda JL, Smaal AC (2002) Physiological response of *Spisula subtruncata* (da Costa, 1778) to different seston quantity and quality. In: *Nutrients and Eutrophication in Estuaries and Coastal Waters*. Orive E, Elliott M, de Jonge VN (eds) p 505–511

Russell BD, Connell SD, Mellin C, Brook BW, Burnell OW, Fordham DA (2012) Predicting the distribution of commercially important invertebrate stocks under Future Climate. PLoS ONE 7:e46554.

Saavedra C, Milan M, Leite RB, Cordero D, Patarnello T, Cancela ML, Bargelloni L (2017) A microarray study of carpet-shell clam (*Ruditapes decussatus*) shows common and organ-specific growth-related gene expression differences in gills and digestive gland. Front Physiol 8:943.

Sakshaug E, Olsen Y (1986) Nutrient status of phytoplankton blooms in Norwegian waters and algal strategies for nutrient competition. Can J Fish Aquat Sci 43:389–396.

Sanford E, Kelly MW (2011) Local adaptation in marine invertebrates. Annu Rev Mar Sci 3:509–535.

Sarà G, Reid GK, Rinaldi A, Palmeri V, Troell M, Kooijman SALM (2012) Growth and reproductive simulation of candidate shellfish species at fish cages in the Southern

Mediterranean: Dynamic Energy Budget (DEB) modelling for integrated multi-trophic aquaculture. Aquaculture 324–325:259–266.

Sarà G, Romano C, Widdows J, Staff FJ (2008) Effect of salinity and temperature on feeding physiology and scope for growth of an invasive species (B*rachidontes pharaonis* - Mollusca: Bivalvia) within the Mediterranean sea. J Exp Mar Biol Ecol 363:130–136.

Schatte Olivier A, Jones L, Vay LL, Christie M, Wilson J, Malham SK (2020) A global review of the ecosystem services provided by bivalve aquaculture. Rev Aquac 12:3–25.

Scholten H, Smaal AC (1998) Responses of *Mytilus edulis* L. to varying food concentrations: testing EMMY, an ecophysiological model. J Exp Mar Biol Ecol 219:217–239.

Scholten H, Smaal AC (1999) The ecophysiological response of mussels (*Mytilus edulis*) in mesocosms to a range of inorganic nutrient loads: simulations with the model EMMY. Aquat Ecol 33:83–100.

Schwaner C, Barbosa M, Connors P, Park T-J, de Silva D, Griffith A, Gobler CJ, Pales Espinosa E, Allam B (2020) Experimental acidification increases susceptibility of *Mercenaria mercenaria* to infection by Vibrio species. Mar Environ Res 154:104872.

Seebacher F, Brand MD, Else PL, Guderley H, Hulbert AJ, Moyes CD (2010) Plasticity of oxidative metabolism in variable climates: Molecular mechanisms. Physiol Biochem Zool 83:721–732.

Seiderer LJ, Newell RC (1979) Adjustment of the activity of a-amylase extracted from the style of the black mussel *Choromytilus meridionalis* (Krauss) in response to thermal acclimation. J Exp Mar Biol Ecol 39:79–86.

Sherker Z, Ellrich J, Scrosati R (2017) Predator-induced shell plasticity in mussels hinders predation by drilling snails. Mar Ecol Prog Ser 573:167–175.

Shimeta J, Jumars PA (1991) Physical mechanisms and rates of particle capture by suspension-feeders. Oceanogr Mar Biol Annu Rev 29:191–257.

Shumway (1982) Oxygen consumption in oysters: An overview. Mar Biol Lett:1–23.

Shumway S, Koehn R (1982) Oxygen consumption in the American oyster *Crassostrea virginica*. Mar Ecol Prog Ser 9:59–68.

Shumway SE, Cucci TL, Newell RC, Yentsch CM (1985) Particle selection, ingestion, and absorption in filter-feeding bivalves. J Exp Mar Biol Ecol 91:77–92.

Shumway SE, Davis C, Downey R, Karney R, Kraeuter J, Parsons J, Rheault R, Wikfors G, others (2003) Shellfish aquaculture–in praise of sustainable economies and environments. World Aquac 34:8–10.

Shumway SE, Gabbott PA, Youngson A (1977) The effect of fluctuating salinity on the concentrations of free amino acids and ninhydrin-positive substances in the adductor muscles of eight species of bivalve molluscs. J Exp Mar Biol Ecol 29:131–150.

Smaal AC, Ferreira JG, Grant J, Petersen JK, Strand Ø (eds) (2019) Goods and services of marine bivalves. Springer International Publishing, Cham.

Smaal AC, Schellekens T, van Stralen MR, Kromkamp JC (2013) Decrease of the carrying capacity of the Oosterschelde estuary (SW Delta, NL) for bivalve filter feeders due to overgrazing? Aquaculture 404–405:28–34.

Smaal AC, Verbagen JHG, Coosen J, Haas HA (1986) Interaction between seston quantity and quality and benthic suspension feeders in the Oosterschelde, the Netherlands. Ophelia 26:385–399.

Smaal AC, Vonck APMA, Bakker M (1997) Seasonal variation in physiological energetics of *Mytilus Edulis* and *Cerastoderma Edule* of different size classes. J Mar Biol Assoc U K 77:817–838.

Smaal AC, Zurburg W (1997) The uptake and release of suspended and dissolved material by oysters and mussels in Marennes-Oléron Bay. Aquat Living Resour 10:23–30.

Snover ML, Commito JA (1998) The fractal geometry of *Mytilus edulis* L. spatial distribution in a soft-bottom system. J Exp Mar Biol Ecol 223:53–64.

Soon TK, Zheng H (2019) Climate change and bivalve mass mortality in temperate regions. Rev Environ Contam Toxicol Vol 251:109–129.

Specht J, Fuchs H (2018) Thermal and viscous effects of temperature on *Mercenaria mercenaria* suspension feeding. Mar Ecol Prog Ser 589:129–140.

Steeves L, Strohmeier T, Filgueira R, Strand Ø (2020) Exploring feeding physiology of *Mytilus edulis* across geographic and fjord gradients in low-seston environments. Mar Ecol Prog Ser 651:71–84.

Stewart BD, Jenkins SR, Boig C, Sinfield C, Kennington K, Brand AR, Lart W, Kröger R (2021) Metal pollution as a potential threat to shell strength and survival in marine bivalves. Sci Total Environ 755:143019.

Strickland JDH, Parsons TR (1968) A practical Handbook of Seawater Analysis. Bulletin of Fisheries Research Board of Canada.

Strohmeier T, Strand Ø, Alunno-Bruscia M, Duinker A, Cranford PJ (2012) Variability in particle retention efficiency by the mussel *Mytilus edulis*. J Exp Mar Biol Ecol 412:96–102.

Strohmeier T, Strand Ø, Alunno-Bruscia M, Duinker A, Rosland R, Aure J, Erga SR, Naustvoll LJ, Jansen HM, Cranford PJ (2015) Response of *Mytilus edulis* to enhanced phytoplankton availability by controlled upwelling in an oligotrophic fjord. Marine Ecology Progress Series 518:139-152

Strohmeier T, Strand Ø, Cranford P (2009) Clearance rates of the great scallop (*Pecten maximus*) and blue mussel (*Mytilus edulis*) at low natural seston concentrations. Mar Biol 156:1781–1795.

Sukhotin AA, Strelkov PP, Maximovich NV, Hummel H (2007) Growth and longevity of *Mytilus edulis* (L.) from northeast Europe. Mar Biol Res 3:155–167.

Sunde BK (2013) Gill and labial palp areas in blue mussels (*Mytilus edulis*) at sites with different food quantity. University of Bergen

Tamayo D, Ibarrola I, Cigarría J, Navarro E (2015) The effect of food conditioning on feeding and growth responses to variable rations in fast and slow growing spat of the Manila clam (*Ruditapes philippinarum*). J Exp Mar Biol Ecol 471:92–103.

Tamayo D, Ibarrola I, Navarro E (2013) Thermal dependence of clearance and metabolic rates in slow- and fast-growing spats of manila clam *Ruditapes philippinarum*. J Comp Physiol B 183:893–904.

Tamayo D, Ibarrola I, Urrutia MB, Navarro E (2011) The physiological basis for inter-individual growth variability in the spat of clams (*Ruditapes philippinarum*). Aquaculture 321:113–120.

Tang F, Kemp JS, Aldridge DC (2020) Life on the edge: Compensatory growth and feeding rates at environmental extremes mediates potential ecosystem engineering by an invasive bivalve. Sci Total Environ 706:135741.

Tangherlini M, Corinaldesi C, Ape F, Greco S, Romeo T, Andaloro F, Danovaro R (2021) Ocean acidification induces changes in virus–host relationships in Mediterranean benthic ecosystems. Microorganisms 9:769.

Tedengren M, André C, Johannesson K, Kautsky N (1990) Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantations. III. Physiology. Mar Ecol Prog Ser 59:221–227.

Telesca L, Peck LS, Sanders T, Thyrring J, Sejr MK, Harper EM (2019) Biomineralization plasticity and environmental heterogeneity predict geographical resilience patterns of foundation species to future change. Glob Change Biol 25:4179–4193.

Thomas Y, Mazurié J, Alunno-Bruscia M, Bacher C, Bouget J-F, Gohin F, Pouvreau S, Struski C (2011) Modelling spatio-temporal variability of *Mytilus edulis* (L.) growth by forcing a dynamic energy budget model with satellite-derived environmental data. J Sea Res 66:308–317.

Thomas Y, Pouvreau S, Alunno-Bruscia M, Barillé L, Gohin F, Bryère P, Gernez P (2016) Global change and climate-driven invasion of the Pacific oyster (*Crassostrea gigas*) along European coasts: a bioenergetics modelling approach. J Biogeogr 43:568–579.

Thomsen J, Stapp LS, Haynert K, Schade H, Danelli M, Lannig G, Wegner KM, Melzner F (2017) Naturally acidified habitat selects for ocean acidification–tolerant mussels. Sci Adv 3:e1602411.

Thyrring J, Rysgaard S, Blicher ME, Sejr MK (2015) Metabolic cold adaptation and aerobic performance of blue mussels (*Mytilus edulis*) along a temperature gradient into the High Arctic region. Mar Biol 162:235–243.

Timbs J, Powell E, Mann R (2019) Changes in the spatial distribution and anatomy of a range shift for the Atlantic surfclam Spisula solidissima in the Mid-Atlantic Bight and on Georges Bank. Mar Ecol Prog Ser 620:77–97.

Trabal Fernández N, Mazón-Suástegui JM, Vázquez-Juárez R, Ascencio-Valle F, Romero J (2014) Changes in the composition and diversity of the bacterial microbiota associated with oysters (*Crassostrea corteziensis*, *Crassostrea gigas* and *Crassostrea sikamea*) during commercial production. FEMS Microbiol Ecol 88:69–83.

Trabal N, Mazón-Suástegui JM, Vázquez-Juárez R, Asencio-Valle F, Morales-Bojórquez E, Romero J (2012) Molecular analysis of bacterial microbiota associated with oysters (*Crassostrea gigas* and *Crassostrea corteziensis*) in different growth phases at two cultivation sites. Microb Ecol 64:555–569.

Tremblay R, Myrand B, Sevigny J-M, Blier P, Guderley H (1998) Bioenergetic and genetic parameters in relation to susceptibility of blue mussels, *Mytilus edulis* (L.) to summer mortality. J Exp Mar Biol Ecol 221:27–58.

Trenberth KE (2011) Changes in precipitation with climate change. Clim Res 47:123–138.

Trestrail C, Walpitagama M, Miranda A, Nugegoda D, Shimeta J (2021) Microplastics alter digestive enzyme activities in the marine bivalve, *Mytilus galloprovincialis*. Sci Total Environ:146418.

Trottet A, Roy S, Tamigneaux E, Lovejoy C, Tremblay R (2008) Impact of suspended mussels (*Mytilus edulis* L.) on plankton communities in a Magdalen Islands lagoon (Québec, Canada): A mesocosm approach. J Exp Mar Biol Ecol 365:103–115.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI (2007) The human microbiome project. Nature 449:804–810.

Turner LM, Alsterberg C, Turner AD, Girisha SK, Rai A, Havenhand J, Venugopal MN, Karunasagar I, Godhe A (2016) Pathogenic marine microbes influence the effects of climate change on a commercially important tropical bivalve. Sci Rep:10.

Urrutia MB, Iglesias JI, Navarro E, Prou J (1996) Feeding and absorption in Cerastoderma eduee under environmental conditions in the bay of Marennesoleron (Western France). J Mar Biol Assoc UK 76(2):431-50.

Vahl O (1972) Efficiency of particle retention in *Mytilus edulis* L. Ophelia 10:17–25.

Vajedsamiei J, Melzner F, Raatz M, Kiko R, Khosravi M, Pansch C (2021) Simultaneous recording of filtration and respiration in marine organisms in response to short-term environmental variability. Limnol Oceanogr Methods:lom3.10414.

Vargas CA, Aguilera VM, Martín VS, Manríquez PH, Navarro JM, Duarte C, Torres R, Lardies MA, Lagos NA (2015) CO2-driven ocean acidification disrupts the filter feeding behavior in Chilean gastropod and bivalve species from different geographic localities. Estuaries Coasts:15.

Vargas CA, Lagos NA, Lardies MA, Duarte C, Manríquez PH, Aguilera VM, Broitman B, Widdicombe S, Dupont S (2017) Species-specific responses to ocean acidification should account for local adaptation and adaptive plasticity. Nat Ecol Evol 1:0084.

Velasco L, Navarro J (2002) Feeding physiology of infaunal (*Mulinia edulis*) and epifaunal (*Mytilus chilensis*) bivalves under a wide range of concentrations and qualities of seston. Mar Ecol Prog Ser 240:143–155.

Velasco L, Navarro J (2005) Feeding physiology of two bivalves under laboratory and field conditions in response to variable food concentrations. Mar Ecol Prog Ser 291:115–124.

Ventura A, Schulz S, Dupont S (2016) Maintained larval growth in mussel larvae exposed to acidified under-saturated seawater. Sci Rep 6:23728.

Vezzulli L, Stagnaro L, Grande C, Tassistro G, Canesi L, Pruzzo C (2018) Comparative 16SrDNA gene-based microbiota profiles of the Pacific oyster (*Crassostrea gigas*) and the Mediterranean mussel (*Mytilus galloprovincialis*) from a Shellfish Farm (Ligurian Sea, Italy). Microb Ecol 75:495–504.

de Villemereuil P, Gaggiotti O, Mouterde M, Till-Bottraud I (2016) Common garden experiments in the genomic era: new perspectives and opportunities. Heredity 116:249–254.

Wang R, He J, Wang J (2016) Heterotrophic bacterial abundance and diversity in the farming environment and guts of the oyster *Crassostrea hongkongensis*. J Shellfish Res 35:343–350.

Wang S, Hou R, Bao Z, Du H, He Y, Su H, Zhang Y, Fu X, Jiao W, Li Y, Zhang L, Wang S, Hu X (2013) Transcriptome sequencing of Zhikong scallop (*Chlamys farreri*)

and comparative transcriptomic analysis with Yesso scallop (*Patinopecten yessoensis*). PLoS ONE 8:e63927.

Wang, W (1995) Assimilation of trace elements ingested by the mussel *Mytilus edulis*: effects of algal food abundance. Mar Ecol Prog Ser:165–176.

Ward E, MacDonald BA (1996) Pre-ingestive feeding behaviours of two sub-tropical bivalves (*Pinctada imbricata* and *Arca zebra*): Responses to an acute increase in suspended sediment concentration. Bull Mar Sci 59(2): 417-432.

Ward JE (1996) Biodynamics of suspension-feeding in adult bivalve molluscs: Particle capture, processing, and fate. Invertebr Biol 115:218.

Ward JE, Levinton JS, Shumway SE, Cucci T (1998a) Particle sorting in bivalves: in vivo determination of the pallial organs of selection. Mar Biol 131:283–292.

Ward JE, MacDonald BA, Thompson RJ, Beninger PG (1993) Mechanisms of suspension feeding in bivalves: Resolution of current controversies by means of endoscopy. Limnol Oceanogr 38:265–272.

Ward JE, Newell RIE, Thompson RJ, Macdonald BA (1994) In vivo studies of Suspension-feeding processes in the eastern oyster, *Crassostrea virginica* (Gmelin). Biol Bull 186:221–240.

Ward JE, Sanford LP, Newell RIE, MacDonald BA (1998b) A new explanation of particle capture in suspension- feeding bivalve molluscs. Limnol Oceanogr 43:741–752.

Waser AM, Deuzeman S, Kangeri AK wa, van Winden E, Postma J, de Boer P, van der Meer J, Ens BJ (2016) Impact on bird fauna of a non-native oyster expanding into blue mussel beds in the Dutch Wadden Sea. Biol Conserv 202:39–49.

Weitzman J, Steeves L, Bradford J, Filgueira R (2019) Far-field and near-field effects of marine aquaculture. In: *World Seas: an Environmental Evaluation*. Elsevier, p 197–220

West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. J Exp Zoolog B Mol Dev Evol 304B:610–618.

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annu Rev Ecol Syst 20:248–278.

Widdows J (1976) Physiological adaptation of *Mytilus edulis* to cyclic temperatures. J Comp Physiol B 105:115–128.

Widdows J (1973) The effects of temperature on the metabolism and activity of *Mytilus edulis*. Neth J Sea Res 7:387–398.

Widdows J, Donkin P, Salkeld P, Cleary J, Lowe D, Evans S, Thomson P (1984) Relative importance of environmental factors in determining physiological differences between two populations of mussels (*Mytilus edulis*). Mar Ecol Prog Ser 17:33–47.

Widdows J, Fieth P, Worrall CM (1979) Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar Biol 50:195–207.

Widdows J, Hawkins AJS (1989) Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding, and growth components. Physiol Zool 62:764–784.

Widdows J, Johnson D (1988) Physiological energetics of *Mytilus edulis*: Scope for Growth. Mar Ecol Prog Ser 46:113–121.

Widdows J, Staff F (2006) Measurement of scope for growth in mussels. ICES Tech Mar Env:1–30.

Wildish D, Kristmanson DD (1997) Benthic suspension feeders and flow. Cambridge University Press.

Willows (1992) Optimal digestive investment: a model for filter feeders experiencing variable diets. Am Soc Limnol Oceanogr 37:829–847.

Wilson JG, Elkaim B (1997) Seasonal and geographical differences in oxygen consumption with temperature of *Cerastoderma glaucum* (Poiret) and a comparison with *C. edule* (L.). Estuar Coast Shelf Sci 45:571–577.

Winter J (1976) A critical review on some aspects of filter-feeding in lamellibranchiate bivalves. Haliotis 7:l–87.

Winter JE (1973) The filtration rate of *Mytilus edulis* and its dependence on algal concentration, measured by a continuous automatic recording apparatus. Mar Biol 22:317–328.

Wold AE, Adlerberth I (2000) Breast feeding and the intestinal microflora of the infant--implications for protection against infectious diseases. Adv Exp Med Biol 478:77–93.

Wong W, Cheung S (2003) Site-related differences in the feeding physiology of the green mussel *Perna viridis*: a reciprocal transplantation experiment. Mar Ecol Prog Ser 258:147–159.

Worrall C, Widdows J (1983) Physiological changes following transplantation of the bivalve *Scrobicularia plana* between three populations. Mar Ecol Prog Ser 12:281–287.

Xu T, Sun J, Watanabe HK, Chen C, Nakamura M, Ji R, Feng D, Lv J, Wang S, Bao Z, Qian P-Y, Qiu J-W (2018) Population genetic structure of the deep-sea mussel *Bathymodiolus platifrons* (Bivalvia: Mytilidae) in the Northwest Pacific. Evol Appl 11:1915–1930.

Yahel G, Marie D, Beninger P, Eckstein S, Genin A (2009) In situ evidence for pre-capture qualitative selection in the tropical bivalve *Lithophaga simplex*. Aquat Biol 6:235–246.

Yan X, Nie H, Huo Z, Ding J, Li Z, Yan L, Jiang L, Mu Z, Wang H, Meng X, Chen P, Zhou M, Rbbani MdG, Liu G, Li D (2019) Clam genome sequence clarifies the molecular basis of its benthic adaptation and extraordinary shell color diversity. iScience 19:1225–1237.

Yang Z, Zhang L, Hu J, Wang J, Bao Z, Wang S (2020) The evo-devo of molluscs: Insights from a genomic perspective. Evol Dev 22:409–424.

Yévenes M, Núñez-Acuña G, Gallardo-Escárate C, Gajardo G (2021) Adaptive differences in gene expression in farm-impacted seedbeds of the native blue mussel *Mytilus chilensis*. Front Genet 12:666539.

Zhang F, Hu B, Fu H, Jiao Z, Li Q, Liu S (2019) Comparative transcriptome analysis reveals molecular basis underlying fast growth of the selectively bred Pacific oyster, *Crassostrea gigas*. Front Genet 10:610.

Zhang G, Fang X, Guo X, Li L, Luo R, Xu F, Yang P, Zhang L, Wang X, Qi H, Xiong Z, Que H, Xie Y, Holland PWH, Paps J, Zhu Y, Wu F, Chen Y, Wang J, Peng C, Meng J, Yang L, Liu J, Wen B, Zhang N, Huang Z, Zhu Q, Feng Y, Mount A, Hedgecock D, Xu Z, Liu Y, Domazet-Lošo T, Du Y, Sun X, Zhang S, Liu B, Cheng P, Jiang X, Li J, Fan D, Wang W, Fu W, Wang T, Wang B, Zhang J, Peng Z, Li Y, Li N, Wang J, Chen M, He Y, Tan F, Song X, Zheng Q, Huang R, Yang H, Du X, Chen L, Yang M, Gaffney PM, Wang S, Luo L, She Z, Ming Y, Huang W, Zhang S, Huang B, Zhang Y, Qu T, Ni P, Miao G, Wang J, Wang Q, Steinberg CEW, Wang H, Li N, Qian L, Zhang G, Li Y, Yang H, Liu X, Wang J, Yin Y, Wang J

(2012) The oyster genome reveals stress adaptation and complexity of shell formation. Nature 490:49–54.

Zhang J, Li Q, Liu S, Yu H, Kong L (2018) The effect of temperature on physiological energetics of a fast-growing selective strain and a hatchery population of the Pacific oyster (*Crassostrea gigas*). Aquac Res 49:2844–2851.

Zhao L, Schöne BR, Mertz-Kraus R, Yang F (2017) Sodium provides unique insights into transgenerational effects of ocean acidification on bivalve shell formation. Sci Total Environ 577:360–366.

Zhao L, Yang F, Milano S, Han T, Walliser EO, Schöne BR (2018) Transgenerational acclimation to seawater acidification in the Manila clam *Ruditapes philippinarum*: Preferential uptake of metabolic carbon. Sci Total Environ 627:95–103.

Zhu X, Liao H, Yang Z, Peng C, Lu W, Xing Q, Huang X, Hu J, Bao Z (2020) Genome-wide identification, characterization of RLR genes in Yesso scallop (*Patinopecten yessoensis*) and functional regulations in responses to ocean acidification. Fish Shellfish Immunol 98:488–498.

Zúñiga D, Castro CG, Aguiar E, Labarta U, Figueiras FG, Fernández-Reiriz MJ (2014) Biodeposit contribution to natural sedimentation in a suspended *Mytilus galloprovincialis* Lmk mussel farm in a Galician Ría (NW Iberian Peninsula). Aquaculture 432:311–320.

Zurel D, Benayahu Y, Or A, Kovacs A, Gophna U (2011) Composition and dynamics of the gill microbiota of an invasive Indo-Pacific oyster in the eastern Mediterranean Sea. Environ Microbiol 13:1467–1476.

de Zwaan A, Wijsman TCM (1976) Anaerobic metabolism in bivalvia (Mollusca) Characteristics of anaerobic metabolism. Comp Biochem Physiol Part B Comp Biochem 54:313–323.