

Abstract

The purpose of this study was to assess the prevalence of trematodes in freshwater gastropods in several aquatic systems at the University of Notre Dame Environmental Research Center (UNDERC). Trematodes, which use gastropods as intermediate host, can have a dramatic effect on the survivorship and reproductive success of gastropods. Prior studies have also suggested that trematode prevalence can also cause an increase in individual shell height (mm). Reduction in the population of gastropod species can have a detrimental impact on any aquatic ecosystem, including those at UNDERC. Three vernal ponds, lakes and streams were sampled. Snails were identified to family, shell height measured in millimeters, and then euthanized and dissected to determine the presence of trematodes. Vernal ponds produced no samples, while in the lakes and streams three Families of gastropods were collected, but only Lymnaeidae and Viviparidae were used in this study. Lymnaeidae was found to a higher trematode prevalence (26.7%) than Viviparidae (14.3%), and streams were found to have a higher prevalence for both families. Though results suggested that system type had an effect on the shell height in both Families, no relationship was established between trematode prevalence and shell height. This study also led to the discovery of the presence of the invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, in several aquatic systems at UNDERC.

Introduction

Snails, Order Gastropoda, can be considered the most widespread organisms of littoral zones in lakes and streams, and play an important role in the aquatic systems that they inhabit which include vernal ponds, lakes, and streams (Brown 1991). Although most species of pulmonate snails are generally terrestrial, a modification in the mantle cavity that acts like a lung has enabled some species to reinvade freshwater habitats (Pechenik 1985). Freshwater gastropods are either herbivores or detritivores, or they may passively consume small invertebrates associated with periphyton (Cuker 1983). More importantly, gastropods serve as a prey species for various fish, birds, mammals, and other invertebrates. These two aspects of the biology of freshwater gastropods predator-prey interactions and their role as grazers in freshwater systems are gaining much attention (Brown 1991). Factors that regulate population size of freshwater gastropods are also considered to be of great concern, as a decline could have a detrimental impact on both the stability and productivity of aquatic systems (Brown 1991).

One potential regulator of snail populations that is generally overlooked is the parasitic larvae of trematodes, or flukes, which use the snails as intermediate hosts (Holmes 1983). Trematode eggs are passed in the feces of small mammals, reptiles, and passerine birds, which serve as the definitive host. Eggs hatched in freshwater produce miracidia, or free-swimming larvae, which must penetrate a suitable snail within hours in order to survive. The miracidia in the snail develop into sporocysts, or a saccular body that is the first asexual reproductive form of a digenetic trematode. The sporocysts bud off cells from its inner surface which then develop into redia, or the larval stage within

the snail. Once the redia matures into a cercaria, a tadpole-shaped larval trematode worm, it leaves the snail searching for its definitive host (Sorenson et al. 2001).

Studies of interactions between Lymnaeid freshwater snails and their larval trematode parasites indicate that parasites can alter snail growth rate or eliminate reproduction (Loker 1979). Minchella et al. (1985) concluded that infected pulmonate of the species *Lymnaea elodes* experienced a temporary increase in growth. However, as infection matured, growth was reduced and reproduction ceased. Infected snails also showed an increased survivorship (Minchella et al. 1985).

Host responses when infected by a parasite may vary. The host and parasite are in a dynamic interaction, the outcome of which depends upon the properties of the parasite and of the host (Todar 2002). A healthy animal will attempt to defend itself against a pathogen, and to such a degree that infection can be prevented entirely (Todar 2002). The defense mounted by *Lymnaea elodes* to its trematode parasites is one of increased growth/survivorship, though it could be deemed an ineffective strategy as trematode parasites tend to outlive this species (Minchella et al. 1985).

Invertebrates cannot easily acquire resistance to parasites, and genotypic frequency-dependent selection may operate to ensure the fitness of any genotypes less vulnerable to a particular trematode (Holmes 1983). Infection can cause a shift in resources from costly reproduction to growth and maintenance and even result in increased survivorship (Minchella et al. 1985). The shift in resources from reproduction to growth effectively leads to a form of castration in pulmonate snails (Minchella et al. 1985). Castration of pulmonate snails due to trematode infection could potentially have a

dramatic impact on aquatic ecosystems (Brown 1988). However, in order to control gastropod populations, trematode prevalence in snails must be fairly high (Holmes 1983).

Prevalence or percentage of infection levels vary from as low as 1% in some populations to as high as 50% in other host-parasite systems (Holmes 1983). In Indiana ponds, prevalence of trematodes in populations varied from 4%-49% (Brown 1988). Prevalence was found to be higher in less productive ponds, as evidence suggests that physiochemical variables, i.e. temperature, pH levels, turbidity, dissolved oxygen, result in longer snail cycles increasing an individual's chance of being invaded by a miracidia (Brown 1991).

The objectives of this study were to 1.) determine the level of prevalence of trematode infection in the pulmonate gastropod species occur at the University of Notre Dame's Environmental Research Center (UNDERC); 2.) determine if infection rates differ among aquatic system types (i.e. lakes vs. vernal ponds vs. streams); and 3.) examine the relationship between prevalence (presence or absence) and snail shell height (mm) which is an indicator of age among pulmonate species (Loker 1979).

I hypothesized that the prevalence of trematodes in snail would be higher in the vernal ponds than in lakes or streams because vernal ponds represent the least productive of the three aquatic systems. As a result, the average snail shell height (mm) in vernal ponds would be significantly much larger than the other aquatic systems sampled. However, the trend in all systems would show that average snail size of infected individuals would be greater than that of uninfected individuals.

Methods

Study Site

Prior studies conducted on the grounds of the University of Notre Dame's Environmental Research Center (UNDERC) in Vilas County, Wisconsin and Gogebic County, Michigan have revealed that there are at least three pulmonate snail species, *Lymnaea stagnalis* (Stahl 1989), *Helisoma trivolvis* (Stahl 1989), and *Gyraulus parvus* (Erhman 1989) residing within the lakes, streams, and vernal ponds at UNDERC, though it is unclear if they cohabitate within these systems. It has been suggested that as many as seven pulmonate species exist on the property (Crowl, personal communication).

Collection

Samples were collected at Brown, Plum, and Tenderfoot Creek; Big, Crampton, and Tenderfoot Lake; Wood Duck pond and vernal ponds #7 and J at UNDERC. At each of these locations, three sampling efforts of ten minutes apiece for a total of thirty minutes will be conducted on three separate occasions, for a total of 90 minutes at each system. For each of the sampling efforts, a new sample site/location was selected and the Universal Transverse Mercator (UTM) coordinates of these sites was recorded using a Global Positioning System (GPS) for a total of nine sample locations at each aquatic system, and the date sampled was recorded. Sites were selected based on preferred pulmonate habitat, i.e. sandy substrate or coble (Brown 1991), with the exception of the vernal ponds that were sampled.

Sampling took place along the shoreline at depths no less than .2 m and no greater than 1.5 m in each system. Drift nets were used to collect snails off the substrate and by hand when individuals were visible on the substrate. A bucket containing water from the

sample location was placed on the shore and used to store samples. Time stopped when samples were brought to the bucket to ensure a full sample effort of ten minutes. After the completion of the third sampling effort, samples will be brought back to the laboratory and placed in an aerated jar containing water from the aquatic system they were collected from until they were processed.

Lab

Survey methods of collected samples were similar to the methods described in Minchella et al. (1985). Samples were brought back and identified to family, and shell height of each individual recorded in millimeters. Individuals were then placed in a Petri dish individually under a florescent light (700,000 lux) for 12 hours. According to Minchella et al. (1985), the florescent light is used to euthanize the snails, and may cause the cercariae in some individuals to emerge from the shell. The individual samples were then examined for emerging cercariae with a stereomicroscope. Snails without emerging cercariae were crushed carefully, as the cercariae lie between the snail and the shell and may be easily damaged. Specimens were then examined under a stereomicroscope for the presence of cercariae. Individuals were then recorded as either “positive” or “negative”, indicating the presence or absence of trematode parasites.

Statistics

Statistical analyses were conducted using SYSTAT 11.0 (SYSTAT Software, Inc.; Point Richmand, CA). For the purpose of this study, all data were grouped by gastropod family for the purpose of data analysis. Trematode prevalence was accessed for system type, i.e. creek vs. lake vs. vernal pond, and also each individual system, i.e. Brown Creek vs. Plum Creek vs. Big Lake. A Pearson chi-square was used to determine

if there was any significant difference in the infection rates between aquatic system types and between individual systems. A two-way ANOVA was run to test for interactions between prevalence and the system type on the shell size of individuals. The effects of trematode prevalence on shell height within individual systems will be analyzed using a one-way ANOVA, with the data being grouped by Family and individual aquatic system.

Results

I collected 334 snails representing three different gastropod Families, Lymnaeidae (n=112), Planorbidae (n=5), and Viviparidae (n=217), across three different streams and lakes (Figures 1 & 2), while vernal ponds were excluded from data analysis as they yielded no individuals. Planorbidae samples were also excluded from data analysis due to the fact that only five individuals were caught and this is not a sufficient number of individuals to obtain any significant results. Although Viviparidae is a member of the Subclass Prosobranchia and this study was designed to look families in the Subclass Pulmonata, it was included in this study as it was found in all three of the lake systems sampled as well as two of the three streams. It was also discovered through personal communication with Dr. Gary Belovsky, that the lone Viviparidae species found at UNDERC happens to be the invasive Chinese Mystery Snail, *Cipangopaludina chinensis*. The will be discussed further in the discussion section.

Trematode prevalence within gastropod families at UNDERC could be classified as moderate for Lymnaeidae species at 27.6% and moderate for Viviparidae species at 14.3% (Figures 3 & 4). Lymnaeidae species were found in one lake, Tenderfoot Lake (N=73), and two streams, Plum Creek (n=2) and Tenderfoot Creek (n=37). Trematode prevalence for this family was much higher in the creek systems, 56.4%, than in

Tenderfoot Lake, 12.3%. For Lymnaeidae, the infection rate was extremely high in Tenderfoot Creek, 56.8%, considering an infection rate of 50% is considered to be very high (Brown 1988) (Figure 5). Though Plum Creek had an infection rate of 50%, this value is suspect as there were only two representatives of this family collected in all sample efforts in this system. Viviparidae, more specifically *Cipangopaludina chinensis* was found in two lakes, Big Lake (n=64) and Crampton Lake (n=3), and two streams, Brown Creek (n=103) and Plum Creek (n=47) (Figure 6). The infection rate for this family was found to be higher in creek systems, 18.7%, than in the lake systems, 4.5%, though neither of these infection rates would be considered to be high (Figure 3).

A Pearson chi-square revealed that according to the data collected there exists a significant difference between system type, lake vs. stream, and trematode prevalence for both Lymnaeidae ($\chi^2=24.676$, $p=.0001$) and Viviparidae ($\chi^2=7.615$, $p=.006$). A comparison between individual systems for Lymnaeidae was unnecessary as the sample size for Plum Creek was small with only two individuals collected in all sample efforts. The chi-square value was similar to the comparison between system type and trematode prevalence and thus yield a significant relationship ($\chi^2=24.719$, $p=.0001$). A chi-square also revealed a significant difference in trematode prevalence between the four individual aquatic systems where Viviparidae ($\chi^2=8.462$, $p=.037$) was found.

The one-way ANOVA showed that system type had a significant effect on the shell height (mm) on both Lymnaeidae (F-ratio=4.591, $p=.034$) and Viviparidae (F-ratio=24.247, $p=.001$). Although the two-way ANOVA did not reveal any significant relationship between shell height (mm) and trematode prevalence, one-way ANOVAs were then run to determine if this relationship between shell height and trematode

prevalence existed within individual aquatic systems (Figures 7 & 8) For Lymnaeidae, a significant relationship did not exist in any aquatic system between shell height (mm) and trematode prevalence, though Tenderfoot Creek was very close (F-ratio=4.0389, $p=.0522$). For Viviparidae, the ANOVAs that were run revealed significant relationships between shell height (mm) and trematode prevalence in Brown Creek (F-ratio=15.5590, $p=.0001$) and Plum Creek (F-ratio=7.1970, $p=.0102$).

Discussion

Due to the fact there were not any individuals collected from the vernal ponds at UNDERC, I would have to reject my overall hypothesis that snails collected in vernal ponds would have a higher trematode prevalence and therefore an increased shell height. Studies conducted by Brown et al. (1988) and Minchella et al. (1985) in vernal ponds located in Indiana provided a sufficient sample size to provide significant results to questions similar to those that were explored in this study. It is unclear why sampling efforts in the vernal ponds were unsuccessful. Many theories could be explored as to why this system type did not yield any samples, such as predation or the short-duration of the vernal ponds. Further investigation into the presence and/or duration of gastropods may be useful for future studies of either gastropods on property or trematode prevalence within gastropods.

The number of individuals of a given family that are sampled should also be standardized within a system. Though the sampling methods in this study could be considered successful, the methods could be revised to improve results if further investigation into this subject were to take place. The sampling methods described in Minchella et al. (1985), where 50 individuals of a given species were collected from a

given site may provide a more accurate look at the prevalence of trematodes in gastropods at UNDERC.

Trematode prevalence would not be classified as extremely high when looking at Families or system type, streams and lakes (Brown 1988). However, it is interesting to note that Lymnaeidae species collected for the purpose of this study had a higher prevalence, 27.6%, than the Viviparidae representatives, 14.3% (Figure 3 &4). This may be due to the operculum, a corneous plate at the opening of the shell and attached dorsally to the foot, that is present in Viviparidae and other families in the subclass Prosobranchia. Prior studies have shown that the operculum in certain species serves as a cover against predators when the snail body is retracted (Yusa et al. 2006, Aditya & Raut 2005). It may be possible that not only does the operculum prevent predation, but it may also aide in resistance to invasion by trematodes. Though there are no such studies looking at the role the operculum may play in parasite resistance, it would be interesting subject to investigate.

Trematode prevalence was also found to be significantly higher in both Families, Lymnaeidae (56.4%) and Viviparidae (18.6%), in the streams at UNDERC as opposed to the lakes. This could be due to the fact that the creeks in general supply more cover for the primary host of trematodes, i.e. birds, amphibians, and mammals, though I have little evidence to support this. This may be due to the increased cover than is provided along the shores of the streams that were sampled.

Though prior studies counted by Brown et al. (1988) and Minchella et al. (1985), found a relationship between increased shell height (mm) and trematode prevalence, the results from this study do not fully support this. Though this relationship was established

in Brown and Plum Creek for Viviparidae representatives, it was not supported in any other system with the same Family or amongst individuals from the Lymnaeidae Family in other systems. Though there was a relationship established in both Families on the effects of system type (lakes or streams) and shell height. Both relationships should be further investigated. It should be of note that Lymnaeidae was the family in which the studies conducted by Brown et al. (1988) and Minchella et al. (1985) discovered the relationship in vernal ponds in Indiana.

Sampling efforts in four aquatic systems (Figure 2) revealed the presence of the invasive Chinese Mystery Snail, *Cipangopaludina chinensis*, at UNDERC. Though there has been little research done investigating the impact this species has on aquatic ecosystems which they have been found, they are still identified as an invasive species by the United States Geological Survey. It has been suggested that their presence may lead to the restructuring of food webs, and also may lead to decline in native species (Mills et al.1993). Further investigation on the presence of this species in UNDERC aquatic systems should be explored.

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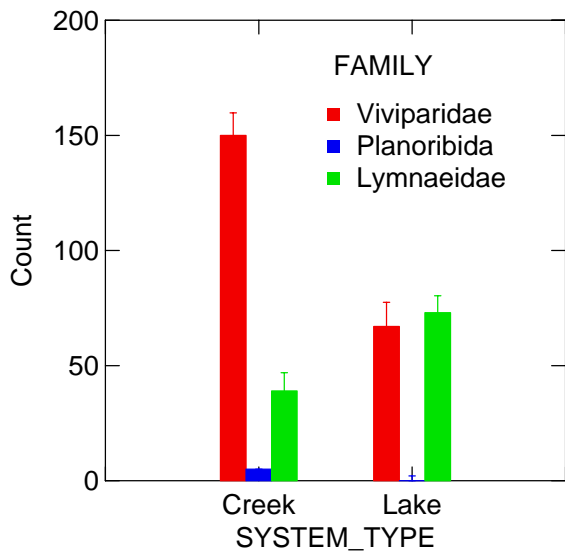


Figure 1. Distribution of the sampled individuals broken down into the three Families that were collected in all sample efforts across aquatic system type they were collected from.

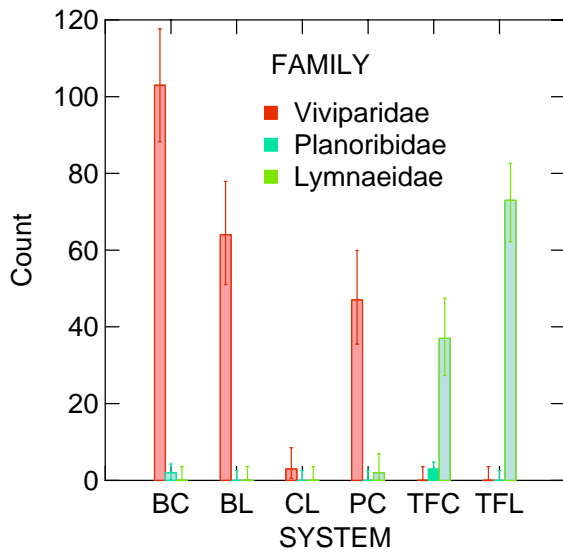


Figure 2. Distribution of the sampled individuals broken down into the three families that was collected in all sample efforts across the individual aquatic systems they were collected from.

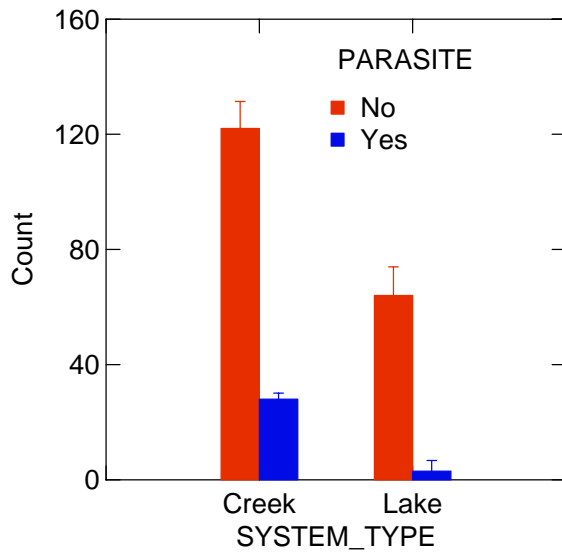


Figure 3. Trematode Prevalence in Viviparidae across aquatic system types

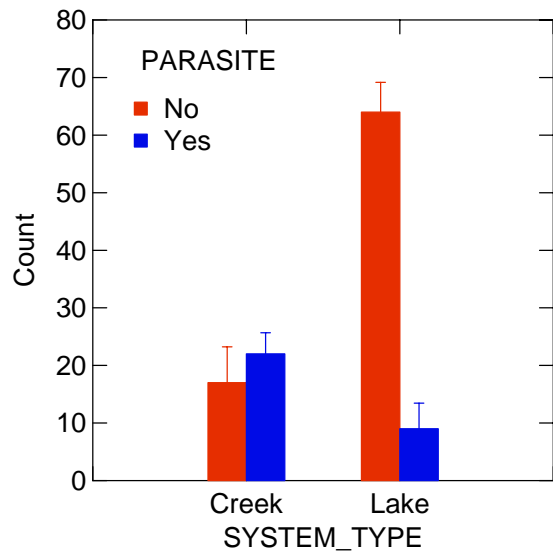


Figure 4. Trematode Prevalence in Lymnaeidae across aquatic system types

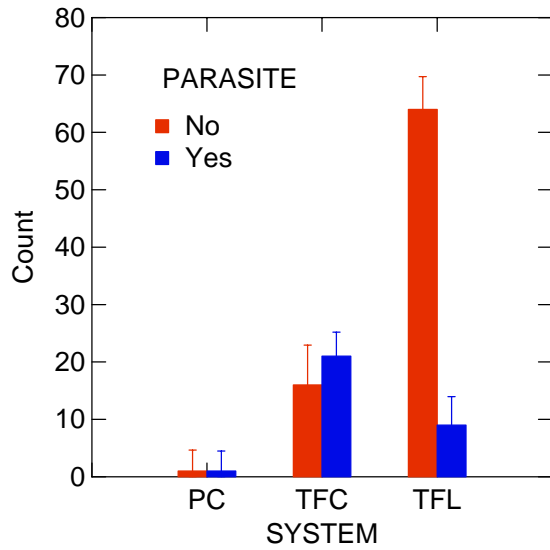


Figure 5. Trematode Prevalence in Lymnaeidae across the four individual systems where the family was collected

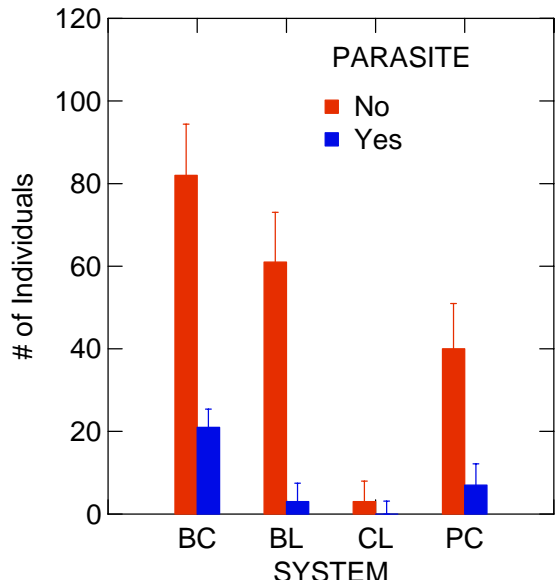


Figure 6. Trematode Prevalence in Viviparidae across the four individual systems where the family was collected

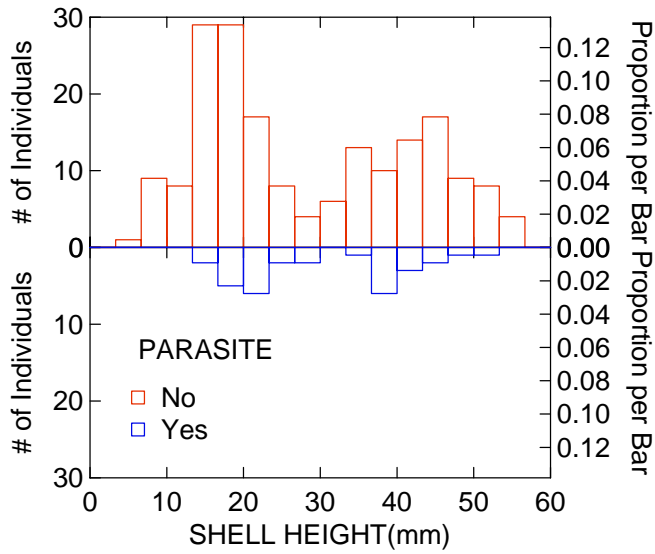


Figure 7. Histogram of the Shell Height (mm) and trematode prevalence in Viviparidae

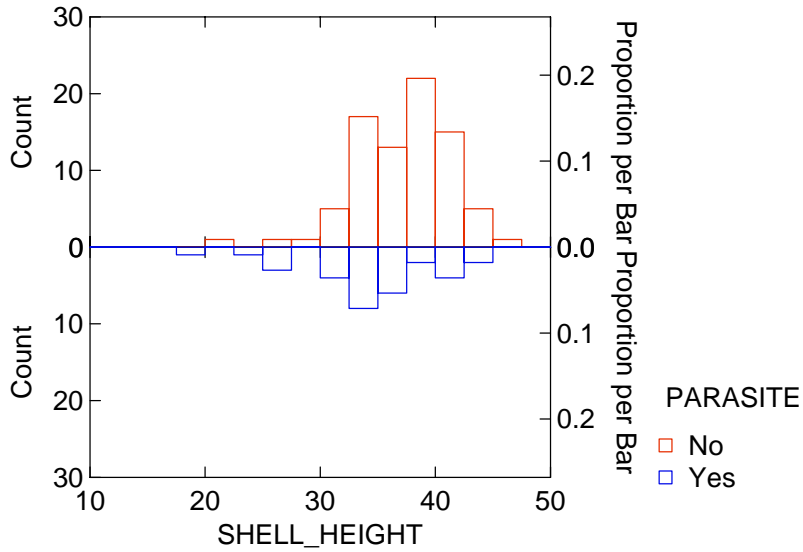


Figure 8. . Histogram of the Shell Height (mm) and trematode prevalence in Viviparidae

Prevalence of Trematodes in Freshwater Gastropods at UNDERC

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