HIGH-RESOLUTION RECORDS OF GROWTH TEMPERATURE AND LIFE HISTORY OF
TWO *NACELLA* LIMPET SPECIES, TIERRA DEL FUEGO, ARGENTINA

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ABSTRACT

Adam Jacob Donnally Nicastro: High-Resolution Records of Growth Temperature And Life History Of Two *Nacella* Limpet Species, Tierra Del Fuego, Argentina
(Under the Direction of Donna Surge)

Nacella deaurata (Gmelin, 1791) and *N. magellanica* (Gmelin, 1791) are common intertidal species of patelloid limpets found along the coast of Tierra del Fuego, Argentina. Stable isotope ratios in patelloid limpets have been previously established as proxies for coastal environmental change at sub-monthly resolution. Proxy records of paleoenvironmental change at high, southerly latitudes are largely interpreted through ice, marine, and lake cores, which, while temporally extensive, fail to capture high-resolution variability in coastal environments. Hence, a proxy record that shows sub-monthly resolution is necessary to understand seasonal variability in coastal environments from high southerly latitudes. Here, we examine $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{shell}}$ values of modern specimens of *N. deaurata* and *N. magellanica* to test the hypotheses that: 1) *Nacella spp.* form their shells in isotopic equilibrium with ambient water; 2) *Nacella spp.* record variation in SST through oxygen isotope ratios; and 3) Prominent growth lines in *Nacella spp.* form annually.

To my family, I couldn't have gotten this far without you.

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LIST OF SYMBOLS AND ABBREVIATIONS

δ	Delta, denotes delta notation, a means of reporting stable isotope ratios relative to a known standard
‰	per mil, the unit of stable isotope ratios reported using delta notation
AAO	Antarctic Oscillation
ACC	Antarctic Circumpolar Current
CONICET	Centro Austral de Investigaciones Científicas
DIC	Dissolved Inorganic Carbon
EPF	Extrapallial Fluid
HMC	High Magnesium Calcite
LMC	Low Magnesium Calcite
MSLP	Mean Sea-Level Pressure
SAM	Southern Annular Mode
SHWW	Southern Hemisphere Westerly Winds
SLAP	Standard Light Antarctic Precipitation
SST	Sea Surface Temperature
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water

HIGH-RESOLUTION RECORDS OF GROWTH TEMPERATURE AND LIFE HISTORY OF TWO *NACELLA* LIMPET SPECIES, TIERRA DEL FUEGO, ARGENTINA

Section 1: Introduction:

Proxy archives are the foundation for extrapolating changes in oceanographic conditions beyond the bounds of the instrumental record. Currently, records of paleoclimate in the Southern Ocean are relatively scarce when compared to records of paleoclimate from other ocean basins. Many of the limited paleoclimate proxy records in the Southern Ocean are derived from ice, marine, or lake sediment cores. These records, while temporally extensive, focus on centennial to millennial timescales. Few of these proxy records capture changes to coastal environments, which are particularly sensitive to changing climate conditions. Even fewer paleoclimate data exist at decadal, annual, and seasonal timescales along coastlines in the Southern Ocean. Hence, a proxy record that can be resolved to sub-monthly resolution is necessary to understand seasonal variability in coastal environments from high southerly latitudes.

Stable isotope ratios in mollusc shells have been used to reconstruct paleoclimate records to sub-monthly resolution. Most molluscs form their shells in isotopic equilibrium with ambient water (Epstein et al., 1951, 1953; Craig, 1957; Grossman and Ku, 1986), but this is not always the case. Previous studies have shown that limpet species from the genus, *Patella*, form their shells in isotopic disequilibrium with ambient water (e.g. Shackleton et al., 1973; Cohen and Tyson, 1995; Fenger et al., 2007; Ferguson et al., 2011; Surge et al., 2013; Parker et al., 2017). In these studies, seawater temperatures were reliably reconstructed after subtracting a consistent, positive offset in $\delta^{18}\text{O}_{\text{shell}}$ values relative to expected equilibrium values. Thus, if such offsets

from expected equilibrium values are consistent in modern specimens, it can be accounted for to reliably reconstruct sea surface temperature (SST) in older (i.e., archaeological or fossil) specimens.

Other studies examined stable isotope ratios in mollusc shells to understand life history and growth patterns (Jones, 1983; Jones et al., 1984, 1989; Jones and Quitmeyer, 1996). In particular, studies of patelloid limpets have noted prominent growth lines that occur annually, possibly due cold climates, reproductive stress, or changes in food supply (Fenger et al., 2007; Surge et al., 2013; Ambrose et al., 2016). As such, prominent growth lines can help to contextualize the growth patterns and lifespans of a shelled animal, provided that the timing of prominent growth formation is understood.

Here, we interpret records of life history and environmental change preserved in modern specimens of two high-southerly latitude limpets, *Nacella deaurata* (Gmelin, 1791) and *N. magellanica* (Gmelin, 1791). Through the analysis of oxygen and stable carbon isotope ratios ($\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{shell}}$, respectively), we test the hypotheses that: 1) these species form their shells in isotopic equilibrium with ambient water; 2) their shells record seasonal variation in SST; and 3) prominent growth lines in their shells form annually.

1.1: Ecology of *Nacella deaurata* and *Nacella magellanica*:

N. deaurata and *N. magellanica* are intertidal patelloid limpets that inhabit coastal areas of the Magellanic Province, which wraps around the southern tip of South America from Chiloé through Cape Horn, southern Patagonia, Tierra del Fuego, and the Falkland Islands (Spalding et al., 2007). The two species can be differentiated using several morphological features such as shell height, sculpture, and mantle tentacle pattern (Valdovinos and Ruth, 2005). *N. magellanica* shells are typically taller than *N. deaurata* shells, with a more rounded sculpture pattern at the

shell margin (Figure 1a). These two species live from 0 to 30 m depth (Valdovinos and Ruth, 2005). *N. deaurata* lives in the lower intertidal and sublittoral zones, while *N. magellanica* lives mostly in the high and middle intertidal zones. As a result, *N. magellanica* are exposed subaerially twice daily for 3-5 hours, whereas *N. deaurata* are exposed once a day only during spring tides. Consequently, *N. magellanica* is subjected to more extreme temperatures ranging from 0 to 20 °C. In contrast, *N. deaurata* experiences temperatures between 4 and 11 °C. (Morriconi and Calvo, 1993; Morriconi, 1999). Both species source their diets primarily from the middle and low intertidal zone, and feed when submerged. *N. deaurata* subsists primarily on macroalgae, whereas *N. magellanica* subsists primarily on invertebrates and microalgae (Rosenfeld et al., 2018). Both species typically have one major spawning event per year. Reproductive organs begin to mature as water temperature starts to rise, with a peak spawning event occurring in late spring (November/December) (Morriconi et al., 1999; Morriconi, 2005). Minor spawning events sometimes occur as water temperatures begin to rise in early spring (Morriconi, 2005).

1.2: Geographic Setting:

The Beagle Channel (54.8-55 °S, 67.2-70 °W) is a narrow waterway in the Tierra del Fuego Archipelago at the southern tip of South America. It extends approximately 240 km from the Argentinian province of Tierra del Fuego in the east to the western coast of the Chilean Province of Magellanes. This study is set in the eastern portion of the Beagle Channel, around 64 km east of Ushuaia, Argentina (Figure 2). Gordillo et al. (2015) reports an average annual range in SST of 4 to 9.5 °C in Ushuaia Bay. Graniero et al, (2016) reports average salinity of 30.8 ± 0.8 psu (practical salinity units) at the study site, with little seasonal variation.

Figure 1: (a) Photographs of *Nacella deaurata* and *N. magellanica*. Note differences in height and sculpture between the two species. Black arrows indicate the anterior growth margin, where monthly-collected shells were sub-sampled. (b) Image of polished cross-section of *N. magellanica* posterior growth axis. (c) Image of cross-sectioned *N. deaurata* shell treated with Mutvei's solution. The red box indicates the enlarged portion of the image to the left. White arrows in the enlarged portion of the image indicate bundles of 10-14 micro-growth lines. *N. magellanica* shows the same packaging of micro-growth increments. (d) Representative image of cross-sectioned *N. deaurata* shell treated with Feigl's solution. The black arrow indicates the aragonitic portion of the shell stained by Feigl's solution. This procedure identified a similar location of the aragonitic layer in *N. magellanica*.

Precipitation averages 570 mm per year, with more precipitation generally occurring in winter than in summer (Gordillo et al., 1992).

Figure 2: Map of study area in the Beagle Channel, Tierra del Fuego, Argentina. The red star indicates the collection site. The red box shows the location of the study area relative to the continent of South America.

Waters of the Beagle Channel are influenced by the Antarctic Circumpolar Current (ACC), the strength of which is determined by the strength of the Southern Hemisphere Westerly Winds (SHWW). The primary cause of climate variation in the study area is the Southern Annular Mode (SAM; Limpasuvan and Hartmann, 1999), also known as the Antarctic Oscillation (AAO, Gong and Wang, 1999). The SAM index is defined as the difference in mean sea-level pressure (MSLP) between 40 °S and 65 °S latitude (Gong and Wang, 1999). During

positive phases of SAM, MSLP is higher at mid latitudes than at high latitudes, causing the SHWW to contract toward Antarctica. During negative phases of SAM, MSLP is higher at high latitudes than at mid latitudes, causing the SHWW to expand toward the southern edges of South America, Africa, and Australia. In southern South America, positive phases of SAM are associated with warmer, drier conditions, while negative phases coincide with colder, wetter conditions (Thompson & Solomon, 2002; Sen Gupta & England, 2006; Gillett et al., 2006; Dätwyler et al., 2018). SAM oscillates between positive and negative phases over periods between several months to approximately five years (Marshall, 2003). SAM oscillation since 1980 is shown in Figure 3, with shell collection years highlighted in red.

Figure 3: Oscillation in SAM from 1980 to 2018. Each data point represents the annual average SAM index for that year. The red symbols indicate SAM index of the years during which shell specimens were collected. Data are provided by the British Antarctic Survey (<https://legacy.bas.ac.uk/met/gjma/sam.html>)

The study area contains a long history of human habitation recorded in archaeological shell and fish middens, of which *N. deaurata* and *N. magellanica* are major constituents (Colonese et al., 2012; Zurro et al., 2017). Midden deposits are generally preserved in normal stratigraphic order, with older material at the bottom and younger material at the top. Archaeological shells within middens provide not only evidence of human harvesting patterns, but also archives of paleoenvironmental change. Middens in the area date from 6500 BP to the 17th century (Estevez et al., 2001). Therefore, archaeological shells in local middens potentially provide a rich archive of late Holocene climate variability in southern South America, as well as providing information on the preferred season of harvest of fish and shellfish of past societies.

Section 2: Methods:

2.1: Shell Collection and Water Measurements:

Live specimens of *N. deaurata* and *N. magellanica* and water samples were collected monthly from the Beagle Channel, Tierra del Fuego, Argentina (54°52'50.07" S, 67°16'25.27" W) from October 2015 to October 2016. The study site was inaccessible due to snow in August of 2016; thus, no shells or water were collected that month. Shells were collected from Outer Camarcres Bay, the seaward side of a narrow isthmus separating the Beagle Channel from an inland lagoon. Between 7 and 10 live individuals of each species were collected at each sampling date. Shells were selected on the basis of size (total height from margin to apex, and length from anterior to posterior), as well as shell thickness. Shells with chipped or cracked margins were avoided.

Limpets were pried from the rocks in the intertidal zone at low tide, and care was taken to not damage the growing edge. The specimens were transported to the laboratories at Centro Austral de Investigaciones Científicas (CONICET), Ushuaia, Argentina. Live specimens were

stored in a refrigerator at 3-4° C for no more than a day before the animal was removed from the shell. Viscera were removed after immersion in hot water for 20 to 30 seconds. Water used to remove the animal from the shell was never hot enough to boil the animal or its shell. Five shells from each collection date were cleaned and sent to the University of North Carolina at Chapel Hill. Temperature of local water was measured at each of the collection dates using a multi-parameter water quality monitor (Hannah Instruments 9828) with a precision of ± 0.15 °C. Salinity was measured at the University of North Carolina at Chapel Hill using a refractometer with an accuracy of ± 0.5 psu. Water for oxygen isotope analysis measurements was collected in 30 ml glass vials and filled to the top to avoid any headspace. Isotopic analytical methods of water samples are described below in section 2.4.

2.2: Analysis of Shell Microstructure and Mineralogy:

Shell growth lines, microstructure, and mineralogy were examined using acetate peels, Mutvei's solution, and reflected-light microscopy. Shells were sectioned from anterior to posterior along the axis of maximum growth using a Buehler IsoMet low-speed saw and mounted onto glass slides using Gorilla Glue clear epoxy. Cross-sectioned shells were ground to a flat surface using a 600 grit silicon carbide grinding paper (BuehlerMet II). Samples were then polished using a 6 μm diamond suspension grit followed by a 1 μm diamond suspension grit (Buehler). Digital images of the sectioned shells were captured using an Olympus DP71 Camera mounted to an Olympus SZX7 microscope, and Olympus StreamEssentials software for Windows v2.2 (Figure 1b). Image resolution was 4080 x 3072 pixels. Acetate peels of the sectioned shells were prepared following the methods described in Carter and Ambrose (1989) to identify growth lines and assess microstructure type.

Shells were treated with Mutvei's solution to enhance the visibility of prominent growth features. Mutvei's solution was prepared according to the methods described by Schöne et al. (2005a). Shell sections were cleaned ultrasonically in deionized water, rinsed in ethanol, and allowed to air dry. Samples were immersed in the Mutvei's solution which was kept at a temperature between 37 and 40 °C under constant stirring for about one hour. The shell sections were then removed from the solution, rinsed with deionized water, and allowed to air dry. Shells treated with Mutvei's solution were viewed and photographed using the same methods described above (Figure 1c).

Feigl's solution was applied to shells that had not been treated with Mutvei's solution to differentiate calcite from aragonite within the shell (Figure 1b). Feigl's solution was prepared according to methods outlined in Feigl (1958). The solution differentiates calcite from aragonite due to the different solubilities of the two minerals. Silver and manganese ions in Feigl's solution react with OH^- ions produced from the dissolution of CaCO_3 in water. This process causes the shell material to change from its original color to a shiny black or silver (Figure 1d). Aragonitic parts of the shell are more soluble and, therefore, change color more quickly than calcitic layers when treated with Feigl's solution. Polished shell sections were immersed in Feigl's solution for 1-2 hours and periodically inspected until changes in shell color were visible.

2.3.1: Sampling along Shell Growth Margin:

Three shells from each collection date were selected for sampling along the growing edge for a total of 33 shells. Shells were selected based on shell thickness and preservation of the shell margin. Specimens showing fragmentation, alteration, or fractures along the anterior growing edge were not selected. Approximately 100 µg of powdered carbonate material was milled from the anterior growth margin (Figure 1a) of each selected shell using a hand-held Volvere VMAX

Dental Drill with a fine-point breaking-contact drill bit (Brasseler Diamond, catalog number: 8859). The milled powder was sent to the University of Arizona's Environmental Isotope Laboratory for stable isotopic analyses. The isotopic analytical methods are described below in section 2.4.

2.3.2: Serial Sampling along Maximum Axis of Growth:

Six shells (three *N. deaurata* and three *N. magellanica*) from the last shell collection date (October 1, 2016) were chosen for serially sampling along the growing axis to produce a sub-monthly isotopic time series. Shells were selected based on visibility of prominent growth lines and shell thickness. Shells were sectioned from anterior to posterior along the axis of maximum growth and prepared following the methods described above. Shell cross-sections were microsampled at 150 to 200 μm intervals from the posterior growth margin toward the apex (9 to 10 cm) using a Merchantek Micromill with a scribe-point drill bit (Brasseler Carbide, catalog number: H1621). Powdered shell material from two shells (specimens ND-1016-4 and NM-1016-3) were sent to the Institute of Geosciences at the University of Mainz for stable isotopic analysis. The remaining four shells (ND-1016-1, ND-1016-3, NM-1016-1, NM-1016-5) were sent to the University of Arizona's Environmental Isotope Laboratory for stable isotopic analyses. The isotopic analytical methods are described below in the next section. Note that specimen identification numbers reflect species (ND = *N. deaurata*, NM = *N. magellanica*), collection month and year (1016 = October 2016), and individual out of the 5 specimens collected that month.

2.4: Geochemical Analyses:

Values of $\delta^{18}\text{O}_{\text{water}}$ were measured on a gas-source isotope ratio mass spectrometer (Finnigan Delta S) at the Environmental Isotope Laboratory, University of Arizona. Samples

were equilibrated with CO₂ gas at approximately 15 °C in an automated equilibration device coupled to the mass spectrometer. Standardization is based on international reference materials Vienna Standard Mean Ocean Water (VSMOW) and Standard Light Antarctic Precipitation (SLAP). Precision is 0.08‰ or better based on replicates of internal standards.

Powdered shell material sent to the University of Arizona's Environmental Isotope Laboratory was measured using an automated carbonate preparation device (KIEL-III) coupled to an isotope ratio mass spectrometer (Finnigan MAT 252). Powdered samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The isotope ratio measurement is calibrated based on repeated measurements of NBS-19 and NBS-18 and precision is ± 0.1 ‰ for $\delta^{18}\text{O}$ and ± 0.08 ‰ for $\delta^{13}\text{C}$ (1σ).

Powdered shell samples sent to the Institute of Geosciences at the University of Mainz were measured on a Thermo Finnigan MAT 253 continuous-flow isotope ratio mass spectrometer equipped with a Gasbench II. Samples were dissolved with concentrated phosphoric acid in He-flushed borosilicate exetainers at 72 °C. Data were calibrated against NBS-19 calibrated Carrara marble distributed by IVA Analysentechnik GmbH & Co.KG (Meerbusch, Germany) ($\delta^{18}\text{O} = -1.90$ ‰; $\delta^{13}\text{C} = +2.01$ ‰). On average, internal precision (1σ) was ± 0.05 ‰ for $\delta^{18}\text{O}$ and ± 0.04 ‰ for $\delta^{13}\text{C}$. Precision is better than 0.04‰ and 0.03‰ for $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, respectively, based on 421 blindly measured NBS-19 samples. Isotope ratios of carbonate are reported as per mil (‰) relative to Vienna Pee Dee Belemnite (VPDB).

2.5: Predicted Shell $\delta^{18}\text{O}$

Predicted shell margin $\delta^{18}\text{O}$ values based on equilibrium fractionation were calculated using the monthly observations of water temperature and $\delta^{18}\text{O}_{\text{water}}$ values, and the equilibrium

fractionation equation for calcite and water (Friedman and O'Neil, 1977, modified from Tarutani et al., 1969):

$$(1) \quad 1000 \ln \alpha = 2.78 * 10^6 * T^{-2} - 2.89$$

where T is temperature in Kelvin and α is the fractionation factor between calcite and water. The terms α and δ are related through the equation:

$$(2) \quad \alpha = (\delta_{shell} + 1000) / (\delta_{water} + 1000)$$

Where δ values are expressed relative to VSMOW. Temperature estimates based on $\delta^{18}\text{O}_{shell}$ values sampled along the axis of maximum growth were calculated assuming a $\delta^{18}\text{O}_{water}$ value of $-1.22 \pm 0.19\text{‰}$. This value represents the average $\delta^{18}\text{O}_{water}$ value and standard deviation during the collection period. Values of $\delta^{18}\text{O}_{shell}$ were then reported relative to VPDB using the following equation (Coplen et al., 1983; Gonfiantini et al., 1995):

$$(3) \quad \delta^{18}\text{O}_{(VPDB)} = (\delta^{18}\text{O}_{(VSMOW)} - 30.91) / 1.03091$$

Section 3: Results:

3.1: Mineralogy and Microstructure:

Acetate peels show four microstructural layers within the shell cross-sections of *N. deaurata* and *N. magellanica* (Figure 4). These layers are named based on their position relative to the myostracum (layer “m”; the muscle attachment site) following terminology in MacClintock (1967) and Carter (1990). The microstructural layers ventral to the myostracum are termed m-1 and are composed of calcitic irregularly foliated microstructure. The microstructural layers dorsal to the myostracum are composed of calcitic regularly foliated (m+1) and irregular simple prismatic (m+2) microstructure. In both species, Feigl's solution only reveals the presence of aragonite in the myostracum near the apex of the shell, which does not constitute a significant amount of shell material. Therefore, only calcitic components of the shell were

sampled for isotopic analyses.

Figure 4: Images of acetate peels of *Nacella deaurata* (left) and *N. magellanica* (right). The myostracum is denoted by the letter “m” and an arrow. Layers above and below are denoted by m+1, m+2, and m-1, depending on their dorsal or ventral position relative to the myostracum following MacClintock (1967).

3.2: Shell Growth Lines:

Prominent growth lines are visible in acetate peels and sometimes in polished shell cross-sections, whereas Mutvei’s solution revealed both prominent and higher order growth lines. Four of the six shells show three prominent growth lines (ND-1016-1, ND-1016-3, NM-106-3, NM-1016-5), and the remaining two specimens (ND-1016-4 and NM-1016-1) show four prominent growth lines. Occasionally, the presence of a prominent growth line was expressed through to the shell’s exterior. Higher order growth lines are spaced between 100 and 200 μm apart and occur in bundles of 10 and 14 micro-growth lines. The time series of $\delta^{18}\text{O}_{\text{shell}}$ values were used to contextualize the location of prominent growth lines with respect to season of formation and are discussed below in section 4.4.

3.3: SST, Salinity, and $\delta^{18}\text{O}_{\text{water}}$ Values:

Water parameters at the time of monthly limpet collection are listed in Table 1. SST ranged from 4.5 to 12.2 °C with an average value of $8.4 \pm 3.0^\circ\text{C}$. Salinity ranged from 29.5 to 31.9 psu with an average value of 30.7 ± 0.73 psu. Values of $\delta^{18}\text{O}_{\text{water}}$ ranged from -1.52 to 0.91‰ , averaging $-1.22 \pm 0.19\text{‰}$.

Table 1: Collection dates of *N. deaurata* and *N. magellanica*, along with SST, salinity, and $\delta^{18}\text{O}_{\text{water}}$ values relative to VSMOW. Average and standard deviation of temperature, salinity, and $\delta^{18}\text{O}_{\text{water}}$ values are reported at the bottom of the table.

Date	Time	SST (°C)	$\delta^{18}\text{O}_{\text{water}}$	Salinity (psu)
10/27/15	13:52	12.2	-1.3	31.9
11/27/15	12:21	11.2	-1.52	30.1
12/31/15	15:55	12.2	-1.45	29.5
1/27/16	12:30	10.5	-1.16	30.6
2/26/16	13:35	12.1	-1.38	30.1
3/29/16	13:15	10.6	-1.22	31.2
4/27/16	13:30	6.3	-1.35	30.2
5/20/16	10:05	5.9	-1.12	31.5
6/23/16	12:15	5.1	-0.91	31.4
7/20/16	11:15	4.5	-1.02	31.2
9/4/16	12:55	6.0	-0.99	30.4
10/1/16	10:40	6.9	-1.30	31.3
Average		8.4 ± 3.0	-1.22 ± 0.19	30.8 ± 0.73

3.4: Stable Isotope Ratios of Nacella Shells:

Monthly Shell Margin Data:

Shell margin $\delta^{18}\text{O}$ values from specimens collected monthly show a sinusoidal trend reflective of seasonal changes in water temperature (Figure 4). Average monthly shell margin $\delta^{18}\text{O}$ values from *N. deaurata* range from $+1.05\text{‰}$ to $+2.79\text{‰}$. The standard deviation of $\delta^{18}\text{O}_{\text{shell}}$ values within each monthly collection date is highest in February, September, and October 2016 ($\pm 0.28\text{‰}$, $\pm 0.25\text{‰}$ and $\pm 0.25\text{‰}$, respectively,) and lowest in March and July 2016 ($\pm 0.08\text{‰}$ and

$\pm 0.07\text{‰}$, respectively). Average monthly $\delta^{18}\text{O}_{\text{shell}}$ values in *N. magellanica* range from $+0.94\text{‰}$ to $+2.86\text{‰}$. In *N. magellanica*, standard deviation of $\delta^{18}\text{O}_{\text{shell}}$ values are highest in December 2015 and October 2016 ($\pm 0.37\text{‰}$ and $\pm 0.40\text{‰}$, respectively,) and lowest in March and July 2016 ($\pm 0.04\text{‰}$ and $\pm 0.02\text{‰}$, respectively).

Figure 5: Monthly shell margin $\delta^{18}\text{O}$ values of 33 specimens of *N. deaurata* and *N. magellanica* collected from October 27, 2015 to October 1, 2016. Red diamonds and blue squares represent the mean shell margin $\delta^{18}\text{O}$ value ($n = 3$) for monthly collected specimens of *N. deaurata* and *N. magellanica*, respectively. Error bars represent ± 1 standard deviation from mean $\delta^{18}\text{O}$ values.

Time Series of $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{shell}}$ Values of Serially-Sampled Specimens:

The time series of $\delta^{18}\text{O}_{\text{shell}}$ values serially sampled along the maximum axis of growth in all six specimens reflect a quasi-sinusoidal trend (Figure 6). Values recorded in *N. deaurata* range from $+0.4\text{‰}$ to $+3.03\text{‰}$ (ND-1016-1), $+1.0\text{‰}$ to $+2.6\text{‰}$ (ND-1016-3), and $+0.36\text{‰}$ to $+2.23\text{‰}$ (ND-1016-4). In *N. magellanica*, values range from $+0.67\text{‰}$ to 2.63‰ (NM-1016-1), $+0.80\text{‰}$ to $+2.54\text{‰}$ (NM-1016-3), and $+0.79\text{‰}$ to $+2.75\text{‰}$ (NM-1016-5). Values of $\delta^{18}\text{O}_{\text{shell}}$ at the posterior shell margin (i.e., the first sample taken along the maximum axis of growth,)

average $2.03 \pm 0.36\text{‰}$, ranging from $+1.45\text{‰}$ to $+2.37\text{‰}$. *N. deaurata* posterior shell margin $\delta^{18}\text{O}_{\text{shell}}$ values average $2.00 \pm 0.19\text{‰}$ ranging from 1.86‰ to 2.22‰ . *N. magellanica* posterior shell margin $\delta^{18}\text{O}_{\text{shell}}$ values average $2.05 \pm 0.53\text{‰}$ ranging from 2.37‰ to 1.45‰ .

Variation in $\delta^{13}\text{C}_{\text{shell}}$ values follow a quasi-sinusoidal trend in some shells but not others (Figure 6). Four of the six shells show $\delta^{13}\text{C}_{\text{shell}}$ values that are roughly in phase with $\delta^{18}\text{O}_{\text{shell}}$ values (ND-1016-3, ND-1016-4, NM-1016-3, NM-1016-5.) Two of four shells show $\delta^{13}\text{C}_{\text{shell}}$ values that are out of phase with $\delta^{18}\text{O}_{\text{shell}}$ values (NM-1016-3, NM-1016-1). *N. deaurata* $\delta^{13}\text{C}_{\text{shell}}$ values range from $+1.07\text{‰}$ to $+1.94\text{‰}$ (ND-1016-1), $+0.89\text{‰}$ to $+1.96\text{‰}$ (ND-1016-3), and $+0.97\text{‰}$ to $+1.67\text{‰}$ (ND-1016-4). *N. magellanica* $\delta^{13}\text{C}_{\text{shell}}$ values range from $+0.85\text{‰}$ to $+1.84\text{‰}$ (NM-1016-1), $+0.82\text{‰}$ to $+2.05\text{‰}$ (NM-1016-3), and $+1.07\text{‰}$ to $+1.94\text{‰}$ (NM-1016-5). At the posterior shell margin, $\delta^{13}\text{C}_{\text{shell}}$ values average $1.36 \pm 0.20\text{‰}$, ranging from $+1.16\text{‰}$ to $+1.62\text{‰}$. *N. deaurata* posterior shell margin $\delta^{13}\text{C}_{\text{shell}}$ values average $1.39 \pm 0.23\text{‰}$ ranging from 1.16‰ to 1.62‰ . *N. magellanica* posterior shell margin $\delta^{13}\text{C}_{\text{shell}}$ values average $1.33 \pm 0.21\text{‰}$ ranging from 1.19‰ to 1.57‰ .

$\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{shell}}$ values show no strong linear correlation, regardless of species or sampling method (i.e., shell margin versus time series) (Figure 7; *N. deaurata* time series: ND-1016-1: $r^2=0.19$, $p < 0.01$, $n=47$; ND-1016-3: $r^2 = 0.34$, $p < 0.01$, $n = 49$; ND-1016-4: $r^2 = 0.01$, $p < 0.01$, $n = 58$; *N. deaurata* shell margin: $r^2 = 0.30$, $p = 0.011$, $n = 33$; *N. magellanica* time series: NM-1016-1: $r^2 = 0.00$, $p < 0.01$, $n = 50$; NM-1016-3: $r^2 = 0.00$, $p < 0.01$, $n = 50$; NM-1016-5: $r^2 = 0.23$, $p < 0.01$, $n = 50$; *N. magellanica* shell margin: $r = 0.09$, $p < 0.01$, $n = 33$). In both species, the variance of data points in Figure 7 is higher in the shell margin data than in the shells sampled along the maximum axis of growth.

Figure 6: $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{13}\text{C}_{\text{shell}}$ values of *N. deaurata* (left column) and *N. magellanica* (right column) sampled along the axis of maximum growth. Specimen ID is indicated at the top of each time series. Blue circles are $\delta^{18}\text{O}_{\text{shell}}$ values. Green squares are $\delta^{13}\text{C}_{\text{shell}}$ values. Black, vertical lines indicate the location of prominent growth lines. Zero mm from growth margin represents the posterior growth margin.

Figure 7: Cross-plots of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of *N. deaurata* (left), and *N. magellanica* (right).

Colored triangles, squares, and circles represent individual specimens sampled along the maximum axis of growth. Black crosses represent monthly values sampled along the anterior growth margin.

Section 4: Discussion:

4.1: Comparison of Observed and Predicted Shell-Margin $\delta^{18}\text{O}$ Values:

We compared measured and predicted shell margin $\delta^{18}\text{O}$ values to determine whether *N. deaurata* and *N. magellanica* precipitate their shell in isotopic equilibrium with ambient water. Measured shell margin values were offset from predicted values by an average of $+1.3 \pm 0.4\text{‰}$ in *N. deaurata* and by $+1.3 \pm 0.3\text{‰}$ in *N. magellanica* (Figure 8a). Although the offset is relatively consistent, and can therefore be accounted for when estimating growth temperature, it is slightly higher in summer months ($1.5 \pm 0.49\text{‰}$ for *N. deaurata*; $1.6 \pm 0.30\text{‰}$ for *N. magellanica*) compared to winter months ($0.90 \pm 0.11\text{‰}$ for *N. deaurata*; $1.03 \pm 0.13\text{‰}$ for *N. magellanica*). We evaluated the effect of this on SST reconstruction in monthly-collected shells by calculating SST using the equilibrium fractionation equation above (Freidman and O'Neil, 1977). Estimated

temperatures from $\delta^{18}\text{O}_{\text{shell}}$ values is lower than observed SST by 5.0 ± 1.4 °C in *N. deaurata* and by 5.2 ± 1.4 °C in *N. magellanica* (Figure 7c). After subtracting 1.3‰ from the measured $\delta^{18}\text{O}_{\text{shell}}$ values of both species to account for the observed offset (Figure 7b), better agreement was reached between calculated and observed temperatures (Figure 7d). Our findings agree with previous studies of patelloid limpets, in which a consistent offset of around +1‰ occurs between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values (Shackleton et al., 1973; Cohen and Tyson, 1995; Fenger et al., 2007; Ferguson et al., 2011; Colonese et al., 2012; Parker et al., 2017). Despite observing a similar, consistent offset between predicted and observed $\delta^{18}\text{O}_{\text{shell}}$ values, Colonese et al. (2012) suggested that the two *Nacella* species precipitate their shells in isotopic equilibrium with ambient water. However, the occurrence of an offset from expected equilibrium values indicates that the two *Nacella* species do not form their shells in isotopic equilibrium with ambient water.

4.2: Possible Mechanisms of Offset from Predicted $\delta^{18}\text{O}_{\text{shell}}$ Values:

Mineralogy: High vs. Low-Magnesium Calcite:

One possible explanation for the positive offset between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values is if calcitic shells are composed of high-magnesium calcite (HMC) rather than low-magnesium calcite (LMC). HMC is defined as calcite in which Mg concentrations are higher than 4 mol-% MgCO_3 and typically approach 15 mol-% MgCO_3 (Tarutani et al., 1969; Prothero and Schwab, 1996). As shown in Carpenter et al. (1991; their figure 5), HMC is ~1‰ more positive than LMC. If calcitic material is composed of HMC, equilibrium fractionation equations require an additional term of 0.06‰ per mol-% MgCO_3 (Tarutani et al., 1969; Carpenter et al., 1991). If unaccounted for, this could yield errors in temperature estimates of 0.26 °C per mol-% MgCO_3 (Fenger et al., 2007). If the calcitic material is composed of LMC, then no additional terms are required in the equilibrium fractionation equations.

Figure 8: a.) Shell margin $\delta^{18}\text{O}$ values before accounting for the consistent offset of $+1.3 \pm 0.4\text{‰}$ in *N. deaurata* and $+1.3 \pm 0.3\text{‰}$ in *N. magellanica*. b.) Shell margin $\delta^{18}\text{O}$ values after subtracting 1.3‰ from observed shell margin $\delta^{18}\text{O}$ values. c.) Temperatures calculated from observed shell margin $\delta^{18}\text{O}$ values using the equilibrium fractionation equation described by Friedman and O’Neil (1977) d.) Temperature estimates after accounting for the consistent offset. Red diamonds and blue squares represent mean shell margin $\delta^{18}\text{O}$ (top) and temperature (bottom) values ($n = 3$). Error bars represent 1 standard deviation from the mean. Gray triangles represent expected $\delta^{18}\text{O}_{\text{shell}}$ values (top) and observed temperatures (bottom). A double-ended, black arrow represents the offset between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values (top) and the offset between calculated and observed measurements of SST (bottom).

N. deaurata and *N. magellanica* would need to contain 22 mol-% MgCO_3 to account for the entire offset of 1.3‰. Previous work reports that *Patella vulgata* shells (a species within the same family as *Nacella*) are composed of LMC (Fenger et al., 2007). Although we did not measure Mg concentration in the two *Nacella* species, it is reasonable to assume that species within the same family have similar mineralogy, and that the calcitic portion of *N. deaurata* and *N. magellanica* shells are composed of LMC. Another possible explanation of positive offsets between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values is the occurrence of a vital effect discussed in the next section.

Vital Effects:

Isotopic disequilibrium can be caused by vital effects, in which an organism's life processes or habits produce isotopic fractionation. Observed departures from equilibrium fractionation are typically negative (e.g., McCrea, 1950; Erez, 1978; Swart, 1983; McConnaughey, 1989a, 1989b; Usdowski and Hoefs, 1993; Spero and Lea, 1996; Spero et al., 1997; Zeebe, 1999; Smith et al., 2000; Adkins et al., 2003), but positive departures from isotopic equilibrium have been observed in benthic algae, foraminifera, non-reef-building corals, barnacles, and marine gastropods (Land et al., 1977; Wefer and Berger, 1980, 1981, 1991; Wefer and Killingley, 1980; Killingley and Newman, 1982; Smith et al., 1988). Schöne et al. (2007) observed no departure from isotopic equilibrium in $\delta^{18}\text{O}$ values of intertidal and marine snails from the North Sea, suggesting that isotopic disequilibrium is not attributable to life habit or taxonomic rank. Instead, it seems that isotopic disequilibrium occurs in some species and not in others. Whether an organism precipitates its shell in isotopic disequilibrium may instead depend on processes of shell formation.

In all molluscs, limpets notwithstanding, shell precipitation occurs in the extrapallial fluid (EPF). The EPF is located in the cavity between the mantle and the shell. Oxygen and carbon are transported to the EPF either through diffusion of CO₂ through the mantle cavity, or through the active transport of CO₃²⁻ and HCO₃⁻ across the shell's epithelial tissue (Wheeler, 1992). Isotopic disequilibrium may be attributable to kinetic or metabolic isotope effects, which affect the fractionation of heavy isotopes of carbon and oxygen.

Kinetic isotope effects are the result of slower reaction rates of ¹⁸O and ¹³C, causing preferential incorporation of the lighter isotope into the EPF (McConnaughey, 1989a, 1989b). As such, kinetic isotope effects would cause depletion of ¹⁸O and ¹³C relative to expected equilibrium values (Erez, 1978; McConnaughey, 1989b). Covariation between δ¹⁸O and δ¹³C values would be expected in materials affected by kinetic isotope effects. However, no such covariation is observed in either *Nacella* species, regardless of sampling method (Figure 7). These results are consistent with previous studies of patelloid limpets by Fenger et al (2007). Therefore, kinetic isotope effects are minimal in *Nacella* shells.

Metabolic isotope effects may explain departures from isotopic equilibrium during shell formation. Metabolic isotope effects include incorporation of respired CO₂ or changes in the pH of the biomineralization medium (Swart, 1983; McConnaughey, 1989b). However, metabolic isotope effects typically produce *negative* departures from isotopic equilibrium, in which observed δ¹⁸O values are *lower* than predicted values (e.g., McCrea, 1950; Erez, 1978; Swart, 1983; McConnaughey, 1989a, 1989b; Usdowski and Hoefs, 1993; Spero and Lea, 1996; Spero et al., 1997; Zeebe, 1999; Smith et al., 2000; Adkins et al., 2003). Other studies have investigated whether positive departures from isotopic equilibrium can be attributed to external factors affecting the isotopic composition of the ambient water.

^{18}O enrichment in shell carbonate relative to isotopic equilibrium may occur through enhanced evaporative conditions of the ambient water, such as in tide pools. Schifano and Censi (1983) attributed ^{18}O enrichment in *Patella coerulea* shells to enhanced evaporative conditions during the time of shell precipitation. However, a later study by Schifano and Censi (1986) observed similar enrichment in ^{18}O in *P. coerulea* inhabiting the subtidal zone. Thus, enhanced evaporation does not explain the positive offset from isotopic equilibrium. Moreover, *N. deaurata* and *N. magellanica* do not inhabit microhabitats strongly influenced by evaporation (i.e., tidal pools). Furthermore, ^{18}O enrichment due to evaporation would likely vary throughout the year and, as such, would produce a variable offset between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values. Because we observe a consistent offset, evaporation is not a reasonable explanation for positive departures from isotopic equilibrium in *N. deaurata* and *N. magellanica*.

Internal processes of the organism may also cause enrichment of ^{18}O relative to equilibrium, resulting in a positive offset between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values. Land et al. (1977) explained positive departures in $\delta^{18}\text{O}_{\text{shell}}$ values relative to equilibrium in non-reef-building corals resulting from the transport of ^{16}O out of the biomineralization medium (i.e., an internal, vital process). A similar process may occur in the EPF of *N. deaurata* and *N. magellanica*. Alternatively, McConnaghey (1989a) attributed enrichment of ^{18}O in barnacles relative to equilibrium through some unknown internal process affecting biomineralization.

The cause of observed offsets between observed and predicted $\delta^{18}\text{O}_{\text{shell}}$ values in *N. deaurata* and *N. magellanica* merits further study. Observation of *N. deaurata* and *N. magellanica* under laboratory conditions may provide further insight. The calibration of these two species as SST archives will be further refined by comparing the high-resolution $\delta^{18}\text{O}_{\text{shell}}$ time series to higher resolution SST measurements. Efforts are underway to acquire a higher

resolution SST record at the study site. Although *N. deaurata* and *N. magellanica* form their shells in isotopic disequilibrium, departures from equilibrium are predictable and consistent, allowing for reliable reconstruction of SST after taking the observed offsets into account.

4.3: Ontogenetic Variation in $\delta^{18}\text{O}_{\text{shell}}$ Time Series:

We compared $\delta^{18}\text{O}_{\text{shell}}$ values sampled along the maximum axis of growth (i.e., high-resolution isotopic time series) among individuals and between species to determine whether *N. deaurata* and *N. magellanica* record similar ontogenetic variation. Both species show reasonable agreement in the amplitudes of the $\delta^{18}\text{O}_{\text{shell}}$ time series (Figure 6). ND-1016-1 records a higher-than-average maximum $\delta^{18}\text{O}_{\text{shell}}$ value of 3.03‰ (average maximum $\delta^{18}\text{O}_{\text{shell}}$ is 2.65 ± 0.26 ‰). This higher-than-average maximum $\delta^{18}\text{O}_{\text{shell}}$ value occurs within the last year of shell growth, but yields temperature estimates which still fall within one standard deviation of observed temperatures during the collection period, ND-1016-4 records a lower-than-average minimum $\delta^{18}\text{O}_{\text{shell}}$ value of 0.36‰ (average minimum $\delta^{18}\text{O}_{\text{shell}}$ is 0.74 ± 0.22 ‰). This lower-than average minimum $\delta^{18}\text{O}_{\text{shell}}$ value occurs after the first year of growth and falls outside of the range of observed temperatures during the collection period (Figure 6, see section 4.4 for a discussion of temperature estimates). General agreement is also observed between $\delta^{18}\text{O}_{\text{shell}}$ values at the posterior growth margin of shells sampled along the maximum axis of growth. Average $\delta^{18}\text{O}_{\text{shell}}$ at the posterior margin of growth in these shells is 2.03 ± 0.36 ‰. SST estimated using this average is $7.54 \pm 1.4^\circ\text{C}$, which is within one standard deviation of observed temperature at the time of shell collection in October of 2016 (6.89°C). One shell (specimen NM-1016-5) records a $\delta^{18}\text{O}_{\text{shell}}$ value at its posterior margin that falls outside of one standard deviation from mean values (1.45‰). We considered several possible explanations for this difference. All shells measured for $\delta^{18}\text{O}_{\text{shell}}$ values along the maximum axis of growth were sampled on the same

collection date (October 1st, 2016), and from the same location along the shore. Therefore, it is unlikely that variation in temperature or oxygen isotope ratios of water would explain the difference in posterior-growth-margin $\delta^{18}\text{O}_{\text{shell}}$ values between NM-1016-5 and the other shells in this study. A damaged shell margin could result in a lack of preservation of the last increment of growth prior to harvest. Inspection of the posterior shell margin of specimen NM-1016-5 showed no such damage. Previous studies have shown that slower growth rates associated with senescence later in ontogeny can produce increased time averaging, and hence, truncated records (e.g.: Jones et al., 1986; Weidman et al., 1994; Goodwin et al., 2003; Surge and Walker, 2006; Fenger et al., 2007). Specimen NM-1016-5 displays a high frequency sinusoidal pattern toward the growth margin, suggesting a slower growth rate during the last year of life (see section 4.6). Therefore, we attribute differences in $\delta^{18}\text{O}_{\text{shell}}$ values at the posterior growth margin of shell in NM-1016-5 relative to that of the other specimens to time averaging associated with slowed growth at senescence. This finding emphasizes the importance high-resolution microsampling of several individuals to obtain the most complete record of variation.

To test agreement between reconstructed and observed SST time series, we calculated temperature using the equilibrium fractionation equation (Friedman and O'Neil, 1977) after subtracting 1.3‰ from measured $\delta^{18}\text{O}_{\text{shell}}$ values to account for the observed offset from equilibrium values and assuming a $\delta^{18}\text{O}_{\text{water}}$ value of $-1.22 \pm 0.09\text{‰}$ (the average and standard deviation of $\delta^{18}\text{O}_{\text{water}}$ values from the shell collection period) (Figure 9). With the exception of ND-1016-4, all shells capture maximum and minimum SST observed during the collection period in the last year of shell growth. Shell NM-1016-4 does not capture minimum winter SST in its last year of growth, which we attribute to increased time-averaging due to senescence (see previous paragraph).

Figure 9: Temperature estimates derived from $\delta^{18}\text{O}_{\text{shell}}$ time series of each of the six specimens sampled along the maximum axis of growth. We subtracted 1.3‰ from measured $\delta^{18}\text{O}_{\text{shell}}$ values prior to calculating temperature to account for the positive offset from isotopic equilibrium. The black line represents estimated temperatures assuming $\delta^{18}\text{O}_{\text{water}}$ values of -1.22‰ , the mean $\delta^{18}\text{O}_{\text{water}}$ value during the calibration period. Gray lines represent temperature estimates made using ± 1 standard deviation from mean water $\delta^{18}\text{O}$ values ($\pm 0.19\text{‰}$). Black, vertical lines represent the location of prominent growth lines. Horizontal, dashed lines represent maximum and minimum SST observed from October 27, 2015 to October 1, 2016.

However, estimates of SST in the last year of growth all fall within the range of observed SST during the collection period, suggesting that SST reconstructions using $\delta^{18}\text{O}_{\text{shell}}$ time series are reliable after accounting for the observed offset.

4.4: Timing of Prominent Growth Lines:

Previous studies of shell growth have documented prominent growth lines that form annually in response to maximum or minimum growth temperature thresholds, reproductive processes, or seasonal food supply (e.g., Pannella and MacClintock, 1968; Lutz and Rhoads, 1977; Jones, 1983; Arthur and Allard, 1987; Jones et al., 1989, 1990; Cerrato et al., 1991; Jones and Quitmyer, 1996; Surge et al., 2001; Schöne et al., 2005b, Fenger et al., 2007, Garcia-March et al., 2011; Surge et al., 2013). Based on the seasonal timing estimated from the reconstructed SST time series, prominent growth lines in *N. deaurata* and *N. magellanica* do not form once annually (Figure 9). Instead, they form roughly twice per year. Prominent growth lines in the two *Nacella* species occur at or near maximum and minimum $\delta^{18}\text{O}_{\text{shell}}$ values, which would imply slowed growth or cessation during both summer and winter. One shell (specimen NM-1016-1) shows a prominent growth line that formed between summer and winter. Prominent growth lines coincident with maximum $\delta^{18}\text{O}_{\text{shell}}$ values (i.e., winter) may result from growth slowdown or cessation due to cold temperature thresholds. The lower number of $\delta^{18}\text{O}_{\text{shell}}$ measurements (i.e., data points) at or near maximum $\delta^{18}\text{O}_{\text{shell}}$ values implies that less material is accreted during colder conditions. Previous studies of patelloid limpets from mid to high latitudes have shown similar growth slowdown or cessation in winter (e.g. Blackmore, 1969; Lewis and Bowman, 1975; Ekaratne and Crisp, 1984; Jenkins and Hartnoll, 2001, Fenger et al., 2007; Surge et al., 2013). Given our observations, temperature thresholds alone cannot explain the timing of prominent growth line formation.

Another possible explanation for the formation of prominent growth lines is growth slowdown or cessation due to seasonal changes in food supply. Variation in $\delta^{13}\text{C}_{\text{shell}}$ values can potentially be used to test whether food supply influences the formation of prominent growth lines. Seasonal variation in $\delta^{13}\text{C}_{\text{shell}}$ values has been linked to changes in primary productivity and respiration (i.e., the decomposition of organic matter) (Arthur et al., 1983; Krantz et al., 1987; Hickson et al., 1999; Schöne et al., 2005b). Primary production preferentially uses the lighter isotope, causing a depletion in ^{12}C of surrounding carbon reservoirs. Conversely, respiration releases ^{12}C into surrounding carbon reservoirs. Therefore, $\delta^{13}\text{C}_{\text{shell}}$ values may indicate higher primary productivity and lower $\delta^{13}\text{C}_{\text{shell}}$ values may represent periods of increased respiration. If food supply controls shell growth, then faster growth would coincide with higher primary productivity, and slower growth would coincide with lower primary productivity (Surge et al., 2013). Consequently, prominent growth lines should form in conjunction with minimum $\delta^{13}\text{C}_{\text{shell}}$ values. This can be seen in summer of the last year of growth in ND-1016-1, ND-1016-3, and ND-1016-4, and in the winter in NM-1016-3 (around 5mm from the growth margin). Seasonal changes in food supply could then explain prominent growth line formation in summer months, particularly in *N. deaurata*. However, it is difficult to determine whether primary productivity influences seasonal variation in $\delta^{13}\text{C}_{\text{shell}}$ values, as this study lacks the records of water parameters necessary to calculate expected $\delta^{13}\text{C}_{\text{shell}}$ values (see section 4.7).

Other studies have suggested that reproductive stress may produce growth slowdown or cessation in molluscs (e.g. Panella and MacClintock, 1968; Ekartne and Crisp, 1984; Surge et al., 2013). Spawning in *N. deaurata* and *N. magellanica* can occur any time after August, as temperatures begins to rise, with a peak spawning event occurring the late spring (November/December) (Morriconi et al., 1999; Morriconi, 2005). Therefore, prominent growth

lines observed at or near minimum or intermediate $\delta^{18}\text{O}_{\text{shell}}$ values (summer and spring/autumn, respectively) may have formed as a result of slowed growth due to reproductive stress. The cause of prominent growth line formation in *N. deaurata* and *N. magellanica* remains unclear, but prominent growth line formation does not seem to occur once annually in either species and, therefore, they cannot be used as annual growth checks to estimate ontogenetic age.

4.5: Minor Growth Increments:

Colonese et al. (2012) suggested that prominent growth lines in *N. deaurata* and *N. magellanica* occur at fortnightly intervals. We observe similar fortnightly growth increments in *N. deaurata* and *N. magellanica* (Figure 1c); however, they are distinct from the prominent growth lines described in section 4.4 and more likely represent higher order growth features. These minor growth increments occur in bundles of 10-14 micro-growth increments and likely represent fortnightly growth due to diurnal tidal frequency. Previous studies have observed similar fortnightly tidal bundles of minor growth increments in limpets (Ekartne and Crisp, 1982, 1984; Fenger et al., 2007; Colonese et al., 2012). Fenger et al. (2007) attributed the formation of fortnightly increments to changes in food abundance associated with tidal oscillation. Because *N. deaurata* and *N. magellanica* primarily source their diets from the low and middle intertidal zone (Sebastian et al., 2018), it is possible that tidal growth is achieved through changes in food availability as the intertidal zone is submerged and exposed.

4.6: Lifespans and Growth Rates:

Although prominent growth lines in *N. deaurata* and *N. magellanica* do not form annual growth checks and cannot be reliably used to estimate ontogenetic age, lifespans can be inferred using the seasonal variation in $\delta^{18}\text{O}_{\text{shell}}$ time series. Based on observed seasonal cycles of $\delta^{18}\text{O}_{\text{shell}}$ variation, *N. deaurata* and *N. magellanica* show observable lifespans of 2-3 years. However,

because the apex of each species contains aragonite and was avoided during micromilling for isotopic analyses, age estimates are underestimated probably by several months. Previous studies of patelloid limpets at northern mid latitudes have observed 5-9 annual cycles in $\delta^{18}\text{O}_{\text{shell}}$ values (Fenger et al., 2007; Surge et al.; 2013). *N. deaurata* and *N. magellanica* have shorter lifespans and grow more slowly than other patelloid limpets. Moss et al. (2016) report that slower growth rates in bivalves may result from cold temperature thresholds at higher latitudes, but that lifespans tend to increase at higher latitudes. *Nacella* spp. occupy the cold-temperate region (as defined by Briggs and Bowen, 2012) along the southern tip of South America, but show faster growth and shorter lifespans than other patelloid limpets from cold-temperate regions (e.g., *Patella vulgata*: Fenger, et al., 2007). Thus, *Nacella* spp. do not follow the expected pattern found in marine bivalves by Moss et al. (2016).

Colonese et al. (2012) report an average growth rate of 18 mm/yr in one year of growth in one specimen of *N. deaurata* and average growth rates of 9 mm/yr and 13 mm/yr in the last two years of growth in one specimen of *N. magellanica*. In a larger sample size, we observed generally slower growth rates, particularly in the third year of shell growth. Specimens ND-1016-1, ND-1016-3, and NM-1016-1 show growth rates of ~7 mm/yr in their first year of growth and a growth rate of ~4 mm/yr in their second year of growth. Specimen NM1016-3 shows annual growth rates of ~4 mm/yr in their second year of growth. NM-1016-5 shows a growth rate of ~2 mm/yr in its second year of growth, and a growth rate of ~8 mm/yr in its first year of growth. ND-1016-4 shows a growth rate of ~3 mm/yr in its second year of growth, and a growth rate of ~1 mm/yr in its third year of growth. Growth rates during the first year of shell growth are difficult to determine in shells ND-1016-4 and NM-1016-3 as records of the first year of growth are truncated due to the aragonitic portion of the shell not being sampled. Previous work by

Colonese et al. (2012) only examined one individual of each species showing only 1-2 years of growth; therefore, our findings illustrate the need for a larger sample size when attempting to determine growth rate.

4.7: Variability in $\delta^{13}\text{C}_{\text{shell}}$ values:

Values of $\delta^{13}\text{C}_{\text{shell}}$ are more difficult to interpret than $\delta^{18}\text{O}_{\text{shell}}$ values due to the lack of relevant water parameters necessary for reconstructing expected $\delta^{13}\text{C}_{\text{shell}}$ values (e.g., chlorophyll, $\delta^{13}\text{C}$ of dissolved inorganic carbon (DIC)). Variation in $\delta^{13}\text{C}_{\text{shell}}$ values appear to follow similar cyclical trends as $\delta^{18}\text{O}_{\text{shell}}$ values in half of observed specimens (ND-1016-1, ND-1016-3, NM-1016-5; Figure 6). This finding suggests some seasonal influence on $\delta^{13}\text{C}_{\text{shell}}$ values in some shells, but not in others. Some studies have shown a correspondence between salinity and $\delta^{13}\text{C}_{\text{shell}}$ values (Surge et al., 2001; Fry, 2002). For example, values of $\delta^{13}\text{C}_{\text{shell}}$ may relate to $\delta^{13}\text{C}_{\text{DIC}}$ through the activity of the enzyme carbonic anhydrase, which has shown changes with increasing salinity in some bivalves (Henry and Saintsing, 1983; Gillikin et al, 2010). However, salinity at the study site is relatively constant throughout the year, and, therefore, cannot explain seasonal $\delta^{13}\text{C}_{\text{shell}}$ variations seen here. It is also possible that variation in $\delta^{13}\text{C}_{\text{shell}}$ is linked to changes in $\delta^{13}\text{C}_{\text{DIC}}$ through changes in primary productivity (see section 4.4). However, without records of relevant water parameters, controls on the $\delta^{13}\text{C}_{\text{shell}}$ values of *N. deaurata* and *N. magellanica* remain a topic of further study.

Section 5: Conclusions:

N. deaurata and *N. magellanica* do not precipitate their shells in isotopic equilibrium with ambient seawater. Instead, $\delta^{18}\text{O}_{\text{shell}}$ values observed in shells are offset from expected equilibrium $\delta^{18}\text{O}_{\text{shell}}$ values by $+1.3 \pm 0.4\text{‰}$ in *N. deaurata* and $+1.3 \pm 0.4\text{‰}$ in *N. magellanica*. These offsets are consistent throughout the shell collection period, and can therefore be

subtracted from observed $\delta^{18}\text{O}_{\text{shell}}$ values to yield reliable estimates of growth temperature. SST calculated from $\delta^{18}\text{O}_{\text{shell}}$ values showed agreement with monthly *in situ* measurements of SST after accounting for this offset. Isotopic disequilibrium is likely not caused by kinetic or metabolic isotope effects, or by external factors such as evaporation or changes in pH of seawater. It is more likely that the observed offset results from an internal, vital process of the organism. Observation of both species under controlled conditions may provide insight into the cause of observed offsets between predicted and observed $\delta^{18}\text{O}_{\text{shell}}$ values. The magnitude of this offset could be further refined with higher resolution SST measurements over a longer period. Prominent growth lines do not form once annually in either species, and therefore cannot be used as annual growth checks to estimate ontogenetic age. Instead, prominent growth lines form approximately twice annually at or near maximum and minimum $\delta^{18}\text{O}_{\text{shell}}$ values (i.e. in summer and winter). Prominent growth lines formed in winter are most likely the result of slowed growth due to colder temperatures, whereas prominent lines formed in summer are most likely due to slowed growth due to reproductive stress. Observation of the growth of the two species under laboratory conditions could reveal the cause of formation of prominent growth lines. Minor growth increments occur in bundles of 10-14 micro-growth lines, likely representing periods of growth due to diurnal tidal influences.

**APPENDIX 1: *NACELLA DEUARATA* ANTERIOR SHELL MARGIN
 $\delta^{18}\text{O}$ AND $\delta^{13}\text{C}$ VALUES:**

Specimen ID	$\delta^{18}\text{O}$ (± 0.10 ‰VPDB)	$\delta^{13}\text{C}$ (± 0.08 ‰VPDB)
ND-1016-2	1.90	2.62
ND-1016-3	1.73	2.41
ND-1016-4	1.56	2.12
ND-0916-5	1.79	2.48
ND-0916-4	2.84	2.92
ND-0916-3	2.41	2.50
ND-0716-1	2.36	2.74
ND-0716-2	2.42	2.87
ND-0716-3	1.96	2.75
ND-0616-4	1.46	2.51
ND-0616-2	1.89	2.56
ND-0616-5	2.10	2.72
ND-0516-5	1.62	2.05
ND-0516-4	1.70	2.24
ND-0516-3	2.23	2.10
ND-0416-5	2.06	1.89
ND-0416-1	1.36	1.46
ND-0416-3	0.86	1.64
ND-0316-3	0.68	1.39
ND-0316-4	0.25	1.22
ND-0316-5	0.73	1.31
ND-0216-1	1.77	1.31
ND-0216-3	1.61	0.76
ND-0216-5	3.15	1.12
ND-0116-1	1.05	1.24
ND-0116-3	0.66	0.81
ND-0116-5	1.79	1.08
ND-1215-2	1.34	1.22
ND-1215-5	1.50	1.12
ND-1215-3	1.19	1.02
ND-1115-1	1.35	1.18
ND-1115-4	1.77	1.36
ND-1115-5	1.31	1.66

**APPENDIX 2: *NACELLA MAGELLANICA* ANTERIOR SHELL MARGIN
 $\delta^{18}\text{O}$ AND $\delta^{13}\text{C}$ VALUES:**

Specimen ID	$\delta^{18}\text{O}$ (± 0.10 ‰VPDB)	$\delta^{13}\text{C}$ (± 0.08 ‰VPDB)
NM-1016-3	2.53	1.76
NM-1016-4	1.93	1.38
NM-1016-5	1.77	1.44
NM-0916-2	2.53	2.27
NM-0916-4	2.69	2.14
NM-0916-5	2.41	2.14
NM-0716-3	2.84	2.22
NM-0716-4	2.87	2.21
NM-0716-5	2.86	2.06
NM-0616-2	2.85	2.48
NM-0616-3	2.63	1.49
NM-0616-5	2.86	2.43
NM-0516-2	2.08	0.56
NM-0516-3	2.08	1.55
NM-0516-5	2.30	2.74
NM-0416-3	1.78	1.29
NM-0416-4	1.86	1.03
NM-0416-5	2.04	1.97
NM-0316-1	1.44	1.59
NM-0316-5	1.38	2.50
NM-0316-4	1.38	1.59
NM-0216-1	1.24	1.49
NM-0216-4	1.42	2.88
NM-0216-5	0.99	1.44
NM-0116-2	1.53	1.95
NM-0116-1	1.38	1.72
NM-0116-5	1.24	1.02
NM-1215-2	1.15	2.22
NM-1215-3	0.52	1.78
NM-1215-4	1.15	2.06
NM-1115-2	1.33	1.41
NM-1115-3	1.32	1.66
NM-1115-4	1.65	1.84

APPENDIX 3: SERIALY-SAMPLED *NACELLA DEAURATA* $\delta^{18}\text{O}$ AND $\delta^{13}\text{C}$ VALUES:

Specimen ID	Distance from Growth Margin (mm)	$\delta^{18}\text{O}$ (± 0.10 ‰VPDB)	$\delta^{13}\text{C}$ (± 0.08 ‰VPDB)
ND-1016-1	0	1.62	1.86
ND-1016-1	0.2	1.39	1.14
ND-1016-1	0.4	1.23	0.83
ND-1016-1	0.6	1.07	1.16
ND-1016-1	0.8	1.15	1.37
ND-1016-1	1	1.37	1.57
ND-1016-1	1.2	1.29	1.28
ND-1016-1	1.4	1.35	1.65
ND-1016-1	1.6	1.59	2.21
ND-1016-1	1.8	1.51	2.46
ND-1016-1	2	1.51	2.48
ND-1016-1	2.4	1.52	2.75
ND-1016-1	2.6	1.71	2.74
ND-1016-1	2.8	1.57	2.71
ND-1016-1	3	2.13	3.03
ND-1016-1	3.2	2.16	3.01
ND-1016-1	3.4	2.13	2.93
ND-1016-1	3.6	2.11	2.84
ND-1016-1	3.8	2.07	2.66
ND-1016-1	4.2	1.92	2.33
ND-1016-1	4.4	2.11	2.39
ND-1016-1	4.6	2.13	2.21
ND-1016-1	4.8	2.02	1.91
ND-1016-1	5	1.92	1.82
ND-1016-1	5.2	1.98	1.79
ND-1016-1	5.4	2.01	1.73
ND-1016-1	5.6	1.80	1.64
ND-1016-1	5.8	1.88	1.52
ND-1016-1	6	1.92	1.54
ND-1016-1	6.2	1.78	1.26
ND-1016-1	6.4	1.93	1.31
ND-1016-1	6.6	1.78	1.14
ND-1016-1	6.8	1.77	1.19
ND-1016-1	7	1.79	0.99
ND-1016-1	7.2	1.81	1.10
ND-1016-1	7.4	1.40	0.92

ND-1016-1	7.8	1.38	0.97
ND-1016-1	8	1.44	1.09
ND-1016-1	8.2	1.69	1.23
ND-1016-1	8.4	1.76	1.19
ND-1016-1	8.6	1.85	1.33
ND-1016-1	8.8	1.94	1.31
ND-1016-1	9	2.00	1.40
ND-1016-1	9.2	1.86	1.52
ND-1016-1	9.4	1.72	1.59
ND-1016-1	9.6	1.53	1.69
ND-1016-1	9.8	1.37	1.83
ND-1016-3	0	1.16	1.94
ND-1016-3	0.2	1.23	1.85
ND-1016-3	0.4	1.20	1.38
ND-1016-3	0.6	0.89	1.09
ND-1016-3	0.8	0.96	1.11
ND-1016-3	1	1.31	1.08
ND-1016-3	1.2	1.74	1.43
ND-1016-3	1.4	1.73	1.80
ND-1016-3	1.6	1.70	2.11
ND-1016-3	1.8	1.55	2.42
ND-1016-3	2	1.59	2.70
ND-1016-3	2.2	1.53	2.65
ND-1016-3	2.6	1.80	2.70
ND-1016-3	2.8	1.89	2.35
ND-1016-3	3	1.74	2.01
ND-1016-3	3.2	1.88	2.02
ND-1016-3	3.4	1.76	1.85
ND-1016-3	3.6	1.96	2.01
ND-1016-3	3.8	1.77	1.75
ND-1016-3	4	1.78	1.55
ND-1016-3	4.2	1.91	1.81
ND-1016-3	4.4	1.85	1.74
ND-1016-3	4.6	1.85	1.69
ND-1016-3	4.8	1.55	1.29
ND-1016-3	5	1.54	1.44
ND-1016-3	5.2	1.38	1.35
ND-1016-3	5.4	1.37	1.38
ND-1016-3	5.6	1.19	1.11
ND-1016-3	5.8	1.09	1.13

ND-1016-3	6	1.04	1.32
ND-1016-3	6.2	0.95	1.06
ND-1016-3	6.4	0.98	1.08
ND-1016-3	6.6	1.17	1.14
ND-1016-3	6.8	1.30	1.02
ND-1016-3	7	1.29	1.00
ND-1016-3	7.2	1.50	1.15
ND-1016-3	7.4	1.75	1.48
ND-1016-3	7.6	1.71	1.40
ND-1016-3	7.8	1.82	1.63
ND-1016-3	8	1.85	1.81
ND-1016-3	8.2	1.71	1.90
ND-1016-3	8.4	1.58	1.94
ND-1016-3	8.6	1.65	2.11
ND-1016-3	8.8	1.81	2.17
ND-1016-3	9	1.89	2.28
ND-1016-3	9.2	1.87	2.38
ND-1016-3	9.4	1.72	2.57
ND-1016-3	9.6	1.61	2.41
ND-1016-3	9.8	1.24	2.36
ND-1016-4	0.00	2.22	1.39
ND-1016-4	0.15	1.99	1.39
ND-1016-4	0.30	1.65	1.36
ND-1016-4	0.45	1.01	1.25
ND-1016-4	0.60	0.98	1.15
ND-1016-4	0.75	1.52	1.11
ND-1016-4	0.90	2.23	1.09
ND-1016-4	1.05	1.76	1.05
ND-1016-4	1.20	2.02	1.09
ND-1016-4	1.35	1.93	1.19
ND-1016-4	1.50	2.06	1.29
ND-1016-4	1.65	1.77	1.29
ND-1016-4	1.80	1.63	1.36
ND-1016-4	1.95	1.39	1.37
ND-1016-4	2.10	1.33	1.47
ND-1016-4	2.25	1.19	1.46
ND-1016-4	2.40	0.84	1.42
ND-1016-4	2.55	1.05	1.46
ND-1016-4	2.70	0.36	1.07
ND-1016-4	2.85	0.80	1.22

ND-1016-4	3.00	0.66	1.04
ND-1016-4	3.15	0.62	1.07
ND-1016-4	3.30	0.51	1.14
ND-1016-4	3.45	0.56	1.23
ND-1016-4	3.60	0.76	1.31
ND-1016-4	3.75	1.15	1.33
ND-1016-4	3.90	1.21	1.23
ND-1016-4	4.05	1.46	1.29
ND-1016-4	4.20	1.55	1.23
ND-1016-4	4.35	1.61	1.25
ND-1016-4	4.50	2.04	1.16
ND-1016-4	4.65	1.19	0.97
ND-1016-4	4.80	1.83	1.21
ND-1016-4	4.95	1.78	1.19
ND-1016-4	5.10	1.59	1.15
ND-1016-4	5.25	1.75	1.24
ND-1016-4	5.40	1.74	1.19
ND-1016-4	5.55	1.44	1.10
ND-1016-4	5.70	1.54	1.26
ND-1016-4	6.00	1.63	1.44
ND-1016-4	6.15	1.59	1.45
ND-1016-4	6.30	1.57	1.62
ND-1016-4	6.45	1.67	1.72
ND-1016-4	6.60	1.67	1.78
ND-1016-4	6.75	1.46	1.67
ND-1016-4	6.90	1.13	1.53
ND-1016-4	7.05	1.13	1.56
ND-1016-4	7.20	1.22	1.56
ND-1016-4	7.35	1.30	1.59
ND-1016-4	7.50	0.86	1.39
ND-1016-4	7.65	1.20	1.55
ND-1016-4	7.80	0.99	1.39
ND-1016-4	7.95	1.06	1.37
ND-1016-4	8.10	1.02	1.34
ND-1016-4	8.25	0.89	1.25
ND-1016-4	8.40	1.09	1.15
ND-1016-4	8.55	0.90	1.07
ND-1016-4	8.70	1.03	1.14

**APPENDIX 4: SERIALY-SAMPLED *NACELLA MAGELLANICA*
 $\delta^{18}\text{O}$ AND $\delta^{13}\text{C}$ VALUES:**

Specimen ID	Distance from Growth Margin (mm)	$\delta^{18}\text{O}$ (± 0.10 ‰VPDB)	$\delta^{13}\text{C}$ (± 0.08 ‰VPDB)
NM-1016-1	0	2.36	1.21
NM-1016-1	0.2	2.28	1.18
NM-1016-1	0.4	2.20	1.24
NM-1016-1	0.6	1.68	1.29
NM-1016-1	0.8	1.02	0.85
NM-1016-1	1	1.18	1.25
NM-1016-1	1.2	1.06	1.31
NM-1016-1	1.4	0.95	1.23
NM-1016-1	1.6	1.15	1.16
NM-1016-1	1.8	1.36	1.18
NM-1016-1	2	1.59	1.54
NM-1016-1	2.2	1.73	1.49
NM-1016-1	2.4	2.33	1.66
NM-1016-1	2.6	2.28	1.61
NM-1016-1	2.8	2.41	1.65
NM-1016-1	3	2.35	1.51
NM-1016-1	3.2	2.61	1.50
NM-1016-1	3.4	2.41	1.31
NM-1016-1	3.6	2.63	1.40
NM-1016-1	3.8	2.56	1.32
NM-1016-1	4	2.30	1.12
NM-1016-1	4.2	2.55	1.43
NM-1016-1	4.4	1.82	1.42
NM-1016-1	4.6	1.59	1.39
NM-1016-1	4.8	1.43	1.42
NM-1016-1	5	1.50	1.51
NM-1016-1	5.2	1.22	1.40
NM-1016-1	5.4	1.14	1.51
NM-1016-1	5.6	1.07	1.44
NM-1016-1	5.8	1.21	1.47
NM-1016-1	6	0.80	1.36
NM-1016-1	6.2	0.84	1.38
NM-1016-1	6.4	0.92	1.32
NM-1016-1	6.6	0.80	1.26
NM-1016-1	6.8	0.89	1.52
NM-1016-1	7	0.80	1.52

NM-1016-1	7.2	0.67	1.61
NM-1016-1	7.4	0.86	1.73
NM-1016-1	7.6	0.86	1.64
NM-1016-1	7.8	1.11	1.76
NM-1016-1	8	1.23	1.74
NM-1016-1	8.2	1.49	1.82
NM-1016-1	8.4	1.51	1.80
NM-1016-1	8.6	1.51	1.74
NM-1016-1	8.8	1.77	1.84
NM-1016-1	9	1.76	1.72
NM-1016-1	9.2	1.91	1.58
NM-1016-1	9.4	2.22	1.55
NM-1016-1	9.6	1.97	1.41
NM-1016-1	9.8	2.56	1.51
NM-1016-3	0.00	2.37	1.57
NM-1016-3	0.15	2.00	1.40
NM-1016-3	0.30	2.15	1.53
NM-1016-3	0.45	2.06	1.50
NM-1016-3	0.60	1.93	1.35
NM-1016-3	0.75	1.77	1.40
NM-1016-3	0.90	1.63	1.48
NM-1016-3	1.05	1.39	1.44
NM-1016-3	1.20	1.41	1.49
NM-1016-3	1.35	1.30	1.46
NM-1016-3	1.50	1.23	1.41
NM-1016-3	1.65	1.19	1.37
NM-1016-3	1.80	1.11	1.45
NM-1016-3	1.95	1.16	1.57
NM-1016-3	2.10	1.14	1.53
NM-1016-3	2.25	1.15	1.62
NM-1016-3	2.40	1.31	1.79
NM-1016-3	2.55	1.35	1.79
NM-1016-3	2.70	1.28	1.86
NM-1016-3	2.85	1.25	1.90
NM-1016-3	3.00	1.49	2.05
NM-1016-3	3.15	1.38	1.54
NM-1016-3	3.30	1.65	1.40
NM-1016-3	3.45	1.95	1.35
NM-1016-3	3.60	2.09	1.39
NM-1016-3	3.75	2.33	1.33

NM-1016-3	3.90	1.55	1.11
NM-1016-3	4.05	2.16	1.31
NM-1016-3	4.20	1.93	1.14
NM-1016-3	4.35	2.26	1.39
NM-1016-3	4.50	2.36	1.46
NM-1016-3	4.65	2.27	1.28
NM-1016-3	4.80	2.33	1.26
NM-1016-3	4.95	2.30	1.13
NM-1016-3	5.10	2.33	1.08
NM-1016-3	5.25	2.42	1.14
NM-1016-3	5.40	2.54	1.34
NM-1016-3	5.55	2.26	1.21
NM-1016-3	5.70	2.21	1.14
NM-1016-3	5.85	1.98	1.05
NM-1016-3	5.85	2.21	1.14
NM-1016-3	6.00	2.09	1.26
NM-1016-3	6.15	1.96	1.20
NM-1016-3	6.30	1.81	1.31
NM-1016-3	6.45	1.56	1.35
NM-1016-3	6.60	1.61	1.33
NM-1016-3	6.75	1.34	1.18
NM-1016-3	6.90	1.66	1.44
NM-1016-3	7.05	1.69	1.45
NM-1016-3	7.20	1.22	1.39
NM-1016-3	7.35	1.39	1.44
NM-1016-3	7.50	0.94	1.26
NM-1016-3	7.65	1.04	1.31
NM-1016-3	7.80	1.25	1.27
NM-1016-3	7.95	1.09	1.11
NM-1016-3	8.10	1.18	1.07
NM-1016-3	8.25	1.00	0.82
NM-1016-3	8.40	1.09	0.91
NM-1016-3	8.55	0.93	0.89
NM-1016-3	8.70	0.80	0.91
NM-1016-5	0	1.45	1.19
NM-1016-5	0.2	1.19	1.07
NM-1016-5	0.4	1.40	1.17
NM-1016-5	0.6	1.89	1.24
NM-1016-5	0.8	2.23	1.36
NM-1016-5	1	2.75	1.54

NM-1016-5	1.2	2.75	1.52
NM-1016-5	1.4	2.60	1.60
NM-1016-5	1.6	2.42	1.66
NM-1016-5	1.8	2.39	1.71
NM-1016-5	2	2.03	1.68
NM-1016-5	2.2	2.06	1.68
NM-1016-5	2.4	1.87	1.68
NM-1016-5	2.6	1.63	1.64
NM-1016-5	2.8	1.71	1.62
NM-1016-5	3	1.61	1.56
NM-1016-5	3.2	1.66	1.52
NM-1016-5	3.4	1.63	1.45
NM-1016-5	3.6	1.40	1.37
NM-1016-5	3.8	1.34	1.33
NM-1016-5	4	1.29	1.30
NM-1016-5	4.2	1.23	1.28
NM-1016-5	4.4	1.15	1.24
NM-1016-5	4.6	1.04	1.19
NM-1016-5	4.8	0.85	1.21
NM-1016-5	5	0.95	1.34
NM-1016-5	5.2	0.79	1.43
NM-1016-5	5.4	0.96	1.69
NM-1016-5	5.6	1.16	1.83
NM-1016-5	5.8	1.53	1.94
NM-1016-5	6	1.48	1.74
NM-1016-5	6.2	1.70	1.75
NM-1016-5	6.4	1.70	1.80
NM-1016-5	6.6	1.69	1.88
NM-1016-5	6.8	1.68	1.76
NM-1016-5	7	1.92	1.88
NM-1016-5	7.2	2.02	1.83
NM-1016-5	7.4	2.24	1.91
NM-1016-5	7.6	2.07	1.91
NM-1016-5	7.8	2.27	1.92
NM-1016-5	8	2.24	1.88
NM-1016-5	8.2	2.31	1.81
NM-1016-5	8.4	2.23	1.81
NM-1016-5	8.6	2.17	1.67
NM-1016-5	8.8	2.53	1.75
NM-1016-5	9	2.50	1.68

NM-1016-5	9.2	2.62	1.72
NM-1016-5	9.4	2.51	1.57
NM-1016-5	9.6	2.58	1.65
NM-1016-5	9.8	2.44	1.61

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