

Stress-induced positive impacts of a predator on prey

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The stress-gradient hypothesis suggests that stress can determine the net sign or magnitude of the effect of one species on another. However, these ideas have not been well studied in the context of predator-prey dynamics where the predator positively impacts its prey, likely through ecosystem engineering. I used the pairwise species interaction between the burrowing nematode *Caenorhabditis remanei* and the bacteria that they consume, *Escherichia coli*, to determine if the net effect of the predator on its prey changed when stress was applied to the system. I measured the amount of bacteria in the presence or absence of predators in an environment that allowed engineering or did not allow engineering for two levels of stress. Colony plate counts of *E. coli* indicated that there was a stress-induced change in the net impact of nematodes on bacteria from neutral to positive, and that predator engineering in the form of burrows resulted in a larger amount of bacteria when stress was applied than when engineering did not occur. An indirect estimate of bacteria density using fluorescence thresholded by pixel brightness indicated that nematodes positively impacted bacteria whether stress was low or high. However, when stress was applied nematodes only benefitted bacteria if engineering occurred. Therefore I conclude that the net impact of a predator on a prey can change when stress is applied to the system and that ecosystem engineering is one mechanism through which this change can occur.

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Table of Contents

Author's declaration	ii
Abstract	iii
Acknowledgements	iv
List of Figures	vii
List of Tables	viii
Chapter 1 How the stress-gradient hypothesis applies to predatory ecosystem engineers	1
1 Introduction	1
2 The stress-gradient hypothesis	2
3 Change in net pairwise species interactions	4
3.1 Positive effects of predators	6
3.2 Predation to mutualism	8
4 A predator-prey model system	9
5 Applications and conclusions	12
Chapter 2 A model system for stress-induced positive impacts of a predator on prey	14
1 Introduction	14
2 Materials and Methods	20
2.1 Study organisms	20
2.1.i <i>Caenorhabditis remanei</i>	20
2.1.ii <i>Escherichia coli</i> (OP50-GFP)	21
2.2 Experimental design	22
2.2.ii Applying prey <i>E. coli</i> to agar filled cuvettes	23
2.2.iii Applying nematode predators	24
2.2.iv Applying stress to the cuvette agar surface	25
2.3 Estimating bacteria population using colony plate counts	25
2.4 Estimating bacteria population using fluorescence	27
2.5 Estimating variation in the number of nematodes	28
2.6 Time blocking and trials	28

2.7 Statistical analyses	30
3 Results	30
3.1 Data cleaning	30
3.2 Predator and stress effects on <i>E. coli</i> population	31
3.3 Treatment effects on nematode number.....	35
4 Discussion	36
4.1 Stress-induced variation in the impact of a predator on prey	37
4.1.i Predator impact on prey under low stress	37
4.1.ii Predator impact on prey when stress was applied	38
4.1.iii Overall change in the net impact of predators on prey.....	39
4.2 The effects of stress on the predator	40
4.3 Conclusions.....	42
Appendix.....	43
A test of the homogenization procedure	43
References.....	45

List of Figures

Figure 1: An interaction diagram for <i>E. coli</i> and <i>C. remanei</i>	11
Figure 2: A photograph of burrows made by <i>C. remanei</i> nematodes in agar (left image) compared to a photograph of agar without nematode burrows (right image).....	17
Figure 3: A representation of the predicted experimental outcomes when a predator is able to benefit its prey through ecosystem engineering.....	19
Figure 4: Chart showing main steps of the experimental procedure by day.....	29
Figure 5: Mean number of <i>E. coli</i> colony forming units per ml of agar with error bars showing +/- one standard deviation.	33
Figure 6: Mean percent of cuvette image with fluorescence above threshold pixel brightness with error bars showing +/- one standard deviation.	35
Figure 7: Linear regression of the estimated number of <i>E. coli</i> colony forming units per ml of cuvette agar by absorbance (OD ₆₀₀) of <i>E. coli</i> culture initially inoculated.	44

List of Tables

Table 1: A table of the eight experimental treatments for factors nematodes, agar, and stress.....	23
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Chapter 1

How the stress-gradient hypothesis applies to predatory ecosystem engineers

1 Introduction

The intensity, frequency, and net effect of pairwise species interactions may vary depending on environmental conditions. Bertness and Callaway (1994) predicted a change in the frequency of positive and negative interactions in plant communities over a gradient of stress, which is now known as the stress-gradient hypothesis. Although still most commonly applied to interactions between plant species (He et al. 2013), the stress-gradient hypothesis has been tested in a few animal-animal and animal-plant interactions (Brooker et al. 2008; Dangles et al. 2013). New interpretations of the hypothesis have also arisen since its formulation, including a prediction that there should be a change in the intensity and net effect of one species on another depending on levels of stress in the environment (Maestre et al. 2009; Kikvidze et al. 2011).

The finding that the intensity and net effect of pairwise species interactions vary with changing levels of environmental stress challenges current methods in ecology that give a static label such as predation, commensalism, or mutualism to interactions. If such stress-induced changes are common, they could make it difficult to categorize pairwise species interactions. A potentially novel interaction arises when considering animal species and the stress-gradient hypothesis. If predators have the potential to positively affect prey species under stressful conditions by modifying the physical habitat (i.e. the predator is an ecosystem engineer as defined by Jones et al. 1994), the benefits of this positive impact may outweigh the negative effects of consumption. Therefore, there could be a change from a net negative impact on the prey (predation) to a net positive impact (mutualism) with increasing stress.

I will discuss literature relevant to two pairwise species interactions that may flip from predation to mutualism as a result of abiotic stress. First, I will provide an overview of the stress-gradient hypothesis and in particular how the hypothesis applies to systems that involve at least one animal species. I will then describe studies that involve one non-plant species in which the authors have demonstrated changes in the intensity or net effect of one species on another. Then I will demonstrate how predatory ecosystem engineers can provide a mechanism for a pairwise species interaction to flip from predation to mutualism as a result of increasing stress. Finally, I introduce a nematode-bacterial model system that I used to test the predictions of the stress-gradient hypothesis in a predator-prey system and present my hypothesis that this system could demonstrate a stress-induced flip from predation to mutualism.

2 The stress-gradient hypothesis

The original formulation of the stress-gradient hypothesis predicts that, in environments where physical conditions are stressful, positive interactions between plant species are common, while under less stressful environmental conditions, competitive interactions dominate (Bertness and Callaway 1994). Positive effects are due to a facilitating species that ameliorates stress on a receptor species. Stress is defined as biotic or abiotic environmental factors that limit the production of biomass (Grime 1977; Brooker et al. 2008). For example, in dry, stressful environmental conditions mature plant species may help maintain soil moisture and provide shade for other establishing seedling species, thus increasing their survival and rate of success (e.g. Svriz et al. 2013).

The stress-gradient hypothesis has been broadly applied to plant-plant interactions (reviewed by Brooker et al. 2008). However, only a handful of authors have examined interactions involving at least one animal species (Bertness et al. 1999; Kawai and Tokeshi 2007;

Daleo and Iribarne 2009; Bulleri et al. 2011; Travers et al. 2011; Barrio et al. 2012; Fugère et al. 2012; Bakker et al. 2013; Dangles et al. 2013). For the animal species in these studies, the definition and application of stress is dependent on the organism (Crain and Bertness 2006) and may depend on resources (e.g., food quality; Fugère et al., 2012) or environmental conditions (e.g., temperature; Kawai & Tokeshi 2007).

Facilitating species studied under the stress-gradient hypothesis are commonly ecosystem engineers: organisms that modify or create physical habitat that positively or negatively affects other species (Jones et al. 1994; Jones et al. 1997). For example, both plants and animals can influence resource availability such as water for other species through engineering. Plants may do this by increasing the retention of soil moisture which will positively impact neighbouring individuals of other species (Dohn et al. 2013). Animals can influence water availability by creating new habitat such as burrows that other species in the system may use as refugia (e.g. Pintor and Soluk 2006). As a more specific example, Travers et al. (2011) found that foraging activity by the Short-beaked Echidna (*Tachyglossus aculeatus*) improved soil quality which had a positive impact on a grass species growing under water stress.

As noted by Crain and Bertness (2006), the intensity and net impact of ecosystem engineers can vary over gradients of stress. In one study, the positive effects of goose barnacles (*Capitulum mitella*) on the survival of sessile mussels (*Septifer virgatus*) increased in intensity with higher levels of wave action and temperature (Kawai & Tokeshi 2007). Positive effects on the less stress-tolerant mussel species were due to amelioration of physical disturbance and thermal stress when growing in a patch mixed with the more stress-tolerant goose barnacles (Kawai & Tokeshi 2004).

3 Change in net pairwise species interactions

When describing the stress-gradient hypothesis, Bertness and Callaway (1994) predicted only a change in the frequency of positive and negative interactions. However, the hypothesis has commonly been interpreted in the literature as a change from a net negative effect of one species on another at a low level of stress to a net positive effect at a high level of stress (Maestre et al. 2009). For example Dohn et al. (2013) conducted a meta-analysis of many tree-grass interactions along a stress gradient of precipitation and found that interactions shifted from competition to facilitation with increasing stress. At high levels of stress the positive effect of the trees species on the grass, due to improved water resources, was greater than the negative effect due to competition for water and nutrients.

As another example of a deviation from the original hypothesis, Kikvidze et al. (2011) suggest that some authors have focused on a change in the intensity, or magnitude, of positive impacts. For example, Dangles et al. (2013) found that the intensity of the positive effect of one moth larvae (*Tecia solanivora*) on another (*Symmetrishchema tangolias*) increased with an increasing gradient of resource-based stress, although the positive effect peaked at intermediate levels of stress. An increase in the magnitude of a positive effect of one species on another with increasing stress could lead to a net change of a pairwise species interaction from negative to positive.

It is important to distinguish between the potentially positive or negative components of a pairwise species interaction and the net effect of one species on another. For example, in the Dangles et al. (2013) study, the two species of moth larvae that feed on potatoes have the potential to negatively impact each other through competition for a shared food resource. Competition is a potential negative component of the interaction. Ecosystem engineering is a

mechanism for a positive impact of *T. solanivora* on *S. tangolias* because *T. solanivora* burrows into potatoes with a thick epidermis which *S. tangolias* has difficulty penetrating. Therefore facilitation is a potential positive component of the interaction (Dangles et al. 2013). The net effect *T. solanivora* on *S. tangolias* will be determined by adding all positive and negative components of the pairwise species interaction. Interestingly, the authors of this paper never found a net negative interaction, but rather an increase in the magnitude of the positive effect of *T. solanivora* on *S. tangolias*.

In some animal-animal and animal-plant experiments, authors have observed a change from net negative to positive interactions over a gradient of stress. In a test conducted by Bulleri et al. (2011) in the intertidal zone, the net effect on macroalgae by tube-building gastropods (*Vermetus triqueter*) ranged from negative to positive with an increasing gradient of consumer pressure. For the low stress treatment, the pairwise interaction was negative-negative due to competition for space. At high levels of stress a net positive effect was due to protection of the macroalgae from other herbivores by the tube structures constructed by the gastropods. Similarly, Bakker et al. (2013) observed the effect of a generalist grazing snail (*Lymnaea stagnalis*) on a specialist grazing aquatic caterpillar (*Acentria ephemerella*) change from net negative to net positive over a nutrient concentration gradient, although the greatest positive effects were seen at intermediate levels of stress. The positive effect at intermediate stress was due to grazing by the snails on filamentous algae that compete for light with macrophytes, the main food source for the caterpillars. The net negative effect at low stress levels could have been due to competition for macrophytes, but the authors were unsure of the exact mechanism.

3.1 Positive effects of predators

It may seem counterintuitive that predators can positively impact their prey, but authors have found evidence for several mechanisms that produce this effect. For example, indirect effects such as intraguild predation may lead to a positive impact of a predator on prey. Wissinger and Mcgrady (1993) report that larval dragonflies which eat one another release other prey invertebrates from consumer pressure. However, this type of indirect effect will likely not lead to a net positive impact of the predator on the prey as negative trophic impacts will outweigh the indirect positive effects. Another mechanism via which predators may benefit their prey is through cultivation. For example, the cultivation of fungus by ant colonies such as in the system studied by Rodrigues et al. (2011) may result in a net effect of a predator on a prey species if cultivation benefits outweigh the predation costs for the fungi. Bichai et al. (2009) found that even direct consumption by a predator can have a positive impact on prey. By exposing nematodes fed *E. coli* bacteria to harmful UV radiation, the authors found that bacteria within the nematode gut were protected from 85% of the UV light. Viable bacteria cells survived in the nematode gut and therefore benefitted from being directly consumed by a predator when exposed to stress.

Ecosystem engineering by the predator is another mechanism through which a predator may benefit its prey (Crain and Bertness 2006). For example, burrowing is a common form of ecosystem engineering. Heemeyer et al. (2012) found that crawfish frog tadpoles (*Lithobates areolatus*) live in crayfish burrows, despite the risk of predation by crayfish. The burrows have a positive impact on tadpole survival by providing access to the water during stressful drought and frost conditions. As another example, burrowing owls (*Athene cunicularia hypugaea*, Bonaparte) have been found to live in abandoned badger burrows (Butts and Lewis 1982; Green and

Anthony 1989). Badgers are thought to be predatory on burrowing owls (Coulombe 1971; Desmond et al. 2000) and therefore, badgers could positively affect their owl prey through the mechanism of ecosystem engineering.

Although some predators have the potential to have a net positive impact on prey through ecosystem engineering, this benefit may only occur in certain situations. High levels of stress could provide the conditions necessary for positive impacts of engineering predators on prey to outweigh the costs of consumption. For example, if crayfish frog tadpoles are caught in stressful drought conditions, the presence of crayfish burrows may be necessary for their survival. If more tadpoles survive when crayfish are present than absent during drought, there would be a net positive impact of crayfish on the tadpoles. Under less stressful conditions the tadpoles may not use the crayfish burrows, and so the tadpoles would do better in the absence of crayfish. As a result, under low stress conditions, the benefits for the prey do not outweigh the costs due to predation. Therefore, the net impact of the predator-prey interaction on the prey species could depend on the level of stress in the system.

Daleo and Iribarne (2009) document such a change in the impact of a predator with a change in the levels of stress. In a manipulative experiment, the species of grass (*Spartina alterniflora*) produced more total biomass in the presence of the herbivorous crab (*Neohelice granulata*) in high stress experimental plots with naturally poor water drainage and low redox potential. In low stress experimental plots, the grass produced more total biomass in the absence of the crab. The positive impact of the crab on the marsh grass was due to burrowing that increased water drainage and soil aeration. The negative impact was due to herbivory by the crab on the grass. At low levels of stress, the net effect of the crab on the marsh grass was negative, while at high levels of stress the net effect was positive.

3.2 Predation to mutualism

Daleo and Iribarne (2009) demonstrated that the net effect of a burrowing crab on a plant species can change from negative to positive with increasing stress. They did not explicitly study the net effect of the plant species on the crab, although it was almost certainly positive because the herbivore would gain nutrients from consuming the plant. If the plant benefits the crab regardless of stress, this means the crab-plant interaction was positive-negative at low stress and positive-positive at high stress. To use pairwise species interaction labels, we could say the crab-plant interaction undergoes a stress-induced flip from predation to mutualism. For a stress-induced flip from predation to mutualism to occur, the predator must benefit its prey in some way, such as through ecosystem engineering, and the benefit must increase in magnitude or only exist when stress is high.

Daleo and Iribarne (2009) propose the question of whether the crab-plant pairwise species interaction was an isolated demonstration of a change in the net effect of a predator on a prey due to stress. This may not be true. For example, Hine's Emerald (*Somatochlora hineana*) larvae are eaten by a predatory, burrowing crayfish (*Cambarus diogenes*). Movement by the prey species could impact the net effect of the pairwise species interaction at high stress because the prey is capable of avoiding being consumed. The dragonfly larvae are very mobile and use the crayfish burrows to survive drought during their long, 4-year maturation period (Pintor and Soluk 2006; Kijowski 2014). Pintor and Soluk (2006) determined that this crayfish will prey on the dragonfly larvae in a laboratory experiment. Therefore the association between *S. hineana* and the burrowing crayfish has a negative trophic component through predation, in addition to the positive, non-trophic, effects of *C. diogenes* as an ecosystem engineer.

Whether the net impact of the crayfish on the *S. hineana* population is positive or negative is unknown and likely depends on environmental conditions such as the wetland water level. When flowing water is present in the wetland (low stress), *S. hineana* are found outside the burrows (Pintor and Soluk 2006), in which case the negative effects of predation may be more intense than any positive effects due to the burrows, leading to a net predatory relationship. In contrast, a low annual rainfall which frequently leads to seasonal drought conditions may result in a net mutualistic relationship because *S. hineana* larvae use crayfish burrows as refuge from desiccation and *C. diogenes* receives trophic benefits (Pintor and Soluk 2006). Therefore, the pairwise species relationship may change from predation to mutualism seasonally. However, conclusive data regarding a shift in the net effect of the species interaction would require manipulative experiments that may be damaging to the endangered *S. hineana* population.

4 A predator-prey model system

I identified a model system of predator and prey that may possess similar properties as the crayfish-dragonfly system and used it to test whether a predator-prey system could flip from predation to mutualism when stress is applied. Model systems are used in ecology to research concepts and hypotheses that are applicable to natural systems and often allow for extensive manipulations that may be otherwise be impossible or impractical, such as when studying an endangered species. Examples of model systems used to study ecological concepts include research about how evolution is directed by environmental conditions using island nematode species (Sommer and McGaughan 2013) and studying how diseases move between host species using a plant-fungus interaction (Antonovics et al. 2002). I used the nematode species *Caenorhabditis remanei* and its prey *Escherichia coli* bacteria as my model system (see Figure 1). Nematodes and bacteria are easy to obtain and culture in a laboratory setting making this

interaction a useful model system for determining if stress can change the net effect of predator-prey pairwise species interactions.

C. remanei consumes *E. coli*. This species of bacteria has been shown to be a suitable food source for nematodes in a laboratory setting (Stiernagle 2006). Some authors, however, have found that nematodes can also positively affect the bacteria that they feed on. In some cases, bacterial biomass or activity was higher in the presence of nematode species at low densities, since grazing by the nematodes kept population growth rate of the bacteria at a maximum level (Traunspurger et al. 1997; Fu et al. 2005). Evidence for a positive, non-trophic effect due to ecosystem engineering was suggested by Jensen (1996). He presented photographic evidence that nematode burrowing in soft agar lead to the inoculation of bacteria within the agar medium. The bacteria were probably spread from bacteria cells on the cuticle of the nematode or from viable cells excreted after consumption (Chantanao and Jensen 1969; Bichai et al. 2009). Therefore, there could be at least one negative and one positive impact of the nematodes on bacteria, similar to that of the crayfish-dragonfly pairwise species interaction.

I propose that the net effect of the nematodes on *E. coli* will depend on the magnitude of the positive effect of ecosystem engineering and the negative effect of nematode predation which can change depending on the level of stress in the system. For example, if an abiotic stress was applied to the surface of the agar that the bacteria grows on, nematodes could benefit their prey by inoculating the bacteria into burrows below the surface of the agar which would provide refugia from stress. A net positive effect of nematodes on bacteria could result if the benefit due to refugia when stress is applied outweighs the negative effect due to predation. Under non-stressful conditions, the negative effect of predation might outweigh the benefit from bacteria

being spread into burrows in the agar, leading to an overall net negative effect. Thus the pairwise species interaction would flip from predation (-/+) to mutualism (+/+).

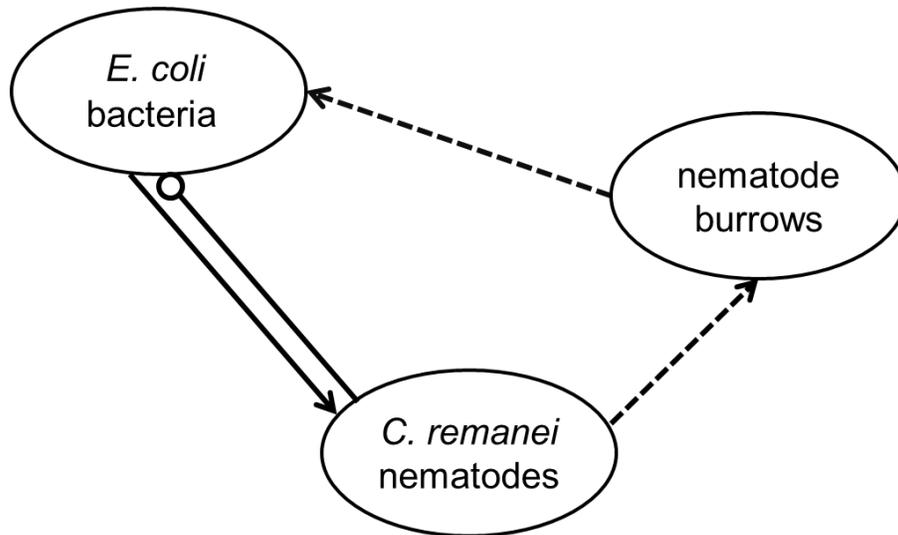


Figure 1: An interaction diagram for *E. coli* and *C. remanei*. Positive effects are shown by arrows and the negative effect of nematodes on bacteria through consumption is shown by the line ending in a circle. The dashed arrows indicate the non-trophic, positive effect of nematodes on *E. coli* by creating burrows which may then provide refuge for the bacteria from stress. The net effect of the nematodes on bacteria will depend on the magnitude of the negative trophic effect and the magnitude of the positive effect due to burrow refugia.

I predict that under highly stressful conditions, the population of *E. coli* will be larger when *C. remanei* are present. The benefit will occur because nematodes burrows will provide the bacteria with refuge from abiotic stress applied to the surface of the agar. Under low stress conditions, I predict that the *E. coli* population will be larger when *C. remanei* are absent because of nematode predation. Thus I predict that the net impact of *C. remanei* on *E. coli* will be negative under low stress and positive under high stress. If we assume that the net impact of

E. coli on *C. remanei* is positive at high and low stress, then this means that the pairwise species interaction could change from predation to mutualism when stress is high due to the mechanism of ecosystem engineering.

5 Applications and conclusions

The net effect and intensity of pairwise species interactions may change over gradients of stress. Researching how stress impacts predator-prey dynamics will be important as our environment changes, leading to stressful conditions for species globally (He et al. 2013). He et al. (2013) conducted a meta-analysis of plant-plant interactions at a global scale and found evidence that pairwise species interactions become more positive with increasing stress. Whether animal-animal or animal-microbial interactions follow a similar pattern predicted by the stress-gradient hypothesis is not currently known.

A change in pairwise species interactions over stress-gradients has important implications for endangered and rare species conservation. Crain and Bertness (2006) proposed that species may be facilitated by ecosystem engineers under stressful environmental conditions that they would otherwise not survive. These beneficial engineering species may be predators of species targeted for management. For example, the endangered Hine's Emerald dragonfly may have an obligate mutualistic relationship with a predatory crayfish under stressful conditions which means a decrease in one will likewise affect the other. A traditional, one-species focused management plan might have been to remove the crayfish, which may have a net negative impact on the survival of the dragonfly under stressful conditions (Pintor and Soluk 2006). Ecosystem engineers may prove to be the key to species survival under changing environmental conditions even if the species they benefit is typically considered a prey.

More generally, the stress-gradient hypothesis as applied to animal-plant or animal-animal interactions will likely be a growing area of research in ecology. We lack a unified conceptual framework for predicting changes in the intensity and net effect of pairwise species interactions over gradients of stress (He and Bertness 2014). Studies that include an animal species may help clarify the current conceptual model of the stress-gradient hypothesis. There are parallels with the plant literature, in that authors have observed a change in pairwise species interactions from competition to facilitation over a gradient of stress. Predation presents a trophic component that is not observed in plant-plant interactions and predatory ecosystem engineers may allow a flip in pairwise species interaction from predation to mutualism. To my knowledge only one demonstrated example of this flip exists in the stress-gradient hypothesis literature (see Daleo and Iribarne 2009). Pintor and Soluk (2006) demonstrated the necessary positive and negative effects of crayfish on dragonflies in their study but did not frame their results in the context of the stress-gradient hypothesis. In this review, I proposed another system that has not yet been studied in the context of the stress-gradient hypothesis. This system is the pairwise species interaction between nematodes and *E. coli* bacteria which may serve as a highly manipulative and representative model system for how stress influences predator-prey dynamics.

Chapter 2

A model system for stress-induced positive impacts of a predator on prey

1 Introduction

The stress-gradient hypothesis was formulated to describe plant-plant interactions over a gradient of stress. Bertness and Callaway (1994) theorized that in low stress environments, interactions between plant species tend to be negative but in high stress environments, interactions tend to be positive. A few authors have applied these ideas to animal-plant or even animal-animal interactions (Bertness et al. 1999; Kawai and Tokeshi 2007; Daleo and Iribarne 2009; Bulleri et al. 2011; Travers et al. 2011; Barrio et al. 2012; Fugère et al. 2012; Bakker et al. 2013; Dangles et al. 2013) and have observed changes in the intensity or net effect of one species on another through the mechanism of ecosystem engineering.

Ecosystem engineering occurs when a species modifies the physical environment in a way that impacts other species (Jones et al. 1994). The intensity or net effect of ecosystem engineering on another species is context dependent, and may vary according to the level of stress in a system (Crain and Bertness 2006). For example, a positive ecosystem engineering effect on a species may become more important when the species is exposed to stressful conditions. Dangles et al. (2013) found that the net positive impact of one species of moth larvae on another increased as their shared food resource, potatoes, became more difficult to consume with increasing potato epidermis thickness. Both moths could feed easily on potatoes with thin epidermises but when the epidermis was thick, one moth species positively impacted the other through ecosystem engineering because it chewed through the potato, which then allowed the other moth species to feed. However, the positive impact did decrease in magnitude at the highest levels of stress. This change in the magnitude of the net effect of one species on another

due to stress is predicted by authors who study the stress-gradient hypothesis (Maestre et al. 2009; Kikvidze et al. 2011).

Stress can also change the net effect of one species on another from negative to positive (see Daleo and Iribarne 2009; Bulleri et al. 2011; and Bakker et al. 2013). Stress could even cause the net effect of a predator on a prey to change from negative to positive if two conditions are met. First, the predator must have a positive impact on a prey such as through ecosystem engineering that provides refuge from harsh environmental conditions. Second, when exposed to environmental stress, the positive impact of the predator on the prey must be greater than the negative impact of consumption. For example, there is a species of dragonfly larvae that are thought to use the burrows of predatory crayfish as protection from desiccation. The larvae are found in higher densities in crayfish burrows during stressful low water conditions than in non-stressful high water conditions (Pintor and Soluk 2006). Therefore, the predator may benefit its prey through ecosystem engineering and under stressful environmental conditions this benefit may be greater than the negative impact due to consumption. Under non-stressful conditions the net impact of the predator on the prey may be negative due to consumption. Therefore, the net effect of the predator on the prey could change from negative to positive with increasing stress.

I am aware of only one study which demonstrates a change in the net effect of a predator on a prey due to ecosystem engineering. In a field experiment, Daleo and Iribarne (2009) found that the net effect of a burrowing crab on its marsh grass prey was negative at low stress but positive at high stress. The net positive effect at high stress was due to burrowing which improved substrate quality for the plant on high stress, poor substrate sites. The authors describe the change in net effect of the crab on the marsh grass as a change from herbivory to facilitation due to increasing stress (Daleo and Iribarne 2009).

Studying the stress-gradient hypothesis in microbes was suggested by He and Bertness (2014) and to my knowledge, no study of the stress-gradient hypothesis has been done on the interaction between two microorganisms. In addition, using species that can be easily cultured in an isolated two-species system will help refine our understanding of the mechanisms involved in stress-induced changes in predator-prey dynamics. For example, the number of predators introduced to the system can be carefully controlled compared to a field study such as that done by Daleo and Iribarne (2009) where predators were either excluded or not from experimental plots but were not themselves counted at any point during the experiment. Variation in the number of predators could impact the measured net effect of the predator on the prey. Studying two microorganisms in the context of the stress-gradient hypothesis will also increase our understanding of how broadly applicable the stress-gradient hypothesis is outside of the plant literature.

I conducted a laboratory study using a burrowing nematode (*Caenorhabditis remanei*) and its prey bacteria (*Escherichia coli*) to determine if the net effect of a predator on its prey can change from negative to positive through the mechanism of ecosystem engineering when stress is applied to the system. The nematode is an ecosystem engineer that can create burrows in an agar substrate (Figure 2). As the nematodes burrow, they spread *E. coli* by passing viable cells in their excrement (Chantanao and Jensen 1969; Bichai et al. 2009) and may transfer cells into the burrows. These burrows may provide refuge for *E. coli* when an abiotic, disturbance stress is applied to the agar surface. Therefore, the nematode has the potential to negatively impact *E. coli* through consumption and positively impact *E. coli* through ecosystem engineering.



Figure 2: A photograph of burrows made by *C. remanei* nematodes in agar (left image) compared to a photograph of agar without nematode burrows (right image).

The net effect of the nematodes on *E. coli* likely depends on the level of stress. At low stress the net effect of *C. remanei* on *E. coli* is probably negative since there is no benefit to the bacteria due to nematode burrowing. Under high stress the net effect may be positive if the benefit due to refugia outweighs the negative effect of consumption. The net effect of *E. coli* on nematodes is positive at both high and low stress because nematodes grow poorly when cultured in the absence of a bacterial food source (Avery 1993; Stiernagle 2006). Therefore the pairwise species interaction may change from predation (+/-) at low stress to mutualism (+/+) at high stress.

To study the effects of stress on the nematode-bacteria interaction, I cultured the bacteria in the presence and in the absence of their nematode predators at high and low stress. In addition, to determine if ecosystem engineering affected bacteria, rather than some other action of the nematodes, I compared treatments where nematodes were able to burrow and where they could

not. I predicted that for low stress, there would be more prey when predators were absent than when they were present which would indicate a net negative impact of the predator on prey. I also predicted that the application of stress would decrease the prey population. When stress was applied, I predicted that there would be more bacteria in the absence than the presence of predators unable to engineer the environment. However, if the predators were able to engineer the environment, I predicted that the prey population would be larger when predators were present than when they were absent. This would indicate a net positive impact of the predator on prey with the application of stress through the mechanism of ecosystem engineering. To summarize, I hypothesized that net effect of the predator on prey changes from negative to positive with the application of stress and that ecosystem engineering is the mechanism for the positive effect of the predator on prey (see Figure 3).

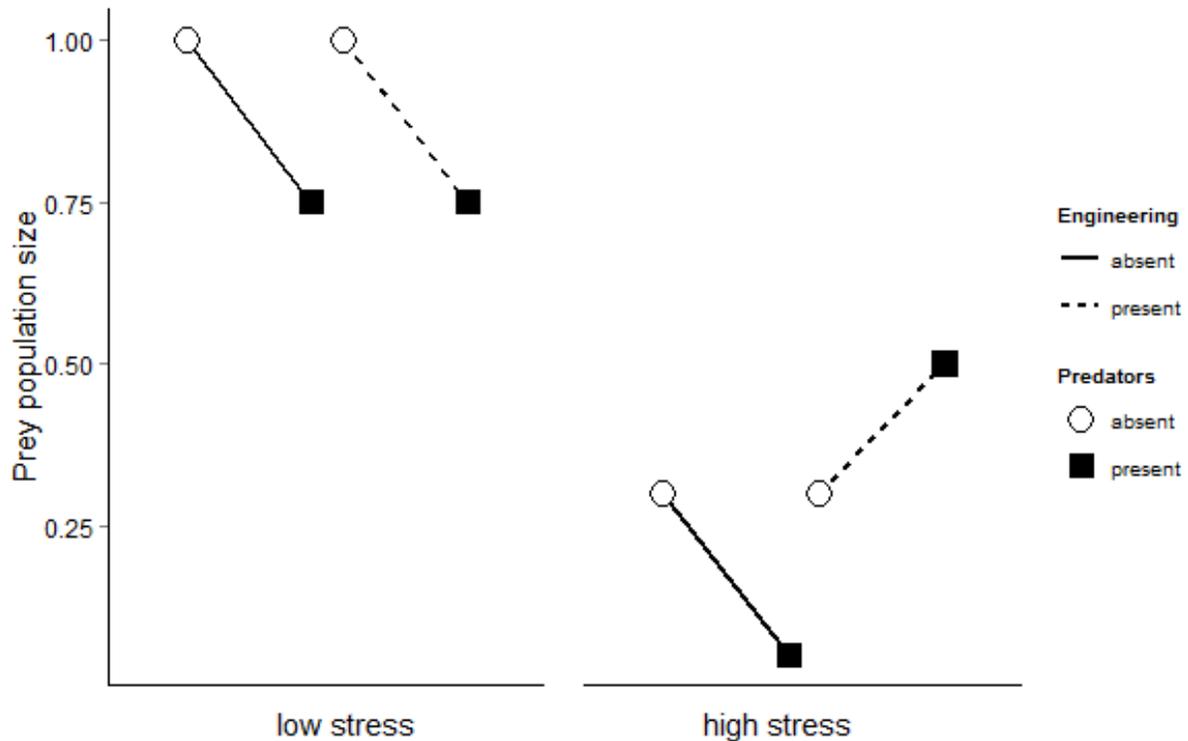


Figure 3: A representation of the predicted experimental outcomes when a predator is able to benefit its prey through ecosystem engineering. Predictions in the absence of predators are represented by hollow circles and solid squares indicate that predators are present. Predictions in an environment that allows predator ecosystem engineering are connected by dashed lines and solid lines connect predictions in the absence of ecosystem engineering. The labels “low stress” and “high stress” indicate the level of stress in the system. For low stress conditions, I predicted that the prey population would be lower in the presence of predators (net negative effect of the predator on prey). For high stress conditions, I predicted that the prey population would also be lower in the presence of predators, but only if there was no ecosystem engineering (net negative effect of the predator on prey). When ecosystem engineering was present and stress was high, I predicted that the prey population would be higher in the presence of predators (net positive effect of the predator on prey).

2 Materials and Methods

2.1 Study organisms

The nematode species *Caenorhabditis remanei* and *Escherichia coli* strains were provided by the Caenorhabditis Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

2.1.i *Caenorhabditis remanei*

The predator that I used in this study, *C. remanei*, is a nematode species with similar biology to the more well-known hermaphroditic *Caenorhabditis elegans*. This species was selected as a predator because it has obligate sexual reproduction between dioecious individuals, which by simple segregation of male and female individuals, allows me to use a population of nematodes that will not increase over the duration of an experimental procedure. In addition to obligate sexual reproduction, *C. remanei* has double the reproductive lifespan and higher lifetime fecundity than *C. elegans* (Kiontke and Sudhaus 2006; Diaz et al. 2008). The lifecycle of *C. remanei* begins when eggs are laid by a female, followed by four molting stages before individuals reach sexual maturity. *C. remanei* has a short generation time when cultured at 20°C with time to maturation averaging 1.25 days after hatching (Diaz et al. 2008). The lifespan of a female is approximately 16 days (Diaz et al. 2008) but may be extended if the nematode enters the dauer stage after the second molt. The dauer stage is induced by stressful conditions such as a lack of food in a stock culture. No development occurs in the dauer stage (Cassada and Russell 1975).

Nematodes are cultured on bacterial lawns of *E. coli* (Kiontke and Sudhaus 2006). This genus of nematode is capable of clearing a bacterial lawn of *E. coli* from a Petri plate (Stiernagle 1999), demonstrating their efficiency as predators on bacteria. In fact, if nematodes are left for

too long on a Petri plate, the population will become starved and dauer larvae will form (Stiernagle 2006; Hu 2007).

Nematodes were maintained on Nematode Growth Medium (Lewis and Fleming 1995, Stiernagle 2006). The culture was maintained by moving a mixed population of nematodes from aged plates to ones freshly inoculated with *E.coli* OP50-GFP (Labrousse et al. 2000). The plates were bleached periodically (approximately every two weeks) to remove contamination (culturing methods adapted from Stiernagle 2006).

2.1.ii *Escherichia coli* (OP50-GFP)

Cultures of nematodes are typically fed *E. coli* OP50 which is a Gram-negative, rod shaped bacteria that can be grown easily in the lab. It is a facultative anaerobe which means that it can grow in the presence or absence of oxygen but grows better when oxygen is present. I used the strain *E. coli* OP50-GFP for this experiment (Labrousse et al. 2000). OP50-GFP has been modified to include a green fluorescent protein (GFP) plasmid (pFPV25.1, Valdivia and Falkow 1996). The green fluorescent protein increased the ease of counting colony forming units (CFU) when determining the amount of bacteria present in each treatment. It also allowed for a second measure of bacterial abundance through the use of fluorescent photographs.

E. coli OP50 is a uracil auxotroph, meaning that it cannot synthesize its own uracil and requires a medium that contains uracil for growth, such as Nematode Growth Medium (Brenner 1974; Stiernagle 2006). *E. coli* OP50 has limited growth on Nematode Growth Medium which produces a transparent bacterial lawn that aids in the visualization of nematodes during transfer (Brenner 1974). OP50-GFP is ampicillin resistant, which allowed me to use this antibiotic as well as the anti-fungal agent nystatin in the agar formulation to discourage the growth of other bacterial and fungal cells.

2.2 Experimental design

To test the hypothesis that the net effect of a predator on prey can change from negative to positive with increasing stress, I determined the net effect of the predator *C. remanei* on prey *E. coli* for two levels of stress. All of the treatments had *E. coli* inoculated on an agar surface in a cuvette. To determine the net effect of nematodes on *E. coli*, I used one set of treatments that had nematodes applied to the agar surface and one set of treatments that did not have nematodes applied to the agar surface. Nematodes will engineer burrows if the surface of the agar has been pierced (Stiernagle 2006). I therefore used one set of treatments with agar modified by piercing and one set without modification to test the hypothesis that nematode burrowing is the mechanism for a positive effect of nematodes on *E. coli* at high stress. To determine if the net effect of nematodes changed with the application of stress, one set of treatments included the application and subsequent removal of a filter paper to the agar surface. This procedure physically removed bacteria. Therefore, the experiment had a three-way factorial design for factors nematode presence or absence, modification of the agar by piercing or unmodified agar as a control, and the application of a filter paper as stress (high stress) or no such application (low stress) for a total of eight treatments (see Table 1).

Table 1: A table of the eight experimental treatments for factors nematodes, agar, and stress. All factors have two levels which are nematodes absent or present, control agar with no modification to allow ecosystem engineering or agar modified to allow ecosystem engineering, and low or high stress.

Treatment	Nematodes	Agar	Stress
1	absent	control	low
2	absent	modified	low
3	present	control	low
4	present	modified	low
5	absent	control	high
6	absent	modified	high
7	present	control	high
8	present	modified	high

Implementation of the experimental design required first inoculating agar-filled spectrofluorometry cuvettes with bacteria, allowing the bacteria to increase, and then adding nematodes to the cuvettes. After allowing time for the nematodes to burrow in treatments with ecosystem engineering, I applied stress to the cuvettes. After a recovery period, I processed the cuvettes to determine the amount of bacteria in each one. Below I describe each step of the methods in more detail (for highly detailed methods see:

http://ecothery.uwaterloo.ca/labwiki/index.php?title=Laura%27s_Methods).

2.2.ii Applying prey *E. coli* to agar filled cuvettes

To inoculate the cuvettes used as experimental replicates with *E. coli*, I created a standardized *E. coli* culture using a sterilized culture tube (16 mm by 150 mm) filled with 10 ml of sterilized broth containing 0.1 mg/ml of ampicillin. The sterile broth was inoculated with *E. coli* OP50-GFP and shaken for 18-20 hours at 37°C on a shaker (Heidolph Rotamax 120) at 175

revolutions per minute. The *E. coli* culture was standardized to an absorbance of 0.800 +/- 0.01 before applying it to the cuvettes.

I filled cuvettes with 2.5 ml of 1.0% agar Nematode Growth Medium (recipe similar to Lewis and Fleming 1995, Stiernagle 2006) using a peristaltic pump under a flowhood (ESCO Laminar Flow Cabinet). Tightly sealed plastic caps (Fisher Scientific) prevented contact with airborne contaminants and were placed on the cuvettes at all times during the experiment unless otherwise mentioned. Cuvettes were stored overnight for 20-24 hours at 20°C. I then removed the cuvette caps under the flowhood and inoculated the center of the agar surface in the cuvettes with 10 µl of standardized OP50-GFP *E. coli* culture in an approximately 3.5 mm radius circle. The *E. coli* was not spread to the edges of the cuvette to discourage nematodes from climbing the sides of the cuvette and to prevent *E. coli* from seeping down between the agar and the cuvette wall.

2.2.iii Applying nematode predators

Feeding behaviour of nematodes on bacteria has implications for my experimental design and has been studied in the similar nematode species *C. elegans* (Avery and You 2012). The pharynx, a muscular pump located at the nematode's anterior opening, grinds bacteria which are then moved to the intestine of the animal. Hungry nematodes will seek food by exploring their growth media while satiated nematodes do not. If I had used nematodes that were satiated in my experiment, they might not have burrowed throughout the agar medium as desired. Therefore I selected nematodes to use for the experiment from one week old nematode stock plates that were initially inoculated with 50 females. These plates were approaching overpopulation and had a bacterial lawn reduced by prior nematode feeding.

I added nematodes to half of the treatments 24 hours after the inoculation of bacteria. For modified agar treatments, I pierced the center of the agar to a depth of 1 mm once prior to adding 7 male nematodes using a flame sterilized platinum wire spatula. Transfers were made with care to make sure no punctures were made in the agar because such breaks in the surface will allow nematodes to burrow. For treatments without nematodes, I touched the spatula to the agar surface of the cuvette three times to mimic the disturbance of the bacterial lawn due to nematode transfer. For all treatments, I unsealed the cuvette caps for 40 minutes while transferring the nematodes. Nematodes were left at 20°C to burrow and interact with the bacteria for 96 hours.

2.2.iv Applying stress to the cuvette agar surface

After 96 hours, I placed a sterilized square filter paper (1 cm by 1 cm) on the agar surface and then removed it after 30 minutes for the high stress treatments. This abiotic, disturbance stress resulted in the removal of bacteria, and may also have removed nematodes from the cuvette. For all treatments, I removed the cap of the cuvette for 5 minutes under the flow hood whether stress was applied or not. After the filter paper was removed, cuvettes were recapped and stored in the 20°C incubator for 24 hours.

2.3 Estimating bacteria population using colony plate counts

I used the colony plate count method to estimate the amount of bacteria in each cuvette at the end of the experiment. The plate count method uses a diluted sample containing viable bacteria to grow colonies on a Petri plate filled with agar (Koch 1994). I counted plates with colonies within the range of 30-300 and used these counts to estimate the amount of bacteria colony forming units per ml of agar in each cuvette.

In order to conduct a colony plate count, I had to homogenize the agar in the cuvettes and perform a dilution series on a sample of the homogenized media. The cuvettes were homogenized using 1.0 ml of sterile saline solution (0.9%), 7 glass beads, and a vortex mixer. Chantanao and Jensen (1969) similarly crushed agar and added saline before performing an *E. coli* colony plate count. I then removed aliquots from each cuvette and performed a dilution series. The dilution series was done aseptically in glass culture tubes (16 by 150 mm) containing sterile saline solution. The culture tubes were vortexed between dilutions to ensure even distribution of bacteria. Dilutions were plated under the flow hood by adding bacteria to Petri plates (100 by 15 mm) containing 15 ml of 1.5% Luria agar (Bertani 1952; Gerhardt et al. 1994). After the plates had solidified they were inverted and incubated at 30°C for 23 hours.

The initial dilution of the series was dependent on the amount of agar in each cuvette which might have varied by treatment, particularly if the application of stress removed agar from the cuvette. Therefore, I weighed the agar in each cuvette after the application of stress and used this value to calculate the initial dilution by replicate. The initial dilution then, assuming a 1:1

ratio of agar weight to volume, was $\frac{\text{agar weight (g)}}{(\text{agar weight (g)} + 1)}$.

I photographed each replicate plate in quarters using a fluorescent microscope to help ensure that the colonies were the OP50-GFP bacteria which were initially inoculated and not another contaminating species. I counted the number of colony forming units using a cell counter plug-in for the computer program ImageJ (De Vos 2010; Rasband 2014). I then added the counts for the four pictures per replicate to get a final number of colony forming units per plate. Finally, I used the following formula to estimate the number of colony forming units per ml (CFU/ml) for

each replicate: $\text{CFU/ml} = \frac{\text{CFU/plate}}{\text{dilution} * \text{amount plated}}$.

2.4 Estimating bacteria population using fluorescence

Fluorescent photographs were taken at three points during the experiment, one day after the *E. coli* culture was initially inoculated on the cuvettes, four days after nematodes were inoculated on the cuvettes, and just prior to homogenization of the cuvettes. The first set of pictures was taken to detect irregularities in the cuvettes such as bubbles in the agar, *E. coli* spread out over the agar surface, or punctures in the agar. Such cuvettes were removed from the experiment. The second set of pictures was taken to identify burrowing by nematodes in the agar control treatments, which should not have included such activity. The third set of photos was taken to determine the effects of nematode presence, agar modification, and the application of stress on the amount of bacterial fluorescence in the cuvettes.

Each cuvette was photographed twice, once on each clear side of the cuvette using a microscope with a UV light (x-cite Q 120, Lumen Dynamics), a filter for the green fluorescent protein (470 nm), and a camera attachment (AxioCam MRc). The cuvette was positioned so that the bottom of the agar was aligned with the left edge of the photograph and the photographs were taken with 4.8 times magnification and a 48.0 mm field of view.

To estimate the amount of fluorescence in the cuvette photographs, I first converted the photographs to 8-bit greyscale images. I then used the software ImageJ to select a threshold pixel brightness. The threshold was set using the method Renyi Entropy (Kapur et al. 1985) from a histogram of all images. I used ImageJ to measure the percent of the area of each image that was above the threshold pixel brightness and used the mean value of the two photographs per cuvette as the final estimate of fluorescence in each cuvette.

2.5 Estimating variation in the number of nematodes

I counted nematodes in the nematode present treatments before applying stress and I counted nematodes in the high stress, nematode present treatments after the application of stress. The nematodes were counted using a dissecting microscope. I counted nematodes before applying stress to determine how many nematodes had crawled up on the sides on the cuvettes, where I had observed them desiccating and dying in preliminary trials of the experiment. This count was taken to ensure that the number of nematodes on the sides of the cuvette was not a large fraction of the total number of nematodes inoculated in the cuvette. I counted nematodes after applying stress in high stress, nematode present treatments to determine how many nematodes remained in the cuvettes following the application of stress. This count was taken to determine if the number of remaining nematodes differed between control and modified agar treatments, because nematodes may have been protected from the application of stress by the burrows that they created in modified agar.

2.6 Time blocking and trials

Each of the previously described experimental steps (summarized in Figure 4) took time. I was only able to complete a total of 64 replicates (8 per treatment) during a 9 day experiment. Therefore the replicates were completed in one of 4 trials from January 5-13, January 20-28, February 4-12, and February 18-26, 2015 for a total of 32 replicates per each of the 8 treatments.

In addition, the steps in each trial took time, for example homogenizing the cuvette agar took approximately one hour to process eight replicates. Therefore it could take up to eight hours to complete one experimental step of one trial. The *E. coli* could increase significantly over an eight hour period which would increase the variation between treatments (Shtonda and Avery 2006; Virk et al. 2012). Therefore replicates were assigned to one hour time blocks with one

replicate of each treatment in each block for a total of eight blocks per trial (32 blocks in the experiment). For each block, I assigned replicates to one of eight treatments using a random number generator, and processed the replicates in the same order for the duration of the experiment.

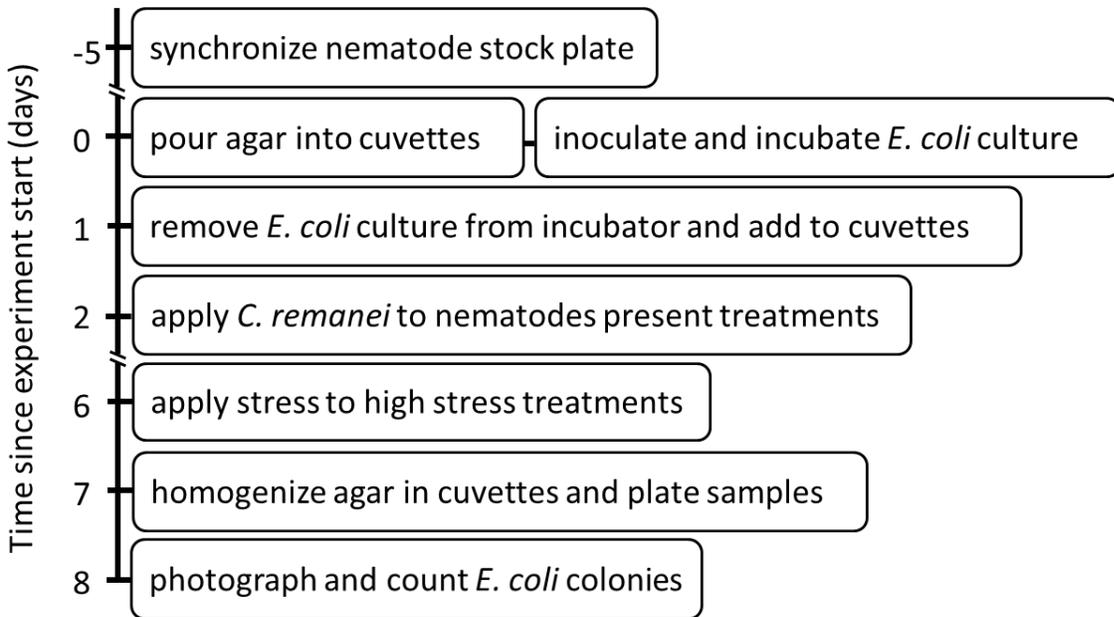


Figure 4: Chart showing main steps of the experimental procedure by day. A stock plate was created one week before male nematodes were moved to create an overcrowded plate. Cuvettes were filled with agar and inoculated with bacteria the following day. Bacteria grew for one day before nematode predators were introduced. Nematodes interacted with the bacteria for four days before stress was applied. The bacteria recovered for one day and then the agar in the cuvettes was homogenized so that dilutions of bacteria could be plated and grown overnight. The fluorescent *E. coli* colony forming units were photographed and counted to estimate the amount of bacteria in each treatment replicate.

2.7 Statistical analyses

After testing the colony plate count data for normality using the Shapiro-Wilk test and homogeneity of variance using the Bartlett test, I constructed a generalized linear model to compare *E. coli* colony forming units between treatments. The colony plate count data were log-normal transformed to obtain normal model residuals. The model was analyzed using the statistical program R (R Core Team 2013) and included the factors nematodes, agar, and stress with blocking by the four trials. I also compared fluorescence between treatments but blocked the data using the 32 time blocks and used a square root transformation. To compare the number of nematodes remaining after stress I used a one factor linear model that was also blocked by the four trials. Treatment means were compared using Tukey's Honestly Significant Difference.

3 Results

Analysis of both colony forming units and cuvette fluorescence suggest that the net effect of nematodes on bacteria was positive when stress was applied to the system. The analysis of colony forming units indicates that this net positive effect under stress was present regardless of the ability of nematodes to engineer the agar, while the analysis of the fluorescence data indicates that the nematodes only positively impacted bacteria when ecosystem engineering was present. There was no net effect of nematodes on bacteria at low stress according to the colony forming unit analysis while the fluorescence data suggested that nematodes positively impacted bacteria at low stress.

3.1 Data cleaning

Some replicates were removed prior to the analysis. First, I removed replicates that had one or more contaminant spores visible in the cuvette agar for all of the statistical analyses.

Second, for the statistical analysis of the plate count estimates of *E. coli*, I removed replicates where the number of colony forming units on all counted Petri plates was outside of the range 30-300. Third, the fourth time block of the first trial was removed from the statistical analysis of the plate count estimates of *E. coli*. The mean number of colony forming units per ml for this block is more than two standard deviations less than the mean for all blocks. In addition, I believe that the initial dilutions during the homogenization experiment for this block received a 50 ul aliquot from the cuvette rather than the 100 ul aliquot required by the experimental design. Finally, one outlier replicate for the treatment where nematodes were present on control agar under high stress was removed from the fluorescence data analysis because it was more than two standard deviations above the treatment mean and model residuals were normal after it was removed (fluorescence of removed replicate = 0.9% of image above threshold, mean fluorescence of treatment = 0.1% of image above threshold). The number of remaining replicates per treatment ranged from 23 to 29 for the plate count estimates of *E. coli* and 26 to 29 for the agar fluorescence estimates of *E. coli*.

3.2 Predator and stress effects on *E. coli* population

Analysis of the log transformed number of colony forming units per ml of agar in the cuvettes indicated there were significant impacts of nematodes and stress on *E. coli* (generalized linear model, Figure 5, $F_{10, 201} = 475.3$, $p < 0.05$). Data were transformed using the natural logarithm to normalize model residuals. There was a significant three-way interaction between the factors nematode presence, agar modification, and stress application when the data were blocked by trial ($F_{10, 201} = 10.3$, $p < 0.05$).

As was expected, stress had a significant negative effect on the number of colony forming units per ml of agar when there were no nematodes. Stress significantly decreased the

number of *E. coli* colony forming units (Tukey HSD, mean = 3.2×10^9 and 5.6×10^8 *E. coli* colony forming units for low stress and high stress, respectively, Figure 5).

Unexpectedly, a Tukey's comparison of means indicated there were no differences between the number of colony forming units with and without nematodes for all four low stress treatments, which indicates that nematode presence had no net negative impact on *E. coli* under low stress. Under high stress conditions, there was no difference in treatments where nematodes were absent (mean = 3.3×10^8 and 3.1×10^8 *E. coli* colony forming units for control agar and modified agar, respectively). However, the means of these treatments but were both significantly lower than treatments with stress and nematodes (mean = 8.7×10^8 and 1.0×10^9 *E. coli* colony forming units for control agar and modified agar, respectively). Therefore, ecosystem engineering may have been the mechanism for this positive impact of nematodes on *E. coli* under high stress. However, nematodes in an environment that did not allow ecosystem engineering still had a lesser, but significant positive impact on *E. coli* under high stress.

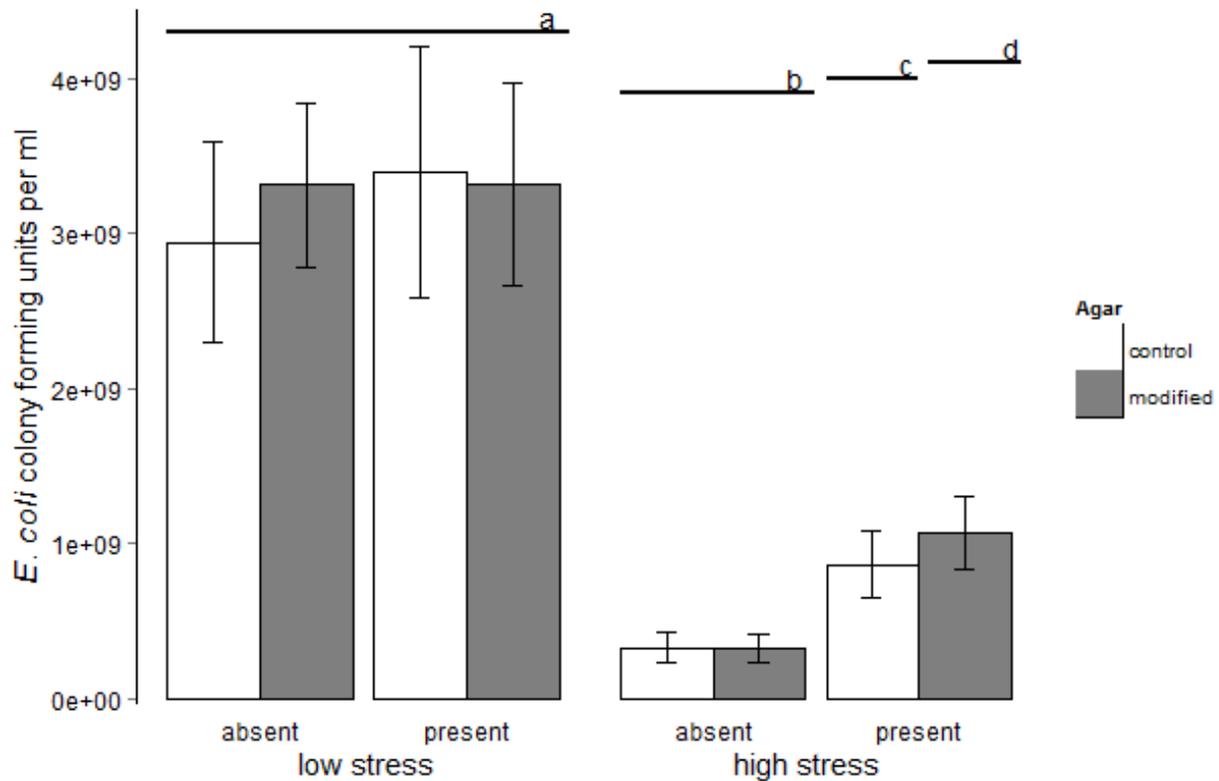


Figure 5: Mean number of *E. coli* colony forming units per ml of agar with error bars showing +/- one standard deviation. Modified agar that allows ecosystem engineering is represented by shaded bars. The labels “absent” and “present” indicate if nematodes were added, while “low stress” and “high stress” indicate if stress was applied in the form of a physical disturbance to the agar surface. Treatments with shared letters do not differ significantly from each other as indicated by a Tukey’s comparisons of means.

There were also significant impacts of nematodes and stress on cuvette agar fluorescence (generalized linear model, Figure 6, $F_{38, 186} = 121.2$, $p < 0.05$). The data were transformed using a square root transform to normalize model residuals, however the variance remained heterogeneous (Bartlett test, Bartlett's $K^2 = 80.1$, $p < 0.05$). Similar to the colony plate count estimates of *E. coli*, there was a significant three-way interaction between the factors nematodes

presence, agar modification, and stress application ($F_{38, 186} = 33.4$, $p < 0.05$), and a significant negative effect of stress (see Figure 6, Tukey HSD, $p < 0.05$).

Contrary to the plate count estimates of the *E. coli* population, a Tukey's comparison of means indicated that nematodes had a significant positive impact on cuvette agar fluorescence under low stress when ecosystem engineering was present and when it was absent, although fluorescence was significantly higher when ecosystem engineering was present (mean = 1.5% and 2.8% of image above threshold pixel brightness for nematodes present on control and modified agar, respectively, compared to mean = 0.6% of image above threshold for nematodes absent on both control and modified agar).

Treatments with agar modification but no nematodes had higher image fluorescence than control cuvettes when stress was applied (mean = 0.1% and 0.2% of image above threshold for control and modified agar, respectively). When nematodes were present, but unable to burrow, the image fluorescence was smaller than any other treatment (mean = 0.04% of image above threshold). In contrast, when nematodes were allowed to create burrows, they had a significant positive impact on fluorescence (mean = 1.1% of image above threshold), which may indicate that ecosystem engineering was the mechanism for the positive impact of nematodes on bacteria. In the absence of stress, fluorescence was 4.7 times higher on modified agar when nematodes were present compared to when they were absent. In the presence of stress, fluorescence was 5.5 times higher on modified agar when nematodes were present compared to when they were absent.

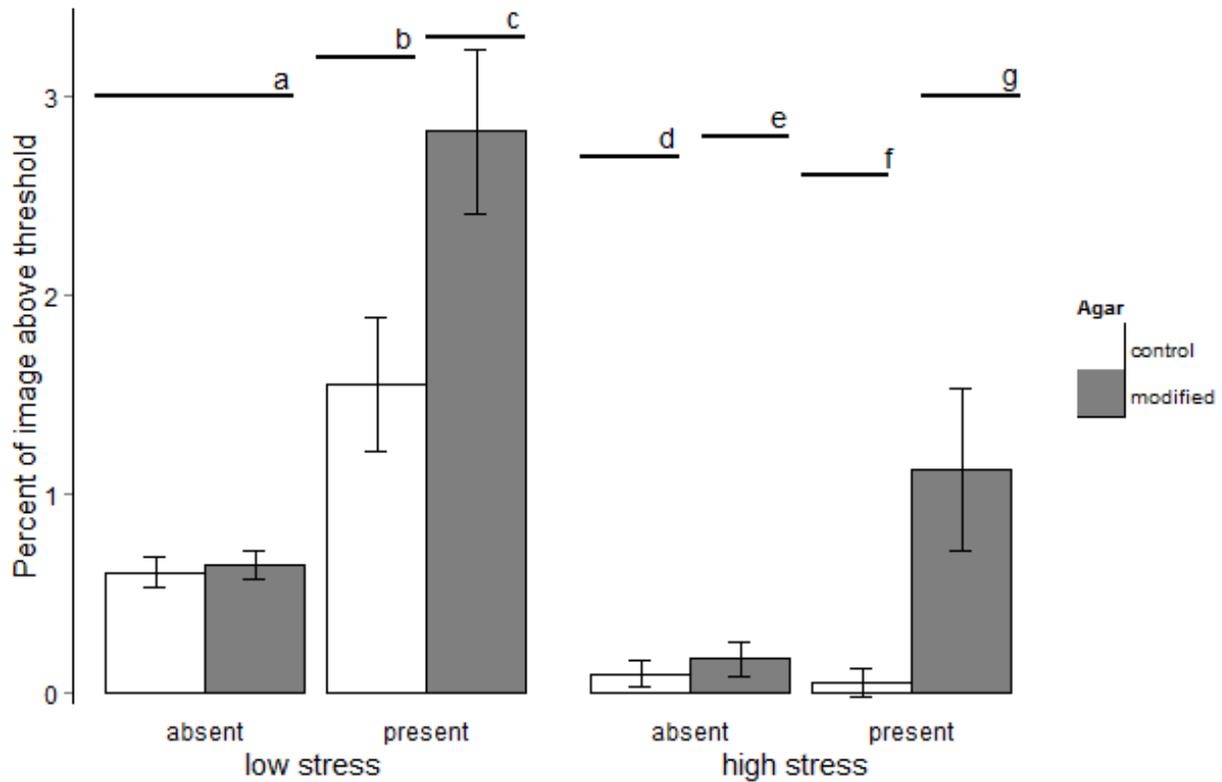


Figure 6: Mean percent of cuvette image with fluorescence above threshold pixel brightness with error bars showing +/- one standard deviation. Modified agar that allows ecosystem engineering is represented by shaded bars. The labels “absent” and “present” indicate if nematodes were added, while “low stress” and “high stress” indicate if stress was applied in the form of a physical disturbance to the agar surface. Treatments with shared letters do not differ significantly as indicated by a Tukey’s comparison of means.

3.3 Treatment effects on nematode number

There may have been an impact of treatment on the number of nematodes present in each cuvette. There was little variation in the number of nematodes that crawled up the sides of the cuvettes during the experiment before the application of stress (68% of the replicates had zero nematodes on the side of the cuvette). However, the number of nematodes observed after the

application of stress differed significantly among treatments ($F_{4, 53} = 3.9$, $p = 0.01$) when data were blocked by trial. Tukey's comparisons of means indicated that there were significantly fewer nematodes remaining in high stress treatments on control agar than on modified agar by approximately one nematode (mean = 2.9 and 2.1 nematodes for control agar and modified agar, respectively).

4 Discussion

Consistent with other studies of the stress-gradient hypothesis, I found that the net effect of one species on another changed depending on the level of stress the species experienced. As was predicted, the analysis of both colony plate counts and the fluorescence data indicated that stress had a negative impact on *E. coli* in absence of nematodes. The net effect of a presumed nematode predator on prey bacteria changed from neutral to positive when an abiotic stressor was applied to the system when effects were measured using colony plate counts. Ecosystem engineering was the mechanism for a higher positive impact of nematodes on bacteria under high stress. The fluorescence data indicate a slightly different result; that nematodes benefitted *E. coli* when stress was not applied to the system, and there was a greater positive impact when ecosystem engineering was present. When stress was applied, fluorescence data indicated that nematodes had a positive impact on bacteria only when ecosystem engineering was present. Therefore, my research provides further evidence that the impact of one species on another through the mechanism of ecosystem engineering can change according the level of stress in the species' environment (Crain and Bertness 2006; Daleo and Iribarne 2009).

4.1 Stress-induced variation in the impact of a predator on prey

4.1.i Predator impact on prey under low stress

As indicated by the analysis of colony plate counts, nematodes had a net neutral impact on *E. coli* when stress was not applied whether ecosystem engineering occurred or not. This perhaps implies that nematodes both positively and negatively impacted bacteria and that these impacts were of the same magnitude and so cancelled out. Positive impacts could have been due to nematode ecosystem engineering that distributed *E. coli* to burrows below the surface of the agar while negative impacts would have been due to predation of nematodes on bacteria. Positive impacts when no engineering occurred could have been due to mechanisms such as the distribution of *E. coli* to other areas on the surface of the agar, which could have released bacteria from local resource limitations.

On the other hand, the fluorescence estimates of the amount of bacteria indicate a net positive impact of nematodes on bacteria at low stress, with a larger positive impact when ecosystem engineering was present. The fluorescence results may be different than the colony plate count results because fluorescence could be an estimate of the distribution of bacteria in the cuvette, rather than an estimate of the number of live cells. A net positive impact of nematodes on bacteria at low stress could have been due to an increase in the distribution of bacteria by nematodes, as the *E. coli* were initially inoculated only in the center of the cuvette and nematodes could have spread bacteria to the edges of the cuvette. There may be more bacterial fluorescence in low stress treatments with nematodes and ecosystem engineering because the bacteria were additionally spread below the surface of the agar into burrows. This would mean that there is a larger distribution of fluorescence in low stress treatments with nematodes present than absent but not necessarily a larger amount of bacteria.

I had predicted that nematodes would have a significant negative impact on *E. coli* at low stress because nematodes are known to use *E. coli* as a food source (Chantanao and Jensen 1969; Stiernagle 1999; Avery and Shtonda 2003; Bichai et al. 2009; Avery and You 2012). However, my data instead indicated that either nematodes had no net impact on bacteria, or a net positive impact. This may be because nematodes can excrete viable bacteria cells (Chantanao and Jensen 1969; Bichai et al. 2009), and food-saturated nematodes may produce a particularly high number of viable cells in their excrement (Chantanao and Jensen 1969). In addition, the number of nematodes that interacted with the bacteria was small. Either a larger number of nematodes or a longer grazing period on *E. coli* could result in a net negative impact of the predator under low stress conditions if the negative impact due to consumption outweighs the positive impacts due to ecosystem engineering. Whether a higher density of nematodes would still result in a positive impact of nematodes on bacteria when stress is applied would be an interesting question for a future study.

4.1.ii Predator impact on prey when stress was applied

The results suggest that ecosystem engineering is one mechanism, but perhaps not the only mechanism, through which predators can positively impact prey under stressful conditions. Colony plate counts indicate that nematodes positively impacted bacteria when stress was applied. This positive impact was larger when nematodes ecosystem engineered burrows than when they did not. The fluorescence data analysis indicates that nematodes positively impacted bacteria when stress was applied only when ecosystem engineering was present, and that nematodes negatively impacted bacteria when ecosystem engineering was not present. The positive impact of ecosystem engineering nematodes on bacteria was likely due to the creation of burrows that provided refuge for bacteria from the application of stress to the surface of the agar.

Future studies should develop methods to identify non-burrowing mechanisms of positive impacts of nematodes on bacteria under stressful conditions.

As indicated by fluorescence, there was a slight positive impact of agar modification on bacteria in the absence of predators when stress was applied. The modification was a small stab in the surface of the agar that may have acted as refuge for the bacteria from the application of stress. This positive impact of agar modification when stress was applied was not indicated by the colony plate counts.

Another discrepancy between the two estimates of bacteria abundance is that the colony plate count results indicate that nematodes on agar that does not allow ecosystem engineering with stress applied have a positive impact on bacteria but the fluorescence results suggest that this impact is negative. This discrepancy could perhaps be explained if the bacteria on control agar with nematodes were recovering from the application of stress and live cells were present but only in a thin bacterial lawn with little fluorescence. However, more research should be done to determine the exact relationship between cuvette fluorescence and the amount of viable bacteria in the cuvette.

4.1.iii Overall change in the net impact of predators on prey

The net impact of nematodes on *E. coli* changed from neutral to positive when an abiotic disturbance stress was applied to the system, as indicated by the results of the colony plate counts. This finding is similar to that of the Dangles et al. (2013) study of two moth species where the net impact of one moth on the other changed from net neutral to net positive with increasing stress. I did not find a change from a net negative impact to a net positive impact of a predator on prey with increasing stress such as was seen by Daleo and Iribarne (2009) in a crab-marsh grass system. The fluorescence data indicate that the net impact of nematodes on *E. coli*

was positive whether stress was applied or not, but only when ecosystem engineering was present. The magnitude of the positive impact did increase slightly when stress was applied, which indicates that stress increased the net positive impact of nematodes on bacteria. An increase in the net positive impact of one species on another due to increasing stress in the form of physical disturbance was also observed by Kawai and Tokeshi (2007) in their study of the net impact of goose barnacles on mussels.

Overall, I did not see the dramatic stress-induced flip from predation to mutualism that I had expected. If we assume that nematodes do indeed benefit from the presence of *E. coli*, then this pairwise species interaction without the application of stress would be classified as commensalism, according to the colony plate count data. The nematodes are benefitting from a food resource provided by the *E. coli* while the *E. coli* are either not affected at all by this relationship (*E. coli* cells pass through the intestine of the nematodes alive) or they are negatively and positively affected by the nematode through some mechanisms with equal magnitudes. The net effect of nematodes on *E. coli* when stress was applied was positive which means that the net interaction under these conditions would be classified as mutualism. Therefore I have perhaps demonstrated a stress-induced flip from commensalism to mutualism for a nematode-bacteria system in the presence or absence of ecosystem engineering. According to the fluorescence data, I have demonstrated an increase in the positive impact of nematodes on bacteria with the application of stress only in the presence of ecosystem engineering.

4.2 The effects of stress on the predator

The physical disturbance stress used in this experiment likely impacted the nematodes in addition to the bacteria, as I counted on average 4 or 5 fewer nematodes after the application of stress than I initially inoculated. The nematode population could have been reduced by a

mechanism other than stress, for example crawling above the surface of the agar and desiccating, but I counted very few nematodes that had done so. It is also possible that nematodes are difficult to count in the cuvettes and so there is not actually a difference in the number of nematodes in high and low stress treatments. Unfortunately, there is no count of nematodes in low stress treatments to make this comparison. In addition, the mean number of nematodes counted after the application of stress on control agar was lower by approximately one nematode when ecosystem engineering was present than when it was absent. The measured difference in the mean number of nematodes could have been due to a bias in counting between the two treatments. The bias could have resulted because nematodes are harder to see on modified agar where they engineer dense burrows compared to the control agar that they cannot penetrate which could have resulted in a higher count of nematodes on control agar.

One could argue that the finding that nematodes have a positive impact on bacteria when stress is high is due to the fact that the overall number of nematodes has been reduced (i.e. the magnitude of the positive impacts on *E. coli* now outweigh the negative impacts because there has been a reduction in predation). However, a reduction in the number of predators could be part of the mechanism through which the predictions of the stress-gradient hypothesis can apply to a predator-prey system. A predator that positively impacts its prey, whether through ecosystem engineering or some other mechanism, is likely to be affected by the same environmental stress that affects its prey. For example, in the crayfish-dragonfly system studied by Pintor and Soluk (2006), the environmental stressor of low wetland water levels likely negatively impacted the crayfish predator in addition to the prey dragonfly larvae. This means that part of the mechanism that allows for a positive impact of a predator on a prey under stressful conditions could be a decrease in the number of predators coupled with an increase in

the relative importance of the positive impact provided by ecosystem engineering. A future study should be conducted that clearly shows how stress affects the predator in addition to the prey to further our understanding of how applicable the stress-gradient hypothesis is to predator-prey systems.

4.3 Conclusions

Overall, the results of this experiment show support for the stress-gradient hypothesis in a predator-prey interaction, as the net effect of a nematode predator on prey bacteria changed depending on the level of stress that the species experienced. Bacteria were negatively impacted by stress in the absence of nematodes and nematodes may also have been negatively impacted by stress. Depending on the method used to estimate bacteria, nematode ecosystem engineers had either a net neutral or net positive impact on bacteria when stress was not applied and had a larger net positive impact on bacteria when stress was applied. Future studies should determine what net impact a higher density of nematodes have on bacteria with and without stress applied, further isolate and identify the mechanisms for positive impacts of nematodes on bacteria, and clarify the relationship between fluorescence and the amount of viable bacteria. To conclude, the nematode-bacteria pairwise species interaction between *C. remanei* and *E. coli* is a model system that is easy to manipulate and can be used to further our understanding of how stress influences predator-prey dynamics.

Appendix

A test of the homogenization procedure

I conducted an experiment to determine if the homogenization procedure produces colony plate counts that accurately quantify bacterial abundance. To do this, I added 1 ml of five known concentrations of bacterial culture inoculated with OP50-GFP to cuvettes according to a randomized block design. The concentrations were 0.00, 0.01, 0.1, 0.4 and 0.8 OD₆₀₀, standardized to a blank of sterile broth. There were three replicates per concentration which were divided by three time blocks with one replicate of each concentration per block. I then added 1.5 ml of 1.67% agar to the cuvettes to make a final agar concentration of 1.0% and a final volume of 2.5 ml to be consistent with the experimental design. An hour after cuvettes were made I followed the homogenization procedure that I used in my experiment with the exceptions that the plates were made using the pour plate technique rather than the spread plate technique (bacteria cells grow within the agar rather than on the agar surface), 1.2 ml of saline was added to the cuvette rather than 1.0 ml, and a 50 ul initial aliquot was taken from the sample rather than 100 ul. I took photographs of two quarters of the Petri plate and counted fluorescent colony forming units using ImageJ. The number of colony forming units per ml was calculated using a standard of 2.5 g of agar for each cuvette, instead of individual cuvette weights as was done in my experimental procedure. To determine if the *E. coli* estimates from the colony plate counts were correlated with the concentration of *E. coli* initially inoculated, I conducted a linear regression with the data blocked by time and checked for normality of model residuals. The results of the linear regression indicated that the homogenization procedure produced colony plate counts that accurately estimated the amount of bacteria in the cuvettes (Figure 7, $F_{3,11} = 18.6$, $p < 0.05$, adjusted $R^2 = 0.8$).

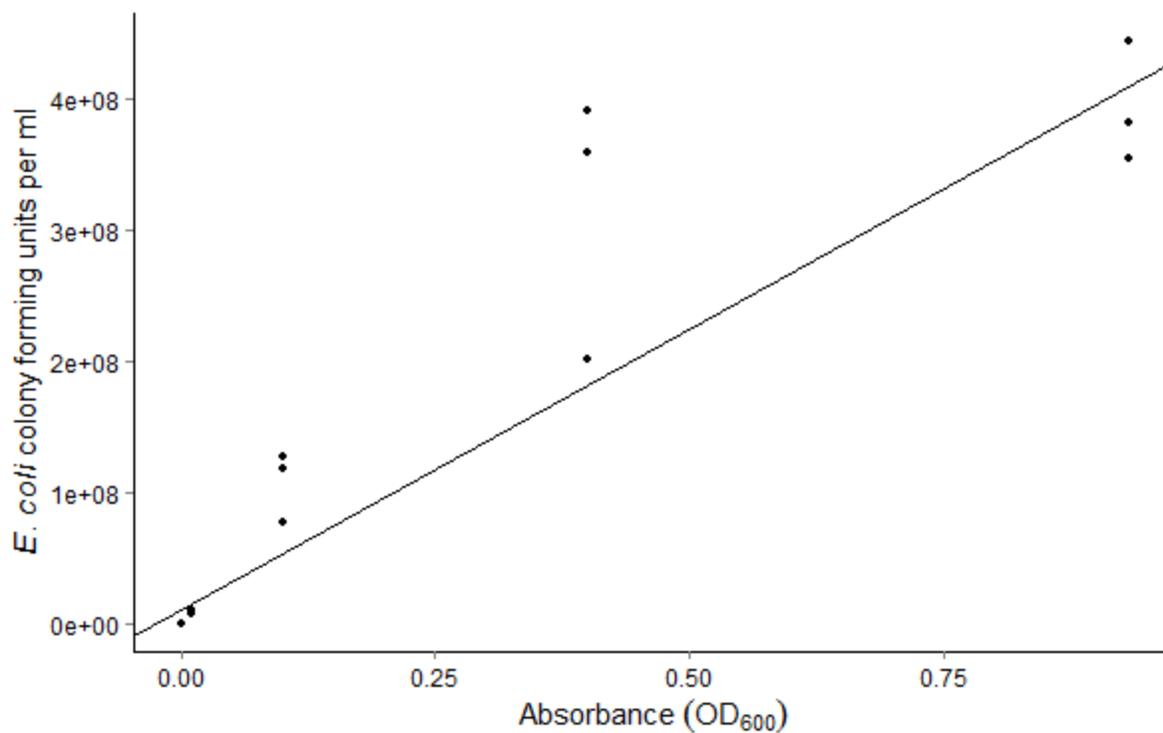


Figure 7: Linear regression of the estimated number of *E. coli* colony forming units per ml of cuvette agar by absorbance (OD₆₀₀) of *E. coli* culture initially inoculated. Colony forming units were estimated using colony plate counts that diluted bacteria following a homogenization procedure similar to that used in my experimental design.

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