The Effect of Aerial Exposure Temperature on *Balanus balanoides* Feeding Behavior

Gracie Thompson* and Matt Goldberg
Monday Afternoon Biology 334A Laboratory, Fall 2014

Abstract

The impact of climate change on many different ecosystems is a growing concern. Intertidal filter feeders, such as barnacles, play an important role in repairing the water quality and contributing to the stability of the ecosystem. This experiment aimed to look at the effect of different aerial exposure temperatures on *Balanus balanoides* feeding behaviors. Barnacles were exposed to 4°C, 12°C, and 20°C air for a 6 hour period and then re-submerged for 2 hours. Feeding rate, percent feeding, and spatial distribution of feeding barnacles were observed. The results showed that warm (20°C) and cool (4°C) exposed barnacles had much lower average feeding rates than the control and 12°C exposed barnacles. The feeding rates of the 12°c exposed barnacles and the control barnacles tended to be 30-50% greater than the other two treatements.The warm and cool exposed treatments also showed a decrease in the percent of barnacles feeding. This is thought to be due to physiological stress caused by the avoidance of desiccation and freezing and a lowering of metabolic rate in the cool-exposed barnacles. There were no significant effects of aerial exposure temperature on the spatial distribution of feeding barnacles.

Introduction

Habitat temperature is a very influential factor that affects all biological and physiological processes in organisms. In ectotherms, the habitat temperature corresponds to their body temperature, and therefore determines their distribution and survival in their environment. Rocky intertidal organisms such as barnacles, sea stars, mussels, anemones and many other organisms, have to withstand harsh conditions due to the fact that they are subjected to wave action and have to survive extreme weather conditions when exposed to the air during low tide.

The vertical zonation of intertidal animals is primarily determined by the tidal heights and competition present in the environment. The upper limit of species (or the highest location in the intertidal at which it can survive) is often determined by physical factors such as temperature, solar radiation, and desiccation while the lower limit (or the lowest location in the intertidal zone at which a species can survive) is primarily determined by competition between species (Connell, 2014). A study discovered that the upper limit of a species is raised in cooler, damper conditions or situations such as north-facing rocks and areas that receive spray from wave action. This shows that the upper limit is primarily restricted by high temperatures and high solar radiation and the upper limit may recede downward to compensate for an increase in temperature or solar radiation (Connell, 2014).

Barnacles are generally in the high tidal zone of a rocky intertidal community due to the fact they are well adapted to avoid desiccation. Specifically, barnacles possess a close-fitting shell which helps them resist desiccation and tissues tolerant to high temperatures and low salinity (Foster, 1971). Because of their adaptations, barnacles are able to live higher up in the tidal column, but due to their location, they are exposed to air for many hours during the day. With increasing air temperatures due to climate change, intertidal organisms exposed to air may be negatively affected. A study on sea stars and foraging behaviors found that sea stars exposed to very warm air (about 26.1°C) for long periods of time had delayed foraging habits when re-submerged and had lower growth rates than sea stars exposed to cooler temperatures (Pincebourde, 2008). A study performed on the barnacles *Balanus balanoides* showed that at increased temperatures, there was increased mortality due to desiccation at the high tidal levels and that young smaller barnacles were more susceptible to desiccation (Foster, 1971). Although temperature and tidal height seem to be a main factor in barnacle survival, it is unknown how exposure temperature affects their feeding habits. Feeding habits have also been found to be linked to the barnacles' placement in relation to one another. A study carried out on barnacle hummocking, or the buildup of barnacles' shells to become taller, showed that those individuals at the peaks of the hummocks or the individuals near the edge of the cluster had much higher feeding rates than the individuals between hummocks and those in the middle of large clusters (Bertness et al., 1998).

The main question of this study was how does aerial temperature during exposure affect the feeding behavior of barnacles after they are re-submerged? Specifically, how does exposure temperature affect barnacle's feeding rate, the percentage of barnacles feeding at a given time, and the spatial distribution of feeding barnacles. It was hypothesized that with increasing air temperature, the higher the feeding rate would be due to the fact that ectotherm's metabolic rate tends to increase with increasing temperature (Clarke and Johnston, 1999) and therefore would increase the barnacle's activity and feeding rate. The opposite would be true for low temperatures, and therefore it was hypothesized that the coolest temperature (4°C) would result in decreased feeding rates. It was also predicted that the percentage of barnacles feeding would be lowest at the high (20°C) and low temperatures (4°C) because barnacles would be actively trying to avoid desiccation in warm temperatures, and freezing in cool temperatures. Therefore, the barnacles would be stressed and less likely to emerge and feed. Finally, it was predicted that barnacles near the edges of the rocks would be less affected by temperature stress based on the study by Bertness et al., and would be the first to begin feeding after being re-submerged.

Materials and Methods

Animal Information

Barnacles of the *Balanus balanoides* species were collected from Hyde Beach in Commencement Bay, Washington. Four rocks (about 6 inches across) covered in barnacles were collected during low tide. The four rocks were placed in a 10 gallon tank containing sea water collected from the Puget Sound. A filter (without filter material) was set up in the tank to provide circulation of the water. When not testing, all the barnacles were kept at about 12°C. The barnacles were fed Kent Marine Zooplex marine zooplankton once a week directly before testing (1 mL zooplankton/37.9 L seawater).

Barnacle Feeding Rate Procedure

There were three experimental groups and a control group. Each group consisted of a rock with many (over 100) barnacles on it. The three experimental rocks were exposed to 4 ° C, 12°C, or 20°C air for a 6 hour period to simulate aerial exposure during low tide. The control rock stayed under water the entire time. After the 6 hour period, the rock was placed back in the water for 2 hours. After the 2 hour period, food was introduced and feeding rate of the experimental rock and the control rock were measured directly after feeding, 10 minutes, 20 minutes, and 30 minutes after feeding. This measurement was done by observing a randomly picked actively feeding barnacle and counting how many “sweeps” or extensions of its cirri it made in one minute. This was done for 10 random barnacles on each rock. At each time period, 10 more barnacles were randomly selected and measured.

Percent Feeding Procedure

The percent of barnacles feeding was also measured for each treatment group. The three experimental rocks were exposed to either 4°C, 12°C, or 20°C for six hours and then submerged for 2 hours. Food was introduced and percent feeding was recorded for each treatment group and the control group immediately after feeding, 10, 20 and 30 minutes after feeding. This was done by drawing out a 2x2 inch square on the tank glass, counting how many barnacles occupied that space, and then observing how many barnacles were actively feeding during three 1 minute periods.

Spatial Distribution Procedure

Spatial orientation of feeding barnacles was measured qualitatively by observing where barnacles began to feed first in relation to their spatial orientation on the rock. Three of the rocks were exposed to either 4°C , 12°C , or 20°C for six hours and then re-submerged. The spatial distribution of feeding barnacles was observed by using a pen to mark on the glass of the aquarium every time a barnacle was observed feeding. A picture was taken of the glass 1 minute and 5 minutes after feeding and observations were recorded.

Statistical Analysis

To analyze the feeding rate and percent feeding data, 2-way ANOVA's were run on the data using the program R-Commander. Because the interaction term was significant for percent feeding, two separate 1-way ANOVA's were run to determine the relationship between exposure temperature and percent feeding, and the relationship between percent feeding and time after feeding. Spatial distribution data was observed visually and recorded as qualitative data. Pictures were taken at 1 and 5 minutes after feeding and these were analyzed for any patterns.

RESULTS

Barnacle Feeding Rate

According to the results, the barnacles' feeding rate was significantly affected by the exposure temperature (2-way ANOVA, $F_{3, 224}$=11.26, $p<0.001$; Figure 1). The control barnacles and the barnacles exposed to 12°C had the highest feeding rate in general. The 4°C exposed barnacles had the lowest feeding rate across all the time periods and the 20°C exposed barnacles had the second lowest feeding rate. At 0 minutes after feeding, it is seen that the 12°C exposed barnacles (35.8±4.6 extensions/min) had about twice the feeding rate as the 20°C exposed barnacles (16.9±2.1 extensions/min) and about three times the feeding rate of 4°C exposed barnacles (11.0±1.9 extensions/min). There seemed to be no significant difference between the feeding rates of the control treatment and the 12°C exposed barnacles. Time after feeding did not seem to have a significant effect on feeding rate ($F_{3,\ 224}$=1.77, p=0.154) and this was seen across all treatment groups ($F_{9,224}$=0.86, p=0.561).

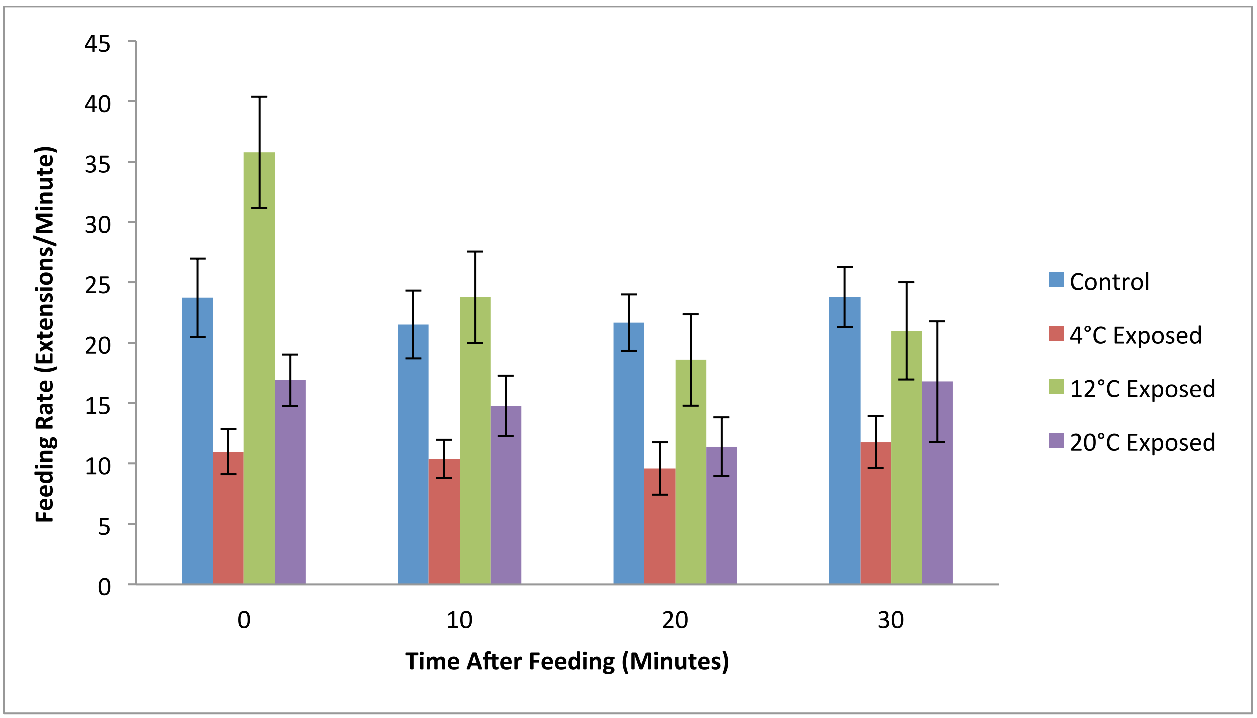

Figure 1. The feeding rate of barnacles in all the treatment groups measured at 0, 10, 20, and 30 minutes after feeding. Control and 12°C exposed barnacles had much greater feeding rates, almost twice that, of the other two treatments (n=40). The temperature of exposure had a significant effect on the feeding rate (p<0.001) but time after feeding did not have a significant effect on feeding rate (p=0.154) and this was seen across all treatment groups (p=0.561).

Barnacle Percent Feeding

The temperature of exposure also had a significant effect on the percent of barnacles feeding at each of the four time periods. The effect of exposure temperature on percent feeding was dependent on the time after feeding (2-way ANOVA, $F_{9, 32}$= 2.28, p=0.042; Figure 2) as seen by the wide variation in data between time periods in Figure 2. For example, at 0 minutes after feeding, the control and 12°C exposed barnacles obviously had higher feeding rates than the other two treatments, but at 20 minutes after feeding, the control, 4°C, and 20°C exposed barnacles did not have significantly different feeding rates, while the 12°C exposed barnacles had much higher feeding rates. There was a significant effect of exposure temperature on percent feeding (1-way ANOVA, $F_{3, 44}$=21.09, p<0.001). The 12°C exposed group had the highest percentage of barnacles feeding at all the time periods, while the barnacles exposed to 4°C had the lowest percentage of barnacles feeding. This difference is most clearly seen at 30 minutes after feeding when the 12°C exposed group (10.1±0.5 %) had a percentage of barnacles feeding that was about 8 times greater than that of the 4°C treatment group (1.3±0.0%). The time after feeding did not have a significant direct effect on percent feeding (1-way ANOVA, $F_{3, 44}$=2.00, p=0.127).

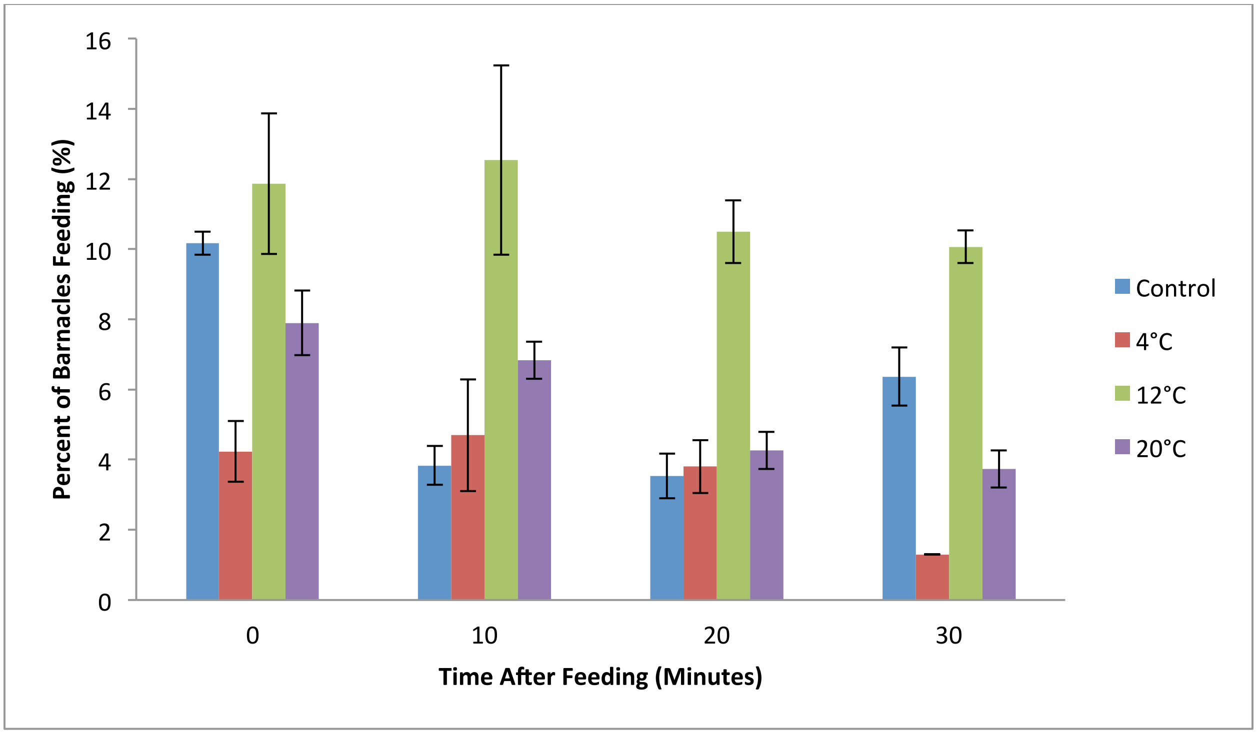

Figure 2. The percent of barnacles feeding in each treatment group at 0, 10, 20, and 30 minutes after feeding (n=40). Exposure temperature had a significant effect on the percent feeding ($p<0.001$) and this effect was dependent on the time elapsed after feeding ($p=0.042$). The time elapsed after feeding did not have a significant effect on the overall percentage of barnacles feeding though ($p=0.127$). The barnacles exposed to 12°C had the highest percentage of barnacles feeding at all time periods while the barnacles exposed to 4°C tended to have the lowest percentage of barnacles feeding at all time periods.

Barnacle Feeding and Spatial Distribution

After analyzing all the treatment groups, there seemed to be no distinct spatial distribution pattern to the barnacles that fed first. At one minute after feeding, only a few barnacles had begun to feed in each treatment group and they were randomly distributed among the rock (Figure 3a). At five minutes after feeding, the actively feeding barnacles were randomly distributed with seemingly no pattern (Figure 3b). Barnacles from the control group began to feed approximately 20 seconds earlier than those barnacles from the exposed rocks. Barnacles from the 4°C exposed treatment group had the longest delay before feeding was observed. The only pattern observed was that across all treatment groups, the barnacles that began feeding first were the small barnacles on the rock.

A **B**

Figure 3. The spatial distribution of feeding barnacles on a 4°C exposed rock after 1 minute (A) and after 5 minutes (B). Red dots represent actively feeding barnacles. These photos represented the randomness in spatial distribution of feeding barnacles of all the treatment groups. There seemed to be no distinct spatial distribution pattern to the barnacles that fed first, although smaller barnacles seemed to start feeding before larger barnacles.

DISCUSSION

In this experiment, we were interested in understanding the effect of the temperature of air exposure on barnacle feeding behavior and habits. It was hypothesized that with an increase in air temperature, there would be an increase in feeding rate. This hypothesis was based on knowledge that as temperature increases, an ectotherm's metabolic rate increases. For the same reason, it was hypothesized that a decrease in temperature would lead to a decrease in feeding rate due to a decrease in metabolic rate. Feeding rate of barnacles, or the beat rate of their cirri, has been measured in several studies. For example, a study on the species *Balanus perforatus* found that their feeding rate was extremely variable, ranging from about 5extensions per minute to about 100 extensions per minute. The hypothesis for our study was supported by some of their data that showed at very warm water temperatures, the barnacles' feeding rate increased to their maximum (about 72 extensions/min). At 23°C, they saw some barnacles cease to feed, showing that this temperature of water could be at or near their threshold temperature (Anderson, 1981).

The results from our experiment contradicted the results discussed by Anderson. Our results suggest that the control group and the barnacles exposed to 12°C air had the highest feeding rates while those barnacles exposed to 4°C and 20°C had the lowest. The discrepancy between this data and Anderson's data could possibly be due to the fact that they were altering the temperature of the water, and not the aerial exposure temperature. Varying temperatures of water would not have the same effect as varying temperatures of air. When submerged, the barnacle's metabolic rate will be affected, but varying air temperature would cause stress on the barnacle as it actively has to avoid desiccation and freezing. The very low feeding rate of the barnacles exposed to 4°C could be due to a decrease in metabolic rate and physiological stress caused by a cold temperature while the feeding rates of the warm exposed barnacles may have been due to the stress caused by the barnacle actively avoiding desiccation for 6 hours. It is also important to note that the control and 12°C exposed barnacles had very similar feeding rates across all time periods. This suggests that air exposure itself does not drastically affect barnacle feeding rate, but the temperature of air exposure does have a significant effect.

It was also hypothesized that the rocks exposed to the low and high aerial temperatures would have a lower percent feeding than the control and 12°C exposed rocks. This was due to the fact that the barnacles exposed to the more extreme temperatures would undergo more stress to avoid desiccation and freezing. A study on intertidal sea stars found that sea stars exposed to very warm temperatures (about 26.1°C) had delayed foraging habits and lower growth rates which could be linked to the physiological stress of avoiding desiccation (Pincebourde, 2008). This hypothesis was supported by the data which showed that the barnacles exposed to 4°C and 20°C had lower percent feeding overall than the control or 12°C. These results suggest that the barnacles exposed to more extreme temperatures experience more physical stress and are therefore less likely to open and feed. The control barnacles had surprisingly low percent feeding measurements at 10 and 20 minutes after feeding. These results could be due to the fact that the rock was submerged throughout the whole experiment and the barnacles therefore had more chance to feed. A study by Ritz and Crisp discovered that *Balanus balanoides* had reduced feeding activity in November after their breeding season. This phenomena was coupled with a loss of gonads and associative organs, an increase in cold tolerance, and a disruption in moulting cycles (Ritz and Crisp, 1970). It is unknown if this is related to our experiment, but the cold-temperature exposed barnacles may have had decreased feeding activity (rate and percent feeding) due to some of these factors.

With changing air temperatures due to climate change, barnacle's feeding behaviors may be affected in the Puget Sound. With more extreme weather patterns becoming prominent, the barnacles may have a hard time adapting. As seen from this experiment, feeding rate and percent of barnacles feeding decreased when the barnacles were exposed to more extreme air temperatures. This could have negative effects on their growth rates and survival of barnacles in the upper intertidal zone. One possible consequence of more extreme weather patterns is the downward recession of barnacles in the intertidal zone. This could cause competition pressures in the intertidal zone among many different species which would have unknown consequences for the ecosystem. One possible reason for lower feeding rates and percent feeding among the extreme temperatures is that the barnacles collected were adapted to a specific temperature range (around 12°C). Future research looking at temperature adaptation in barnacles and different species from different geological locations would provide some very valuable information.

In regards to spatial distribution, it was hypothesized that barnacles near the edges of the rock would begin feeding first due to hummocking effects. Although the rocks that were collected did not have significant hummock peaks, we hypothesized that hummocking effects may still have an effect on the barnacles towards the middle of clusters and therefore these barnacles would begin feeding later than the "edge" barnacles. A study done looking at feeding behavior of barnacles in relation to hummocks found that those barnacles at the hummock peaks and at the edges fed more actively. This was because flow velocities were higher near the edges and at the peaks of the hummocks, allowing for more particle capture. The barnacles in the troughs between hummocks did not receive nearly as much water flow and therefore fed less (Bertness et al., 1998). Our results showed that there was no influence of air temperature on spatial distribution. When testing, we did notice that smaller barnacles were the first to emerge and begin feeding. This is an interesting result, because a study done on the *Balanus balanoides* barnacles found that smaller, younger barnacles were more susceptible to desiccation due to an increase in surface to volume ratio (Foster, 1971). It is unclear why they would be the first to begin feeding, but it could be because smaller barnacles are more pressured to grow larger and therefore feed more, due to increased survival rates in larger barnacles.

In the future, it would be beneficial to do more research on temperature adaptions of barnacles and how certain species' feeding behaviors may be affected differently by temperature variations. It would also be interesting to study how seasonal variation plays a role in barnacle feeding habits. For example, a study could be designed where a certain species of barnacles was collected during 4 different times of the year and their feeding behaviors were measured after being exposed to different aerial temperatures. The data from this experiment provides interesting preliminary research into the effects of aerial exposure temperature on the feeding behaviors of a primary intertidal filter feeder. The health of barnacles play an important role in the health of the intertidal ecosystem as filter feeders such as barnacles have been found to play four major roles in the ecosystem which are; repairing the water quality by removing harmful toxins, contributing to the reliability and stability of the ecosystem, contributing to habitat heterogeneity, and contributing to the migration of chemical elements (Ostroumov, 2005). Knowing more about these organisms and how our actions may affect their health is important to understanding the health of the entire marine ecosystem

Literature Cited

Anderson, D. T. "Cirral Activity and Feeding in the Barnacle Balanus Perforatus Bruguiere (Balanidae), With Comments on the Evolution of Feeding Mechanisms in Thoraciean Cirripeds." *Philosophical Transactions of the Royal Society of London* 291.1053 (1981): 411-49. Print.

Bertness, Mark, Steven Gaines, and Su Ming Yeh. "Making Mountains out of Barnacles: The Dynamics of Acorn Barnacle Hummocking." *Ecology* 79.4 (1998): 1382-394. Print.

Clarke, Andrew, and Nadine Johnston. "Scaling of Metabolic Rate with Body Mass and Temperature in Teleost Fish." *Journal of Animal Ecology* 68.5 (1999): 893-905. Print.

Connell, Joseph. "Community Interactions on Marine Rocky Intertidal Shores." *Annual Review of Ecology and Systematics* 3 (1972): 169-92. Print.

Foster, B. A. "On the Determinants of the Upper Limit of Intertidal Distribution of Barnacles." *Journal of Animal Ecology* 40.1 (1971): 33-48. Print.

Ostroumov, S. A. "Some Aspects of Water Filtering Activity of Filter-feeders." *Developments in Hydrobiology* 180 (2005): 275-86. Print.

Pincebourde, Sylvain, Eric Sanford, and Brian Helmuth. "Body Temperature during Low Tide Alters the Feeding Performance of a Top Intertidal Predator." *Limnology and Oceanography* 53.4 (2008): 1562-573. Print.

Ritz, D. A., and D. J. Crisp. "Seasonal Changes in Feeding Rate in Balanus Balanoides." *Journal of Marine Biological Association of the United Kingdom* 50.1 (1970): 223-40. Print.