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The Toxicity of Dopamine on Salish Sea Phytoplankton

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The Toxic Effects of Dopamine on Salish Sea Phytoplankton

By

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of the Requirements for the Degree
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Master's Thesis

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Allyson Lombardo

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The Toxic Effects of Dopamine on Salish Sea Phytoplankton

A Thesis
Presented to
The Faculty of
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In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Allyson Lombardo
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Abstract

In the Salish Sea, blooms of the intertidal macroalgae, *Ulvaria obscura*, are common and can achieve extraordinarily high biomass. Upon desiccation and subsequent rehydration from incoming tides, *U. obscura* releases dopamine. Previous studies showed that dopamine negatively affects other macroalgal species and can deter grazers. However, the effects of dopamine on co-occurring phytoplankton remains unknown. This study explored the toxicity of dopamine on four phytoplankton known to inhabit the Salish Sea: the haptophyte, *Isochrysis galbana*; the chlorophyte, *Dunaliella tertiolecta*; the dinoflagellate, *Heterocapsa triquetra*; and the diatom, *Thalassiosira* sp. Over the course of 8 days, phytoplankton growth was monitored across six dopamine concentrations ranging from 0 to 240 μM dopamine. This concentration range of dopamine represents concentrations observed in laboratory experiments. Dopamine reduced phytoplankton growth in all species; however, the concentration at which intrinsic phytoplankton growth rates were reduced was species-specific. Based on IC50 estimates, *H. triquetra*, *Thalassiosira* sp., and *I. galbana* were the most and equally sensitive to dopamine, while *D. tertiolecta* was the least sensitive. The intrinsic growth rates in *Thalassiosira* sp. and *H. triquetra* recovered after four days of dopamine exposure in the high dopamine treatments. Results from this study showed that dopamine exposure significantly decreased phytoplankton intrinsic growth rates for all species tested, and that after an initial decline in growth, two species recovered and achieved pre-exposure intrinsic growth rates. This suggests that in the presence of dopamine, phytoplankton community structure may be influenced by species-specific sensitivity to dopamine, whereby dopamine-tolerant species come to dominate these communities.

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Introduction

In marine environments, microalgae and macroalgae are known to produce and release toxic chemicals (Gross, 2003). These toxins can elicit deleterious effects in other co-occurring algae, invertebrates, fish, and humans (Ribalet et al., 2007; Chia et al., 2015; Cassotti et al., 2005; Rountos et al., 2019; Mello et al., 2018). Harmful phytoplankton blooms regularly employ allelopathy, which is the release of secondary metabolites into the environment by an organism that reduce the fitness of the target organism by inhibiting its germination, growth, survival, behavior, or reproduction (Rice, 1984). For example, toxins from harmful algal blooms have been shown to decrease fish survival and growth (Rountos et al., 2019) and reduce the growth and photosynthesis in co-occurring algae (Chia et al., 2019; Mitrovic et al., 2004).

Harmful algal blooms are not exclusive to phytoplankton. Ulvacean macroalgae can form toxic blooms known as green tides. Green tides are blooms of macro green algae that occur in coastal systems, and are recognized as harmful algal blooms. These blooms are an increasing concern due to their rising frequency (Ye et al., 2011; Smetacek & Zingone, 2013) and negative ecological effects. Green tides create anoxic conditions, alter the chemistry of surrounding seawater (Valiela et al. 1997; Van Alstyne et al. 2015), overgrow important seagrass habitats (McGlathery, 2001), and release toxic chemicals into the environment (Nelson, 2003).

Ulvacean macroalgae green tides release exudates from desiccated tissues, and these compounds are known to be toxic to many marine organisms. For example, exudates from the chlorophyte, *Ulva lactuca*, reduced grazing in the oyster, *Crassostrea virginica*, and the annelid, *Spinoidae* sp., (Warkus et al., 2010; Green-Gavrielidis et al., 2018), while exudates from the chlorophyte, *Ulva compressa*, reduced grazing in the abalone, *Haliotis rubra*, and *C. virginica*

(Green-Gavrielidis et al., 2018; Huggett et al., 2005). These chemicals can also elicit toxic effects on surrounding autotrophs. Ulvacean exudates can significantly inhibit the growth of co-occurring phytoplankton (Budzalek et al., 2021; Tang & Gobler, 2011; Jin et al., 2005), common harmful algal bloom cyanobacteria (Tang & Gobler, 2011; Gharbia et al., 2017), and other macroalgae (Xu et al., 2012).

U. obscura is an abundant macroalga that blooms in Washington State, United States, (Nelson et al., 2003). Exudates of *U. obscura* contain dopamine, a compound with known toxic potential (Van Alstyne et al., 2011, 2014). Dopamine is released from *U. obscura* after its desiccation and subsequent rehydration during tidal exchanges (Van Alstyne et al., 2011). Its presence in *U. obscura* tissues is thought to function primarily as an antiherbivore defense (Van Alstyne et al., 2006) and may protect the alga from excess light (Van Alstyne, 2018). However, dopamine also negatively affects the growth and survival of marine invertebrates. For example, dopamine decreased the survival rates of *Metacarcinus magister* crab zoeae (Van Alstyne et al., 2014). Further, it negatively affected the growth of juvenile oysters and sand dollars, contributing to smaller shells in the oyster, *Crassostrea gigas*, and shorter archenterons (the precursor to stomach-like organs) in the sand dollar, *Dendraster excentricus* (Rivera Vázquez et al., 2017). Furthermore, dopamine inhibited the germination of *Fucus distichus* rockweed zygotes, and limited the growth rate of another dominant chlorophyte, *U. lactuca* (Van Alstyne et al., 2014).

In seawater, dopamine quickly oxidizes to form reactive oxygen species (ROS), dopamine-quinones, and melanin (A1). Dopamine-quinones and ROS can be toxic to marine life. Dopamine forms several quinones during its molecular breakdown, including aminochrome (DAC) (Sun et al., 2018). Sun et al. (2018) hypothesized that DAC is the most toxic dopamine-

quinone because of its persistence and accumulation in seawater. However, they attributed hydrogen peroxide, an ROS, as the primary toxic agent of dopamine oxidation because it persists longer in the environment compared to DAC. Some of these dopamine-quinones can alter the development and habitat settlement of larval invertebrates. (Bonar et al. 1990; Dobretsov and Qian 2003; Adams et al. 2011). Furthermore, ROS are known to inhibit multiple functions in phytoplankton by permeating the cell membrane (Halliwell, 1992). For example, ROS altered the photophysiology of the diatom, *Thalassiosira pseudonana* (Li et al., 2021), reduced freshwater cyanobacteria populations (Lusty & Gobler, 2020), and inhibited photosynthesis in marine cyanobacteria and chlorophyte phytoplankton (Leunert et al., 2014).

Dopamine's toxic effects on marine invertebrates in the Salish Sea (Van Alstyne et al., 2014; Rivera Vázquez et al., 2017), and the effects of Ulvacean exudates on phytoplankton growth (Budzalek et al., 2021; Tang & Gobler, 2011; Jin et al., 2005) have been examined; however, little is known of dopamine's effects on phytoplankton that are sympatric with dopamine-producing macroalgae. Phytoplankton significantly contribute to primary production, carbon sequestration, and the marine food web in the Salish Sea. As such, a pressing question is whether dopamine negatively affects members of this seminal functional group. The purpose of this study was to test whether dopamine has a toxic effect on commonly occurring phytoplankton species in the Salish Sea.

Methods

Experiment Overview

The objectives of this study were to (1) quantify phytoplankton species' intrinsic growth rates (d^{-1}) across a range of dopamine concentrations; (2) determine if any observed differences in a phytoplankton's intrinsic growth rates were due to the toxic effects of dopamine; (3) determine if reduced phytoplankton intrinsic growth rates increased (i.e. recover) after dopamine exposure; (4) determine the dopamine concentration at which the intrinsic growth rate of each phytoplankton species is inhibited by 50% (IC50); (5) compare the IC50s, no observed effect concentrations (NOEC), and lowest observed effect concentrations (LOEC), all metrics of dopamine sensitivity, across the phytoplankton species; and (6) compare time-to-effect over 24 hour periods to determine how quickly intrinsic growth rates are affected by dopamine in each species.

Phytoplankton culturing

The phytoplankton used in this study were *Isochrysis galbana*, a marine haptophyte; *Dunaliella tertiolecta*, a marine chlorophyte; *Heterocapsa triquetra*, a marine dinoflagellate; and *Thalassiosira* sp., a marine diatom. These phytoplankton were either provided by the laboratory of Dr. Suzanne Strom (*I. galbana, H. triquetra, D. tertiolecta*) or isolated from the Salish Sea by Dr. Brady Olson. Prior to experiments, cultures were maintained in autoclaved filtered seawater (AFSW) amended with f/2 nutrients at 15°C under a 14:10 L:D cycle. Cultures were diluted frequently to maintain exponential growth.

Experiment Setup

To test the effects of dopamine on the intrinsic growth rates of phytoplankton, a concentration range of dopamine treatments were made. The dopamine treatments used in this study were 0, 15, 30, 60, 120, and 240 µM. These concentrations were chosen based on the Van Alstyne et al. (2011) and van Hees et al. (2013) laboratory studies , which showed that *U. obscura* releases dopamine into the environment at concentrations ranging from 3 to 563 µM. The 240 µM treatment was chosen as the highest dopamine concentration because preliminary experiments showed that the intrinsic growth rates of *I. galbana* and *D. tertiolecta* were reduced at 100 and 200 µM dopamine. A potential confounding predictor of phytoplankton growth under this experimental design was light limitation. When dopamine oxidizes over time, it produces melanin, a black particulate that settles on the walls of the experimental bottles and inhibits ambient light penetration. Therefore, to test whether changes in intrinsic growth rates were the result of dopamine-induced shading, a set of controls were used to test for the effects of light-limitation on phytoplankton intrinsic growth rates. To achieve this, a series of experimental bottles were wrapped with layers of neutral density screening to reduce incident irradiance by 50% and 25%. These levels were chosen based on preliminary experiments that showed high concentrations of dopamine shaded bottles to similar ambient light levels.

For the experiments, the control and dopamine treatments were replicated in triplicate. To begin, AFSW was amended with f/4 nutrient concentration and was dispensed into 60 mL Nalgene polycarbonate bottles. Each phytoplankton species was added to individual bottles to achieve low cell concentrations that would allow for exponential growth under optimal conditions. A 1 mM primary dopamine stock solution was made by dissolving 93.8 mg of 3-hydroxytyramine hydrochloride into 500 mL of AFSW amended with f/4 nutrients. Immediately

after the dopamine stock solution was made, the appropriate volume of dopamine stock solution was added to bring the bottles to the desired dopamine concentration per treatment. The dopamine stock solution was added last to minimize oxidation before sampling. The same procedure was followed with the controls, except dopamine was not added. Immediately following dopamine inoculation, all treatment and control bottles were inverted several times to homogenize cells within the solution, and 5 mL of sample was removed from each replicate and preserved in glass scintillation vials containing a volume of acid Lugol's that achieved a final Lugol's concentration of 5%. After sampling, all the bottles were placed haphazardly into an environmental incubator set at 15°C under a 14:10 L:D cycle with incident irradiance set at 200 µm $m^{-2}sec^{-1}$. Once in the incubator, a QSL-100 light probe (Biospherical Instruments) was used to measure incident light in all bottles from day 0 to day 2. A previous experiment showed that on day 2, dopamine is completely oxidized to melanin, so incident light levels in the bottles remain constant after day 2 (A2, A3). Under these conditions, the cultures were allowed to grow for 8 days. On days 0, 1, 2, 3, 4, 6 and 8, 5 mL of sample was collected from each bottle and preserved as described above.

Cell Counts

To determine phytoplankton intrinsic growth rates, phytoplankton from all samples were manually counted using a compound microscope at 40X magnification and a hemocytometer *(I. galbana* and *D. tertiolecta*) or a Sedgewick rafter chamber (*H. triquetra* and *Thalassiosira* sp.), or by using an inverted microscope at 20X magnification and a 6 cell well plate when *Thalassiosira* sp. were in low abundance. A minimum of 200 cells were counted per sample to achieve low sampling error (Harris et al., 2000). Cell numbers (mL^{-1}) per day from each control and treatment replicate were natural-log transformed and plotted over time. The intrinsic growth

rate for each control and treatment replicate was determined from the slope of the linear regression, when R-squared values were greater than 0.80. During the *I. galbana* and *D. tertiolecta* experiments, ambient light in the incubator was increased shortly before day 1 samples were taken and incident light levels were measured. Given this change, intrinsic growth rates were determined from day 1 to day 8 for these two species. *H. triquetra* intrinsic growth rates in the two highest dopamine concentrations declined from day 0 to day 4, then increased after day 4. Therefore, the intrinsic growth rates of *H. triquetra*, for all controls and treatments, were determined from day 0 to day 4 and from day 4 to day 8. *Thalassiosira* sp. intrinsic growth rates in the 120 μM dopamine concentration declined from day 0 to day 4, then increased after day 4. Therefore, the intrinsic growth rates of *Thalassiosira* sp., for all controls and treatments, were determined from day 0 to day 4. *Thalassiosira* sp. cultures reached a stationary growth after day 6 in the 100% light level/0 μM dopamine control, 50% light level control and the 15 μM dopamine treatment. Therefore, *Thalassiosira* sp. intrinsic growth rates from day 4 to day 8 were only calculated for the two highest dopamine concentrations. Given that intrinsic growth rates among species were determined over different time scales, the intrinsic growth rates used to calculate IC50s were determined from day 1 to day 4 to account for the time scale discrepancies among species. Time-to-effect, i.e. the amount of elapsed time until a dopamine effect was observed, was determined by calculating intrinsic growth rates in every 24 hour time block from day 1 to day 4 at the 0 μM dopamine control, and the 60, 120, and 240 μM dopamine treatments. Intrinsic growth rates over these specific 24-hour blocks were determined by $\mu = \ln(t_f/t_0)/t$, where t_f and t_0 are cell concentrations at the end and the beginning of a 24 hour time block, respectively, and t is the time block. To standardize time to effect, each species' intrinsic growth

rate under dopamine exposure was divided by their intrinsic growth rate under 0 μM dopamine for the respective time block.

Statistical Analysis

Differences in intrinsic growth rates for treatments and controls were tested using a one-way ANOVA, with $p < 0.05$ as the threshold of significance. To test for normality, box and whisker plots were made to visually determine normal distributions (A9, A13, A18, A19, A24, A28). Equal variance in data was tested using Levene's analysis (A8, A12, A16, A17, A23, A27). When significant differences were observed between treatments, Dunnett post-hoc tests were used to determine which dopamine treatments and light level controls differed from the 100% light level/0 μM dopamine control.

Dopamine dose-response curves for each species were created using the best-fit model (LL.4 model) in *R: A Language and Environment for Statistical Computing*, and from these, the IC50 for each species was determined from their intrinsic growth rates at each dopamine concentration. Significant differences in IC50 values were determined based on overlap of 95% confidence intervals, where no overlap indicated significance when the range of values was positive. NOECs and LOECs were determined using a one-way ANOVA with $p < 0.05$ as the threshold of significance. NOECs represent the highest tested dopamine concentration that did not decrease intrinsic growth rates, whereas LOECs represent the lowest tested dopamine concentration where intrinsic growth rates were significantly lower than the 0 μM dopamine control. When significance was observed between treatments, Dunnett post-hoc tests were used to determine which dopamine treatments differed from the 0 μM dopamine control.

Results

Effects of Light Levels and Dopamine on Phytoplankton Intrinsic Growth Rates

Isochrysis galbana

Incident light levels from each dopamine treatment fell within the range of the incident light levels from the shaded controls during the *I. galbana* experiment (Figure 1). *I. galbana* intrinsic growth rates were the same across the control light levels, (Figure 2, A10: $p > 0.05$), indicating that dopamine shading should not have affected *I. galbana* intrinsic growth rates.

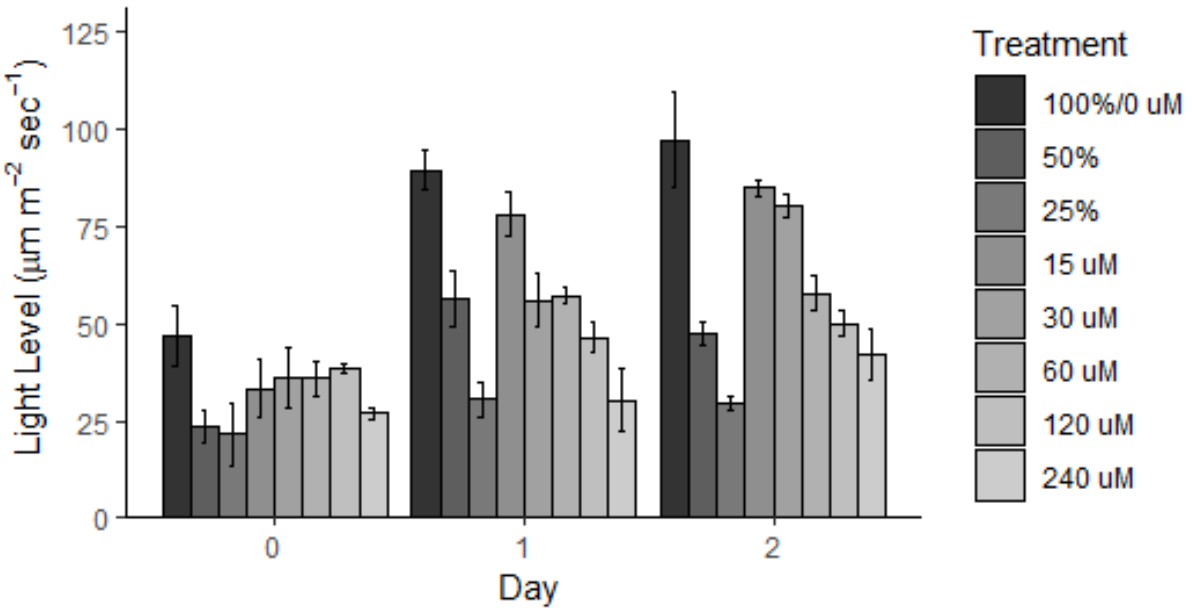


Figure 1: Mean incident light levels for each treatment and control (n=3) for days 0, 1, and 2 for *I. galbana*. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Ambient light in the incubator was increased shortly before day 1 samples were collected and light levels were measured.

Significant differences in *I. galbana* intrinsic growth rates were observed across dopamine treatments (Figure 2: $p << 0.001$). *I. galbana* intrinsic growth rates were equal across dopamine concentrations ranging from 0 to 30 µM, averaging 0.35 d^{-1} (Figure 2) *I. galbana* intrinsic growth rates at 120 and 240 µM were significantly lower than the 0 µM dopamine control, and in both cases, intrinsic growth rates were negative (Figure 2, A10: $p < 0.05$). The intrinsic growth rates of the 240 µM treatment declined 52% more than the 120 µM dopamine intrinsic growth rates.

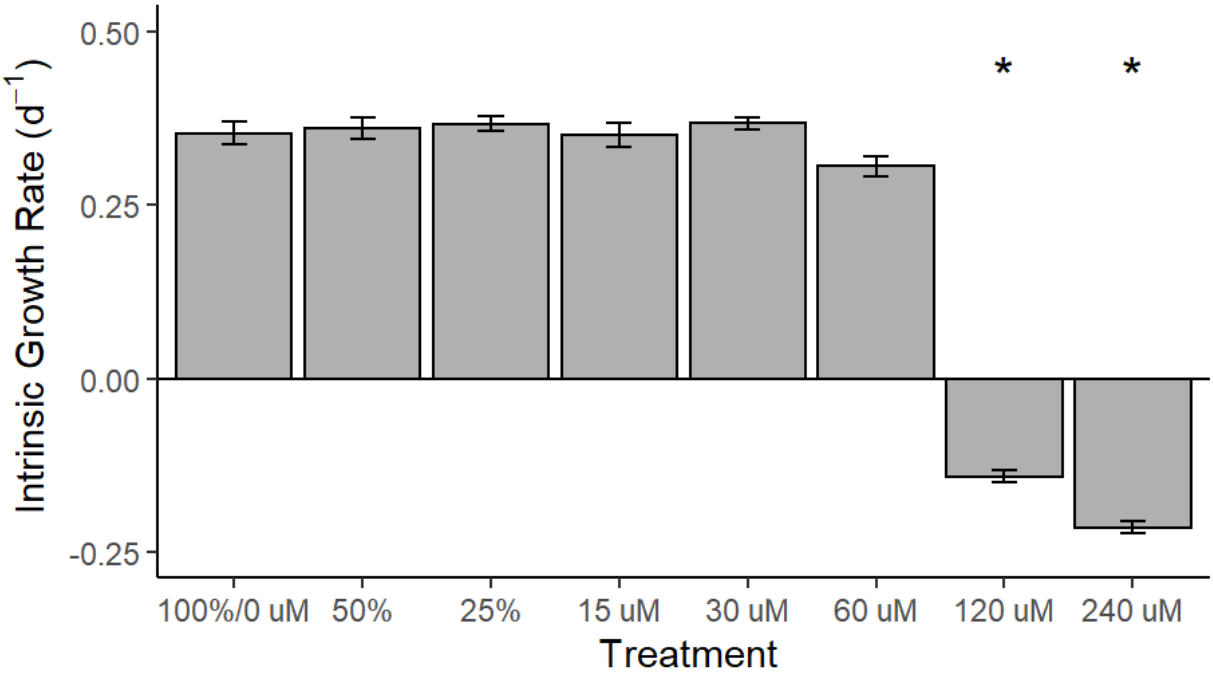


Figure 2: Mean intrinsic growth rates of *I. galbana* (n=3) across dopamine treatments and light controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisks denote significant differences in intrinsic growth rates between the specified dopamine treatment and the 100%/0 µM control, determined from Dunnett's post hoc test.

Dunaliella tertiolecta

Incident light levels from each dopamine treatment fell within the range of the incident light levels from the shaded controls in the *D. tertiolecta* experiments (Figure 3, A11). *D. tertiolecta* intrinsic growth rates were the same across control light levels (Figure 4: $p>0.05$), indicating dopamine shading should not have affected *D. tertiolecta* intrinsic growth rates.

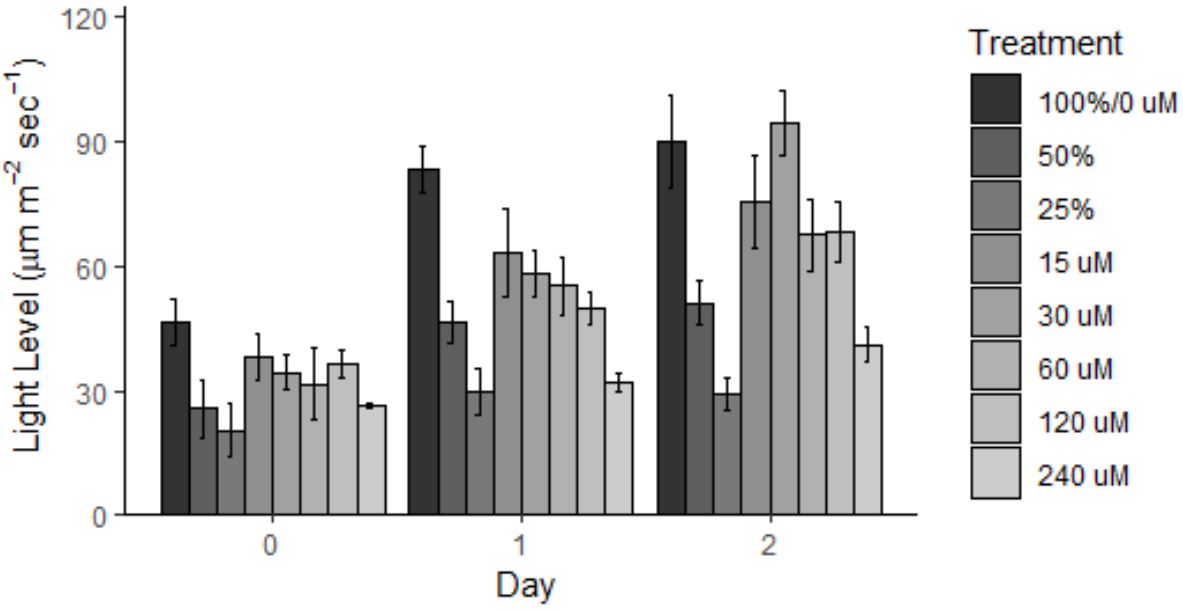


Figure 3: Mean incident light levels for each treatment and control (n=3) for days 0, 1, and 2 for *D. tertiolecta*. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Ambient light in the incubator was increased shortly before day 1 samples and light levels were collected.

Significant differences in *D. tertiolecta* intrinsic growth rates were observed across dopamine treatments (Figure 4: $p << 0.001$). Intrinsic growth rates were equal to the 0 µM dopamine control across dopamine treatments ranging from 15 to 120 µM, averaging 0.36 d^{-1} (Figure 4). At 240 µM, *D. tertiolecta* intrinsic growth rates were significantly reduced by 45% compared to the 0 µM dopamine control (Figure 4, A14: $p << 0.01$).

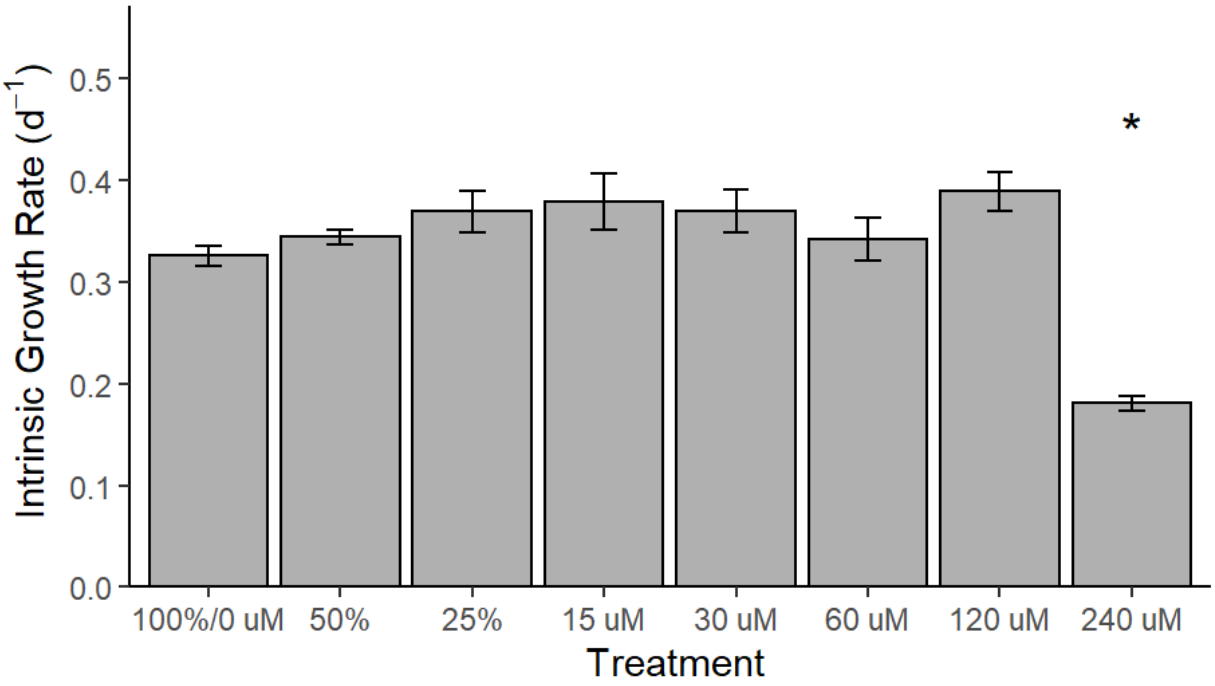


Figure 4: Mean intrinsic growth rates of *D. tertiolecta* (n=3) across dopamine treatments and light controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisk denotes a significant reduction in intrinsic growth rates compared to the 100%/0 µM control, determined by Dunnett's post hoc test.

Heterocapsa triquetra

Incident light levels from each dopamine treatment fell within the range of the incident light levels from the shaded controls, except for the day 1 incident light levels from the 240 μM dopamine treatment (Figure 5, A15). On day 1, the incident light levels from the 240 μM treatment were slightly, yet significantly, lower than the incident light levels from the 25% light level control ($p= 0.0487$). Intrinsic growth rates in the 50% light level control were significantly greater than the 100%/0 μM dopamine control (Figure 6: $p= 0.0134$), possibly suggesting photoinhibition in *H. triquetra* at the ambient unshaded experimental light level. However, the incident light levels of the 50% shaded control were most similar to the 15 μM dopamine incident light levels (A15). Unlike the 50% light level control, the intrinsic growth rates of the 15 μM treatment were not significantly higher than the 100%/0 μM intrinsic growth rates (Figure 6: $p= 0.513$), despite having similar incident light levels to the 50% light level control. This suggests photoinhibition was not the cause of reduced intrinsic growth rates at the 100% incident light level.

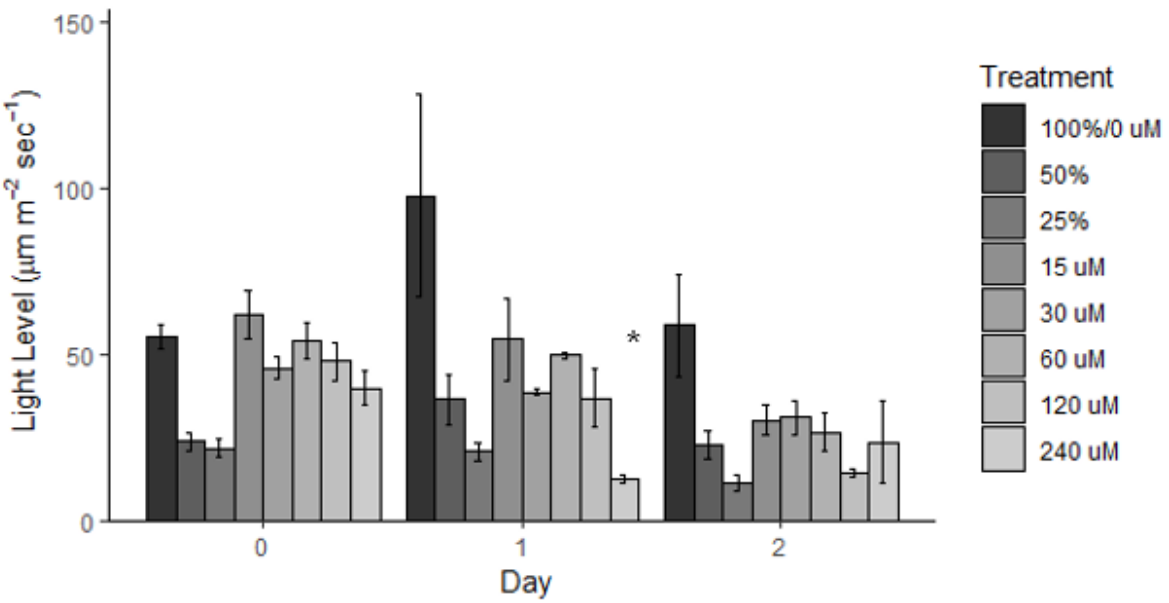


Figure 5: Mean incident light levels for each treatment and control (n=3) for days 0, 1, and 2 for *H. triquetra*. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisk denotes a significant difference in the mean incident light levels compared to the lowest light level treatment.

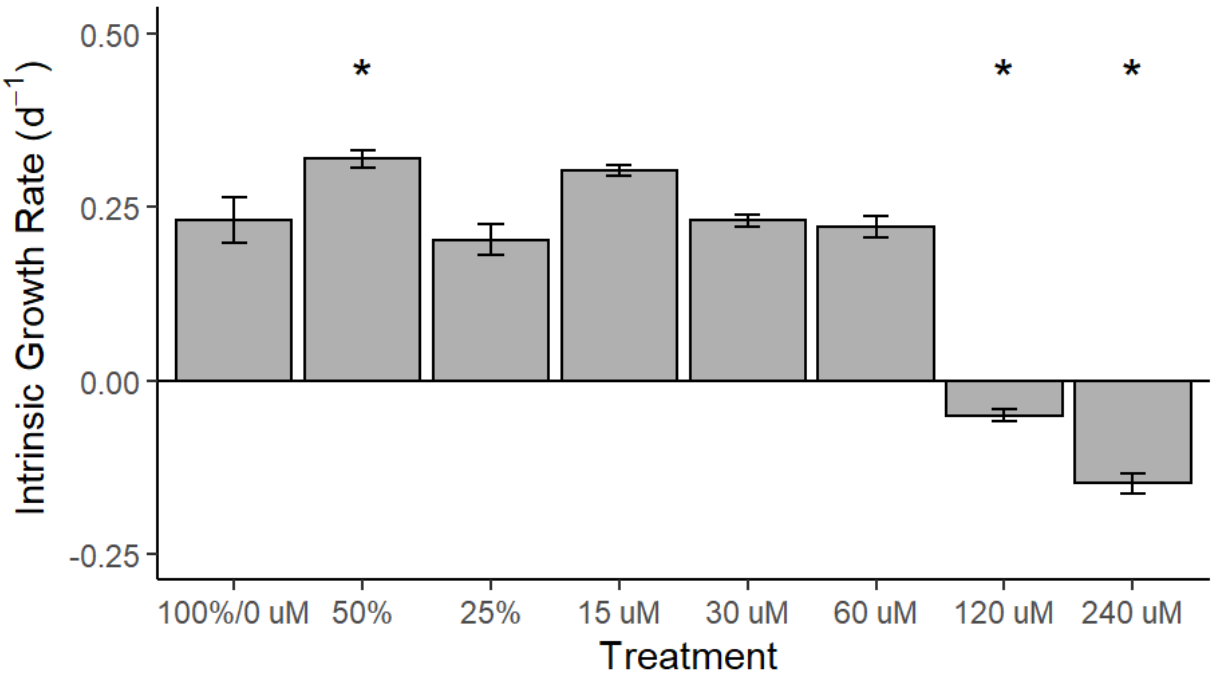


Figure 6: Mean intrinsic growth rates of *H. triquetra* (n=3) across dopamine treatments and light controls from day 0 to day 4. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisks exclusively denote significant differences in intrinsic growth rates between the specified light level control or dopamine treatment and the 100%/0 µM control, determined from Dunnett's post hoc test.

Significant differences in *H. triquetra* intrinsic growth rates were observed across dopamine treatments from day 0 to day 4 (Figure 6: $p << 0.001$). Intrinsic growth rates were equal to the 0 µM dopamine control across dopamine concentrations ranging from 15 to 60 µM, averaging 0.25 d^{-1} (Figure 6: $p > 0.05$). *H. triquetra* intrinsic growth rates at 120 and 240 µM dopamine were significantly lower than the 0 µM dopamine control, and in both cases, intrinsic growth rates were negative (Figure 6, A20: $p < 0.05$). The intrinsic growth rates at 240 µM dopamine declined 194% more than the intrinsic growth rates at 120 µM dopamine from day 0 to day 4.

Light level did not have a significant effect on intrinsic growth rates in *H. triquetra* from day 4 to day 8 (Figure 7, A21: $p > 0.05$), indicating that dopamine shading should not have affected *H. triquetra* intrinsic growth rates during these later time stages. Significant differences in *H. triquetra* intrinsic growth rates were observed across dopamine treatments from day 4 to day 8 (Figure 7: $p < 0.01$). *H. triquetra* intrinsic growth rates from 15 to 120 µM dopamine were equal to the 100%/0 µM control, averaging 0.31 d^{-1} After day 4, the intrinsic growth rates of both the 120 and 240 µM treatments were positive, and increased to an average rate of 0.39 d^{-1} and 0.17 d^{-1}, respectively. However, the 240 µM dopamine intrinsic growth rates were significantly lower than the 120 µM dopamine intrinsic growth rates ($p < 0.01$) and were 45% lower than the intrinsic growth rates for the 100%/0 µM control from day 4 to day 8 (Figure 7, A21: $p < 0.01$).

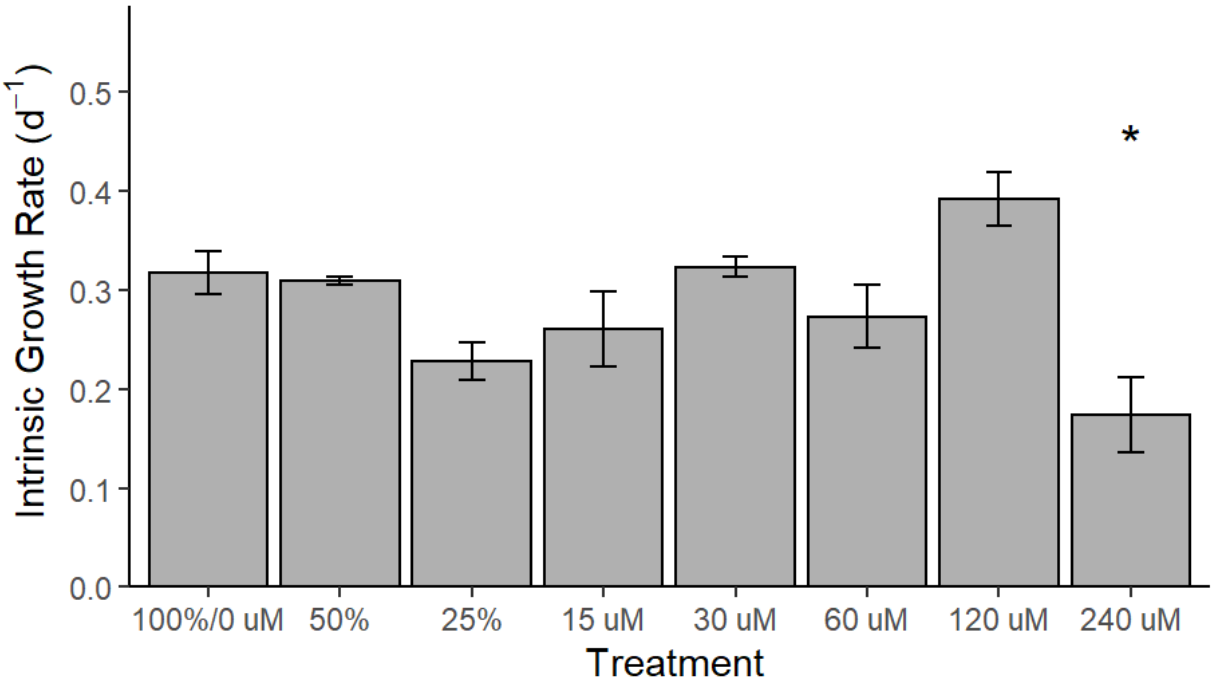


Figure 7: Mean intrinsic growth rates of *H. triquetra* (n=3) across dopamine treatments and light controls from day 4 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars were determined from standard error. Asterisk denotes a significantly lower intrinsic growth rate compared to the 100%/0 µM dopamine control, determined from Dunnett's post hoc test.

Thalassiosira sp.

Incident light levels for each dopamine treatment fell within the range of light level controls (Figure 8, A22). Shading significantly decreased *Thalassiosira* sp. intrinsic growth rates at the 25% incident light level from day 0 to day 4 (Figure 9: $p<<0.01$). The reduction of *Thalassiosira* sp. intrinsic growth rates in the 25% light level control, compared to the 100%/0 μM (Figure 9, A25: $p<<0.01$), suggests that the reduced intrinsic growth rates of *Thalassiosira* sp. at the 30 and 60 μM dopamine concentrations may be due to shading. However, the incident light levels of the 30 and 60 μM dopamine treatments most closely reflect the incident light levels measured in the 50% light level control (Figure 8, A22), and there was no significant difference in the intrinsic growth rates of the 50% light level control compared to the 100%/0 μM control. The incident light levels measured in the 120 and 240 μM dopamine treatments were similar to the incident light levels of the 25% light level control, yet the 25% light level control intrinsic growth rates were positive, whereas the intrinsic growth rates of 120 and 240 μM dopamine treatments were negative. All considered, this suggests that shading should not have affected the intrinsic growth rates of *Thalassiosira* sp. in dopamine treatments from day 0 to day 4.

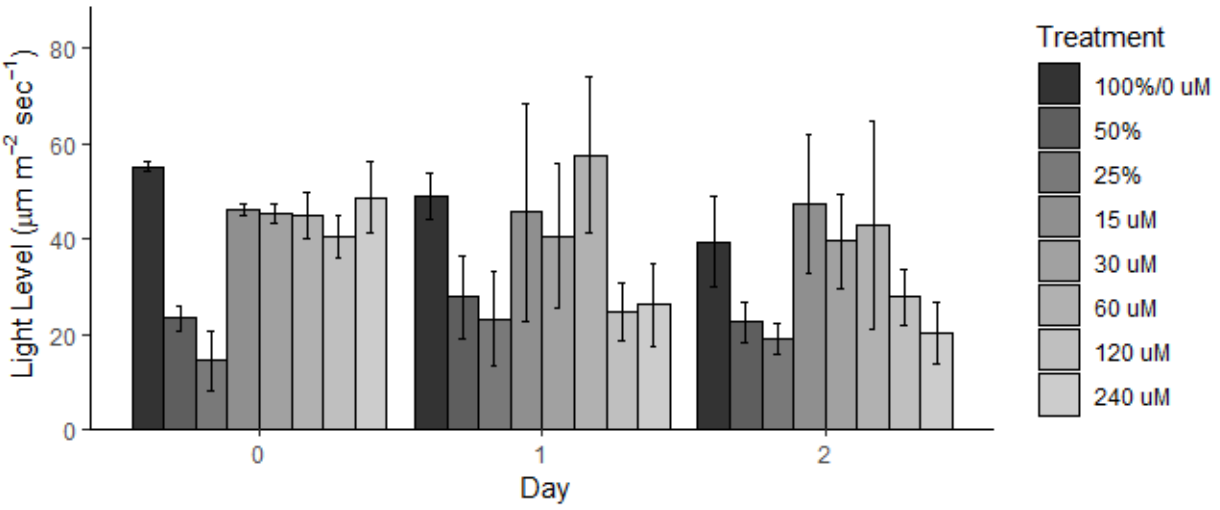


Figure 8: Mean incident light levels from each dopamine treatment and light level control (n=3) for days 0, 1, and 2 for *Thalassiosira* sp. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars were determined from standard error.

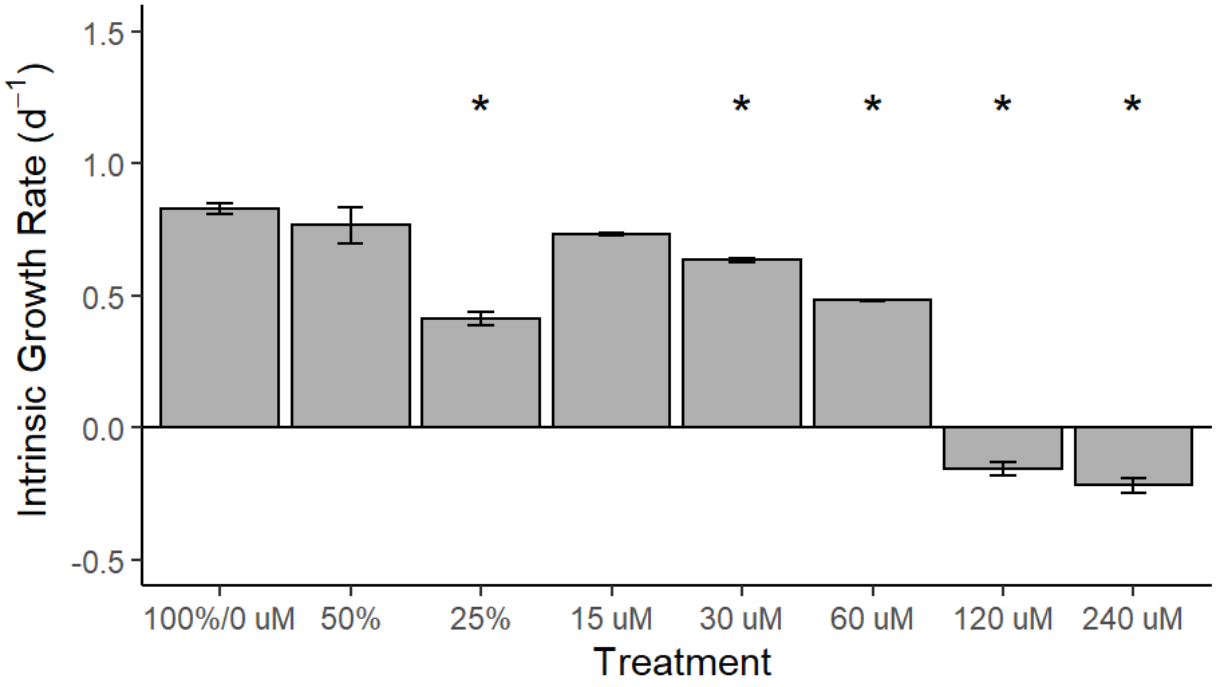


Figure 9: Mean intrinsic growth rates of *Thalassiosira* sp. (n=3) across dopamine treatments and light controls from day 0 to day 4. 100%, 50%, and 25% represent the incident light levels measured in each light level control. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisks exclusively denote significant differences in intrinsic growth rates between the specified light level control or dopamine treatment and the 100%/0 µM control, determined from Dunnett's post hoc test.

Significant differences in *Thalassiosira* sp. intrinsic growth rates were observed across dopamine treatments from day 0 to day 4 (Figure 9: $p<<0.001$). *Thalassiosira* sp. intrinsic growth rates in the 15 μM dopamine concentration were equal to the 100%/0 μM control, averaging 0.78 d^{-1} (Figure 9: $p= 0.292$). *Thalassiosira* sp. intrinsic growth rates across dopamine concentrations from 30 to 240 μM dopamine were significantly lower than the 0 μM dopamine control (Figure 9, A25: $p< 0.05$). In both the 120 and 240 μM dopamine treatments, intrinsic growth rates were negative from day 0 to day 4 (Figure 9). From day 4 to day 8, the intrinsic growth rates were positive in the 120 μM dopamine treatment and increased to an average of 0.39 d^{-1}. Unlike the 120 μM dopamine treatment, intrinsic growth rates for *Thalassiosira* sp. in the 240 μM dopamine treatment remained negative.

Comparing Dopamine Sensitivity Between Species

The estimated IC50s for *I. galbana*, *H. triquetra*, *D. tertiolecta*, and *Thalassiosira* sp. from day 1 to day 4 were 76.8 μM, 99.7 μM, 316 μM, and 75.2 μM dopamine, respectively (A26). There were no significant differences in IC50s between *I. galbana*, *H. triquetra*, and *Thalassiosira* sp. (A26). By this metric, *D. tertiolecta* was the least sensitive to dopamine, while *I. galbana*, *H. triquetra*, and *Thalassiosira* sp. were equally sensitive to dopamine from day 1 to day 4. *D. tertiolecta* intrinsic growth rates declined significantly only at the 240 μM dopamine concentration, modeling a non-logarithmic curve (Figure 10) in which the IC50 estimate resulted in a large 95% confidence interval (IC50= 316, LL= -1100, UL= 1730). Therefore, NOEC and LOEC values were also used as a metric of species sensitivity.

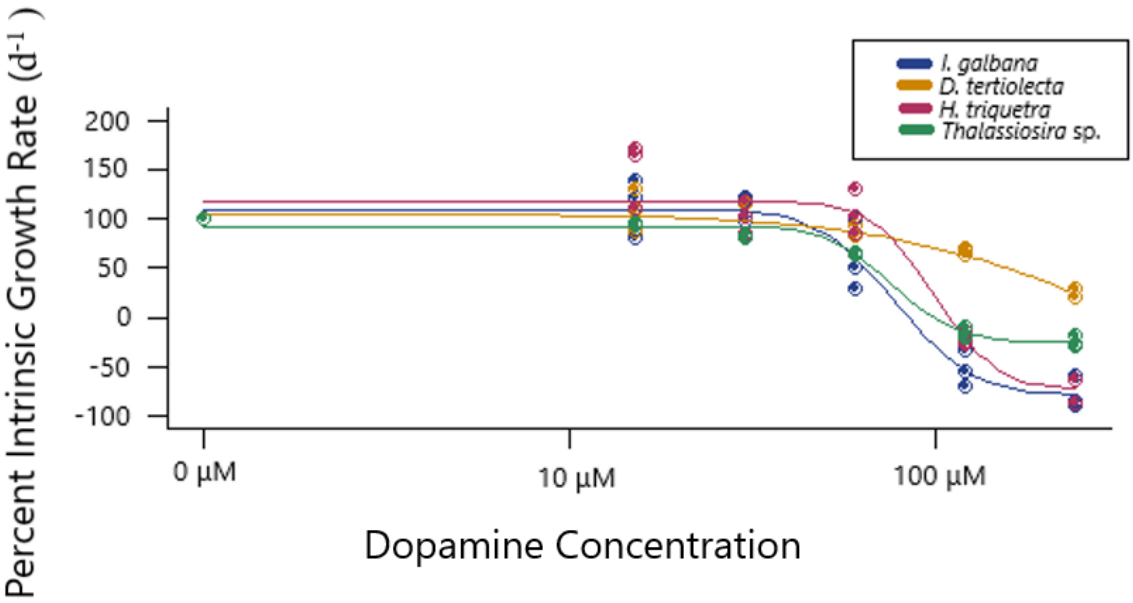


Figure 10: Dose-response curves of the intrinsic growth rates (n=3) from day 1 to day 4 for *I. galbana*, *H. triquetra*, *D. tertiolecta*, and *Thalassiosira* sp. determined by the best-fit LL.4 model. Date points are intrinsic growth rates for the dopamine control and dopamine treatments from day 1 to day 4. Intrinsic growth rates are represented as a percentage of the intrinsic growth rate of the average intrinsic growth rate of the 0 µM dopamine control by species.

All phytoplankton species showed a significant difference in intrinsic growth rates from day 1 to day 4 across dopamine concentrations (Figure 11). The NOEC and LOEC for *I. galbana*, *D. tertiolecta*, and *H. triquetra* were 60 and 120 µM, respectively, while the NOEC and LOEC for *Thalassiosira* sp. were 15 and 30 µM, respectively (Figure 11). By this metric, *I. galbana*, *D. tertiolecta*, and *H. triquetra* were equally sensitive to dopamine, while *Thalassiosira* sp. was the most sensitive species. Although the NOEC and LOEC of *D. tertiolecta* were the same as *I. galbana* and *H. triquetra*, *D. tertiolecta* intrinsic growth rates were not negative at the

120 and 240 µM dopamine concentrations as they were in *I. galbana* and *H. triquetra* (Figure 11).

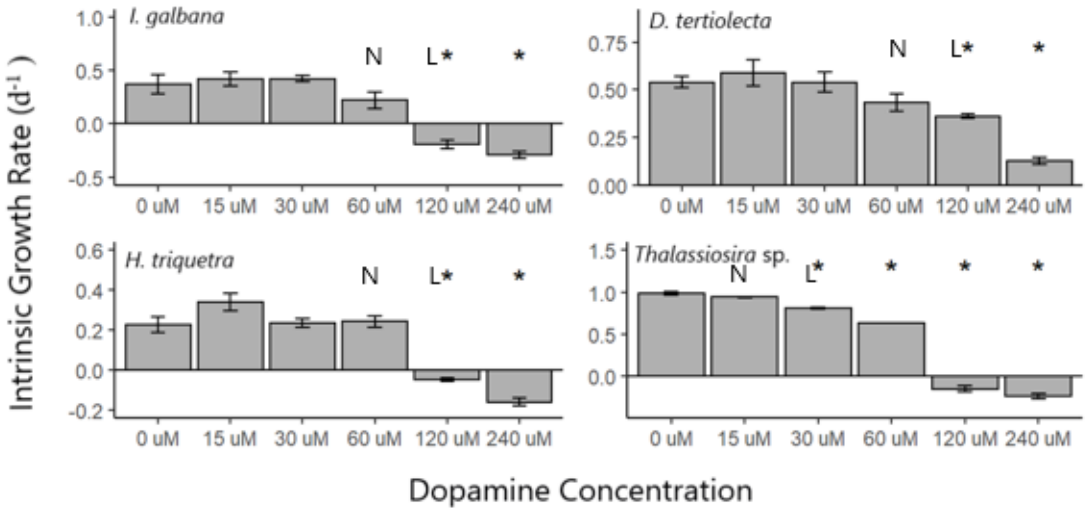
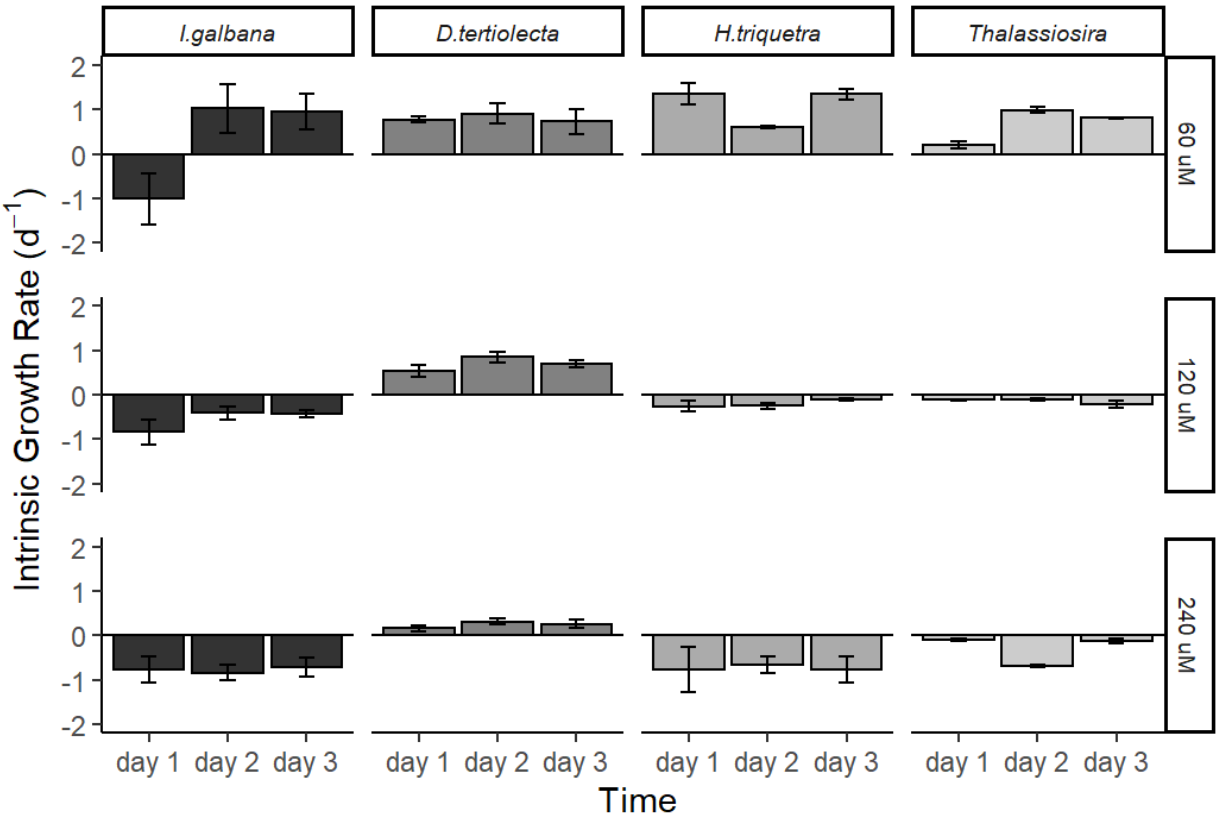


Figure 11: Mean intrinsic growth rates (n=3) of *I. galbana* (top left), *D. tertiolecta* (top right), *H. triquetra* (bottom left) and *Thalassiosira* sp. (bottom right) across dopamine treatments from day 1 to day 4. 0, 15, 30, 60, 120, and 240 µM represent the concentrations of dopamine in each treatment. Error bars show standard error. Asterisks exclusively denote significant differences in intrinsic growth rates between the dopamine treatment and the 0 µM dopamine control, determined from Dunnett's post hoc test. 'N' and 'L' indicate the NOEC and LOEC, respectively. Note differences in scale of Y-axes.

Phytoplankton species were also analyzed for time-to-effect, i.e. the time at which each species responded to dopamine toxicity (Figure 12). At the 60 µM dopamine concentration, all phytoplankton intrinsic growth rates were positive except *I. galbana* from day 1 to day 2. At the 120 and 240 µM dopamine concentrations, intrinsic growth rates for all species declined at each time period, except for *D. tertiolecta*. At the 120 µM dopamine concentration, *I. galbana* populations decreased at the greatest rate over all time periods. At the 240 µM concentration, *I. galbana* and *H. triquetra* populations declined at the same rate across all time points. *Thalassiosira* sp. populations declined at the lowest rate at the 120 µM dopamine concentration

from day 1 to day 2 and from day 2 to day 3. At the 240 µM dopamine concentration, *Thalassiosira* sp. populations declined at the lowest rate from day 1 to day 2, and from day 3 to day 4. The rate at which dopamine affects *I. galbana*, *H. triquetra*, and *Thalassiosira* sp. populations at the 120 and 240 µM concentrations remained constant within each species between 24 hour periods from day 1 to day 4.

Figure 12: Normalized intrinsic growth rates of each phytoplankton species within a 24 hour period across the 60, 120, and 240 µM dopamine concentrations. To standardize time to effect,



each species' intrinsic growth rate was divided by their intrinsic growth rate under 0 µM dopamine. X-axis represents the starting day of the 24-hour period. Error bars show standard error.

Discussion

The results of this study showed that dopamine has a toxic effect on Salish Sea phytoplankton. These results add to the body of literature showing the deleterious effects of dopamine on disparate marine organisms (Van Alstyne et al., 2014; Rivera Vázquez et al., 2017). By using a range of different phytoplankton species and functional groups, these results also showed that phytoplankton respond to dopamine differently, with some species showing higher sensitivity, while others showed the ability to recover after initial declines in intrinsic growth rates. Of the species tested, *D. tertiolecta* was the most tolerant to dopamine toxicity. Intrinsic growth rates of *D. tertiolecta* declined only at the highest dopamine concentration but remained positive across all dopamine treatments. This contrasts with *I. galbana*, *H. triquetra*, and *Thalassiosira* sp., which all showed more acute responses to dopamine toxicity and demonstrated negative growth rates under high dopamine concentrations.

Variation in phytoplankton size and physiology between species may be a factor in determining dopamine tolerance. Smaller cells, like *I. galbana*, have a larger surface area to volume ratio than larger cells. This allows for increased acquisition of toxicants (Tato & Beiras, 2019), which may render *I. galbana* vulnerable to dopamine's toxic effects. This sensitivity to toxicants is why *I. galbana* is commonly used for algal toxicity tests over other phytoplankton species (Tato & Beiras, 2019). *I. galbana* shows higher sensitivity to metals (Satoh et al., 2005), herbicides, surfactants, and microbiocides compared to other phytoplankton species (Tato & Beiras, 2019). Although *D. tertiolecta* is similar in size to *I. galbana*, it was the least sensitive to dopamine. Further, *H. triquetra* and *Thalassiosira* sp. are larger than *D. tertiolecta*, and both showed higher degrees of dopamine toxicity compared to *D. tertiolecta*. This suggests size is not the only factor dictating toxicant sensitivity. Stauber & Florence (1990) found that *D. tertiolecta*

was highly tolerant to heavy metal toxicity compared to 10 other algal species in different taxonomic groups. Furthermore, other studies have shown that the genus *Dunaliella* is more fit than other phytoplankton species in waters with high concentrations of metals (Moreno-Garrido et al., 2005; De Kuhn et al., 2006). This tolerance to other toxicants could explain why *D. tertiolecta* was less sensitive to dopamine compared to the other species tested and suggests mechanisms beyond size explain its tolerance.

The sensitivity of the comparatively larger *H. triquetra* and *Thalassiosira* sp. to dopamine may be reflective of similarities in their dopamine chemical receptor sites and signaling pathways to those found in higher organisms. In humans, dopamine binds to transmembrane G-protein coupled receptors (GPCRs), causing a signal cascade mediated by G-proteins or by calcium ion channels. This binding is highly efficient, with dopamine signals firing at 0.2-10 Hz to control important processes like motor function and reward-based learning (Liu & Kaeser, 2019). Analogs of GPCRs have been identified in both diatoms and dinoflagellates. Port et al. (2013) identified GPCRs in *Thalassiosira pseudonana*. Using comparative transcriptomic analysis, Mojib and Kubanek (2020) identified primal GPCRs in 81 diatoms and 36 dinoflagellates and found that the diatoms analyzed shared approximately 41-69% similarity in their signaling protein sequence to those in humans, while the dinoflagellates shared approximately 25-37% similarity to humans. In animals, Ca^{2+} channels and GPCRs are utilized in chemical detection (Hilger et al. 2018), and similar mechanisms have been observed in diatoms and dinoflagellates. For example, intracellular Ca^{2+} levels and GPCR expression changed in the diatom, *Skeletonema marinoi*, when exposed to herbivore cues (Port et al., 2013). Furthermore, Mojib & Kubanek (2020) found that diatoms and dinoflagellates predominately expressed GPCRs like those involved in neurotransmitter signaling in humans. These signaling

molecules found in diatoms and dinoflagellates, and their similarity to those in humans, may help explain the sensitivity to dopamine in *H. triquetra* and *Thalassiosira* sp. Because dopamine bonds to receptors quickly in humans, it is likely that dopamine was able to bond efficiently to the similar GPCR proteins in *Thalassiosira* sp. and *H. triquetra*. *Thalassiosira* sp. may be more sensitive to dopamine compared to *H. triquetra*, as diatom signaling molecules are closely related to the ones found in humans, whereas dinoflagellate signaling molecules share fewer similarities to those in humans (Mojib & Kubanek, 2020).

Dopamine's toxic potential on phytoplankton in the environment may depend on length of exposure. This study found that sensitivities to dopamine, and the ability to recover from initial exposure, were time and species dependent. For example, *I. galbana* was unable to recover from dopamine exposure at high concentrations, whereas for *H. triquetra* and *Thalassiosira* sp., after an acute negative response from days 0 to 4, they were able to recover and expressed positive intrinsic growth rates.

The observed differences in dopamine toxicity over time may be related to time-dependent detoxification potential. The International Organization for Standardization established that standard algal toxicity tests should take place over 48 to 72 hours, excluding metals toxicity tests, to limit the chance of nutrient limitation and detoxification of the tested analyte (Nyholm & Kallqvist, 1989). This may be why dopamine sensitivities in *I. galbana*, *Thalassiosira* sp., and *H. triquetra* were different when IC50s were analyzed from day 1 to day 4 compared to the response over 8 days. It is possible that *Thalassiosira* sp. and *H. triquetra* have a greater detoxification potential than *I. galbana.* This would explain the observation that *I. galbana* was the most dopamine-sensitive species when analyzed over the 8 days, whereas from day 1 to day 4, *H. triquetra*, *Thalassiosira* sp., and *I. galbana* were equally sensitive to

dopamine. Comparing toxicity metrics, determination of species sensitivity using IC50 values compared to NOEC and LOEC values differed due to the scope of each analysis. Dose-response curves estimate IC50 values by creating a model of the effects of dopamine over a continuous concentration scale, whereas NOEC and LOEC values determine dopamine sensitivity from only the concentrations tested, narrowing the scope of analysis. This is likely why the NOEC and LOEC values determined that *D. tertiolecta* was equally sensitive to dopamine as *I. galbana* and *H. triquetra*, while the IC50 values determined that *D. tertiolecta* was the least sensitive to dopamine.

Ecological Implications

As mentioned, dopamine in the Salish Sea is a product derived from the chlorophyte macroalga, *U. obscura*. Highest measured concentrations of dopamine in laboratory experiments were measured by Van Alstyne et al. (2011) and van Hees et al. (2013), who showed that dopamine can achieve concentrations greater than 500 µM upon desiccation and subsequent rehydration of *U. obscura*. Currently, it is unknown whether dopamine exists in significant concentrations in pelagic waters, and if so, at what concentrations. Given this, dopamine's ecological impact on phytoplankton would likely be greatest on benthic diatoms and tidepool phytoplankton communities. While dopamine's potential toxicity to benthic diatoms was not tested in this study, all tested phytoplankton species, residing in different taxonomic groups, responded to dopamine exposure. It is therefore likely that dopamine would negatively impact other species of phytoplankton, including benthic diatoms, which, in part, support intertidal ecosystems.

Any ecological impact of dopamine may be exacerbated by climate change. The Salish Sea will likely be impacted by climate change more severely than other marine environments. Cold-water ecosystems, like the Salish Sea, are more susceptible to ocean acidification because carbon dioxide dissolves more readily into cold water compared to warm water ecosystems (McNeil and Matear, 2008). Further, the Salish Sea experiences high rates of respiration due to its high productivity, and as such, experiences diel and seasonal variation in pH that exacerbates ocean acidification (Cai et al., 2011). Increased temperature and pCO_2 predicted for future marine environments were shown to increase *Ulva rigida* germination and specific growth rate (Gao et al., 2017), and increase the relative growth and photosynthetic rate of *Ulva prolifera*, *Ulva linza*, and *Ulva compressa* (Wang et al., 2011). It is likely that the dopamine-producing chlorophyte, *U. obscura*, will increase growth and photosynthetic rates under climate change (Nelson et al., 2003). If true, dopamine production in intertidal regions may increase. Additionally, desiccation rates may increase under magnified UV-radiation and higher temperatures resulting from climate change. This could increase dopamine production, as dopamine is released due to desiccation stress in *U. obscura*.

Conclusion

This study adds to the body of knowledge on the toxic potential of marine macroalgae exudates. Studies have investigated the toxic potential of *U. obscura* exudates containing dopamine to invertebrate larvae; however, this is the first study to examine the toxicity of dopamine to phytoplankton. This study illuminates the toxic effect of dopamine on disparate phytoplankton functional groups. Understanding drivers of reduced phytoplankton production is crucial in maintaining the health and productivity of marine ecosystems. Further investigation is needed to determine the mechanism of action of dopamine on phytoplankton intrinsic growth rates, to determine the effects of dopamine on phytoplankton community composition, and to model the effects of dopamine on phytoplankton under predicted future climates.

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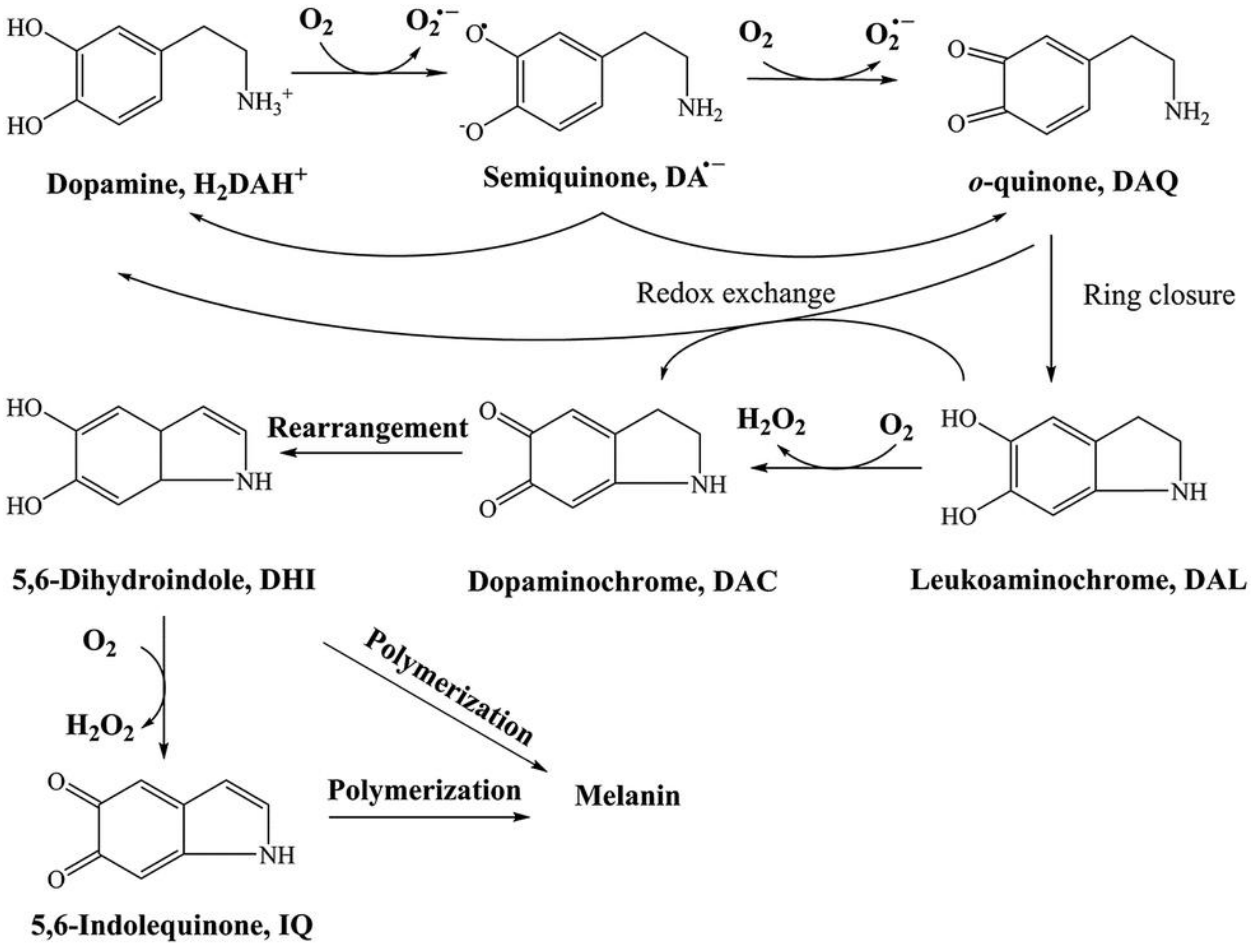
Appendix

Dopamine Oxidation



A1: Dopamine oxidation in seawater to dopamine-quinones, reactive oxygen species (ROS), and melanin (Credit: Van Alstyne et al., 2011).

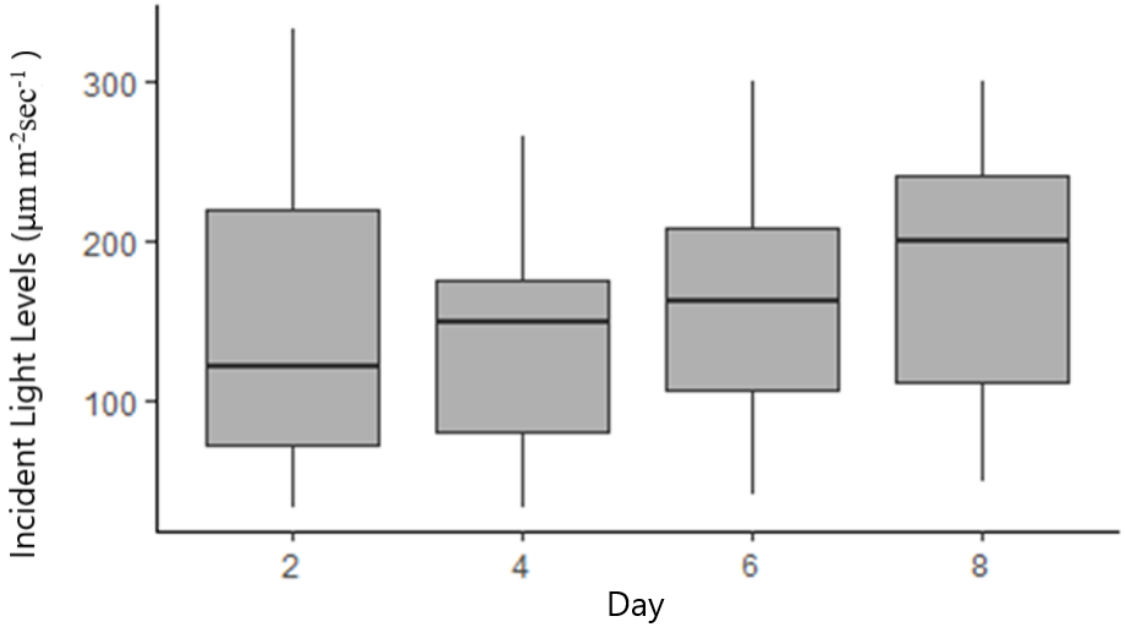
Preliminary Light Level Experiment

A2: Incident light levels from a preliminary experiment (n=2) from day 0 to day 8 for each dopamine treatment (0 μM 15 μM, 30 μM, 60 μM, 120 μM, and 240 μM dopamine) and light level control. 100%, 50%, and 25% represent the incident light levels measured in each light level control.

Treatment	100% light level/ 0 μM	50% light level	25% light level	15 μM	30 μM	60 μM	120 μM	240 μM
Light Levels ($\mu m\ m^{-2}sec^{-1}$) Day 0	232.4, 298.8	116.2, 132.8	41.5, 49.8	365.2, 282.2	332.0, 232.4	265.6, 249	166, 199.2	149.4, 149.4
Light Levels ($\mu m\ m^{-2}sec^{-1}$) Day 2	315.4, 332.0	74.7, 83.0	33.2, 49.8	232.4, 249	215.8, 166.0	166.0, 124.5	116.2, 99.6	66.4, 49.8
Light Levels ($\mu m\ m^{-2}sec^{-1}$) Day 4	265.6, 199.2	83.0, 83.0	33.2, 49.8	166.0, 215.8	215.8, 166.0	166.0, 166.0	132.8, 166.2	58.1, 74.7
Light Levels ($\mu m\ m^{-2}sec^{-1}$) Day 6	199.2, 199.2	99.6, 83.0	41.5, 49.8	265.6, 298.8	232.4, 166.0	166.0, 232.4	157.7, 157.7	107.9, 132.8
Light Levels ($\mu m\ m^{-2}sec^{-1}$) Day 8	298.8, 298.8	99.6, 116.2	49.8, 49.8	232.4, 282.2	232.4, 215.8	232.4, 256.6	149.4, 182.6	149.4, 91.3

A3: Levene's analysis of equal variance output table for incident light levels from day 2 to day 8 from a preliminary experiment with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where $p > 0.05$.

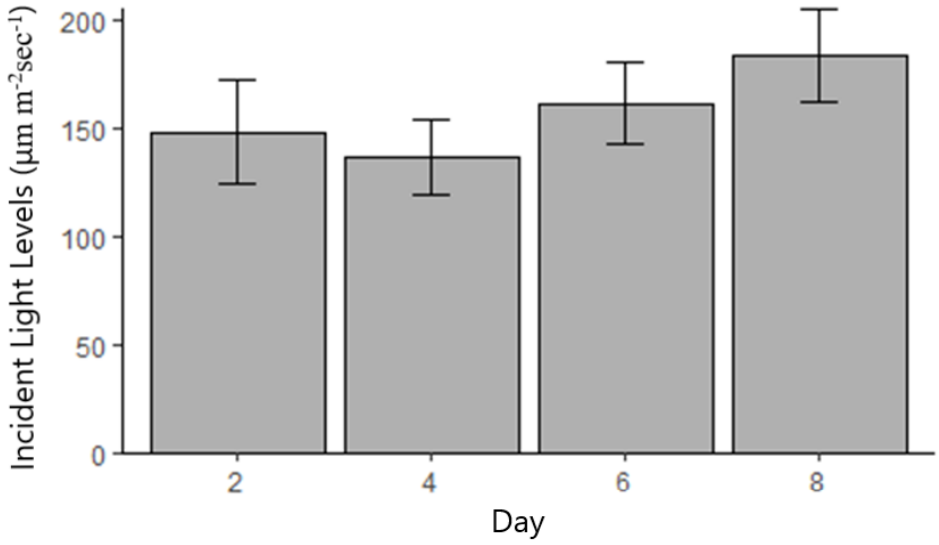
Levene's analysis	df= 3	F= 0.708	p= 0.551



A4: Distributions of incident light levels in all dopamine treatments and light level controls from a preliminary experiment (n=2). Data meets assumption of normality.

A5: One-way ANOVA output for incident light levels at days 2, 4, 6, and 8 (n=2) from a preliminary experiment with degrees of freedom (df), F-value, and p-value, where α= 0.05.

One-way ANOVA	df= 3	F= 0.986	p= 0.405



A6: Mean incident light levels in all dopamine treatment and light level controls for day 2 through day 8 from a preliminary experiment (n=2). Error bars are standard error.

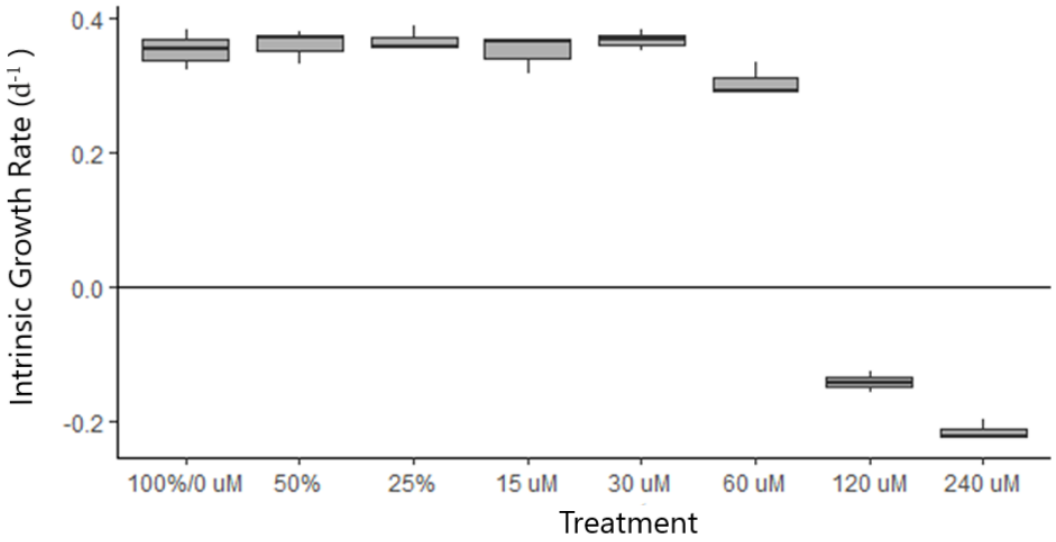
Isochrysis galbana

A7: Range of incident light levels measured in *I. galbana* bottles (n=3) from each dopamine treatment and light level control from day 0 to day 2. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Asterisk denotes that the lowest incident light level recorded in the 240 µM dopamine treatment on day 1 fell below the lowest incident light level measured in the light level controls on day 1

Treatment	100% light level/ 0 µM	50% light level	25% light level	15 µM	30 µM	60 µM	120 µM	240 µM
Light Level Range (µm $m^{-2}sec^{-1}$) Day 0	32.5-59.0	14.8-27.8	11.7-37.3	21.7-47.0	20.5-44.6	27.1-42.2	38.6-41.0	25.3-30.1
Light Level Range (µm $m^{-2}sec^{-1}$) Day 1	84.3-99.4	44.6-69.3	22.9-38.6	72.3-89.8	47.0-69.3	53.6-60.2	41.6-54.2	16.9-44.6*
Light Level Range (µm $m^{-2}sec^{-1}$) Day 2	75.3-116.9	42.8-53.0	25.9-31.9	81.3-88.6	74.7-84.9	50.0-65.7	36.1-54.8	34.9-54.8

A8: Levene's analysis of equal variance output table for *I. galbana* intrinsic growth rates across all tested dopamine concentrations and light level controls (n=3) from day 1 to day 8 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where p> 0.05.

Levene's analysis	df= 7	F= 0.149	p= 0.992



A9: Distribution of intrinsic growth rates for *I. galbana* across all tested dopamine concentrations and light level controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Data meets assumption of normality.

A10: Dunnett test output for *I. galbana* intrinsic growth rates across all tested dopamine concentrations and light level controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments and light level controls were tested for significance against the 100% light level/ 0 μM dopamine control, where $\alpha = 0.05$.

Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
50%-100%/0 μM	-0.0459	0.0608	0.998
25%-100%/0 μM	-0.0399	0.0668	0.959
15 μM -100%/0 μM	-0.0563	0.0504	1.00
30 μM -100%/0 μM	-0.0391	0.0676	0.946
60 μM -100%/0 μM	-0.101	0.00556	0.0888
120 μM -100%/0 μM	-0.548	-0.441	<<0.00100
240 μM -100%/0 μM	-0.621	-0.514	<<0.00100

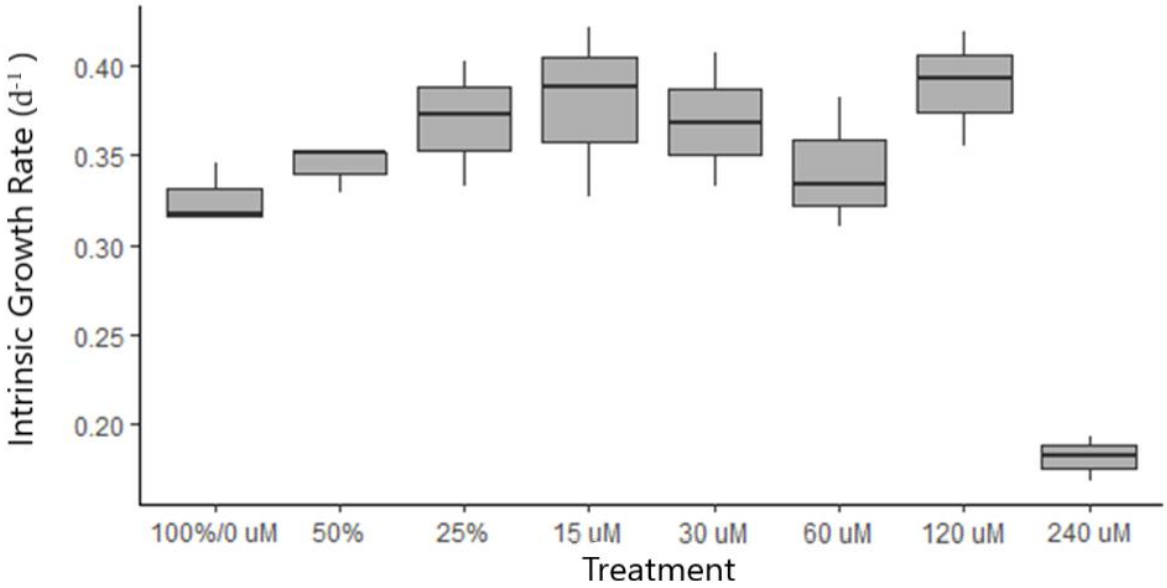
D. tertiolecta:

A11: Range of incident light levels measured in *D. tertiolecta* bottles (n=3) from each dopamine treatment and light level control from day 0 through day 2. 100%, 50%, and 25% represent the incident light levels measured in each light level control.

Treatment	100% light level/ 0 μM	50% light level	25% light level	15 μM	30 μM	60 μM	120 μM	240 μM
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 0	36.1-55.4	12.0-36.1	10.0-31.3	32.5-49.4	30.1-43.4	18.1-48.2	30.1-41.0	25.3-27.7
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 1	72.3-90.4	36.7-52.4	21.7-40.4	42.2-78.3	51.8-69.3	47.0-53.0	45.2-57.8	28.9-36.1
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 2	73.5-111.4	41.6-60.2	24.7-36.7	53.6-91.5	85.5-109.6	51.2-81.3	53.6-77.1	34.3-48.8

A12: Levene's analysis output for *D. tertiolecta* intrinsic growth rates across all dopamine treatments and light level controls (n=3) from day 1 to day 8 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where $p > 0.05$.

Levene's analysis	df= 7	F= 0.576	p= 0.765



A13: Distribution of intrinsic growth rates in *D. tertiolecta* across all dopamine treatments and light level controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Data meets assumption of normality.

A14: Dunnett test output for *D. tertiolecta* intrinsic growth rates across all dopamine treatments and light level controls from day 1 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments and light level controls were tested for significance against the 100% light level/ 0 μM dopamine control, where $\alpha = 0.05$.

Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
50%-100%/0 μM	-0.0572	0.0936	0.966
25%-100%/0 μM	-0.0321	0.119	0.419
15 μM -100%/0 μM	-0.0227	0.128	0.242
30 μM -100%/0 μM	-0.0318	0.119	0.413
60 μM -100%/0 μM	-0.0594	0.0915	0.982
120 μM -100%/0 μM	-0.0121	0.139	0.120
240 μM -100%/0 μM	-0.221	-0.0700	<0.00100

H. triquetra:

A15: Range of incident light levels measured in *H. triquetra* bottles (n=3) from each dopamine treatment and light level control from day 0 through day 2. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Asterisk denotes that the lowest incident light level recorded in the 240 μM dopamine treatment on day 1 fell below the lowest incident light level measured in the light level controls on day 1.

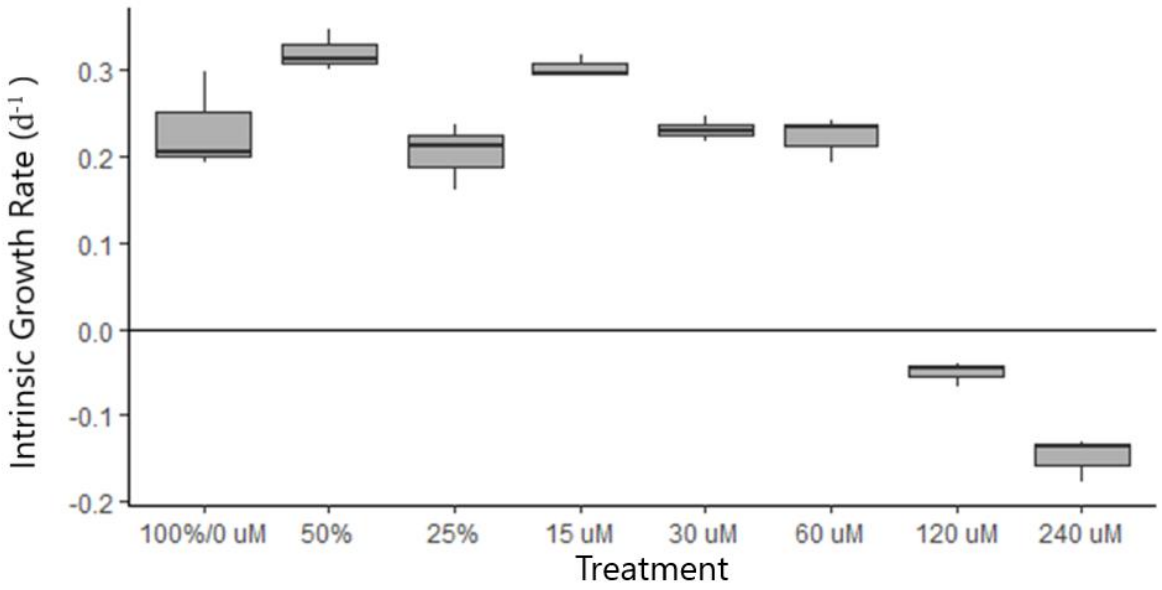
Treatment	100% light level/ 0 μM	50% light level	25% light level	15 μM	30 μM	60 μM	120 μM	240 μM
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 0	48.2-60.2	19.3-28.9	18.1-27.7	48.2-72.3	39.8-50.6	45.2-63.2	37.3-57.2	31.3-48.2
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 1	45.8-150.6	21.7-44.0	16.3-25.3	41.0-79.5	36.7-40.4	48.2-51.8	22.9-53.0	10.2-14.4*
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 2	34.9-87.3	14.4-28.3	7.53-15.7	23.5-38.6	22.3-40.4	15.7-34.9	12.6-16.9	8.73-48.2

A16: Levene's analysis output for *H. triquetra* intrinsic growth rates across all dopamine treatments and light level controls (n=3) from day 0 to day 4 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where $p > 0.05$.

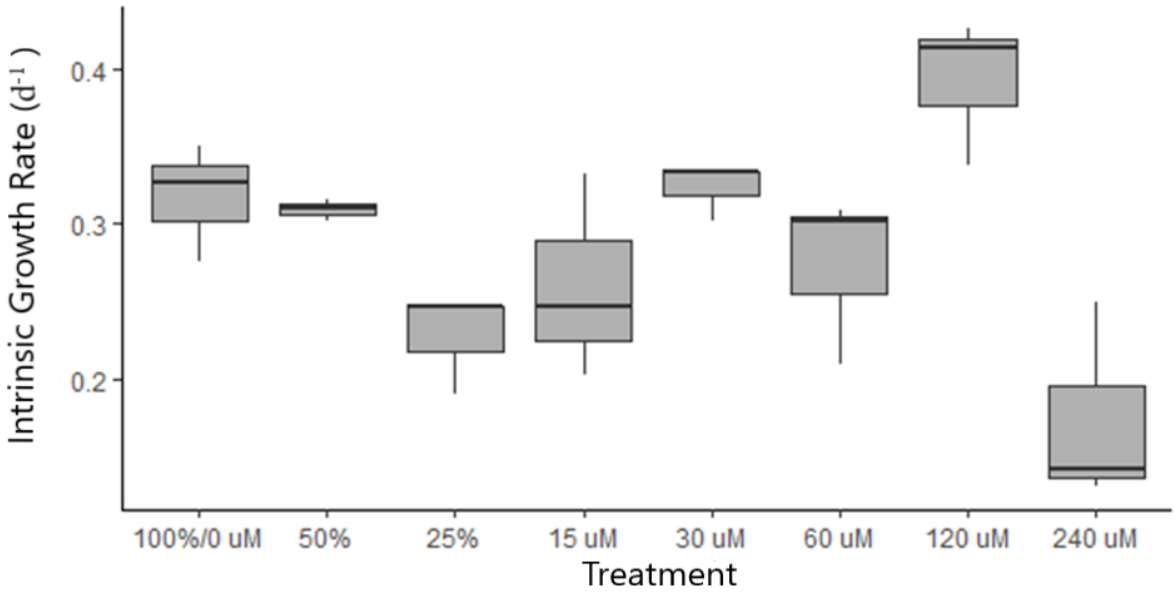
Levene's analysis	df= 7	F= 0.414	p= 0.879

A17: Levene's analysis output for *H. triquetra* intrinsic growth rates across all dopamine treatments and light level controls (n=3) from day 4 to day 8 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where $p > 0.05$.

Levene's analysis	df= 7	F= 0.371	p= 0.906



A18: Distribution of intrinsic growth rates in *H. triquetra* across all dopamine treatments and light level controls (n=3) from day 0 to day 4. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Data meets assumption of normality.



A19: Distribution of intrinsic growth rates in *H. triquetra* across all dopamine treatments and light level controls (n=3) from day 4 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Data meets assumption of normality.

A20: Dunnett test output for *H. triquetra* intrinsic growth rates across all dopamine treatments and light level controls from day 0 to day 4. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments and light level controls were tested for significance against the 100% light level/ 0 μM dopamine control, where $\alpha = 0.05$.

Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
50%-100%/0 μM	0.0162	0.159	0.0134
25%-100%/0 μM	-0.100	0.0427	0.747
15 μM -100%/0 μM	-0.000648	0.142	0.0524
30 μM -100%/0 μM	-0.0723	0.0705	1.00
60 μM -100%/0 μM	-0.0811	0.0617	0.999
120 μM -100%/0 μM	-0.353	-0.210	<<0.00100
240 μM -100%/0 μM	-0.451	-0.308	<<0.00100

A21: Dunnett test output for *H. triquetra* intrinsic growth rates across all dopamine treatments and light level controls from day 4 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments and light level controls were tested for significance against the 100% light level/ 0 μM dopamine control where $\alpha = 0.05$.

Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
50%-100%/0 μM	-0.117	0.101	1.00
25%-100%/0 μM	-0.198	0.0193	0.132
15 μM -100%/0 μM	-0.166	0.0520	0.513
30 μM -100%/0 μM	-0.103	0.115	1.00
60 μM -100%/0 μM	-0.153	0.0648	0.741
120 μM -100%/0 μM	-0.0342	0.184	0.258
240 μM -100%/0 μM	-0.252	-0.0343	<0.0100

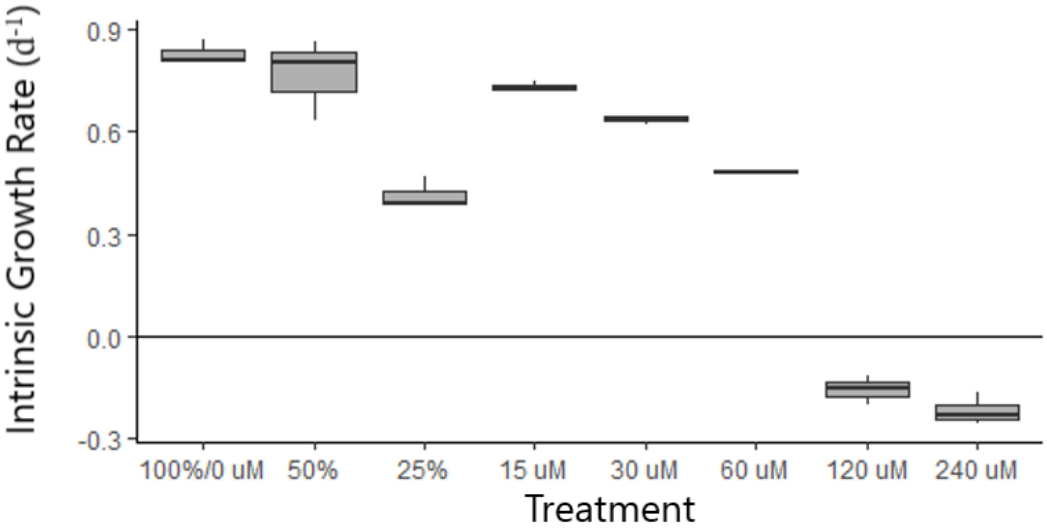
Thalassiosira sp.:

A22: Range of incident light levels measured in *Thalassiosira* sp. bottles (n=3) from each dopamine treatment and light controls for day 0 through day 2. 100%, 50%, and 25% represent the incident light levels measured in each light level control.

Treatment	100% light level/ 0 μM	50% light level	25% light level	15 μM	30 μM	60 μM	120 μM	240 μM
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 0	54.2-57.2	18.1-26.5	16.9-28.9	44.6-48.2	42.2-49.4	38.6-54.2	32.5-48.2	37.3-63.2
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 1	41.6-57.8	18.7-45.2	11.4-42.8	14.4-90.3	13.8-66.3	28.3-84.3	13.2-33.7	16.3-43.4
Light Level Range ($\mu m\ m^{-2}sec^{-1}$) Day 2	20.5-50.6	18.0-31.3	12.6-22.9	30.1-75.9	23.5-57.8	17.5-86.1	17.5-37.3	13.2-33.1

A23: Levene's analysis output for *Thalassiosira* sp. intrinsic growth rates across all dopamine treatments and light level controls (n=3) from day 0 to day 4 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where $p > 0.05$.

Levene's analysis	df= 7	F= 1.07	p= 0.425



A24: Distribution of intrinsic growth rates in *Thalassiosira* sp. across all dopamine treatments and light level controls (n=3) from day 0 to day 4. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Data meets assumption of normality.

A25: Dunnett test output for *Thalassiosira* sp. intrinsic growth rates across all dopamine treatments and light level controls from day 0 to day 8. 100%, 50%, and 25% represent the incident light levels measured in each light level control. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments and light level controls were tested for significance against the 100% light level / 0 µM dopamine control, where $\alpha = 0.05$.

Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
50%-100%/0 µM	-0.188	0.0641	0.571
25%-100%/0 µM	-0.540	-0.288	<<0.00100
15 µM -100%/0 µM	-0.222	0.0306	0.183
30 µM -100%/0 µM	-0.319	-0.0665	<0.0100
60 µM -100%/0 µM	-0.473	-0.220	<<0.00100
120 µM -100%/0 µM	-1.11	-0.858	<<0.00100
240 µM -100%/0 µM	-1.17	-0.921	<<0.00100

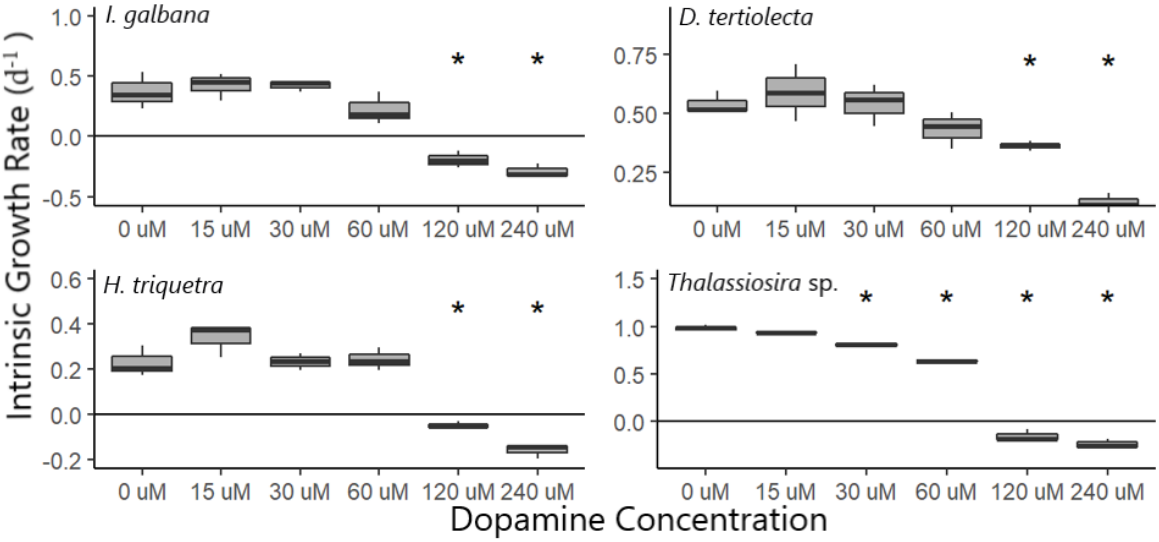
Species Sensitivity Comparisons

A26: Inhibitory concentration results (IC50s) for each tested species from day 1 to day 4. IC50s were determined from an LL.4 model and calculated from the intrinsic growth rates of each species (n=3) from day 1 to day 4 at 0 µM , 15 µM , 30 µM , 60 µM, 120 µM, and 240 µM dopamine. Upper and lower confidence intervals are at 95% confidence.

Species	IC 50	Standard Error	Lower Confidence Interval	Upper Confidence Interval
I. galbana	76.80	9.90	55.60	98.1 0
D. tertiolecta	316.00	659.00	-1100.00	1730.00
H. triquetra	99.70	11.40	75.40	124.00
Thalassiosira sp.	75.20	3.34	68.10	82.40

A27: Levene's analysis output for each tested species' intrinsic growth rates across dopamine concentrations 0 µM , 15 µM , 30 µM , 60 µM , 120 µM , and 240 µM from day 1 to day 4 with degrees of freedom (df), F-value, and p-value. The assumption of equal variance is met where p> 0.05.

Species	df	F-value	p-value
I. galbana	5	0.510	0.764
D. tertiolecta	5	0.926	0.497
H. triquetra	5	0.390	0.846
Thalassiosira sp.	5	0.509	0.764



A28: Distribution of intrinsic growth rates in *I. galbana* (top left), *D. tertiolecta* (top right), *H. triquetra* (bottom left), and *Thalassiosira* sp. (bottom right) across all dopamine treatments (n=3) from day 1 to day 4. Data meets assumption of normality.

A29: One-way ANOVA output of the intrinsic growth rates of each species (n=3) from day 1 to day 4 at all tested dopamine concentrations, where α= 0.05. Df is degrees of freedom.

Species	df	F-value	p-value
I. galbana	5	28.5	<<0.00100
D. tertiolecta	5	16.3	<0.00100
H. triquetra	5	44.7	<<0.00100
Thalassiosira sp.	5	718	<<0.00100

A30: Dunnett test output for all tested species' intrinsic growth rates across all dopamine treatments from day 1 to day 4. Upper and lower confidence intervals are at 95% confidence. All dopamine treatments were tested for significance against the 0 μM dopamine control, where α= 0.05.

I. galbana				***D. tertiolecta***			
Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value	Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
15 μM-0 μM	-0.195	0.300	0.956	15 μM-0 μM	-0.124	0.223	0.873
30 μM-0 μM	-0.195	0.300	0.954	30 μM-0 μM	-0.173	0.174	1.00
60 μM-0 μM	-0.396	0.0991	0.331	60 μM-0 μM	-0.280	0.0669	0.312
120 μM-0 μM	-0.816	-0.321	<0.00100	120 μM-0 μM	-0.351	-0.00385	0.0447
240 μM-0 μM	-0.910	-0.4145	<0.00100	240 μM-0 μM	-0.586	-0.239	<0.00100
H. triquetra				***Thalassiosira* sp.**			
Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value	Comparison	Lower Confidence Interval	Upper Confidence Interval	p-value
15 μM-0 μM	-0.00824	0.233	0.0713	15 μM-0 μM	-0.127	0.0412	0.469
30 μM-0 μM	-0.114	0.126	1.00	30 μM-0 μM	-0.256	-0.0870	<0.00100
60 μM-0 μM	-0.106	0.135	0.995	60 μM-0 μM	-0.433	-0.264	<<0.00100
120 μM-0 μM	-0.396	-0.155	<0.00100	120 μM-0 μM	-1.22	-1.05	<<0.00100
240 μM-0 μM	-0.509	-0.268	<<0.00100	240 μM-0 μM	-1.30	-1.14	<<0.00100