Interactive effects of temperature and food availability on the growth of *Arctica islandica* (Bivalvia) juveniles

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Abstract (150)

The interest in *Arctica islandica* growth biology has recently increased due to the widespread use of its shell as a bioarchive. Although temperature and food availability are considered key factors in its growth, their combined influence has not been studied so far under laboratory conditions. We tested the interactive effect of temperature and food availability on the shell and tissue growth of *A. islandica* juveniles (9-15 mm in height) in a multi-factorial experiment with four food levels (no food, low, medium, and high) and three different temperatures (3, 8, 13 °C). Shell and tissue growth were observed in all treatments, with significant differences occurring only among food levels (2-way ANOVA; P-value < 0.05). Siphon activity (% open siphons), however, was affected by temperature, food, and the interaction between them (2-way ANOVA; P-value < 0.05). Siphon observations, as indication of feeding activities, played a key role to better understand the growth variation between individuals.

1. Introduction

In sclerochronology, shell growth increments are used for retrospective climate studies, in the same way as growth rings of trees are used in dendrochronology (Jones, 1980; Witbaard et al., 1994; Karney et al., 2011). Based on *Arctica islandica* annual growth increments, it is possible to distinguish periods of rapid and slow growth to create shell-growth chronologies (Schöne and Gillikin, 2013). These chronologies can be coupled with local environmental records, providing insight into past environmental and ocean climatic conditions (Schöne et al., 2003; Witbaard et al., 2003; Butler et al., 2013; Mette et al., 2016).

The long-living bivalve *A. islandica* can be found on both sides of the North Atlantic: from Cape Hatteras to the Canadian arctic, and from the North Sea to the Barents Sea, including Iceland (Jones, 1980; Dahlgren et al., 2000). This species has an optimal thermal range between 6-16 °C (Golikov and Scarlato, 1973; Cargnelli et al., 1999; Zettler et al., 2001; Begum et al., 2009), but tolerates temperatures between 0 and 20 °C (Kraus et al., 1992; Witbaard et al., 1997a; Hippler et al., 2013). With a lifespan of up to 507 years (Marchitto et al., 2000; Schöne et al., 2005a; Wanamaker et al., 2008; Butler et al., 2013), its growth rate is characterized by a sharp decrease after the first 20 years of life (Thompson et al., 1980; Kennish et al., 1994). Shell length can reach a maximum size of ~14cm (Ropes, 1985). However, size may not be a strong indicator of age since there is geographical variation in the growth of the species (Ropes, 1985; Witbaard et al., 1999).

There is a lack of consensus about the intra-annual timing of shell growth in *A. islandica*, the period during which the annual growth increment is formed, and the main environmental forces regulating its growth. The growing season has been defined from eight months (Weidman et al., 1994; Schöne et al., 2005b; Dunca et al., 2009; Ballesta-Artero et al., 2017) to twelve months (Jones, 1980; Wanamaker et al., 2008; Mette et al., 2016). Further, the shell growth rate has been assumed both as being constant throughout the growing season (Weidman et al., 1994; Marchitto et al., 2000), or, alternately, exhibiting intra-annual variability (Witbaard et al., 2003; Witbaard and Hippler, 2009; Schöne et al., 2005b; Dunca et al., 2009; Wanamaker et al., 2008; Mette et al., 2016). Lastly, the cessation of the main growing season has been proposed as occurring during autumn/winter (Jones, 1980; Murawski et al., 1982; Weidman et al., 1994; Schöne et al., 2005b), early spring (Mette et al., 2016), or late summer (Witbaard et al., 2003; Dunca et al., 2009; Ballesta-Artero et al.,

2017). Studies from different locations, e.g. from the New Jersey Coast (Jones, 1980), Middle Atlantic Bight (Murawski et al., 1982), Nantucket Shoals (Weidman et al., 1994), Fladen Ground (Witbaard et al., 1997b; Witbaard et al., 2003; Butler et al., 2009), North and Baltic Seas (Schöne et al., 2005b), Gulf of Maine (Wanamaker et al., 2008), Swedish West Coast (Dunca et al., 2009), and Northern Norway (Mette et al., 2016; Ballesta-Artero et al., 2017), suggest that different environmental conditions play key roles in explaining the variability in the length or timing of the growing season. Therefore, the specific mechanistic link between shell growth and the environment has to be well understood, to not limit the utility of this species as a retrospective monitor of ocean conditions.

In situ observational studies on juvenile *A. islandica* in the Baltic Sea and the Gulf of Maine showed variable shell growth. In Maine, growth rates ranged between 0.50-76.67 µm d^{-1} (Lutz et al., 1983; Kraus et al., 1992; Kennish et al., 1994). In the Baltic Sea, Brey et al. (1990) found growth rates between 12.60-36.71 µm d^{-1}. Hippler et al. (2013) found a maximum growth rate of 157 µm d^{-1} with Baltic specimens cultivated in the NIOZ harbor (Texel Island, the Netherlands). All these authors hypothesized that the different growth rates were mainly the result of different environmental conditions, highlighting the importance of food availability and water temperature in the study areas.

Under laboratory conditions, Witbaard et al. (1997a) tested the effect of five different food levels at 9° C, and the effect of five different temperatures at optimal food conditions (>10x10^6 cells L^{-1}; Winter, 1969) on the growth of *A. islandica*. There was a tenfold increase in shell height between 1°C and 12 °C, with a maximum growth rate of 74 µm d^{-1} (Witbaard et al., 1997a; Hippler et al., 2013). Witbaard et al. (1997a) also reported an increase of siphon activity at higher food concentrations, corresponding with greater shell and tissue growth. Other laboratory studies tested the combined effects of temperature and salinity as well as temperature and acidification on specimens collected from the Baltic Sea (Hiebenthal et al., 2012; Hiebenthal et al., 2013). The average growth rate per treatment was between 2.86- 25.71 µm d^{-1} (Hiebenthal et al., 2012; Hiebenthal et al., 2013). These studies showed that acidification did not have an influence on *A. islandica* shell growth; however, high temperature (16 °C) and low salinity (15) resulted in decreased growth rate. Milano et al. (2017) tested the effect of temperature (10 and 15° C) and diet (three types) on the microstructural organization of *A. islandica* shells. In their food experiment (performed on

shells from Baltic Sea), they found higher growth rates (41.22 µm d^{-1}) at the most biodiverse diet, i.e., the one composed of different phytoplankton species. In their temperature experiment (executed on shells from Maine), the specimens at 10 °C had the highest growth rate reported so far under experimental conditions i.e. 295.21 µm d^{-1} (Milano et al., 2017).

The combined effects of temperature and food availability have not yet been studied in a single multi-factorial experiment. Most existing studies have focused on a single parameter and did not take into account the possible interaction between environmental factors (e.g., Witbaard et al., 1997a; Milano et al., 2017). Therefore, to improve paleoclimatic reconstructions based on *A.islandica* shell chronologies, a better understanding of the relationship between food, temperature and growth is necessary. Thus, we analyzed the interactive effects of temperature and food availability on the shell and tissue growth of *A. islandica*. Furthermore, we studied siphon activity as an indication of feeding activity. We aim to clarify the diversity of results found on *A. islandica* growth rate relative to two key environmental factors: temperature and food availability.

2. Methods

2.1 Experimental setup

Living juveniles of *Arctica islandica* (< 20mm; 1-3 years old) were collected in July 2014 from Kiel Bay, Baltic Sea (54° 32’ N, 10° 42’E). They were transferred to the Alfred Wegener Institute (AWI, Bremerhaven) where they were kept at 7ºC and salinity 21 for 6 months. During this period, the individuals were fed with a microalgae mix of *Nannochloropsis* sp*., Isochrysis galbana*, and *Pavlova lutheri.* In January 2015, they were transported to NIOZ (Texel) under refrigerated conditions. They were placed in small aquaria with aerated seawater inside a controlled climate room (air temperature 9 ºC). Seawater temperature varied between 8 and 10 ºC and salinity between 29 and 30. *A. islandica* specimens were fed twice per week with a commercial mix of marine microalgae: *Isochrysis sp., Tetraselmis sp., Paulova sp., Thalassiosira sp.* and Nannochloropsis spp (Mixalgae; www. acuinuga.com). They were kept under these conditions for a year.

In February 2016, 250 individuals (of ~400) were randomly selected for the growth experiment and subdivided among four 15-liters aquaria for acclimation to their target

temperatures (about 1°C change per day). These animals were fed with Mixalgae twice per day during one month (February 22nd to March 22nd, 2016) to achieve similar body conditions. Optimal food concentration level (10 -11 x10^{6}cells L^{-1}; Winter, 1969) was maintained until the beginning of the experiment**.** Cell concentration of the water was measured using a BD Accuri C6 flow cytometer.

The starting shell height of the experimental animals ranged between 7.86 and 15.48 mm (± 0.01). Prior to the start of the experiment, the specimens were soaked in a calcein solution of 125mg L^{-1} for 24 hours (Linard et al., 2011; Ambrose et al., 2012). Calcein is a non-toxic fluorescent dye which becomes incorporated in the shell and that gives a shell time marker for growth studies (Linard et al., 2011; Ambrose et al., 2012). The experiment was done under dimmed light conditions, and took place during spring (March 22nd to June 23rd, 2016; 14 weeks) to avoid undesirable effects on growth due to autumn/winter physiological state of the specimens (Hiebenthal et al., 2013; Ballesta-Artero et al., 2017).

2.2 Experimental design

The experimental set-up had 12 treatments: all combinations of four food levels (no ; low: 0.5 x10^{6} phytoplankton cells L^{-1}; medium: 5 x10^{6} cells L^{-1}; and high:15x10^{6} cells L^{-1}) and three different temperatures (3°C, 8°C, and 13 °C; see Table 1 for more details). There were 3 replicates per treatment (3 aquaria), which meant a total of 36 aquaria (4 food levels x 3 temperatures x 3 replicates). Five *A. islandica* juveniles were randomly assigned to each aquarium, amounting to a total 180 *A. islandica* specimens. Since individual specimens within one experimental unit (aquarium) are interdependent pseudo-replicates, single average values for mortality, siphon activity, shell and tissue growth were calculated for each aquarium separately. Thus, we present the average response of all individuals within each aquarium (replicate); in particular, the specific combination of food and temperature of that aquarium.

Each aquarium measured 30x40x17 cm (polypropylene; www.hulkenberg.nl) and contained 15 liters of aerated seawater. This volume was refreshed at a rate of 600 mL h^{-1} (~100 % d^{-1}) with fresh filtered seawater taken from the Marsdiep tidal inlet. Suspended material was filtered out over sandbed filters. Fresh water was cooled or heated before it

arrived to the experimental set up so that the right temperature was constantly maintained in the experimental aquaria.

Table 1: Summary of treatments.

	3°C	8°C	13°C
No food			
Target concentration (cells L^{-1} x 10^6)	0	0	0
Actual concentration (cells L^{-1} x 10^6)	0.04 ± 0.03	0.04 ± 0.00	0.03 ± 0.02
Mg dry weight ind^{-1} d^{-1}	-	-	-
Low food			
Target concentration (cells L^{-1} x 10^6)	0.50	0.50	0.50
Actual concentration (cells L^{-1} x 10^6)	0.85 ± 0.43	0.22 ± 0.02	0.53 ± 0.18
Mg dry weight ind^{-1} d^{-1}	0.62	0.62	0.62
Medium food			
Target concentration (cells L^{-1} x 10^6)	5	5	5
Actual concentration (cells L^{-1} x 10^6)	6.19 ± 0.64	2.34 ± 0.17	1.53 ± 0.05
Mg dry weight ind^{-1} d^{-1}	5	5	5
High food			
Target concentration (cells L^{-1} x 10^6)	15	15	15
Actual concentration (cells L^{-1} x 10^6)	24.56 ± 2.85	12.99 ± 0.82	6.45 ± 2.08
Mg dry weight ind^{-1} d^{-1}	14	14	14
Actual temperature (°C)	2.49 ± 0.02	7.94 ± 0.07	13.11 ± 0.05
Salinity	30.26 ± 0.10	30.38 ± 0.08	29.39 ± 0.24

To reach the desired micro-algal concentrations (Table 1), the estimated filtration rate per individual (~350 mL h^{-1}; Winter, 1969) and the water flux (600 mL h^{-1}) were considered as loss factors, from which the amounts to be added to each treatment were determined. Due to the importance of a constant food supply on bivalve's growth (Langton and McKay, 1976; Winter and Langton, 1976), food was provided eight times per day, every three hours using a peristaltic pump and a timer. During the entire experiment, the amount of added food was kept constant at each food level (Table 1). Every second day, a new food batch was

prepared. The amount of concentrated algal suspension needed (for the three replicates of each treatment) was diluted with seawater based on the flux of the peristaltic pump and the required concentration in each aquarium.

Mixalgae ($2x10^9$ cells mL^{-1}; 18% dry weight) were used for this experiment based on a previous *A. islandica* growth experiment, which showed highest shell growth rates (41.22 µm d^{-1}) at the most biodiverse diet (Milano et al., 2017). This phytoplankton mix provided a particle size range from 3 to 16 µm and a balanced fatty acid composition (Lipids 16%: 16% EPA and 10% ARA), ensuring an optimal nutritional profile (Widdows, 1991; Milano et al., 2017). Each shell was placed in a numbered plastic jar of 7-cm of diameter and 4-cm high. Each jar was filled with micro glass beads (www.kramerindrustriesonline.com) to avoid undesirable food input and provide a uniform sediment for all shells. The average size of the beads was 350 µm (Ballesta-Artero et al., 2017).

A BD Accuri C6 flow cytometer was used to evaluate the differences in the number of cells between treatments and replicates, and a portable multipara-meter (HI98192; www.hannainst.com) to check temperature and salinity values during the entire experimental period. Numbers of cells were counted once per week, while temperature and salinity were checked once daily.

2.3 Shell growth

Shell size (height, length, and width) was measured three times: at the beginning, mid-term, and at the end of the three-month experiment. Measurement error might be relatively large due to the small size of the animals, and we therefore triplicated each measurement and calculated the average. The electric caliper error was ± 0.01 mm and the average measurement error (over all measurements and individuals) for height, length and width was: 0.07, 0.06, and 0.06 mm respectively (standard deviation). Shell growth per individual was determined based on the difference in the shell sizes between the representative periods of the experiment (beginning, midterm and end).

To verify the reliability of the externally-measured growth determined with the caliper, 73 shells were cross-sectioned to accurately identify the shell portion that grew during the experimental period on the basis of the calcein mark. The right valve of each specimen was cut into one 1.5-mm thick section along the axis of maximum growth (saw Buehler Isomet

1000). Given the small size and fragility of the juvenile shells, the valves were fully embedded in a block of Struers EpoFix (epoxy). All samples were ground at different grit sizes (P320, P600, P1200, P2500, and P4000) and then polished with a Buehler diamond polycrystalline suspension (3-µm). The calcein marks were located under a fluorescence light microscope (Zeiss Axio Imager.A1m microscope), to enable measuring the newly formed shell portion (for more details refer to: Milano et al., 2017). Subsequently, a comparison was made between the externally- and internally-measured shell growth.

2.4 Body Mass Index (BMI)

To calculate the reference Ash Free Dry Weight ($AFDW = Dry\ Weight - Ash\ Weight$), twenty animals of the 250 preconditioned shells were sacrificed at the start of the experiment. Dry weight was determined after drying the soft tissue at 60 ºC for 3 days. Then, the dried flesh was incinerated at 540 ºC for 4 hours to obtain the ash weight. Based on these AFDW and the individual shell heights, we determined a height-weight relationship. This relationship was subsequently used to estimate the weights of the experimental animals at the beginning of the experiment. AFDW data was also used to calculate the Body Mass Index of the animals ($BMI = AFDW/H^3 * 1000$) as a fitness parameter.

After 45 days, 36 individuals were sacrificed, one per aquarium, for which the AFDW and BMI were determined. At the end of the experiment, which lasted 93 days, the AFDW and BMI of the rest of individuals (89) was measured (Table 2). The BMI was compared between the representative periods of the experiment.

Table 2: Summary of individuals used.* Due to the small size of the individuals their shells were quite fragile.

Specimens	Number	Comments
Starting individuals	180	5 x 36 aquaria
Dead individuals	53	37 dated + 16 not dated
Sacrificed individuals at midterm	36	34 BMI (two specimen broken*)
Remaining individuals at midterm	127	126 used (one outlier removed)
Remaining individuals at the end	90	89 (one specimen broken*)

2.5 Siphon activity (%)

Siphon activity is an indication of feeding (Møhlenberg and Riisgård, 1979; Newell et al., 2001a; Riisgård and Larsen, 2015) and thus, it may be linked to growth (Witbaard et al., 1997a; Ballesta-Artero et al., 2017). Here siphon activity is expressed as the percentage of observations with open siphons relative to the total number of observations per specimen. The siphon activity of all individuals was checked at least twice per week at randomly-selected times. The data were recorded as 0 and 1, closed and open siphon, respectively. The average per aquarium (experimental unit) was calculated after the experimental period and used in the subsequent analyses.

2.6 Mortality (%)

At the end of the experiment, the percentage of dead specimens was calculated per aquarium (replicate). During the experiment, dead specimens were replaced by new ones to keep the density constant, and thus the loss of food by filtration. However, these new individuals were not considered in the statistical analyses of shell and tissue growth (Table 2).

2.7 Data analysis

Response variables were transformed to obtain normality and homogeneity of variance. Percentages, that is siphon activity and mortality, were logit transformed (Warton and Hui, 2011); shell growth and BMI were log-transformed. Data were analyzed by a 2-factorial ANOVA with a significance level of α=0.05. Afterwards, a residual analysis was used to study the relationship between initial size and shell growth. Differences between treatment levels were calculated by Tukey's HSD post hoc test. Pearson correlation coefficients were used to study the strength of the relationship between the response variables. All analyses were performed using R version 3.2.2 (www.r-project.org).

3. Results

3.1 Shell growth

Visual inspection of the experimental animals identified new shell growth during the experiment because the new deposited shell had a darker colored periostracum (Fig. 1a). Five outliers were identified based on the relationship between external and internal shell

growth, possibly due to external measurement error. Therefore, the outliers were corrected based on the linear relationship of the rest of individuals, $y = 1.0742x$ (R^2 = 0.94), where y and x were the external and internal shell height, respectively (Fig. 1b).

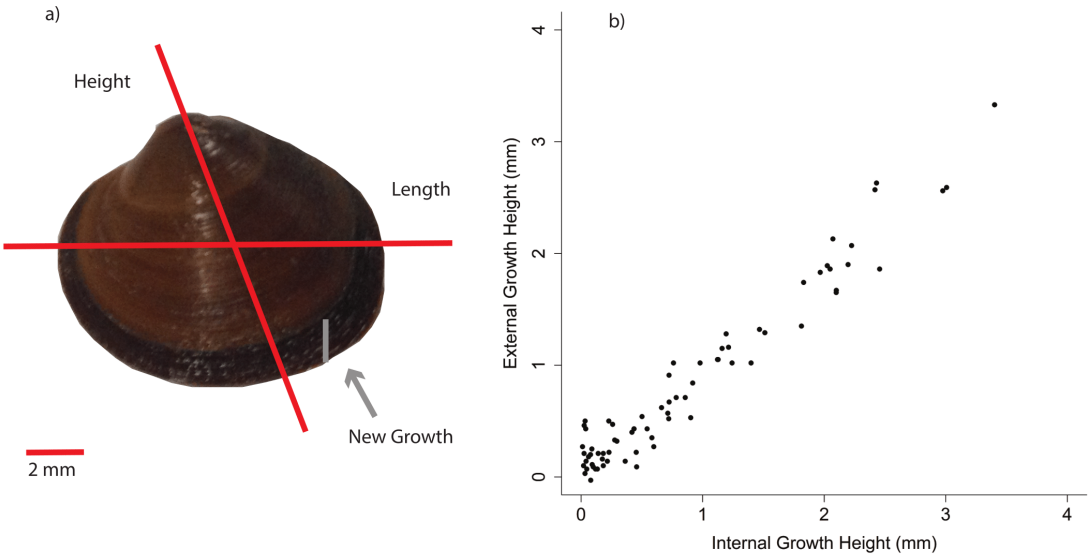

Fig. 1. a) Visible shell growth in one specimen of *Arctica islandica* due to change of periostracum color b) Relationship between external and internal growth in height (GH in mm; n=73). Internal growth was determined on the basis of calcein mark.

At the end of the experiment, externally-measured shell growth varied between 0.21 and 2.29 mm (Fig. 2) while internal shell growth varied between 0.04 and 1.88 mm (Table 3). The difference in absolute values between external and internal growth indicated a small difference on the inclination of the measurements axis relative to the axis of maximum height. Nevertheless, the significant and high correlation (r= 0.90, P-value < 0.01; Table 4) between internal and external growth verified the accuracy of the 'external' measurements taken with the caliper. To keep results comparable with findings from other studies, the rest of the analysis is based on the 'external' measurements.

Table 3: Summary of all response variable per treatment (mean ± SD). Note: GH means growth in height, BMI is body mass index, and AFDW is ash free dry weight.

	3°C	8°C	13°C
No food			
External GH midterm (mm)	0.11 ± 0.06	0.58 ± 0.82	0.19 ± 0.16
External GH end (mm)	0.30 ± 0.23	0.21 ± 0.00	0.28 ± 0.22
Internal GH (mm)	0.17 ± 0.12	0.18 ± 0.00	0.04 ± 0.03
BMI midterm (mg AFDW/mm3)	3.96 ± 0.29	5.48 ± 0.88	4.59 ± 1.76
BMI end (mg AFDW/mm3)	3.03 ± 0.90	3.34 ± 0.00	2.45 ± 0.10
% Siphon Activity	65.00 ± 21.51	27.97 ± 8.33	10.68 ± 3.08
% Mortality	44.67 ± 11.55	73.33 ± 11.55	26.67 ± 23.09
Low food			
External GH midterm (mm)	0.26 ± 0.22	0.08 ± 0.03	0.43 ± 0.22
External GH end (mm)	0.43 ± 0.22	0.30 ± 0.20	0.55 ± 0.16
Internal GH (mm)	0.39 ± 0.28	0.28 ± 0.22	0.39 ± 0.45
BMI midterm (mg AFDW/mm3)	5.30 ± 2.25	4.48 ± 0.27	4.64 ± 0.61
BMI end (mg AFDW/mm3)	3.67 ± 0.36	2.55 ± 0.53	2.79 ± 0.55
% Siphon Activity	71.12 ± 29.11	83.61 ± 8.13	39.90 ± 12.13
% Mortality	13.00 ± 11.55	33.00 ± 11.55	33.33 ± 23.09
Medium food			
External GH midterm (mm)	0.43 ± 0.48	0.50 ± 0.19	0.47 ± 0.09
External GH end (mm)	0.83 ± 0.28	1.50 ± 0.40	1.63 ± 0.37
Internal GH (mm)	0.91 ± 0.24	1.66 ± 0.35	1.82 ± 0.52
BMI midterm (mg AFDW/mm3)	4.64 ± 1.17	8.99 ± 1.90	9.54 ± 1.82
BMI end (mg AFDW/mm3)	6.12 ± 0.23	4.83 ± 0.67	5.28 ± 0.24
% Siphon Activity	77.07 ± 6.47	89.38 ± 7.63	71.65 ± 9.07
% Mortality	33.33 ± 23.09	6.67± 11.55	13.33 ± 11.55
High food			
External GH midterm (mm)	0.09 ± 0.09	0.84 ± 0.53	1.19 ± 0.21
External GH end (mm)	0.75 ± 0.36	1.17 ± 0.43	2.29 ± 0.92
Internal GH (mm)	0.72 ± 0.39	1.34 ± 0.40	1.48 ± 0.05
BMI midterm (mg AFDW/mm3)	5.26 ± 2.27	8.11 ± 2.90	9.33 ± 1.46
BMI end (mg AFDW/mm3)	6.24 ± 2.86	7.89 ± 2.43	7.77 ± 1.05
% Siphon Activity	68.49 ± 13.75	77.08 ± 9.11	63.70 ± 15.54
% Mortality	26.67 ± 23.09	20.00 ± 20.00	26.67 ± 11.55

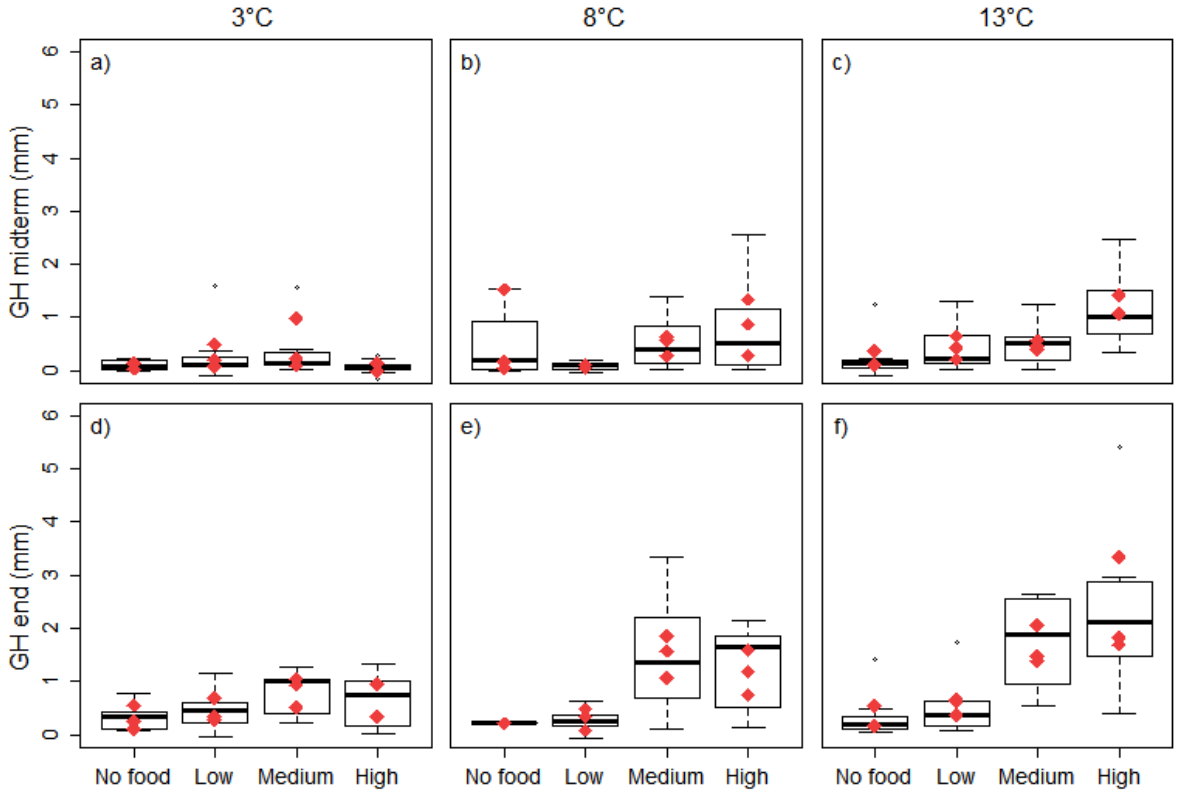

Fig. 2. Shell growth in height (GH; mm) at midterm (45 days): a) 3°C, b) 8°C, c) 13°C, and at the end of the experiment (93 days) d) 3°C, e) 8°C, f) 13°C. Red diamonds indicate average per aquarium (data used for the statistical analysis) and boxplot showed the inter-specimen variation (n=126).

At midterm (after 45d), the average external shell growth was < 1 mm in all treatments (Fig. 2; Table 3). At the end of the experiment, the maximum growth rate per day was 58.26 µm d^{-1} at 'high' food and a temperature of 13°C. The externally-measured growth in shell height showed significant differences at different food levels at the end and midterm (2-way ANOVA, P-value < 0.05; Table 5). Residual plots showed no relationship (no pattern) between initial shell size and total shell growth. The effect of temperature on shell growth was almost significant at mid-term (P-value=0.06; Table 5) and it was associated with higher growth rates at the two highest food levels (medium & high; Fig. 2; Table 3). Such temperature effects could not be detected at the two lowest food levels (no food & low food; Fig. 2; Table 3). A one factorial Tukey HSD test showed that shell growth in the treatments 'no' food and 'low' significantly differed from 'medium' and 'high' (one-factorial TUKEY P-value < 0.01).

Table 4: Correlation among the different response variables and the factors food and temperature (Temp): Height growth in mm at midterm (GHmid; 45d) and the end of the experiment (GH; 93 d), internal growth in height (IntGH), siphon activity (S.Act.), body mass index at midterm (BMImid) and the end of the experiment (BMI), and mortality (Mort). The top right part shows the P-values of the corresponding correlations.

	Log(GH)	Log (GHmid)	Logit (S.Act)	Log (BMI)	Log(BMI mid)	Sqrt (intGH)	Logit (Mort)	Temp	Food
Log(GH)		0.000	0.024	0.000	0.000	0.000	0.111	0.155	0.000
Log(GHmid)	0.76		0.638	0.001	0.000	0.000	0.321	0.027	0.002
Logit(S.Act)	0.39	0.08		0.006	0.092	0.000	0.139	0.022	0.002
Log(BMI)	0.78	0.55	0.46		0.002	0.000	0.482	0.538	0.000
Log(BMImid)	0.68	0.59	0.29	0.52		0.000	0.775	0.044	0.004
Sqrt(IntGH)	0.90	0.62	0.57	0.83	0.66		0.148	0.446	0.000
Logit(Mort)	-0.33	-0.25	-0.22	-0.14	-0.15	-0.29		0.718	0.039
Temp	0.25	0.37	-0.38	-0.11	0.35	0.14	-0.02		1
Food	0.73	0.51	0.49	0.82	0.49	0.75	-0.35	0	

3.2 Fitness Condition

The weights (AFDW) of all the experimental animals at the beginning of the experiment (y) was calculated as $= 0.0008e^{0.1702x}$, where x was equal to the height of the individuals at the start of the experiment ($R^2 = 0.54$). Then, AFDW data was used to calculate the BMI of these animals which had a mean value of 4.89 mg mm^{-3}.

Food level significantly affected the BMI of *Arctica islandica* (2-way ANOVA, P-value < 0.05; Table 5) over the entire experiment. Temperature, was significant only at midterm (P-value = 0.02; Table 5). At most of the treatments, the mean BMI per treatment decreased from the mid-term to the end of the experiment (Fig. 3; Table 3). Since the quantity of food added to each treatment did not change, this is an indication of asynchrony between shell and tissue growth. Individuals at higher temperatures had increased BMIs at food levels 'medium' and 'high', but there was almost no BMI change between temperatures at food levels 'no' and 'low' (Fig. 3). In food-limited treatments ('no' and 'low' food), most of the individuals had a decrease in BMI between the midterm and the end of the experimental period (Fig. 3).

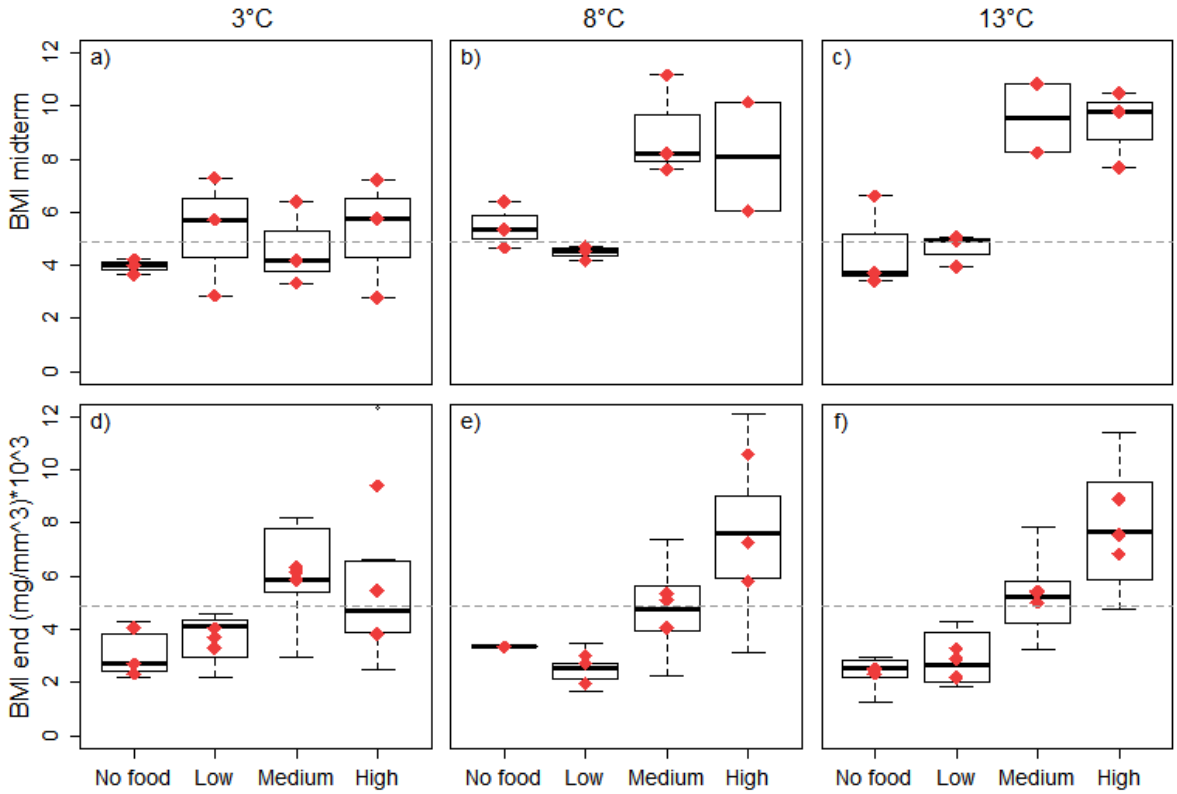

Fig. 3. Body Mass Index (BMI) per treatment: at midterm (45 d; n=34): a) 3°C, b) 8°C, c) 13°C, and at the end of the experiment (93 d) d) 3°C, e) 8°C, f) 13°C. Horizontal line denotes average BMI at the start of the experiment (reference BMI). Red diamonds indicate average per aquarium (data used for the statistical analysis) and boxplot show the inter-specimens variation ($n_{midterm}$=34,n_{end}= 89).

At the end of the experiment, tissue growth, as well as shell growth, was only significantly affected by food level and not by temperature. 'No' and 'low' food treatments were significantly lower from those of 'medium' and 'high' food (one-factorial TUKEY P-value < 0.01).

3.3 Siphon activity

Siphon activity varied significantly with temperature, food level, and the interaction between them (2-way ANOVA, P-value < 0.05; Table 5). Mean siphon activity increased at higher food levels (except for the highest concentration): between 11 and 77% at temperature 13°C (64% at the highest concentration), between 28 and 89% at 8 °C (77% at the highest concentration); and between 65 and 78% at 3 °C (68% at the highest concentration; Fig. 4a, b, c; Table 3). The differences in siphon activity between food levels were far smaller at 3 °C than at the other temperatures (8 and 13 °C, Table 3; Fig. 4a, b, c).

Moreover, the starving animals ('no' food) had a much lower average siphon activity (35%) than the rest of treatments (65-79%, one-factorial TUKEY P-value < 0.01; Fig. 4a, b, c). Only at 3 °C, the starving animals had a relatively high siphon activity (65%; Table 3).

Siphon activity was significantly correlated with shell growth (r=0.39, P-value < 0.05; Fig 4d; Table 4) and marginally significant with tissue growth (r=0.46, P-value = 0.06; Fig. 4e; Table 4).

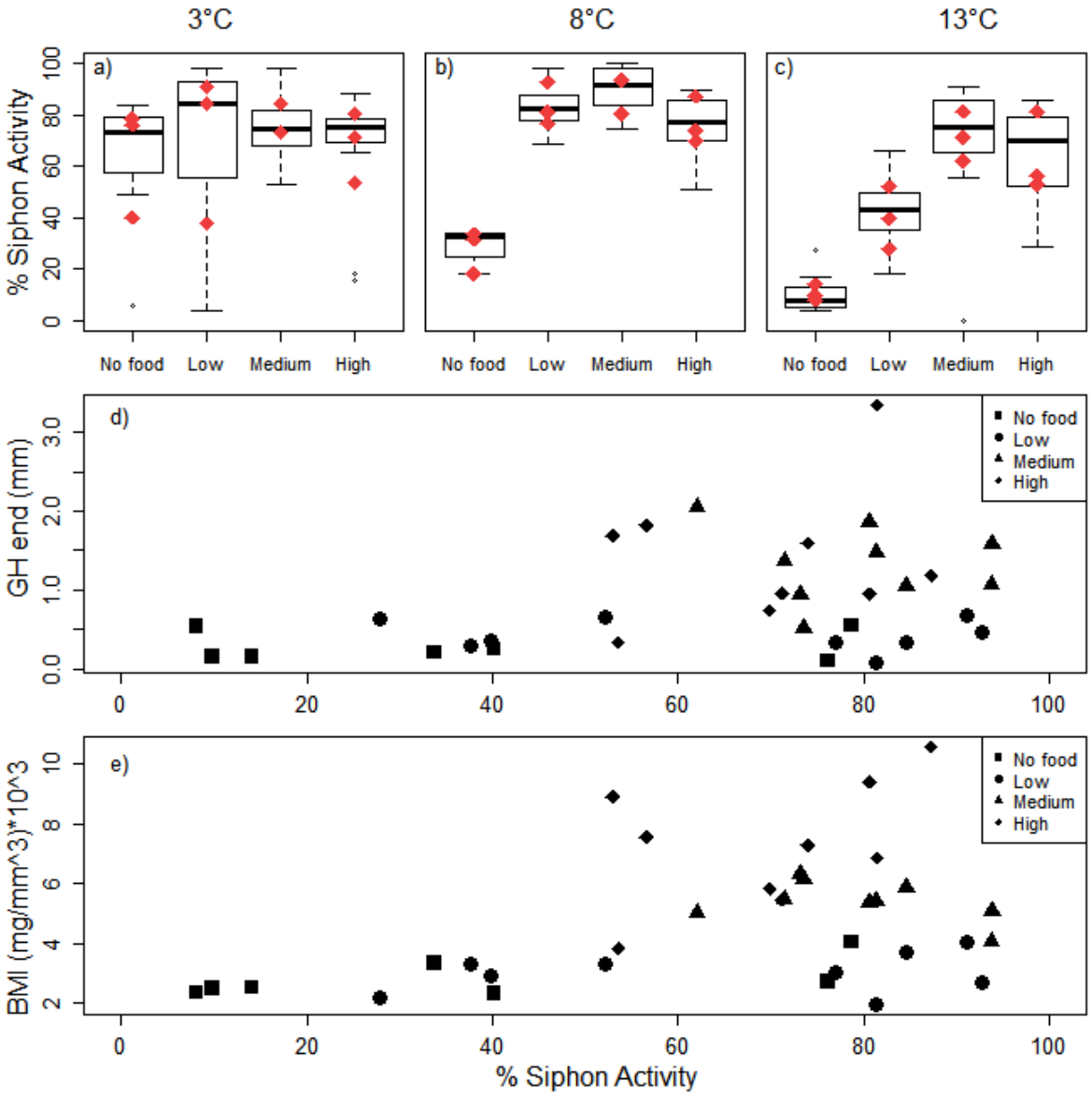

Fig. 4. Percentage of average siphon activity at: a) 3°C, b) 8 °C and c) 13°C. Red diamonds indicate average per aquarium (data used for the statistical analysis) and boxplots show the inter-specimen variation (n=126). Relationship between average siphon activity and d) external growth in height (GH per aquarium; n=36) e) Body Mass Index (BMI) per aquarium (n=36).

Table 5: Two-way ANOVA table testing on the effects of temperature and food level on the different response variables. Significant factors per model are highlighted in italic (P-value < 0.05).

Variable response	Effect	df	Sum Sq	Mean Sq	F-value	Pr (> F)
log(GHmid)	*Food (F)*	3	1.9824	0.6608	4.835	0.0094
	Temperature (T)	2	0.8886	0.4443	3.251	0.0571
	Interaction F*T	6	1.2275	0.2046	1.497	0.2234
	Residuals	23	3.1417	0.1367		
log(BMImid)	*Food*	3	0.27517	0.091725	5.7569	0.0046
	Temperature	2	0.17364	0.086819	5.4490	0.0120
	Interaction F*T	6	0.16106	0.026844	1.6848	0.1719
	Residuals	22	0.35052	0.015933		
log(GH)	*Food*	3	3.198	1.0660	18.709	2.94E-06
	Temperature	2	0.357	0.1783	3.129	0.0637
	Interaction F*T	6	0.393	0.0655	1.149	0.3680
	Residuals	22	1.253	0.0570		
log(BMI)	*Food*	3	0.99031	0.33010	36.9768	9.09E-09
	Temperature	2	0.01138	0.00569	0.6373	0.5382
	Interaction F*T	6	0.09588	0.01598	1.7900	0.1477
	Residuals	22	0.19640	0.00893		
logit(Activity)	*Food*	3	26.489	8.8296	16.4803	4.99E-06
	Temperature	2	13.144	6.5718	12.2662	0.0002
	*Interaction F*T*	6	10.208	1.7014	3.1756	0.0194
	Residuals	24	12.858	0.5358		
logit(Mortality)	*Food*	3	13.70	4.567	2.955	0.053
	Temperature	2	0.41	0.204	0.132	0.877
	Interaction F*T	6	20.51	3.418	2.211	0.077
	Residuals	24	37.10	1.548		

3.4 Mortality (%)

There was a 29% mortality during the entire experimental period over all treatments and replicates. There were only significant differences in mortality among food levels (2-way ANOVA; P-value < 0.05; Table 5 and Fig. 5a, b, c). More individuals died at 'no' food (n=22) than at the other food treatments (low, n=12; medium, n=8; high, n=11; Fig 5d). A few specimens were not included in Fig. 5d because we could not precisely determine the date

of death (Table 2). The observed mortality was not significantly correlated to any of the other response variables (Table 4).

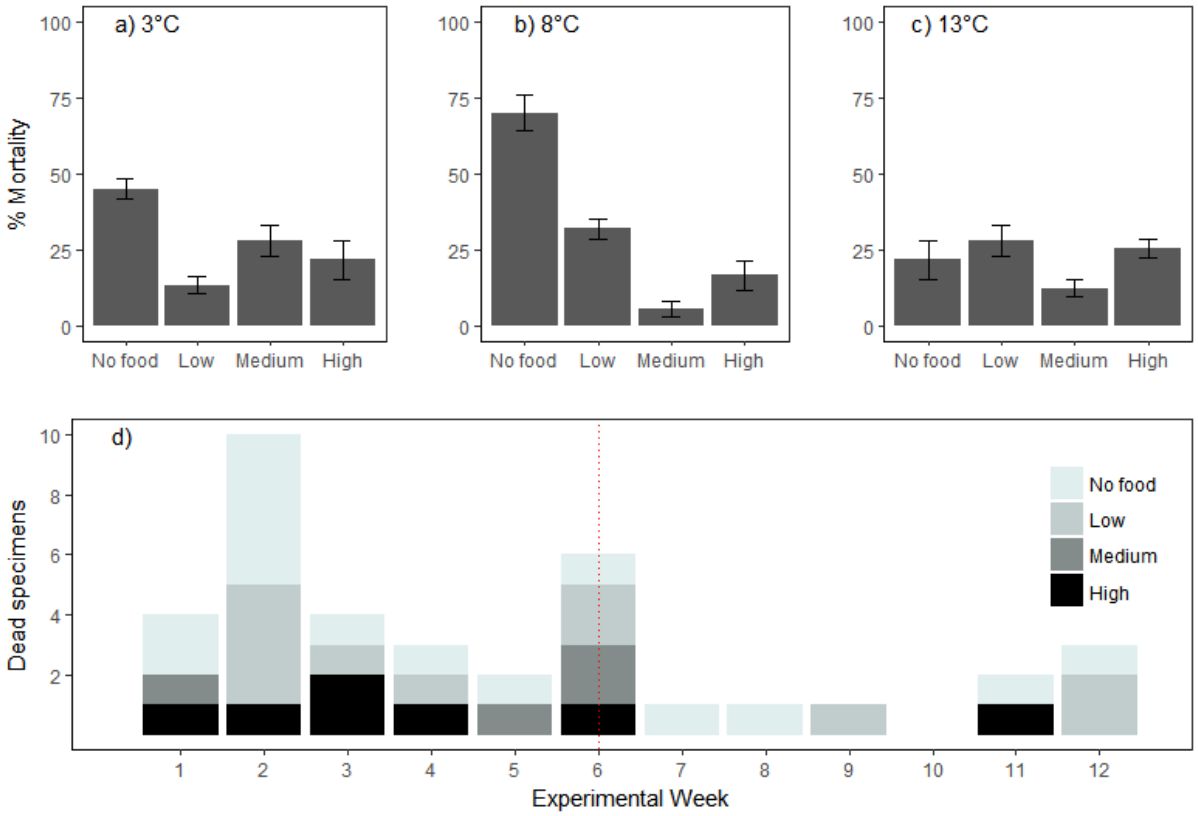

Fig. 5. Percentage of average mortality at: a) 3 °C, b) 8 °C and c) 13°C. d) Number of dead specimens per food level and experimental week. Vertical line indicates the week that the midterm measurements were done. NOTE: No specimens died at week 10.

4. **Discussion**

Within the temperature range tested (3-13°C), only food level significantly affected shell and tissue growth of *Arctica islandica* juveniles. Siphon activity, however, was affected by both factors as well as by the interaction between them. Siphon observations played a key role to better understand the growth differences among individuals (Fig. 4).

4.1. Food availability and growth

We found increased shell and tissue growth at higher food concentrations (all temperatures; Fig. 2 & 3). More growth at elevated food levels have been also reported in other bivalve species such as *Mytilis edulis, Tapes japonica* or *Pinctada margaritifera* (Winter and Langton,

1976; Langton et al., 1977; Linard et al., 2011; Thomsen et al., 2013; Joubert et al., 2014). Thus, our findings support that bivalve growth is strongly influenced by food supply. Our growth rate measurements (0.32 -58.26 µm d^{-1}) were in the range of growth rates found in other laboratory growth experiments (Witbaard et al., 1997a; Hiebenthal et al., 2012; Hiebenthal et al., 2013; Hippler et al., 2013; Stemmer et al., 2013), although lower than *A. islandica* growth rates reported *in situ* (Hippler et al., 2013; Milano et al., 2017). The minimal shell growth found at all treatments without food (all replicates at all temperatures) has to be interpreted with caution. All specimens could have grown on the basis of the energy reserves that were accumulated during the acclimation time (average reference BMI=4.9; Fig. 3). They were fed at optimal conditions and thus had reserves to survive some period of starvation.

Mean BMIs of most treatments decreased at the end of the experiment with respect to mid-term (Fig. 3). Since the quantity of food added to each treatment did not change, this is an indication of asynchrony between shell and tissue growth. Figure 6 supports that finding because it shows how the concentration of phytoplankton cells per treatment decreased or remained almost equal through time. This indicated equal or higher food intakes at the second part of the experiment. Moreover lower levels of phytoplankton cells coincided with higher siphon activity at most of the treatments (Fig.6).

At the food-limited treatments, 42% of the animals died (11 of 26, Fig. 5d) in the second week of the experimental period. At the 'medium' and 'high' food level, 27 % of the animals died (3 of 11) and mortality was more spread over the duration of the experiment (Fig. 5d). Those results suggest that mortality is related to food level and the duration of starvation (P-value < 0.05). In the food-limited treatments, mortality decreased with time and the observations suggest that this might be due to a decrease in feeding activity as evidenced by a lower siphon activity (Fig. 6). Lower siphon activity means lower energy cost and thus, energy conservation by which the specimens could survive starvation for longer periods of time. We furthermore, observed that after measuring the specimens at mid-term, for 1-2 weeks the specimens changed their 'normal' siphon activity registered until that moment (Fig. 6) and were seen less with open siphons. Similar reduction in gape activity had been observed in *in-situ* valve gape measurements where specimens also needed up to two weeks to return to their previous valve gape level after disturbance (Ballesta-Artero et al., 2017).

These findings clearly illustrate the disturbing effect on the behavior of these clams caused by manipulation.

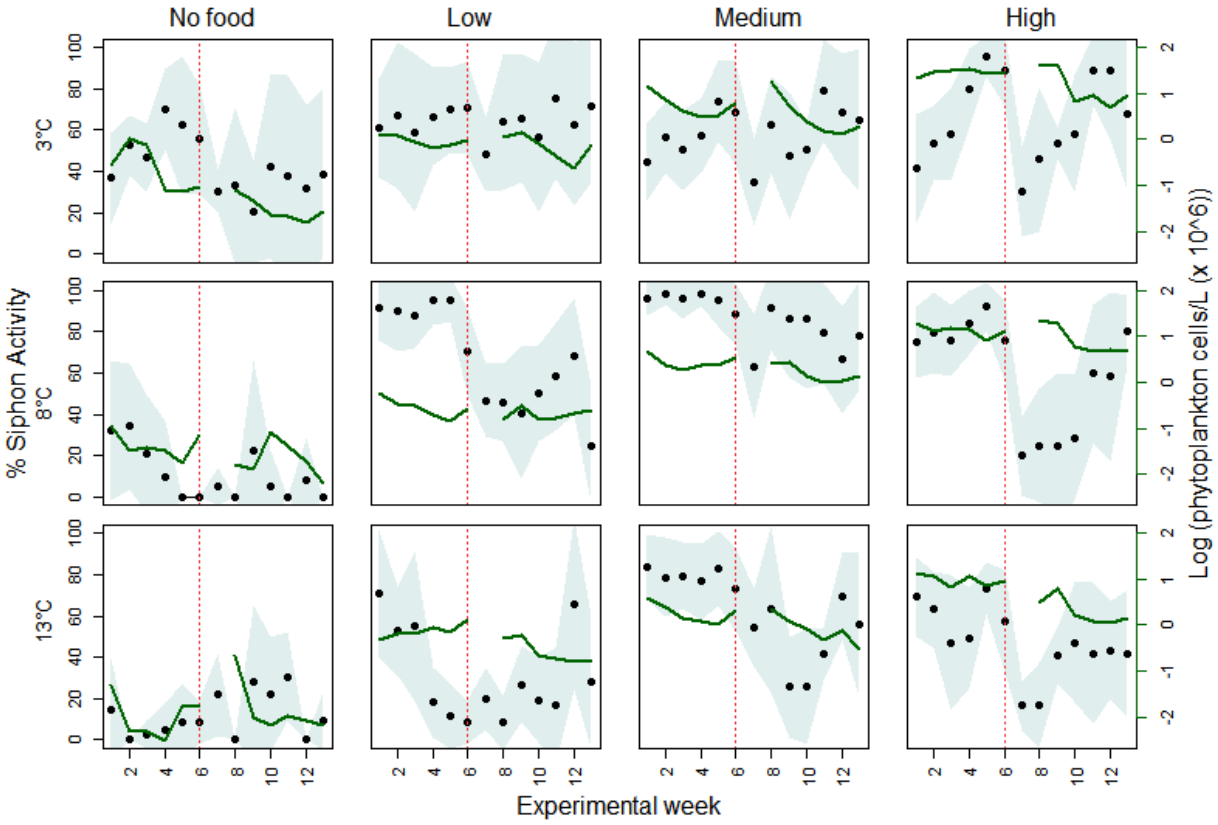

Fig. 6. Percentage of siphon activity per treatment (dots) and mean phytoplankton cells concentrations (solid line) during the entire experimental period. Grey shadow represents siphon activity standard deviation (all specimens considered). Dashed vertical line marks midterm.

4.2 Temperature and growth

Our results are in agreement with Begum et al. (2010) who constructed a growth and energy budget model for six different North Atlantic populations of *Arctica islandica* (temperature range 4-10 °C). Since the Q_{10} for *A. islandica* respiration is about 2.5, they predicted that temperature should have an effect on shell and tissue growth (measured as tissue and shell AFDW). Nevertheless, they could not find such a temperature effect, and they postulated that site-specific effects (for example differences in salinity) could have obscured the temperature effect on their results (Begum et al., 2010). Likewise, food availability could be the reason for this. In previous laboratory experiments conducted in the range of 1 and 12 °C, faster shell and tissue growth at higher temperatures was reported (Witbaard et al.,

1997a). Other studies conducted between 10 and 16°C found, that shell and tissue growth decreased at increasing temperatures (Hiebenthal et al., 2012; Milano et al., 2017). Field studies also showed conflicting results regarding the effect of temperature on the growth of *A. islandica* (Witbaard et al., 1996; Marchitto et al., 2000; Epplé et al., 2006; Wanamaker et al., 2008; Stott et al., 2010; Marali and Schöne, 2015). Different authors reported occasions where *A. islandica* growth rate was lower than expected on the basis of temperature alone, and suggested that food availability determined growth rate within the optimal temperature range of the species (Witbaard et al., 1996; Witbaard et al., 1997b; Witbaard et al., 1999; Schöne et al., 2003; Witbaard et al., 2003; Strahl, 2011; Ballesta-Artero et al., 2017). We only used specimens from one population, Kiel Bay, and we cultivated them at constant controlled environmental conditions (Table 1). The results suggest that the temperature between 3 and 13 ° C had a limited effect on shell and tissue growth when compared to the role of food availability. On basis of our results, however, we cannot exclude the role of temperature. Exclusion of outliers in the statistical analyses turned temperature into a significant factor. Future experiments at higher temperatures and/or broader temperature range (0–20° C) could better elucidate its effect on *A. islandica* growth.

We observed that at the same food level the algal concentrations tended to be lower at higher temperatures, suggesting that filtration rate increases with temperature. The typical filtration rate curve in bivalves shows how filtration rate increases up to the optimal species-specific temperature. Above that optimal temperature, filtration rate collapses (Winter, 1978). Moreover, larger body size of the specimens as well implicate higher filtration rate (Walne, 1972; Winter, 1978). Consequently, only at the coldest temperature, the mean of phytoplankton concentration measured in the aquaria was higher than the desired target level for each of the treatments (Table 1). Based on the phytoplankton concentration measurements at the different temperatures (Table 1), we did not find an indication of an abrupt reduction in their filtration rate. The absence of that abrupt change suggests that the highest experimental temperature remained still equal or below the optimum for filtration. Therefore, our study together with results obtained in other laboratory growth experiments suggest that between 1 and 13 °C, the growth rate of *A. islandica* increases (Witbaard et al., 1997a), but that temperatures above 14-15 °C could be suboptimal (Hiebenthal et al., 2012; Milano et al., 2017), leading to a decrease in filtration and growth. These findings are in

agreement with earlier studies which found that *A. islandica* distribution limit follows the 16 °C isocline (Mann, 1982; Cargnelli et al., 1999; Weinberg et al., 2002).

4.3 Siphon activity and growth

We noticed that siphon activity at the 'high' food levels was lower, than at the 'medium' food levels (Fig. 4; Table 3). This may indicate that the food concentration of the highest food level was close to gut capacity, making the specimens to regulate their filtration rate in such a way that the amount of food ingested is kept constant (Winter, 1978). If this is true, our findings would support the idea that *Arctica islandica,* similar to *Mercenaria mercenaria* and *Cardium edule,* controls ingestion primarily by reducing filtration rate instead of producing copious pseudofaeces such as *Mytilus edulis* and *Crassostrea virginica* (Bricelj and Malouf, 1984). The reduction of filtration rate above a critical algal concentration prevents overloading of the feeding system and is a way to optimize the ingestion rate and growth (Møhlenberg and Riisgård, 1979; Riisgård, 1991). These critical thresholds, however, may vary depending on the diet or pre-experimental conditions of the individuals (Maire et al., 2007) and may be different for the species in other situations.

Ballesta-Artero et al. (2017) reported that *A. islandica* in a natural setting had the highest valve gape coinciding temporally with the highest ambient levels of chlorophyll-*a* (Chl-*a*). This result agreed with earlier studies arguing that the main driver for opening valves in bivalve species as *Mytilus edulis*, *Austrovenus stutchburyi*, and *Crassostrea virginica*, was the occurrence of Chl-*a* (Higgings, 1980; Williams and Pilditch, 1997; Riisgård et al., 2006). Open valves are needed for siphon extension in filter-feeding bivalves. Therefore, open siphons (and open valves) can be used as an indicator of feeding activity. Our laboratory results not only confirm above field experiment (Ballesta-Artero et al., 2017) but also confirm the findings of a previous *A. islandica* growth experiment (Witbaard et al., 1997a), where siphon activity ranged from 12% in the individuals that did not receive any food to 76% with the highest food concentration (from 11 to 89% in our study). A low activity in the absence of food (% open siphons and gaping activity) can be interpreted as a physiological mechanism that allows *A. islandica* to save energy and survive starvation periods in nature (Taylor, 1976; Witbaard et al., 1997a; Riisgård et al., 2006; Tang and Riisgård, 2016; Ballesta-Artero et al., 2017). In the experiment reported here, we observed that specimens at 8 and 13 °C showed a marked change in their siphon activity between the no-food and food treatments.

466 However, this change did not occur at 3°C (Fig. 4), suggesting that the metabolic energy cost
467 for the species is lower at 3°C (Abele et al., 2002; Abele and Puntarulo, 2004; Hiebenthal et
468 al., 2013; Milano et al., 2017), and could allow for higher siphon activity at all tested
469 concentrations.

470 Finally, we found, similarly to Witbaard et al. (1997a), a strong correlation between shell
471 and tissue growth, and siphon activity (Fig. 4d, 4e; Table 4). Therefore, the inter-specimen
472 variation in growth of *A. islandica* can partly be explained by differences in their siphon
473 activity, i.e., in their individual feeding activities. Food density seems to be driving the
474 siphon/gaping activity of this species, and with that, shell and tissue growth (this study;
475 Witbaard et al., 1997a; Ballesta-Artero et al., 2017). The present study supports this link
476 between valve gape, open siphons, and shell growth in the bivalve *A. islandica* (Witbaard et
477 al., 1997a; Ballesta-Artero et al., 2017).

478 4.4 Application to paleoclimate studies
479 Paleoclimate studies based on *Arctica islandica* chronologies usually use old and large
480 specimens to reconstruct past environmental conditions. Such large specimens have
481 extremely low growth rates (Thompson et al., 1980; Murawski et al., 1982; Kennish et al.,
482 1994) which hampers experimental growth studies with them. It is virtually impossible to
483 reliably measure size differences over short (experimental) time spans. That is much easier
484 when juvenile specimens are used, but they might respond differently to variations in food
485 and temperature when compared to adults. The *A. islandica* valve gape study done with
486 adults (up to 9-cm height; Ballesta-Artero et al., 2017) showed, however, a similar link
487 between shell growth and valve gape (siphon activity) as we report here. This supports the
488 idea that adults and juveniles have the same behavioral response to variations in food and
489 temperature. On basis of this similarity, we think that our experimental results will help with
490 the interpretation of growth line records in adults, being of great utility for the
491 sclerochronology community.

492

493 **5. Conclusions**
494 Our study helps to understand the role of food and temperature on the growth rate of *A.*
495 *islandica*. Within the temperate range tested (3-13°C), the interaction between feeding

conditions and temperature did not have a significant effect on the growth of the species. The concentration of algal food was the main factor driving siphon activity and with that shell and tissue growth. Very low and very high algal concentrations led to shell closure and reduction (or cessation) of filtration in *A. islandica*. Therefore, paleoclimatic reconstructions based on *A. islandica* shell chronologies should not only consider temperature but also food supply of the area under study.

5. Compliance with Ethical Standards

The authors declare that they have no conflict of interest. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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