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GAMETOGENESIS IN THE SUNRAY VENUS *MACROCALLISTA NIMBOSA* (BIVALVIA: VENERIDAE) IN WEST CENTRAL FLORIDA IN RELATION TO TEMPERATURE AND FOOD SUPPLY

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ABSTRACT In Florida, culture of the sunray venus *Macrocallista nimbosa* is currently limited by seed supply. Hatcheries have not been able to condition and spawn brood stock on a predictable and consistent basis. The objective of this study was to determine the relative effects that temperature and diet have on the natural gametogenic cycle of this species so that improved conditioning protocols can be established for this species. The sunray venus *M. nimbosa* from west central Florida (Anna Maria Island) reached sexual maturity at a shell length >35 mm (age 6–8 mo). Small clams (mean shell length: 60 mm) had a 1:1 sex ratio, whereas larger clams (mean shell length: 129 mm) were predominantly female. This species exhibited a poorly defined annual reproductive cycle, and development was not synchronous between the sexes. Males developed mostly over the cooler months and spawned in the spring and early summer. Females exhibited two periods of relatively greater development: June to October and December to February, with relative little development occurring in November and from March to May. Nonetheless, mature individuals of both sexes were found throughout the year. All of these suggest that spawning within this population is almost continuous and that as females develop mature ova, they are released sporadically and fertilized by male clams, and a new generation of oocytes is rapidly produced. Given the large time over which gametes are produced, temperature does not seem to be critical. Instead, it is likely that *M. nimbosa* responds to fluctuations in food supply by rapidly developing gametes, spawning, and redeveloping. Future conditioning attempts should therefore focus on diet quality and quantity.

KEY WORDS: gametogenesis, clams, temperature, food supply, *Macrocallista*

INTRODUCTION

The sunray venus clam *Macrocallista nimbosa* (Lightfoot, 1786) ranges from North Carolina to Florida and Texas in the Gulf of Mexico (Abbott 1974). Within that range, it is found in sandy, coastal habitats. It has been considered a potential commercial species in Florida for over 50 y (Akin & Humm 1959). Harvesting of natural stocks using hydraulic dredges was explored by Stokes et al. (1968) and Jolley (1972), but a commercial industry never developed.

Shellfish aquaculture in Florida has increased rapidly since its beginning in the 1980s. The value of hard clam and oyster production in Florida was $11.6 million in 2012 (USDA 2014). Florida hatcheries are now producing juvenile northern quahogs (=hard clams) *Mercenaria mercenaria*; oysters *Crassostrea virginica*; and bay scallops *Argopecten irradians* (for stock enhancement). In spite of considerable interest within the industry, commercial production of the sunray venus *Macrocallista nimbosa* has been limited by an inability to consistently and predictably produce juvenile (seed) clams.

A comprehensive understanding of the reproductive biology of any species is necessary for successful commercial culture. Little is known, however, about the reproductive biology of *Macrocallista nimbosa*, including its age (size) at sexual maturity, sexuality (whether it is protandric), and its natural gametogenic cycle and the environmental factors that regulate it. Haines (1976) examined gonadal tissue from 10 to 23 clams collected monthly from St. Joseph Bay, FL, for a year and reported that spawning began in July for males and August for females and continued through November. No corresponding environmental data (water temperature, salinity, or food availability) were collected.

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Food and temperature are generally considered the primary exogenous factors regulating gametogenesis in marine bivalves (Sastry 1979, Barber & Blake 2016). Thus, the primary objective of this project was to gain an understanding of the roles that food supply and temperature play in the control of reproduction in *Macrocallista nimbosa*. We examined the annual reproductive cycle of a population of clams in west central Florida and correlated specific gametogenic events with water quality parameters, including temperature, salinity, and food availability.

MATERIALS AND METHODS

Clams were collected from a natural population near the mouth of Tampa Bay (Anna Maria Island), on the west coast of Florida (27° 31′ 51.03″ N; 82° 43′ 27.92″ W). From August 2014 to July 2015, up to 5 small clams (<80 mm shell length) and 15 large clams (>90 mm shell length) were collected monthly by hand (probing the sediment with a thin bladed knife) at low tide from a subtidal sand bar. Clams were placed on ice in a cooler and transported to Eckerd College for processing. Clam shell length was measured (±0.1 mm) with calipers and recorded. Gonadal tissue contained in a section of visceral mass was removed from each clam and fixed in 10% formalin in seawater, dehydrated and cleared, and embedded in Paraplast. Thin sections (6 μm) were placed on microscope slides and stained with Harris' hematoxylin and eosin (Howard et al. 2004).

On each collection date, water temperature (±0.5°C, mercury thermometer) and salinity (±0.5 psu, refractometer) were recorded. In addition, a qualitative plankton sample was obtained using a 20-μm mesh plankton net (Sea Gear Corporation). Diatoms were identified to lowest practical taxon by examining fresh material on return to the laboratory. In addition, permanent mounts of plankton were made by filtering about 10 ml of sample through a 25-mm membrane filter,

placing half onto each of two microscope slides, clearing the filter with a drop of immersion oil overnight, and mounting a coverslip.

Finished slides were examined using a Nikon Labophot-2 compound microscope (×100 magnification). For each clam, the sex and stage of gametogenesis (inactive, early developing, late developing, mature, early postspawning, or late postspawning) was determined. Stages were defined as follows (Hesselman et al. 1989, Barber et al. 2005):

Early Developing: follicles small but expanding within gonadal connective tissue; mostly spermatocytes or primary oocytes present.

Late Developing: follicles expanded with thin walls; mostly spermatocytes, spermatozoa or secondary oocytes present.

Mature: follicles and gametes occupying gonadal tissue completely; mostly spermatozoa or ova present.

Early Postspawning: follicle walls ruptured; space evident in the lumen; redevelopment evident.

Late Postspawning: follicles shrunken; infiltration of hemocytes and resorption of remaining gametes; no redevelopment occurring.

Inactive: follicles shrunken; very few residual gametes; no development occurring.

In addition, gametogenesis in female clams was quantified by measuring the diameters of 50 oocytes per individual using an Amscope MU1000 digital camera and ToupView 3.2 image analysis software. This approach has been previously shown to be effective by Barber and Blake (1983), Maloy et al. (2003), and Barber et al. (2005). Mean monthly oocyte diameters were statistically compared using one-way analysis of variance followed by Mann–Whitney pairwise *post hoc* tests, using the PAST statistical software package (Hammer et al. 2001).

Gametogenesis in male clams was quantified as the percentage of gonadal area occupied by spermatozoa using the Fiji image analysis software (Schindelin et al. 2012). This is the first application of this approach to quantification of gametogenesis in marine bivalves. A color image of fixed, stained gonadal tissue from each male clam was converted to grayscale. Using the green channel, the dark staining (hematoxylin) spermatozoa color was isolated using thresholding. The thresholded areas (percentages) within three regions of each gonad were averaged for each clam. Mean monthly gonadal areas (%) were statistically compared using one-way analysis of variance and Mann–Whitney pairwise *post hoc* comparisons; percent values were transformed (arcsin) prior to analysis.

RESULTS

"Small" clams ranged in size from 35.4 to 79.2 mm with an overall mean shell length of 60.3 mm. "Large" clams ranged from 93.6 to 151.7 mm with an overall average shell length of 129.0 mm (Table 1). Small clams were encountered less frequently than large clams because they were less numerous or were simply not detected using the collecting method.

Water temperature declined steadily from 30.8°C in August 2014 to a minimum of 14.5°C in February 2015. From there, temperature increased rapidly and reached a maximum in June 2015 (Fig. 1). Salinity ranged from 31.5 to 36.5 psu (Fig. 1), with the lower readings occurring in the summer months and higher readings in the winter months (December 2014 and January 2015).

TABLE 1.

Mean shell length (mm) and number of individuals (*Macrocallista nimbosa*) collected between August 2014 and July 2015.

	Small	Clams	Large	Clams
Sampling date	Shell length	Number	Shell length	Number
August 13, 2015	59.9	5	123.3	15
September 13, 2015	65.9	5	132.6	15
October 12, 2015	61.9	5	121.5	15
November 25, 2015	55.9	4	132.6	15
December 26, 2015	69.5	5	122.1	15
January 25, 2016	64.7	5	133.4	15
February 22, 2016	54.1	5	128.2	15
March 20, 2016	51.0	2	132.1	15
April 17, 2016	49.5	2	128.1	15
May 18, 2016	61.2	1	135.8	15
June 16, 2016	65.7	3	126.2	15
July 12, 2016	64.6	4	131.6	15

Nineteen diatom taxa were collected and identified over the study period (Table 2). The most common species, *Paralia sulcata*, was found in 10 of the 12 monthly samples. Diatom diversity (number of taxa) ranged from 2 in August 2014 to 11 in December 2014 (Fig. 2).

Small clams had equal numbers of both sexes. Of the 46 small clams examined, 22 were males, 23 were females, and 1 was undifferentiated. The sex ratio of small clams (0.956:1) was not significantly different from 1:1 ($P \leq 0.8815$). Large clams were predominantly female. The 180 large clams included 65 males and 115 females, resulting in a sex ratio of 0.565:1, which was significantly different from 1:1 ($P \leq 0.0002$).

For small clams, the proportion of visceral mass occupied by gonad was considerably less than that of large clams. Follicles of both male and female clams were smaller and contained fewer gametes than large clams. All small clams were differentiated except for the smallest (35.4 mm), collected in November 2014. There were not enough small clams to determine seasonal trends in gametogenesis.

Large female clams had a poorly defined annual cycle and exhibited little synchrony within the population from month to month (Fig. 3). For example, mature clams were present in 7 out of 12 mo (August, September, October, and December 2014 as well as February, April, and June 2015). Inactive clams occurred in every month between November 2014 and May 2015. In December 2014, February 2015, and April 2015, both mature and inactive females occurred simultaneously.

In spite of the general lack of synchronous development in female clams, there were two major periods of oogenic activity, which included development and spawning of oocytes along with redevelopment (Fig. 3). The first period of oogenic activity extended from August to October 2014; in November 2014, clams were either spawned out or inactive (showing no signs of redevelopment). Oogenic activity resumed in December 2014 and continued through February 2015; in March 2015, clams were again either spawned out or inactive. From May to July 2015, clams were mostly developing in preparation for another summer spawning period.

These two periods of relatively active oogenesis followed by a brief period of inactivity are reflected by the mean oocyte

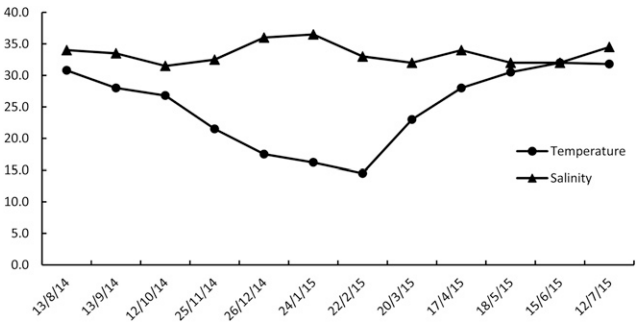

Figure 1. Water temperature (°C) and salinity at the field collection site (Anna Maria Island, FL) at the time of monthly collection of *Macrocallista nimbosa*, August 2014 to July 2015.

measurements (Fig. 4). Mean diameters were greatest in September 2014 and February 2015 and lowest in November 2014 and March 2015, when the greatest number of inactive females were present. Mean oocyte diameter in November 2014 was significantly lower than in September 2014 ($P \leq 0.01$) and mean oocyte diameter in February 2015 was significantly greater than in November 2014 ($P \leq 0.006$). Values in March 2015 were significantly lower than in January and February 2015 ($P \leq 0.03$). These two significant decreases in mean oocyte diameter between October and November 2015 and February and March 2015 suggest there were two primary spawning periods.

Gametogenesis among large male clams was more synchronous than that in female clams. In addition, there was a more clearly defined seasonal cycle of development. Mean percent gonadal area increased from a low of 32% in September 2014 to a maximum of 68% in April 2015 (Fig. 5). Mean gonadal area in April 2015 was significantly greater than in August and September 2014 ($P \leq 0.003$). Significant declines in percent gonadal area in May and again in July 2015 ($P \leq 0.03$) indicated that most spawning of male clams occurred in the spring and summer months.

Histologically, it was observed that male clams had spermatozoa throughout the year. Thus, even at the point of lowest overall development (September 2014), over 30% of the gonad contained spermatocytes and spermatozoa.

DISCUSSION

Out of a total of 616 clams examined histologically, there was only one that was undifferentiated (i.e., the sex could not be determined). This individual was the smallest one (shell length 35.4 mm) found in November 2014. Based on the age to size relationship provided by Stokes et al. (1968), *Macrocallista nimbosa* can reach a length of 73 mm within 1 y. Accordingly, this undifferentiated clam was approximately 6–8 mo old. It is thus safe to conclude that sexual maturity in *M. nimbosa* occurs at an age of less than 1 y.

Small *Macrocallista nimbosa* (shell length 35.4–79.2 mm) had an equal number of males and females. Large clams, (shell length 93.6–151.7 mm), however, had significantly more females than males, suggesting that *M. nimbosa* may be protandric. The only other explanation for this change in sex ratio with age would be differential mortality between sexes. Another member of the Veneridae, the northern quahog *Mercenaria mercenaria*, is known to be protandric. Younger clams are predominantly male, some of which develop as females in later years (Eversole 2001).

In spite of the lack of developmental synchrony among female clams, there appeared to be two periods of relatively greater oogenic activity involving development, spawning, and redevelopment. One was from August to November 2014 and the second was from December 2014 to March 2015. Males exhibited a more

TABLE 2.

Dominant species of diatoms present at the clam, *Macrocallista nimbosa*, collection site on the 12 sampling dates, from August 2014 to July 2015.

	2014						2015					
Taxon	August	September	October	November	December	January	February	March	April	May	June	July
Bellerochea horologicalis		x	x		x	x		x				
Bellerochea malleus				x		x				x		
Chaetoceros affinis				x						x		
Chaetoceros danicus					x	x	x			x		
Chaetoceros sp. A								x			x	x
Cocconeis spp.					x							
Coscinodiscus spp.		x	x	x	x	x				x		
Diploneis spp.		x	x		x						x	
Gyrosigma/*Pleurosigma* spp.					x	x	x	x	x	x	x	x
Leptocylindrus minimus							x	x				
Navicula lyra					x							
Paralia sulcata	x	x	x	x	x		x		x	x	x	x
Pennales spp.			x									x
Proboscia indica				x								
Rhizosolenia pungens				x	x		x	x		x		
Rhizosolenia sp. A	x	x					x					
Skeletonema costatum					x				x	x	x	
Thalassionema nitzschioides											x	x
Triceratium favus					x							

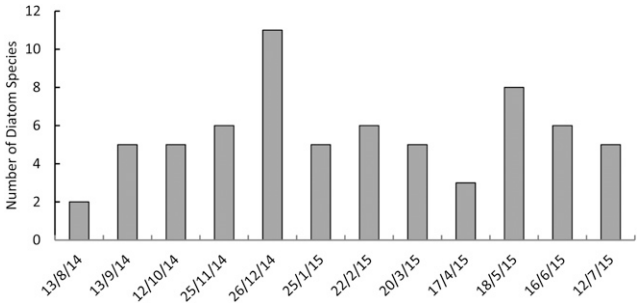

Figure 2. Number of species of diatoms identified at the clam collection site (Anna Maria Island, FL) between August 2014 and July 2015.

defined annual cycle of gametogenesis, with most development occurring between September 2014 and April 2015, with spawning occurring in May and July 2015. In spite of the lack of obvious developmental synchrony between sexes, there was ample evidence that at least some males and some females in this population were mature and capable of spawning viable gametes throughout the year. Thus, the population examined (Anna Maria Island, FL) is sustainable without a well-defined annual gametogenic cycle. Instead, a strategy of more frequent, low-level spawning is supported for this population.

The only other examination of gametogenesis in *Macrocallista nimbosa* was that of Haines (1976), who studied a population from St. Joseph Bay, FL (approximately 355 km to the northwest of Anna Maria Island). That study found that female clams were mostly mature in June and July and spawned from August to November. Males were ripe in June and spawning occurred from August to November. Haines (1976) concluded that most spawning (both sexes) occurred in October and November, making *M. nimbosa* a fall spawner. At the more southern Anna Maria Island location, it was found that spawning occurs over a longer portion of the year, with spawning peaks in both the fall and spring. Haines (1976) also noted that mature female clams were found in 9 out of 12 mo and ripe male clams were present in all months, which is similar to what was found in this study.

With decreasing latitude, gametogenic cycles of marine bivalves tend to become more prolonged or bimodal (Barber & Blake 1983, Hesselman et al. 1989, Eversole 2001). This is undoubtedly related to the fact that on an annual basis, tropical

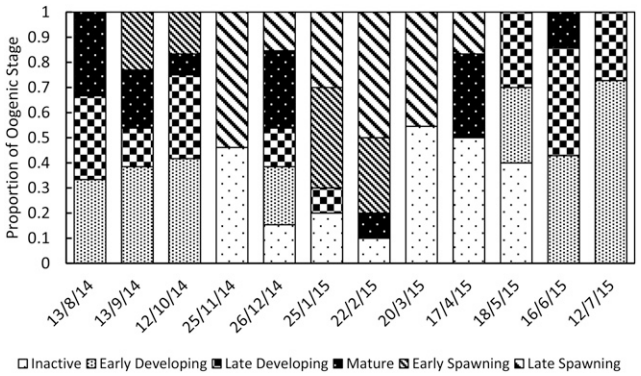

Figure 3. Proportion of female clams (*Macrocallista nimbosa*) in each stage of oogenic development on each of the monthly collection dates between August 2014 and July 2015; $n = 6$–13.

environments are less variable than temperate environments with respect to day length, phytoplankton production, and water temperature. Thus, the strategy of almost continuous gametogenesis with incomplete spawning and redevelopment is common for tropical bivalves *Donax trunculus* (Gaspar et al. 1999), *Pinctada margaritifera* (Pouvreau et al. 2000), and *Perna viridis* (Kripa et al. 2009). Barber et al. (2005) compared gametogenesis between the native scorched mussel *Brachidontes exustus* and the nonnative green mussel *Perna viridis* in Tampa Bay, FL, and found that both species exhibited a prolonged period of development, partial spawning, and resorption similar to that described here for *Macrocallista nimbosa*.

It has long been established that the two most critical factors regulating gametogenesis in marine bivalves are temperature and food (Sastry 1979, Barber & Blake 2016). A critical threshold temperature and food level are required for initiation of gametogenesis; completion of gametogenesis to maturity and fecundity depend on a minimum temperature and adequate food supply being maintained. Fecundity, or the total amount of gametogenic material produced, is a function of both stored reserves and amount of food available during the developmental period. For example, a population of bay scallops *Argopecten irradians* near Tarpon Springs, FL, underwent cytoplasmic growth in July when water temperature was near maximal (Barber & Blake 1983). In contrast, *Mercenaria* spp., in the Indian River, FL, initiated gametogenesis in the fall, when water temperature was decreasing (Hesselman et al. 1989). Gametogenesis in populations of blue mussels *Mytilus* spp. was closely associated with increasing water temperature in Cobscook Bay, ME (Maloy et al. 2003). In Tampa Bay, FL, spawning of the native scorched mussel *Brachidontes exustus* and the nonnative green mussel *Perna viridis* occurred primarily in the spring, in association with increasing water temperature and in the fall, in association with decreasing water temperature (Barber et al. 2005).

The lack of a clear annual reproductive cycle in *Macrocallista nimbosa*, as found in this study (especially for females), makes it difficult to correlate specific gametogenic events with environmental parameters such as temperature, salinity, and food availability. Temperature reached a low of 14.5°C in February but was over 30°C from May to August. Even though the period of least active oogenesis occurred in March, after the lowest temperature of the year, the two periods of active oogenic development, spawning, and redevelopment took place during both the summer (maximal temperature) and winter (minimal temperature) periods. Most development in males occurred with decreasing water temperature (September to February) and most spawning occurred after water temperature exceeded 30°C (May to July). Thus, gametogenesis in female clams was influenced less by temperature than gametogenesis in male clams.

Relatively few studies have correlated food (phytoplankton) abundance with gametogenesis in marine bivalves. Reproductive development in *Mercenaria mercenaria* was positively correlated with phytoplankton abundance (reviewed by Eversole 2001). Darriba et al. (2004) reported that for razor clams *Ensis arcuatus*, gametogenesis occurred when food was less abundant, but was supported by energy reserves stored during periods of upwelling and high primary productivity. The role of food in regulating gametogenesis in scallops was reviewed by Barber and Blake (2016). Gonadal differentiation in *Placopecten magellanicus* occurred when temperature was low but food was abundant

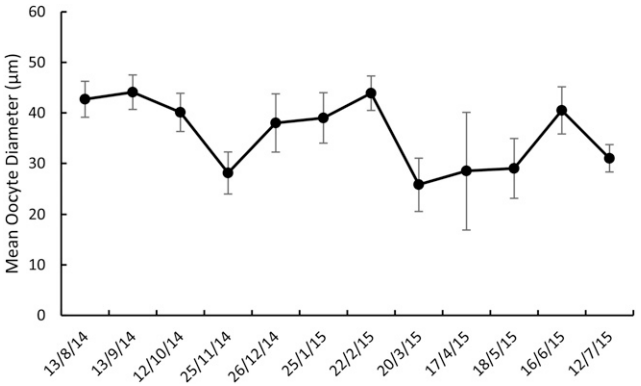

Figure 4. Mean oocyte diameters of female *Macrocallista nimbosa* collected monthly between August 2014 and July 2015. Error bars = ±1 SE; *n* = 6–13.

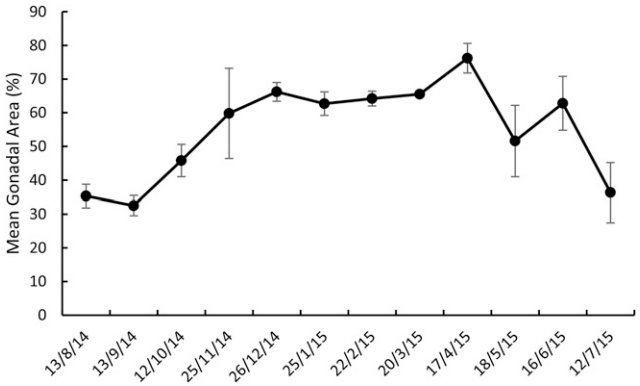

Figure 5. Mean gonadal area occupied by spermatocytes in male clams, *Macrocallista nimbosa*, collected monthly from August 2014 to July 2015. Error bars = ±1 SE; *n* = 2–9.

(Thompson 1977). The high food levels that occurred at the end of spring were necessary for oocyte maturation in *Pecten maximus* (Lubet et al. 1987). Gametogenesis in *Chlamys islandica* was initiated after spawning when food was abundant, was arrested in autumn as food supply decreased, but was completed the following spring as food supply reached a maximum (Thorarinsdóttir 1993). Similarly, gonad growth in *Argopecten gibbus* was greatest when both temperature and food supply were low, but vitellogenesis and maturation of oocytes were dependent on food supply (Sarkis et al. 2006).

Although the examination of diatom species conducted in this study was not comprehensive or quantitative, it did provide a comparison of the diversity of algal species available to clams over the course of the year. The dominant species identified in this study were also noted by previous investigators (Steidinger & Gardiner 1982, Badylak et al. 2007), and included *Chaetoceros* spp. and *Skeletonema costatum*, both of which have been found to support growth and conditioning of marine bivalves (Utting & Millican 1997, Helm et al. 2004). In addition, it is generally accepted that optimal nutrition is best provided by multiple species of microalgae. Thus, it is interesting to note that the greatest diversity (number of species) of diatoms in this study occurred in December and May, both of which preceded periods of oogenic development in female clams. In August, diatom diversity was the lowest (dominated by *Rhizosolenia* spp.), which correlated with minimal gonadal development in male clams.

Because of the prolonged period of active gametogenesis exhibited by both male and female clams from Anna Maria Island, it is not likely that temperature plays a critical role. Salinity varied little over the year and was not obviously correlated to any aspect of gametogenesis in *Macrocallista nimbosa* at the Anna Maria Island site. On the other hand, food supply and more importantly food composition may be responsible for supporting active periods of gametogenesis in both sexes. The more continual, but lower level of gamete production and release of gametes seen here could logically be explained by a food (energy) supply that varies little seasonally. The strategy used by *M. nimbosa* in west central Florida might thus be to develop and spawn at least a few gametes quickly when adequate food is available. Future attempts to condition the sunray venus should therefore focus on diet quality and quantity, rather than temperature.

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