

Cold Temperature Effects on Byssal Thread Production by the Native Mussel *Geukensia demissa* versus the Non-Native Mussel *Mytella charruana*

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ABSTRACT: *Mytella charruana* is a Central/South American mussel that has been found as an introduced species along the southeastern Atlantic coast of the United States since 2004. Previous experiments have determined the lethal thermal minimum of *M. charruana* to be between 6–9° C. Continuous byssal thread production is essential for mussel survival as it is used for attachment to substrates in natural environments, but may decrease when environmental conditions deviate from their optimum. We sought to determine whether *M. charruana* exhibits a non-lethal, thermal response involving a reduction in byssal thread production. *Mytella charruana* and, for comparison, a native mussel species, *Geukensia demissa*, were collected for this study. Water temperature was manipulated with chillers to near lethal temperatures (10°, 13° C) and newly produced byssal threads of all mussels were counted and cut daily for seven days. Both mussel species had lower byssal thread production at the colder tested temperatures (10°, 13° C) than in the control (23° C). However, *G. demissa* produced some threads at 10° C while *M. charruana* produced none. Also, *G. demissa* showed no difference in mean byssal thread production between 13° and 23° C. *Mytella charruana* had significantly fewer byssal threads at 13° C than at 23° C. Our results suggest that *G. demissa* can withstand colder temperatures than *M. charruana*, owing to their increased ability to produce byssal threads at lower temperatures. These results have implications for the survival and future spread of non-native *M. charruana*.

KEY WORDS: invasive species; marine mussels; byssal threads; thermal limits; temperature

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INTRODUCTION

Invasive species pose numerous ecological and economic threats (Vitousek et al. 1996, Pimentel et al. 2005). Ecologically, they may induce changes in existing ecosystems via competition or by predator-prey interactions (Vitousek et al. 1996). These factors can have negative impacts on the ecosystem and may result in the displacement of native species (Townsend 1996, Holway 1999, Pimentel et al. 2005, Mooney et al. 2005). The zebra mussel, *Dreissena polymorpha*, for example, has outcompeted and displaced native mussel species in the Great Lakes area (Wilson 1999). Indeed, approximately 42% of species listed as “threatened” and “endangered” are thought to be threatened as a consequence of interactions with non-native species (Wilcove et al. 1998, Pimentel et al. 2005). Invasive species are also well known for their economic costs, which are estimated to be \$120 billion per year in the United States (Pimentel et al. 2005). These costs include repairing damage caused by invasive species as well as eradication and control (Pimentel et al. 2005).

Invasive species may be introduced by several means. They may be intentionally or unintentionally released from domestic aquaria (Verlaque and Fritayre 1994, Whitfield et al. 2002, Semmens et al. 2004). One example of this type of introduction is the Indo-Pacific lionfish, *Pterois volitans*, which has invaded the western Atlantic Ocean (Whitfield et al. 2002). This species has significantly reduced native reef fish populations, including species that control seaweed overgrowth on corals (Albins and Hixon 2008). The intentional release of invasive species for ornamental purposes is another method of introduction, as seen with the Brazilian pepper tree *Schinus terebinthifolius* (Morton 1978, Curnutt 1989). This South American plant has spread throughout peninsular Florida and displaced native plants due to its dense stands and allelopathic properties (Morgan and Overholt 2005, Donnelly et al. 2008, Florida Department of Environmental Protection 2009). A third leading cause of invasions is via the ballast water of ships, which unintentionally transports many marine invasive species, including planktonic and fouling benthic species (Williams et al. 1988, Hicks and Tunnell 1995). The introduced charru mussel *Mytella charruana* (d’Orbigny, 1846), which is the focus of this study, is thought to have been transported in this way (Lee 1987, Boudreaux and Walters 2006).

Mytella charruana is a marine mussel native to the

Atlantic and Pacific coasts of South America (Keen 1971, Carlton 1992, Szefer et al. 1998, Boehs et al. 2004, Boudreaux and Walters 2006). It has a light green to black shell, and reaches up to 46 mm in length (Keen 1971, Szefer et al. 1998, Pereira et al. 2003). It also has a unique capability to change sex, which has not been observed in *G. demissa*, a native mussel that inhabits areas where *M. charruana* has been introduced (Stenyakina et al. 2010). *Mytella charruana* was first introduced to the United States in Jacksonville, Florida in 1986 and was found clogging intake pipes of a power plant generator (Lee 1987). In 2004, this species appeared on reefs of the eastern oyster *Crassostrea virginica* in central Florida (Boudreaux and Walters 2006). *Mytella charruana* has since been found along the Atlantic coast from Jupiter, Florida, to South Carolina, and is currently known to occur from New Smyrna Beach, Florida, to northern Georgia (Gillis et al. 2009, Spinuzzi et al. 2010). This species has also been found fouling a variety of man-made and natural substrates (Boudreaux and Walters 2006). To predict *M. charruana*’s potential distribution, we previously examined the temperature limits for survival of this species. Through a series of experiments, we found the thermal minimum for *M. charruana* to be between 6-9° C and the thermal maximum between 31-36° C (Brodsky et al. 2009). During these temperature trials, we noticed that at lower temperatures (9 and 11° C), some *M. charruana* survived (21%) after two weeks but were not actively producing byssal threads.

Byssal threads are produced by the foot of a mussel, which releases an aromatic protein compound that hardens on contact with water or a substrate (Van Winkle Jr. 1970). This creates a strong attachment that can be detached and reproduced when necessary (Yonge 1949). The production of these threads can be a determining factor for the establishment of mussels in certain habitats (Van Winkle Jr. 1970, Mackie 1991). As Mackie (1991) noted of the zebra mussel, *Dreissena polymorpha*, the tight attachment afforded by byssal threads can also be used as a means of defense against predators. Byssal thread attachment is especially important in areas of high water motion, where competition for space may be intense (Bell and Gosline 1996). For these reasons, we suggest that the production of byssal threads is important for the establishment and maintenance of mussel communities. Several factors such as salinity, temperature, tidal regime, seasonality, and agitation may affect the formation of byssal threads (Van Winkle Jr. 1970, Young 1985, Clarke and McMahan 1996, Rajagopal et al. 1996, Masilamoni et al. 2002). It

has been observed that byssal thread formation in mussels (*Dreissena polymorpha*, *Brachidontes striatulus*, *Mytilus edulis*) decreases when approaching lethal temperature limits (Van Winkle Jr. 1970, Rajagopal et al. 1996, Masilamoni et al. 2002).

One trait common to many successful invasive organisms is the capability to survive in a wide range of physical conditions (Morton 1997, Marchetti et al. 2004, Rajagopal et al. 2006). Therefore, an assessment of physiological tolerance limits is essential because it aids in predicting where invasive species may be able to disperse and colonize (Whitfield et al. 2002, Kimball et al. 2004). Many studies of physiological limits have focused on lethal environmental parameters, such as lethal temperature (Storey and Churchill 1995, Masilamoni et al. 2002, Kimball et al. 2004, Jost and Helmuth 2007). However, limits that coincide with functional ability (e.g., growth, reproduction) of a species are also important determinants of distribution. For example, Kimball et al. (2004) found that the mean lethal minimum temperature for the lionfish (*Pterois volitans/miles* complex) was 10° C, but the mean minimum temperature for feeding was 16.1° C. Although the thermal limits for survival of *M. charruana* are between 9–31° C in a laboratory setting (Brodsky et al. 2009), the environmental limits at which important functions such as growth, feeding, and reproduction become impaired are unclear.

In addition to *M. charruana*, we tested temperature effects on byssal thread production in *G. demissa*, a native species that has been found living in close proximity to introduced populations of *M. charruana* (S. Brodsky, pers. obs.). *Geukensia demissa* (Dillwyn, 1817) is a large mussel species (up to 100 mm in length) that is distinguishable from *M. charruana* by its ribbed, dark brown shell (Coen and Walters 2006). It is native to the North American Atlantic coast from the Gulf of St. Lawrence to central Florida, a distribution which overlaps with *M. charruana*'s current introduced range (Knopf 1981, Cohen 2005, Boudreaux and Walters 2006, Coen and Walters 2006, Gillis et al. 2009). Coinciding with its large geographical range, *G. demissa* tolerates temperatures ranging from 0° C to approximately 45° C (Storey and Churchill 1995, Jost and Helmuth 2007). *Geukensia demissa* is a fundamental component of salt marsh and oyster reef communities, where it is often found in syntopy with *M. charruana* (Bertness 1984, Coen and Luckenbach 2000, Meyer and Townsend 2000, Coen and Walters 2006, Boudreaux and Walters

2006). For these reasons, we tested *G. demissa* in addition to *M. charruana*. The focus of this study was to investigate the effects of environmental stress on byssal thread production, which is important for the attachment of mussels to a substrate, and also an easily measured and quantified parameter (Van Winkle Jr. 1970, Mackie 1991, Bell and Gosline 1996). The goal of this project was to determine cold temperature effects on byssal thread production of both *M. charruana* and *G. demissa* to better understand how both species respond to low temperatures. We accomplished this objective by manipulating water temperature and counting the number of byssal threads produced.

METHODS

The following experiments were completed in a laboratory setting. The *Mytella charruana* trial began on 3 July 2009 and the *Geukensia demissa* trial began on 11 June 2009. Fifty adult-sized *M. charruana* (mean length: 23.5 ± 0.6 mm) were collected from the dock at Jacksonville Fire Station 38 Marine Unit (30° 23.232' N, 81° 38.305' W). Fifty adult-sized *G. demissa* (mean length: 51.4 ± 1.4 mm) were collected from a sea wall in New Smyrna Beach, Florida (28° 59'22.57" N, 80° 52'06.09"W). Following collection, mussels were cleaned and placed in aerated tanks at room temperature (23°C) with a filter and seawater from the collection location. The mussels were kept in the tanks for thirteen days to acclimate to laboratory conditions and were monitored daily to remove any dead individuals. Trials for both species were run with identical methodologies.

Plastic aerated aquarium tanks were placed in a laboratory setting at 23°C at the University of Central Florida. These tanks (29 x 20 x 20 cm) were prepared with 50% collection site water and 50% deionized water mixed with Instant Ocean® salts. Salinity was adjusted to match the conditions of the collection site (Jacksonville—10 ppt, New Smyrna Beach—30 ppt). Three replicate tanks were then prepared for each temperature treatment. Four mussels were placed in each tank on a plastic surface, and then individually labeled with bee tags (The Bee Works, Orillia, Ontario).

There were three temperature treatments: 10°, 13°, and 23° C. Water temperature was manipulated with chillers and water pumps. Experimental temperatures were reached by adjusting temperature 2° C/day for one week following acclimation. After the last temperature adjustment, all byssal threads were cut with a disposable

scalpel. The number of new byssal threads was counted and cut each day for seven days, which was the total duration of the experiment. Mussel care included daily monitoring of mortality and 100% water change after three days. An algal paste solution, which contained *Phaeodacylum tricornerutum*, *Chaetocerus-B*, and *Nannochloropsis oculata*, was fed to the mussels daily (1mL/mussel).

At the end of the experimental trials, we tested for significant differences in the mean number of byssal threads among treatments and over time using a repeated measures Analysis of Variance (ANOVA) using SPSS (version 17.0.2, 2009). No transformations were used because all data met the assumptions of ANOVA. A Tukey's *post-hoc* comparison was then used to delineate differences among treatments. A one-way ANOVA using SPSS (version 17.0.2, 2009) was also run at the end of the *M. charruana* trial to look for significant differences in mean number of byssal threads over time at the 23° C treatment. A Tukey's *post-hoc* comparison test was then used to determine differences among days.

RESULTS

Temperature had a significant effect on byssal thread production for both *M. charruana* ($F= 56.73$, $p<0.0001$; Figure 1) and *Geukensia demissa* ($F=10.16$, $p=0.012$; Figure 2). *Geukensia demissa* had a higher survival rate at 10° C than *M. charruana*, but *M. charruana* produced twice as many byssal threads than *G. demissa* at the highest tested temperature. Also, *M. charruana* showed a difference in byssal thread production over time while *G. demissa* did not.

Survival of *M. charruana* was 100% in the control (23° C) and 13° C treatments. At 10° C, there was 66% survival. At 23° C, *M. charruana* had a mean of 10.8 ± 1.6 byssal threads per day over seven days (Figure 1A). This was significantly higher ($p<0.0001$) than the 13° and 10° C treatments, which had 0.05 ± 0.04 and 0 byssal threads per day, respectively (Figure 1A). At 13° C, only 4 of 12 individuals produced byssal threads. Specifically, two mussels produced one byssal thread on one day over the seven day trial, one mussel produced two new threads on two separate days of the trial, and one mussel made three new threads on two separate days.

Results of the one-way ANOVA for the *M. charruana* trial at 23° C showed that time was a significant factor for production of byssal threads ($F= 5.604$, $p=0.004$

(Figure 1B). Byssal thread production on Day 2 was significantly different from Days 1, 4, 6, and 7 (Figure 1B). No other days were different from one another.

Geukensia demissa had 100% survival in all treatments. Temperature had a significant effect on the mean number of byssal threads produced by *G. demissa* ($F=10.16$, $p=0.012$) (Figure 2A). At 23° C, *G. demissa* produced a mean of 5.78 ± 0.84 byssal threads per day (Figure 2A). This was not significantly different from the number of threads produced at 13° C, which had a mean of 4.26 ± 0.35 (Figure 2A). At 10° C there was a significant reduction in the number of byssal threads produced (mean of 2.90 ± 0.29 byssal threads per day over seven days; $p=0.013$) from the 23° C treatment (Figure 2A). However, time was not a significant factor for byssal thread production ($F=2.072$, $p=0.081$) (Figure 2B).

DISCUSSION

We found that both *M. charruana* and *G. demissa* produced fewer byssal threads at colder temperatures. *Mytella charruana* at 23° C produced ten times more byssal threads than at lower temperatures (13 and 10° C); this difference was significant. At 13° C, survival of *M. charruana* was 100%, and some mussels (25%) produced new byssal threads, although the number of threads was ten times lower than the control (23° C; Figure 1A). Byssal thread production significantly varied on the second day of the control (23° C) in *M. charruana*, but we have no reason to believe this had any biological significance. We expect *M. charruana* to have continued survival at 13° C, even though the amount of byssal threads produced was lower than controls, and we base this conclusion on two lines of evidence. The first was that *M. charruana* created at least two byssal threads at 13° C, which has been documented to be the smallest number of threads needed for attachment in a similar size mussel; two such threads can be formed in as little as one hour (Lesin et al. 2006). It has also been documented (in other mussels) for individual byssal threads to retain their integrity and remain attached to a surface for 4-6 weeks (Carrington 2002). Based on this data, we suggest that the actual thermal minimum of *M. charruana* is at or very close to 13° C. Our data suggest that *M. charruana* may experience difficulty surviving for extended periods of time in natural habitats at 10° C. In addition to only 66% survival in our laboratory trial, no byssal threads were produced at 10° C (Figure 1A). Our results suggest that if *M. charruana* survived and was dislodged at this temperature, they would not be able to re-attach. This

would especially pose a problem for *M. charruana* living on vertical substrates, since attaching near the surface of the water is often necessary for mussels to gain sufficient food and avoid benthic predators, such as crabs (Johnsen and Jakobsen 1987). Therefore, *M. charruana* would not be able to withstand 10° C for long periods of time. These results may also explain why populations of *M. charruana* decrease during months of low temperatures, which includes a severe cold weather event that occurred from December 2009 to February 2010 when water temperatures in Jacksonville, Florida, repeatedly dropped below 13° C for 48 days (A. Godwin, unpublished data).

At 10° C, *Geukensia demissa* produced fewer byssal threads than other tested temperatures, with an overall mean of 2.9 ± 0.3 threads per day (Figure 2A). Unlike *M. charruana*, *G. demissa* had 100% survival at this temperature, which suggests that *G. demissa* does not have difficulty surviving and can remain attached at this temperature. These results suggest that *G. demissa* may simply be more cold tolerant than *M. charruana*. In studies on the invasive freshwater mussel *Dreissena polymorpha* and the blue mussel *M. edulis*, byssal thread production was found to increase proportionally with temperature, which coincides with what we have found in *M. charruana* and *G. demissa* (Young 1985, Clarke and McMahon 1996).

Although temperature influences the survival of these two species differently, this does not dismiss the idea of competition between them. At 23° C, *M. charruana* had an overall mean of 10.84 ± 1.60 byssal threads, which was twice as high as the 5.78 ± 0.84 threads produced by *G. demissa* (Figures 1A, 2A). In warmer months, growth rates of these two species are similar, with an average increase in shell length of 5 mm per month for *M. charruana* and 4.4 mm for *G. demissa* (Brousseau 1984, A. Godwin unpublished data). Problems may arise for native species such as *G. demissa* since mussels compete for limited space, which can intensify during times of high growth and settlement (Bayne 1976). A study by Boudreaux et al. (2006) also found that *M. charruana* attaches to substrates quicker than *G. demissa*, which could increase survival of *M. charruana*. Future competition studies between these species will be useful in assessing the extent to which *M. charruana* might become a threat to other native species, such as the economically and environmentally important eastern oyster *Crassostrea virginica* (Boudreaux and Walters 2006).

Expansion of *M. charruana*'s invasive range will depend on its ability to produce byssal threads at varying temperatures. In previous laboratory studies, 21% of *M. charruana* were able to survive at 9° C, and its thermal minimum was predicted to be between 6 and 9° C (Brodsky et al. 2009). However, based on the results from our current study, the thermal minimum is predicted to be at least 3 degrees higher, at or near 13° C. Temperature would thus create a barrier to where *M. charruana* could establish, and it is likely that it would not be able to survive in habitats where water temperatures are lower than 13° C for extended periods of time.

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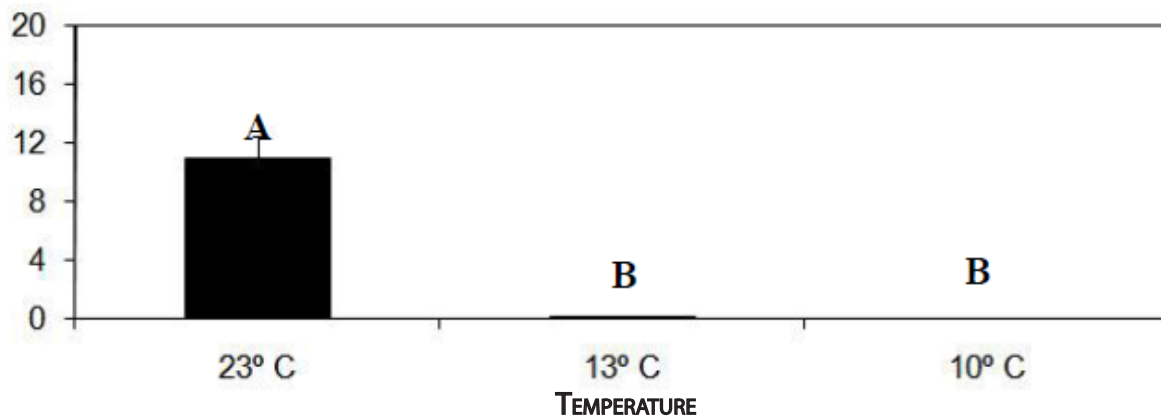
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FIGURES

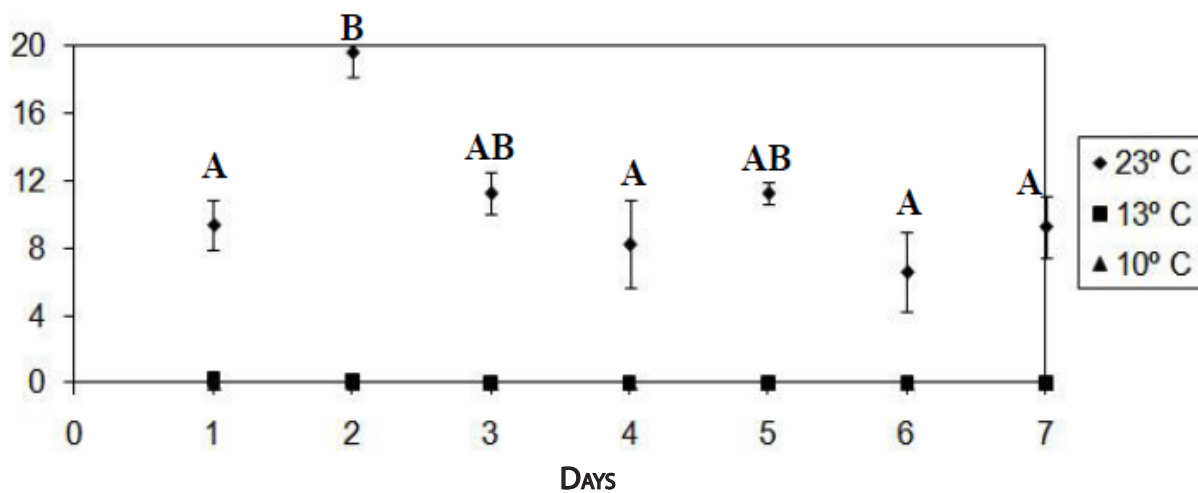
FIGURE 1: *MYTELLA CHARRUANA*

A)

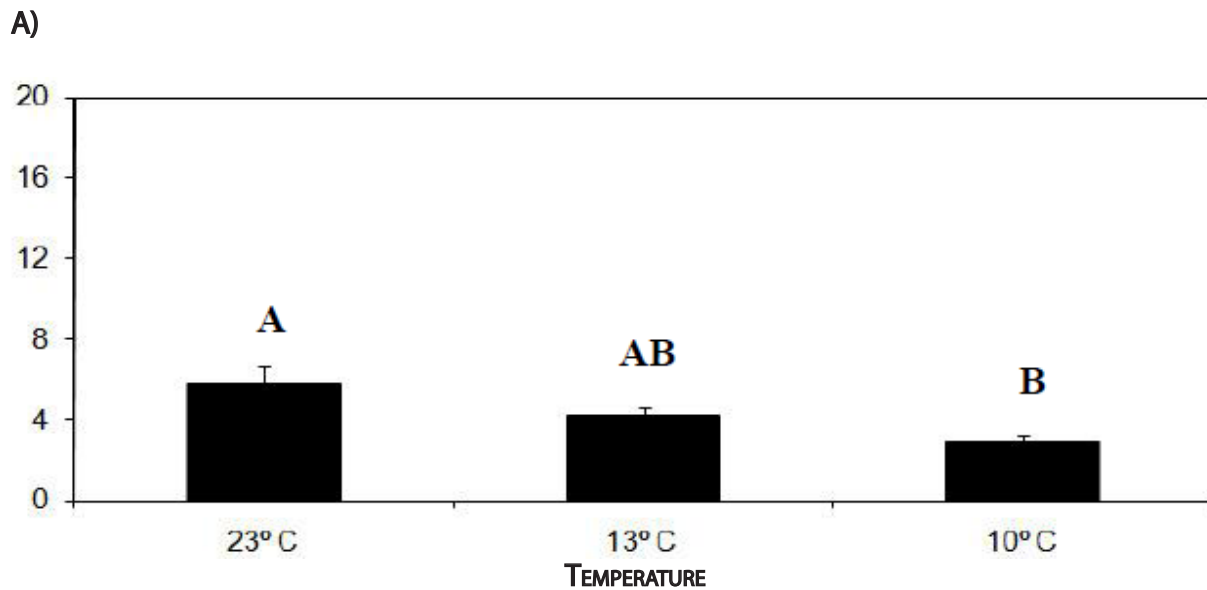


Mean number of byssal threads per day (averaged across seven days) produced by *Mytella charruana* when exposed to three different temperature treatments (23°, 13°, and 10° C). Letters indicate significant differences and error bars represent standard errors.

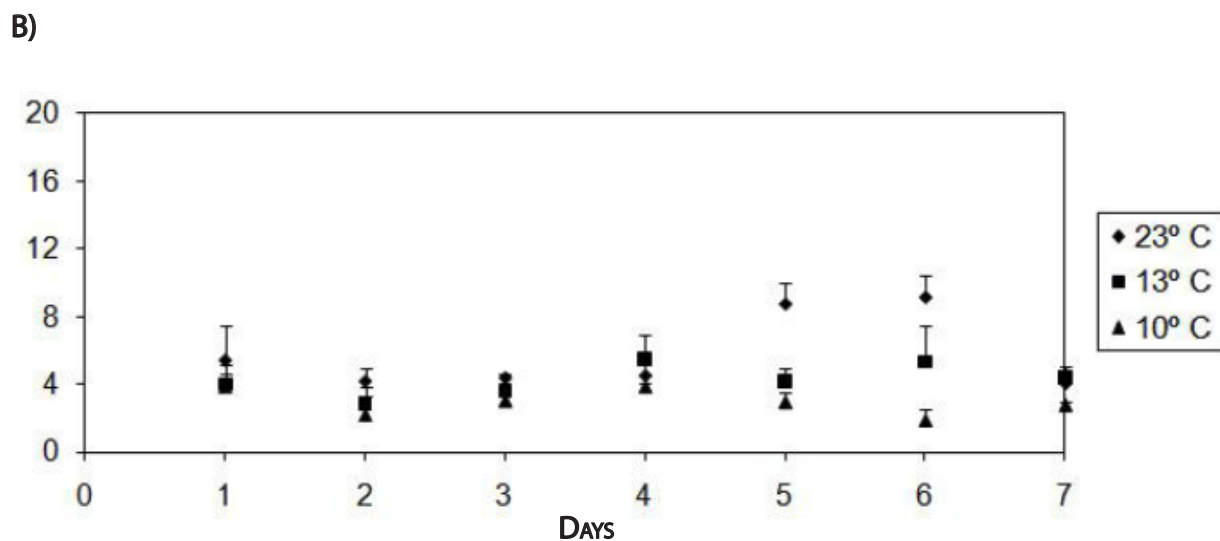
B)



Mean number of byssal threads produced per day by *M. charruana* when exposed to three different temperatures (23°, 13°, and 10° C) over a seven-day period. Letters indicate significant differences.

FIGURE 2: *GEUKENSIA DEMISSA*

Mean number of byssal threads per day (averaged across seven days) produced by *Geukensia demissa* when exposed to three different temperature treatments (23°, 13°, and 10° C). Letters indicate significant differences and error bars represent standard errors.



Mean number of byssal threads produced per day by *G. demissa* when exposed to three different temperatures (23°, 13°, and 10° C) over a seven-day period.