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Exploring feeding physiology of *Mytilus edulis* across geographic and fjord gradients in low-seston environments

Laura Steeves[1,*], Tore Strohmeier[2], Ramón Filgueira[2,3], Øivind Strand[2]

[1]Dalhousie University, Department of Biology, NS B3H 4R2, Canada
[2]Institute of Marine Research, Bergen, Nordnes, Bergen 5871, Norway
[3]Dalhousie University, Marine Affairs Program, Halifax, NS B3H 4R2, Canada

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.*

KEY WORDS: *Mytilus edulis* · Capture efficiency · Pumping rate · Ingestion · Plasticity · Acclimation

1. 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 it also has 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 knowledge gap; 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

*Corresponding author: laura.steeves@dal.ca

overall growth and ecosystem interactions of widely distributed species.

Ingestion rate in bivalves is a function of 4 components: food concentration, pumping rate (PR), capture efficiency (CE), and rejection rate. PR 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 particle 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 (e.g. low-seston environments, usually below 2.5–5 mg l^{-1}; Widdows et al. 1979), ingestion in bivalves is a function of food concentration, PR, 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 in *Mytilus edulis*, including this asymptote (4 µm; Møhlenberg & Riisgård 1978), the 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 (see Rosa et al. 2018 for review). Most variability in CE occurs with small particle sizes (~1–4 µm); 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 is widely distributed on a global scale (Sukhotin et al. 2007), making it a model species for exploring the effects of localized conditions on the response of CE and PR. These responses may be plastic, e.g. operate in the short term and be reversible, or adaptive, causing long-term irreversible changes. Many feeding and growth traits of bivalves are highly plastic, particularly PRs, 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 to the environment over long periods of time and may not easily respond to short-term environmental changes (e.g. salinity tolerance; Riginos & Cunningham 2005). 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 compared among 3 populations of mussels, and within 2 groups along 2 fjord gradients. Using natural seston, the CE, PR, and ingestion rate were measured in all 5 sampling locations, which covered a broad range of environmental conditions reflecting the diverse habitats in which *M. edulis* grow. Given that differences in CE were observed in *M. edulis* within the same population along a fjord gradient, mussels were reciprocally transplanted between these 2 locations along the fjord to determine if these differences were driven by short- or long-term changes in the environment. This study aimed to provide a clearer understanding of particle capture, PR, and ingestion in filter-feeding bivalves.

2. MATERIALS AND METHODS

2.1. Experimental design

Two sequential experiments were carried out between April and June 2018 in Norway. In Expt 1, feeding trials were conducted at 5 field sites (Fig. 1; Austevoll, Hardangerfjord, Flødevigen, and 2 sites within Åfjord), covering a geographic range from 58–63° N, and 2 fjord gradients, from inner to outer area (Hardangerfjord–Austevoll and Åfjord 1–Åfjord 2) (Fig. 1). Subsequently, in Expt 2, mussels were transplanted between 2 sites along a fjord gradient that had previously been sampled in Expt 1, Austevoll and Hardangerfjord. These mussels were acclimated for 3 wk, and feeding trials were conducted, measuring both native and transplanted mussels at each site.

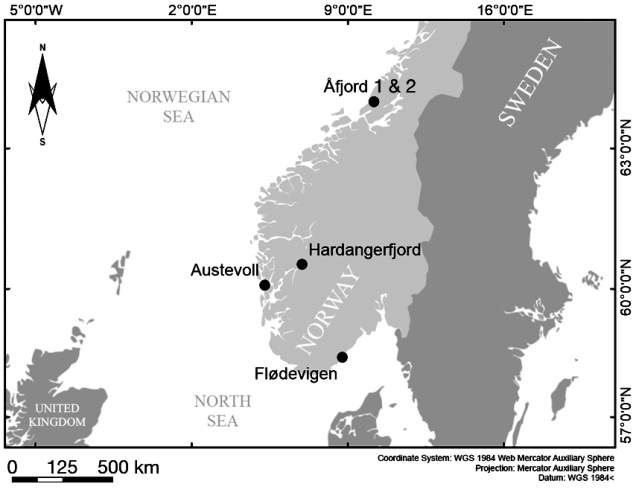

Fig. 1. Location of the 5 field sites used in this study. For Expt 1, measurements were taken at Austevoll (60° 6′ 45.77″ N, 5° 11′ 23.95″ E) on 16–19 April; Hardangerfjord (60° 32′ 38.86″ N, 6° 56′ 47.60″ E) on 24–25 April; Flødevigen (58° 25′ 34.42″ N, 8° 45′ 16.09″ E) on 8–9 May; and Åfjord (63° 56′ 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

2.2. Water quality measurements

At each field site for both Expts 1 and 2, measurements were taken to describe water and seston characteristics. A CTD (SAIV A/S Model 204) was deployed in the header tank to record temperature and salinity. Each day that a feeding trial was run, water characteristics were determined at the beginning, middle, and end of the feeding trial by collecting water from the pump supplying water to the trial. To measure chlorophyll *a* (chl *a*) concentration, 250 ml 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 (POC) was measured by filtering 150 ml of water onto a 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 were estimated as equivalent spherical diameter (ESD, µm). Using these counts (in triplicate) and associated size (ESD, µm), particle volume by size class was determined.

2.3. Feeding trials

Feeding trials were conducted using the static method to measure mussel CE and PR (Cranford et al. 2016). At each site, the day before sampling began, wild mussels were graded for length (50.1 ± 4.3 mm for all mussels), cleansed of epibionts, and held at 3 m depth. For each experiment, 40 individual mussels were sampled. During trials, mussels were held in a tank provided with flowing water pumped from 3 m depth. CE was measured following Cranford et al. (2016). This technique is based on continuous monitoring, at high temporal resolution (30 s), 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 (0.98 l 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 s 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 1 h.

2.4. Estimation of CE, PR, 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:

$$CE_{size} = \frac{\lambda_{sample,\,size} - \lambda_{control,\,size}}{\lambda_{average}} \quad (1)$$

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; and $\lambda_{average}$ 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 8.5–11.5 µm. All particle sizes are expressed as ESD (µm). To further describe the CE of mussels at each sampling site, CE for particles at 4 ESD (µm), and the minimum particle size at which CE reached 1 were calculated from the group average, and group average + SD, respectively, at each sampling site in both Expts 1 and 2.

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:

$$CE = \frac{1}{1 + e^{[-phi2 \times (size - phi3)]}} \quad (2)$$

where 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 CE 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, PR (l h^{-1}), the volume of water moved across the gill per unit time, can be calculated as:

$$PR = \lambda_{average} \times V \times 60 \times 60 \quad (3)$$

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

$$VIR = \sum_{size\,=\,1.75}^{9.5}(PR \times CE_{size}) \times (particle\ count_{size} \times particle\ volume_{size}) \quad (4)$$

where $particle\ count_{size}$ and $particle\ volume_{size}$ are the number of particles of a given size, and the 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 calculated 2 additional ways, using POC and subsequently chl *a* as other measures of food concentration:

$$Ingestion = PR \times (POC\ or\ chl\ a) \quad (5)$$

where POC is in units of mg l^{-1}, chl *a* is µg l^{-1}, and ingestion rate is either in mg h^{-1} or µg h^{-1}.

2.5. Standardization of PR and ingestion rate

PR was standardized to average gill area (GA) using the following formula:

$$PR_{std} = PR \times \left(\frac{GA_{std}}{GA_{ind}}\right) \quad (6)$$

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 Expts 1 and 2), and GA_{ind} is the gill area for the individual being standardized. For Expt 1, GA 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 the inner organs and mantle on both sides of the shell, leaving 2 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 4 gills were equal in size, a picture was taken of a shell half containing 2 stacked gills. A top-down view of a shell half with 2 gills in it shows half of the surface area of one gill. This area was measured using freehand selections in ImageJ. This area (in cm^2) was then multiplied by 8 (2 sides of 4 gills), to estimate total GA. Average GA in Expt 1 was 23.3 cm^2, which is equivalent to a length of 51.4 mm. For Expt 2, GAs were estimated using the relationship between GA and length of Expt 1 from each respective group — Austevoll: ($GA\ [cm^2] = 0.0004 \times length\ [mm]^{2.85}$, $r^2 = 0.68$, n = 10); Hardangerfjord: ($GA\ [cm^2] = 0.0027 \times length\ [mm]^{2.26}$, $r^2 = 0.79$, n = 27). The average estimated GA for Austevoll mussels in Expt 2 was 31.0 cm^2, equivalent to a length of 51.6 mm. The average estimated GA for Hardanger-

fjord mussels in Expt 2 was 158 cm^2, equivalent to a length of 46.3 mm. GA was standardized to the average GA of both groups (21.8 cm^2).

2.6. Statistics

Parametric tests (ANOVA or Student's *t*-tests) were employed to compare environmental parameters and feeding physiology measurements (PR, VIR). A 1-way ANCOVA was conducted to test for differences in GA by location while controlling for length. Assumptions of parametric tests were examined, 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).

3. RESULTS

3.1. Expt 1: water quality parameters

Temperature ranged from 5.5–13.8°C (Austevoll and Åfjord 2, respectively), and salinity ranged from 20.8–31.6 (Flødevigen and Austevoll, respectively) (Table 1). Chl *a* was highest at Hardangerfjord ($F = 19.78$, $df_{4,21}$, $p < 0.05$; Fig. 2A), followed equivalently by Åfjord 2, Åfjord 1, and Flødevigen ($df_{4,21}$, $p > 0.05$; Fig. 2A). Austevoll had the lowest chl *a* levels, significantly lower than both Hardangerfjord and Åfjord 2 ($df_{4,21}$, $p < 0.05$; Fig. 2A). POC levels were highest at Åfjord 2, followed equivalently by Åfjord 1, Hardangerfjord, and Flødevigen ($F = 41.76$, $df_{4,21}$, $p < 0.05$; Fig. 2B). Austevoll again had the lowest levels of POC, lower than both Åfjord 1 and 2 ($df_{4,21}$, $p < 0.05$; Fig. 2A). Volume of particles for each size class (ESD, µm) varied with particle size (Fig. 2C), with a notable peak in the Hardangerfjord data between 4 and 6 µm and Austevoll at 4 µm (Fig. 2C).

3.2. Expt 1: feeding trials

No pseudofaeces production was observed during any of the feeding trials. When controlling for length, GA varied significantly between populations ($F_{4,115} = 31.2$, $p < 0.001$), where the Hardangerfjord mussels had significantly smaller gills than any other group of mussels ($df_{4,115}$, $p < 0.001$). The Hardangerfjord mussels had an uncharacteristic peak of high CE values for particles between 2 and 3 µm ESD (Fig. 3B). The steepness of the curves (i.e. phi2) differed among all groups, with Flødevigen and Hardangerfjord being the highest and lowest, respectively ($p < 0.001$; Fig. 4A). The particle size when CE is at 0.5 (i.e. phi3) was lowest for Åfjord 1 ($p < 0.05$) followed by Hardangerfjord and

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

	Austevoll	Hardangerfjord	Flødevigen	Åfjord 1	Åfjord 2
Temp. (°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

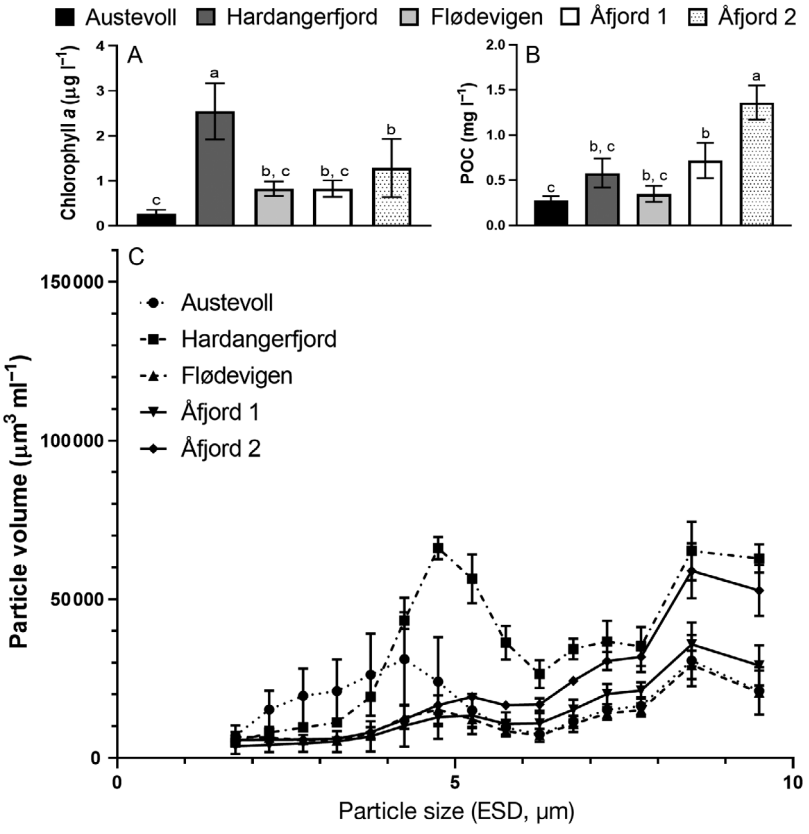

Fig. 2. Water quality measurements (±SD) from all locations sampled in Expt 1: (A) chl *a*, (B) particulate organic carbon (POC), and (C) total particle volume for each size class measured (equivalent spherical diameter, ESD). Particle volume was estimated using 0.5 µm diameter steps, excluding the last 2 measurements (8.5 and 9.5 µm) which used 1 µm steps due to low particle counts. Letters denote statistical significance at $\alpha = 0.05$

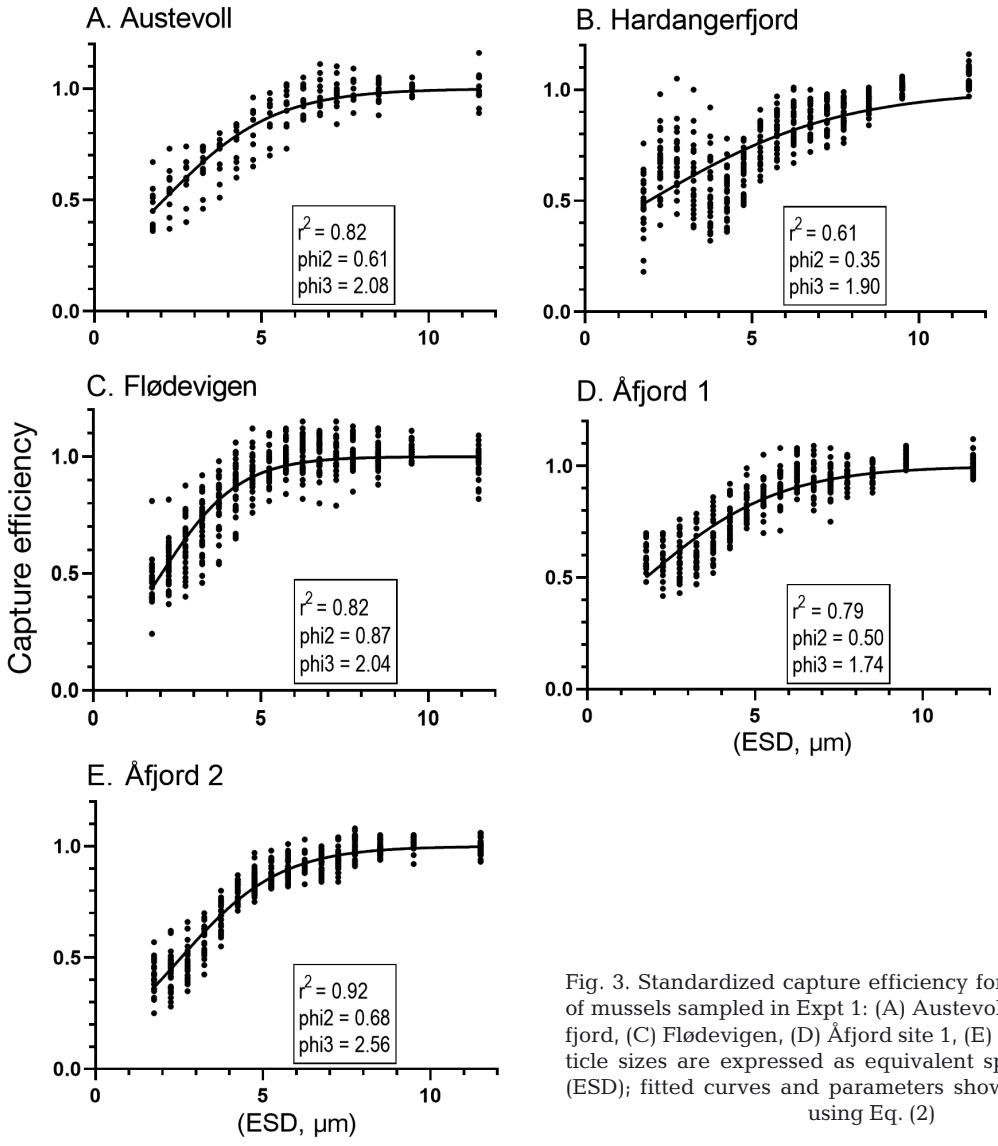

Fig. 3. Standardized capture efficiency for each population of mussels sampled in Expt 1: (A) Austevoll, (B) Hardangerfjord, (C) Flødevigen, (D) Åfjord site 1, (E) Åfjord site 2. Particle sizes are expressed as equivalent spherical diameter (ESD); fitted curves and parameters shown are calculated using Eq. (2)

Flødevigen, which were statistically similar ($p > 0.05$, Fig. 4B). For Austevoll, phi3 was not significantly different from Åfjord 2 (the highest), or Hardangerfjord and Flødevigen ($p > 0.05$; Fig. 4B). 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) ($p < 0.05$; Fig. 4C). There were no significant differences for CE at 4 µm ESD between the other populations (Austevoll, Åfjord 1, Åfjord 2) ($F = 35.48$, $df_{4,120}$, $p > 0.05$; Fig. 4C). For all populations, CE of 1 was reached at different particle sizes; the highest was Hardangerfjord (9.5 µm ESD) and the lowest was Flødevigen (4.75 µm ESD) (Fig. 4D).

PR (l h^{-1}) was significantly lower for both Austevoll and Hardangerfjord compared to all other populations ($F = 16.49$, $df_{4,120}$, $p < 0.05$; Fig. 5A). VIR was highest for Hardangerfjord and Åfjord 2 ($F = 55.88$, $df_{4,120}$, $p > 0.05$; Fig. 5B) and lowest for all other locations ($df_{4,120}$, $p > 0.05$; Fig. 5B). Åfjord 1 had the third highest VIR (mm^3 h^{-1}), followed by Flødevigen and Austevoll ($df_{4,120}$, $p < 0.05$; Fig. 5B). Ingestion rates calculated using POC and chl *a* did not provide additional relevant information for either Expt 1 or 2 (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m651p071_supp.pdf).

3.3. Expt 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). Chl *a* and POC were both higher in Hardangerfjord ($df_{5,24}$, $p < 0.05$; Fig. 6A,B).

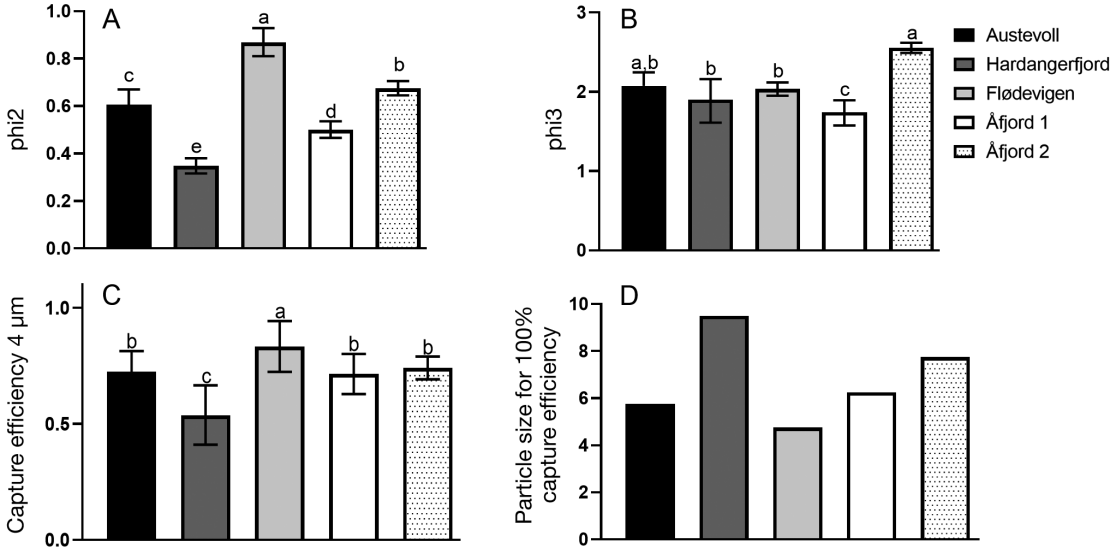

Fig. 4. Mathematical descriptions of the capture efficiency (CE) curves shown in Fig. 3: (A) steepness of the curve (phi2) (1 / [equivalent spherical diameter {ESD}, µm]), (B) particle size when CE = 0.5 (phi3) (ESD, µm), (C) CE values for 4 µm particle size, and (D) particle size when CE first reaches 1 µm. Error bars show ±SD and letters denote statistical significance at α = 0.001 (A,B) and 0.05 (C)

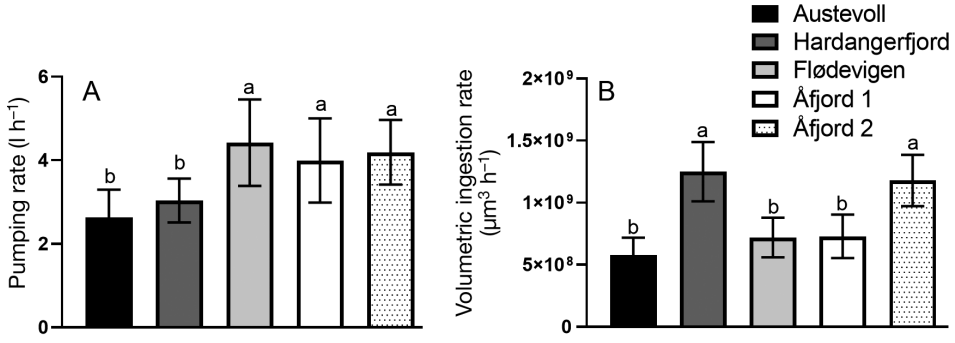

Fig. 5. (A) Pumping rate and (B) volumetric ingestion rate from populations of mussels from Expt 1. Error bars show ±SD and letters denote statistical significance at α = 0.05

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 (Fig. 6C).

3.4. Expt 2: feeding trials

CE values were similar within each sampling location, regardless of the origin of the of *Mytilus edulis* sampled (Fig. 7A–D). Phi2 was significantly greater for mussels sampled in Austevoll compared to those sampled in Hardangerfjord ($p < 0.001$; Fig. 8A). In addition, phi3 was higher for mussels sampled in Austevoll (Fig. 7A,B, respectively) compared to those sampled in Hardangerfjord (Fig. 7C,D, respectively) ($p < 0.001$; Fig. 8B). For both phi2 and phi3, no differences were found based on the effect of origin. Despite general differences in CE curves, all mussels sampled in Expt 2 had similar values for CE at 4 µm ESD: 0.89 ± 0.08 ($F = 0.93$, $df_{3,44}$, $p > 0.05$; Fig. 8C). Particle size at which CE reached 1 was similar for both groups, 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 (Fig. 8D).

PR (l h^{-1}) varied by both sampling location and origin; it was highest for Hardangerfjord mussels in Austevoll, followed by Hardangerfjord mussels in Hardangerfjord ($F = 54.42$, $df_{3,44}$, $p < 0.05$; Fig. 9A). There were no statistical differences in PR between the Austevoll mussels in Austevoll and Austevoll mussels in Hardangerfjord ($df_{3,44}$, $p > 0.05$; Fig. 9A). Within each location, Austevoll mussels consistently had statistically lower PRs than Hardangerfjord mussels.

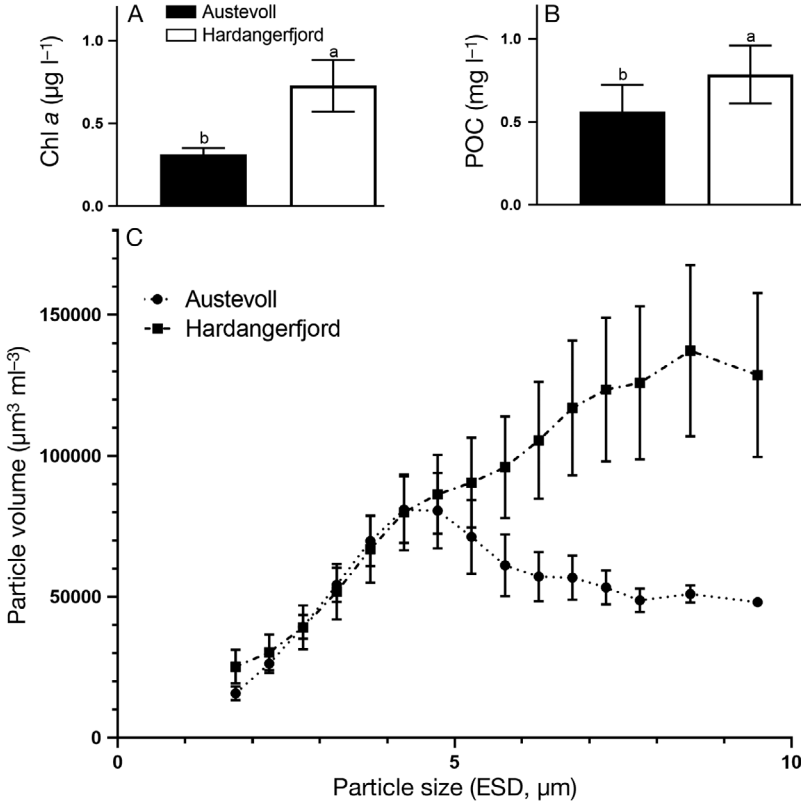

Fig. 6. Water quality measurements (±SD) from all locations sampled in Expt 2: (A) chl *a*, (B) particulate organic carbon (POC), and (C) total particle counts for all size classes measured. Letters denote statistical significance at $\alpha = 0.05$

VIR (μm^3 h^{-1}) was highest for Hardangerfjord mussels in Hardangerfjord, followed by Hardangerfjord mussels in Austevoll ($F = 56.70$, $df_{3,44}$, $p < 0.05$; Fig. 9B). Austevoll mussels in both locations had the lowest and statistically similar VIRs ($df_{3,44}$, $p > 0.05$; Fig. 9B).

4. DISCUSSION

This study demonstrated that CE, PR, and ingestion rate of *Mytilus edulis* all varied both between populations and along fjord gradients. CE differed among 3 geographically distinct populations of mussels and changed temporally within 2 populations. Further, when mussels were reciprocally transplanted along a fjord gradient, individuals of different origin had similar CEs when placed in the same location, suggesting that CE is primarily driven by environmental cues. Ingestion rates were not similar between and within populations of *M. edulis*. Further, when mussels were transplanted between 2 locations, both pumping and ingestion rates were driven by both origin and environmental cues.

4.1. Capture efficiency

The CE of *M. edulis* generally increased with particle size to an asymptote, beyond which particles were completely captured. However, the CE of small particles differed among the 3 populations and 5 sampling sites in Expt 1. Additionally, in Expt 1 the Hardangerfjord mussels had a CE for particles ~4 µm of under 50%, which is unusual for this species (Møhlenberg & Riisgård 1978, Cranford et al. 2016) although it has been previously observed (Strohmeier et al. 2012). In Hardangerfjord, the CE of *M. edulis* 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 chl *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. Although GA did not appear to play a role in differences in CE between other locations, the Hardangerfjord mussels did have proportionally smaller gills when controlling for length. Previous studies have indicated that there is plasticity in gill morphology of bivalves in response to environmental change (Tuttle-Raycraft & Ackerman 2019), but it is assumed that the structure of the gill does not change with size for adult mussels (Cannuel et al. 2009). Further, when the Hardangerfjord mussels were sampled again in Expt 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 Expt 1 was driven by environmental cues.

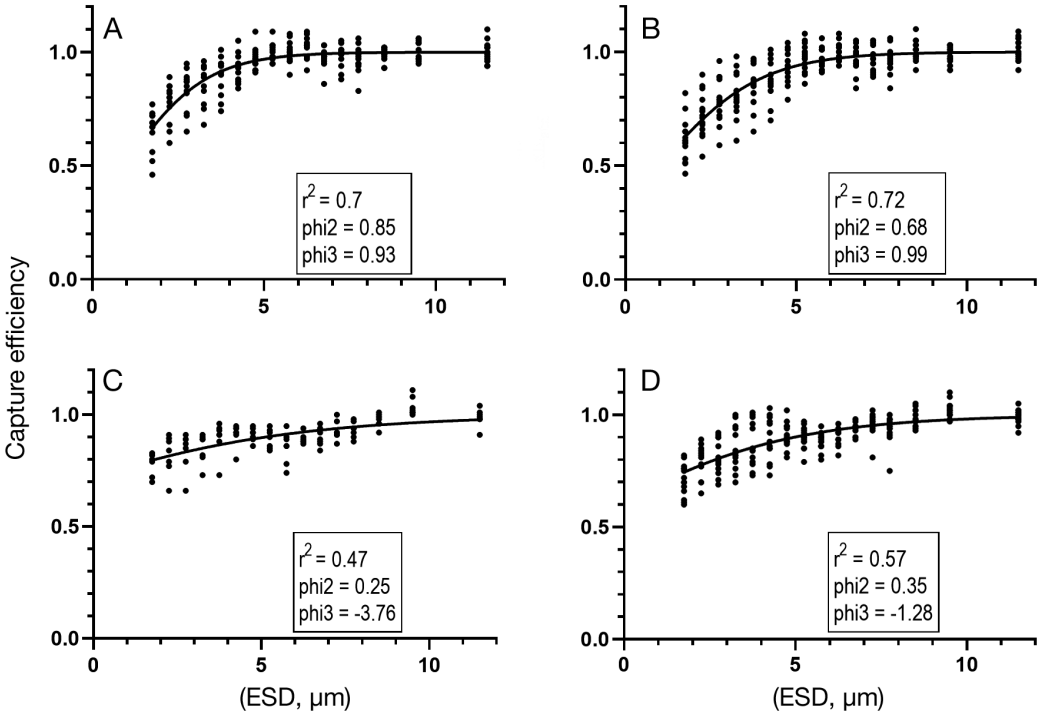

Fig. 7. Standardized capture efficiency for each population of mussels sampled in Expt 2: (A) Austevoll mussels in Austevoll, (B) Hardangerfjord mussels in Austevoll, (C) Austevoll mussels in Hardangerfjord, and (D) Hardangerfjord mussels in Hardangerfjord. Particle sizes are expressed as equivalent spherical diameter (ESD); fitted curves and parameters shown are calculated using Eq. (2)

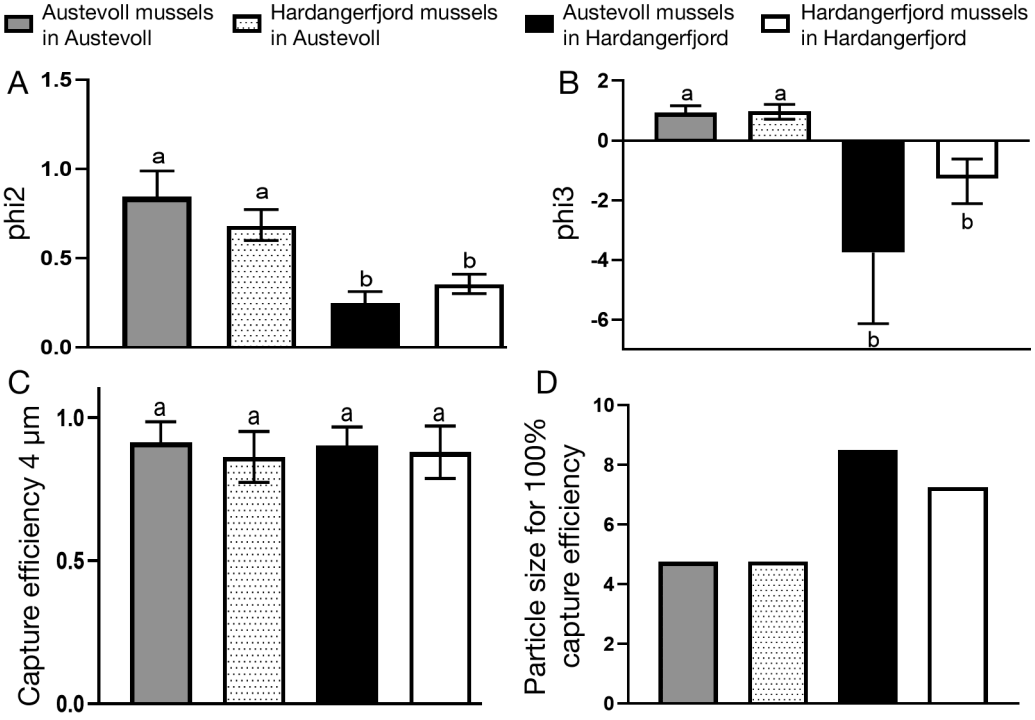

Fig. 8. Mathematical descriptions of the capture efficiency (CE) curves shown in Fig. 7: (A) steepness of the curve (phi2) (1 / [equivalent spherical diameter {ESD}, µm]), (B) particle size when CE = 0.5 (phi3) (ESD, µm), (C) CE values for 4 µm particle size, and (D) particle size when CE first reaches 1 (µm). Error bars show ±SD and letters denote statistical significance at $\alpha = 0.001$ (A,B) and 0.05 (C)

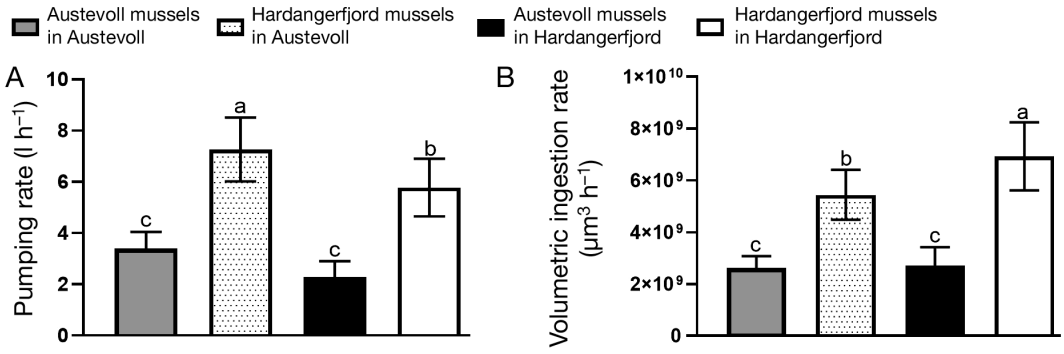

Fig. 9. (A) Pumping rate and (B) volumetric ingestion rate from populations of mussels from Expt 2. Error bars show ±SD and letters denote statistical significance at $\alpha = 0.05$

CE of mussels sampled at the same location in the Hardangerfjord changed over 3 mo during Expts 1 and 2. Strohmeier et al. (2012) measured CE of one population of *M. edulis* over 4 mo 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 mo, 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 ability. 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 3 mo was not explained by changes in concentration of small particles (following Strohmeier et al. 2012), seston volume, chl *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.

An 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.

4.2. Ingestion rates

Ingestion rates, as measured by seston volume, POC, and chl *a*, differed in several of the sampling locations of Expt 1. Further, ingestion rates in the Hardangerfjord mussels varied between Expts 1 and 2 over a 3 mo period. No compensatory mechanisms between CE and PR 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 a good indicator of energy (T. Strohmeier et al. unpubl. data), 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 9.5 µm 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 chl *a* provided similar results to volumetric ingestion estimations (Fig. S1). 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 groups in the transplant experiment (Fig. S2), indicating that 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. Finally, differences in GA may affect ingestion rates disproportionately to overall metabolic rates, emphasizing the importance of considering other physiological rates, including respiration, for a full understanding of individual bioenergetics. Although different ingestion rates were observed between populations of *M. edulis*, it could not be determined if those differences were driven by the internal state, environmental conditions, or local adaptation.

4.3. 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 Expt 2. After the 3 wk acclimation period in Expt 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 d 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 d, *M. chilensis*) in another transplant experiment revealed 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 *M. galloprovincialis* to a laboratory setting and determined scope for growth of both populations. After 15 d, 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 PR or overall ingestion rates.

As a primarily sessile organism that grows in diverse environments, *M. edulis* is a good model species to explore plastic and adaptive traits. Changes in CE within a single group of *M. edulis* transplanted along a fjord gradient 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 by both 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. For example, seasonal changes in gill morphology in response to turbidity have been observed in bivalves (Dutertre et al. 2017), and it is possible that for an individual of a certain size, GA may influence feeding behaviour. 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 of 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 under different environmental conditions.

4.4. 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. Further consideration should be given to morphological gill plasticity in transplant experiments, for its potential role in modulating feeding physiology and bioenergetics. Although the use of natural seston is imperative to understand 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. Understanding the mechanisms of changes in CE would contribute to the development of a mechanistic model for ingestion — a current limitation in predicting growth of bivalves without local model calibration.

This study demonstrated that feeding physiology, measured as CE, PR, and ingestion rate, was 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*, and results indicate that CE seems to be primarily driven by environmental cues. These findings further corroborate that the 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 may vary for individual processes. An accurate characterization of CE and PR is 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.

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