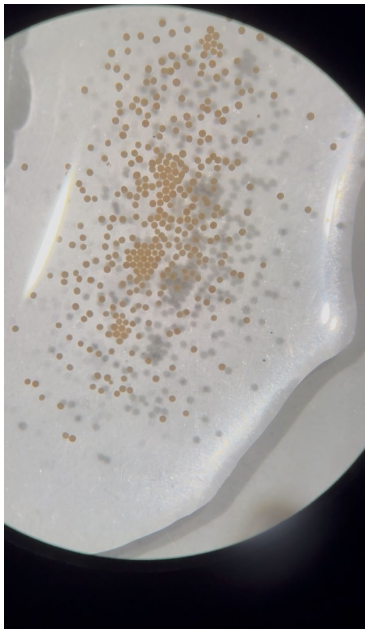
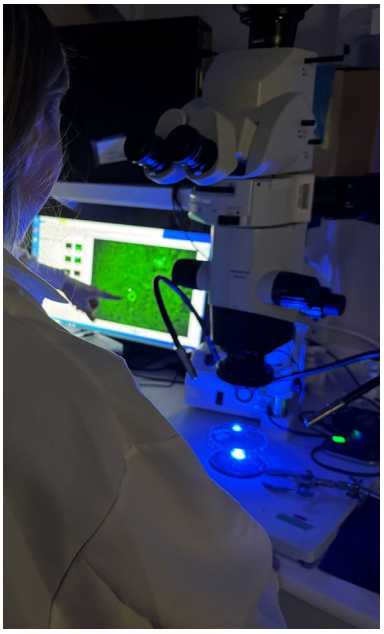
Determining Protective Nutrient Thresholds for the Early Life Stages of Corals

Determining Protective Nutrient Thresholds for the Early Life Stages of Corals

Final Report

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Management Summary (300 words or less)

Multiple concentration-response experiments were conducted to determine the impact of the phosphate, nitrate, ammonium and urea on the fertilization and larval survival/settlement of six critical coral species (*Colpophyllia natans*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, *Orbicella faveolata*, *Orbicella franksi* and *Pseudodiploria strigosa*). The results of these experiments revealed that two species were susceptible to elevated nutrients. First, cell division during fertilization of *Diploria labyrinthiformis* was found to be impacted by increased levels of phosphate. The Half Maximal Effective Concentration (EC_{50}), or the phosphate concentration at which the number *Diploria labyrinthiformis* eggs achieving a 4-cell state (i.e. undergoing 2 cell divisions) had fallen to 50% of the initial numbers was determined to be 0.33 µmol L^{-1}. Second, larval survivorship during settlement for *Montastraea cavernosa* was found to be impacted by increased levels of nitrate. The EC_{50}, or the nitrate concentration at which the number of surviving *Montastraea cavernosa* larvae (either swimming or settled) had fallen to 50% of the initial numbers was determined to be 11.5 µmol L^{-1}. The EC_{50} values determined by this study provide a benchmark for the mitigation of phosphate and nitrate levels along Florida's Coral Reef Tract.

Executive Summary (max 1 page)

High levels of nitrogen and phosphorus in the marine environment are contributing to a loss of water quality and concomitant decline in the health of Florida's Coral Reef. The numerous impacts of eutrophication in coastal marine waters have been documented on for coral reef ecosystems, including increased macroalgal growth and phytoplankton blooms that outcompete corals for space and light, changes to coral physiology, increased disease susceptibility, and shifts in reef community composition. Consequently, mitigating the threat of nutrient pollution has become an overarching resource management priority.

To date, most of the documented toxicant impacts of high nutrient exposures on corals have been demonstrated in adult life stages, yet empirical studies show evidence of population and physiological changes along nutrient gradients thus illustrating the importance of understanding how the more vulnerable early life stages may be affected by similar exposures. Comprehending the nature of these vulnerabilities provides resource managers with critical tools for reducing the likelihood of larval recruitment failures that prevent coral reefs from naturally recovering from disturbances and pollution.

The objective of this study was to examine the toxicant effects of the macronutrients phosphate, nitrate, ammonium and urea on two early life stages of six priority corals: *Colpophyllia natans*, *Diploria labyrinthiformis*, *Montastraea cavernosa*, *Orbicella faveolata*, *Orbicella franksi* and *Pseudodiploria strigosa.* A series of concentration-response experiments were conducted to determine the impact of the target macronutrients on the fertilization and larval survival/settlement of the coral species.

The results of these assays showed that two species were susceptible. First, cell division during fertilization of *Diploria labyrinthiformis* was found to be impacted by increased levels of phosphate. The Half Maximal Effective Concentration (EC_{50}), or the phosphate concentration at which the number *Diploria labyrinthiformis* eggs achieving a 4-cell state (i.e. undergoing 2 cell divisions) had fallen to 50% of the initial numbers was determined to be 0.33 µmol L^{-1}. Second, larval survivorship during settlement for *Montastraea cavernosa* was found to be impacted by increased levels of nitrate. The EC_{50}, or the nitrate concentration at which the number of surviving *Montastraea cavernosa* larvae (either swimming or settled) had fallen to 50% of the initial numbers was determined to be 11.5 µmol L^{-1}.

Together, these EC_{50} values provide a benchmark for the mitigation of phosphate and nitrate levels along Florida's Coral Reef Tract. Lowering phosphate and nitrate levels towards these values will be beneficial to the early life stages of *Diploria labyrinthiformis* and *Montastraea cavernosa,* and the coral reef ecosystem in general.

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1. INTRODUCTION

1.1. Overview

In Florida, as with much of the world, high levels of nitrogen and phosphorus in the marine environment are thought to contribute to degrading water quality and the resulting decline in coral reef health (Szmant & Forrester 1996; Lam et al. 2015; Whitall et al. 2019; Lesser 2021; Thanopoulou et al. 2022). Moreover, numerous impacts associated with eutrophication in coastal marine waters have been documented on coral reef ecosystems such as increased macroalgal growth and phytoplankton blooms that outcompete corals for space and light, changes to coral physiology, increased disease susceptibility, and shifts in reef community composition. Consequently, mitigating the threat of nutrient pollution has become an overarching management priority.

In corals, most of the documented toxic impacts of high nutrient exposures have been demonstrated in adult stages, and include effects on calcification, photosynthetic efficiency, and fecundity (see review by Lesser 2021 and references therein). The sensitivity of corals in empirical studies and the evidence of population and physiological changes along nutrient gradients illustrate the importance of understanding how the more vulnerable early life stages may be affected by similar exposures (Tomascik 1991; Wittenberg & Hunte 1992; Hunte & Wittenberg 1992; Kitchen et al. 2020). A complicating biogeochemical factor is that different nutrient forms (e.g. nitrate vs ammonium vs urea as a source of nitrogen) can have differing impacts on organisms and ecosystems, and potentially coral (Burkepile et al. 2020).

Many factors can interrupt the process of coral reproduction, larval survival, and successful recruitment onto the reef, and the failure of these processes in critical coral species warrants dedicated focus in the research and management communities. For reef ecosystems, whose persistence relies on the successful recruitment of new individuals, understanding any potential interruption to early life stages in corals is fundamental to the perpetuation of the reef ecosystem. Moreover, there is a paucity of species-specific data in the scientific literature on how perturbations in nutrient levels impact early life stages of corals, which in turn represents a significant limitation in providing resource management outcomes. Thus, the overarching objective of this project was to determine if elevated nutrient levels were toxic to early life stages of corals and establish benchmarks that can be used as protective thresholds against which nutrient mitigation efforts could be measured.

1.2. This Study

The macronutrients examined for this study were phosphate and the nitrogen sources nitrate, ammonia/ammonium and urea. The nutrient concentrations used for the assays were based on a review of the literature (see Table 1). The list of studies presented in Table 1 is a representative selection of studies looking at nitrate, ammonium and phosphate. It is by no means a comprehensive or exhaustive listing. The range of used in

this study concentrations was selected such that the lowest concentrations were close to the limit of detection for the analytical methods used and the highest concentration was well in excess of concentrations associated with polluted reefs.

While considerably less data are available on distribution of urea in coral reef systems, Crandall & Teece (2012) point to urea as being a dynamic source of bioavailable nitrogen for coral reefs. These authors reported ambient water column concentrations on the order of 0.5 µmol-N L^{-1} along the northern Florida Keys, consistent with other open ocean and reef studies (0.3-3.4 µmol-N L^{-1}; Wafar et al. 1986, Painter et al. 2008). Crandall & Teece (2012) also observed higher "hotspot" concentrations associated with seagrass beds (up to 11.2 µmol-N L^{-1}) and schooling fish (up to 13 µmol-N L^{-1}). In addition, runoff from agricultural and urban lawncare activities may also represent a critical source of urea to the coastal environment. A number of studies have found elevated urea levels associated with urban "finger" canals and water management canals, often in conjunction with episodically increased water flow from precipitation events such as thunderstorms and hurricanes or managed releases. For example, Revilla et al. (2005) reported urea levels in Florida Bay ranging up to 4.94 µmol-N L^{-1}, Ivey et al. (2020) observed periodic spikes in urea concentrations between 4 and 12.5 µmol-N L^{-1} in Sarasota Bay, whilst Crandall & Teece (2012) reported urea levels in Largo Canal (Key Largo) ranging up to 19.1 µmol-N L^{-1}. Whether or not the elevated levels of urea observed in terrestrial runoff represents a significant source of urea for offshore coral reefs and potential toxicant to the early life stages of coral remains unclear. They do, however, represent a tangible benchmark for evaluation during toxicological assays.

***Table 1**: List of representative maximum concentrations reported in the literature for nitrate, ammonium and phosphate in coral reef ecosystems and enrichment experiments involving corals.*

Study	Location	Site Description	NO_3^-	NH_4^+	PO_4^{3-}
Tomascik (1985)	Barbados	Pristine	0.45	0.56	0.06
	Barbados	Polluted	4.42	2.7	.21
Hoegh-Guldberg (1989)	Enrichment Study			45.8	
Dubinksy (1990)	Enrichment Study			100	10
Szmant (1996)	FKNMS* (Marinas/Canals)	Surface	1		1
	FKNMS* (Offshore, +500 m)	Surface	< 0.25	< 0.1	> 0.2
	FKNMS* (Marinas/Canals)	Interstitial		> 150	
	FKNMS* (Offshore, +500 m)	Interstitial		> 50	
Lapointe (1992)	FKNMS* (Marinas/Canals)		~ 4	~ 6	
	FKNMS* (Offshore, +2 km)		< 1	< 2	
Lapointe (1996)	FKNMS*			24	
Lapointe (1997)	Jamaica		27.9***	0.49	
	KJCAP**		1.22***	2.42	
Leichter (2003)	FKNMS*		4	2.2	0.3
Fabricius (2005)	Multiple Global Locations (Review)		20	20	4
Briceno (2013, 2022)	FKNMS*	Surface	1.49	8.01	3.9
	FKNMS*	Bottom	2.73	3.9	1.19
Lipp (2020)	FKNMS*	Water Column	1.11	7.95	2.49
Buckingham (2022)	Enrichment Study		~4.5		~5.7

Key: * FKNMS = Florida Keys National Marine Sanctuary; ** KJCAP = Kristin Jacobs Coral Aquatic Preserve; *** denotes measurement includes nitrate plus nitrite.

2. METHODS

2.1. Overview

The impact of each nutrient was examined using eight concentrations across six nutrient (regimes) combinations as outlined in Table 1. Since it is likely that both nitrogen and phosphorus will be present in most natural waters that coral gametes will develop, both nitrogen and phosphorus were added to the media although concentration levels for only one nutrient at a time were altered for the dose-response testing (as indicated in Table 2). The concentrations selected typify the range of nutrients typically encountered in the waters of the Florida Reef Tract with the highest levels being representative of highly impacted coral reefs (Szmant & Forrester 1996; Lam *et al*. 2015; Whitall *et al*. 2019). Up to 5 seawater controls were used to assess impact of the unamended aquarium seawater and natural seawater samples (Table 3).

Nutrients samples were collected from the seawater media used to grow the coral larvae at the end of each experiment. All nutrient samples were filtered through a 0.2 µm Acrodisc (25 mm) syringe filter and frozen until analysis. Nitrate and nitrite were determined using the copper-cadmium reduction method (US-EPA Method 353.2). Ammonium was analyzed using the alkaline phenol/hypochlorite method (US-EPA Method 350.1 revision 2). Urea was measured using the diacetyl monoxime colorimetric method of Chen *et al.* (2015). Phosphate (Orthophosphorus) was analyzed using the antimony-phosphomolybdate complexation method (US-EPA Method 365.1).

2.2. Media and Nutrient Supplementation.

Synthetic seawater based on Enriched Seawater Artificial Water (ESAW; Harrison *et al*. 1980, Berges *et al*. 2001) was used as the base media for the nutrient amendments used in both the fertilization and settlement assay. This formulation allowed for the creation of a seawater mix that contained no nitrogen or phosphate sources. For these experiments, the quantities of the salts were adjusted proportionally to achieve a final salinity of 36 parts per thousand by weight. The basic ESAW media was supplemented with pre-prepared silicate, trace elements and vitamin solutions based on L1 media (20% final concentration; Guillard & Hargraves 1993) to ensure a more representative suite of macro- and micro-nutrients were present. Primary stock solutions for nitrate and phosphate were prepared according to Guillard & Hargraves (1993) with ammonium and urea primary stock solutions made to the same molarity using ammonium chloride and urea. The primary stocks were then used to prepare secondary solutions for the nutrient amendments in the assays. All chemicals used were Certified ACS grade (or equivalent or better).

2.3. Gamete Collection.

All gametes used in this project were collected from broodstock held at the Florida Coral Rescue Center (FCRC). For the fertilization assays, intact gamete bundles were collected from the coral parent immediately after bundle release to ensure that no mixing of the eggs and sperm occurred. Gametes were collected from *Colpophyllia natans* (CNAT), *Diploria labyrinthiformis* (DLAB), *Montastraea cavernosa* (MCAV),

Orbicella faveolata (OFAV)*, Orbicella franksi* (OFRA), and *Pseudodiploria strigosa* (PSTR). For each species, 3+ genotypes/parents were collected (see Appendix 3, Section 6.3, Tables 4-6). In the case of the larvae used for the settlement assays, gametes from 2+ genotypes were collected and batched together (see Appendix 4, Section 6.4, Tables 7-10).

2.4. Fertilization Assays

After sufficient bundles had been collected, the bundles were gently washed with SW water to separate the eggs and sperm (where applicable). Once separated, the eggs were visually examined by microscopy to verify that they had not already started dividing (i.e. had already been fertilized). This critical step is required for the fertilization assays to verify that the eggs have not been fertilized prior to their addition to the nutrient-amended media thereby allowing an assessment of the impact of nutrients on the fertilization process. A batch sample was then generated from the individual genotypes with the volumes of eggs and sperm from each genotype adjusted to ensure that each genotype was represented equally in the batch sample.

Eggs (~100) were then added to an acid-washed vial containing 4 mL of media followed by the sperm (to a final concentration of ~1 x 10^6 mL^{-1}) and the mixture then allowed to fertilize for between 1.5 and 4 hours depending on the species. The eggs were monitored for development and fixed with 4 mL for Z-fix solution once most of the eggs had undergone 2+ divisions. The samples were then returned to the HML for counting by microscopy. Each sample was assessed for unfertilized eggs, 2 cell stage, 4 cell stage and 8+ cell stage. Fertilization efficiency was determined as being the proportion of total number of eggs experiencing cell division.

During the August spawning, visual examination of the MCAV eggs showed that fertilization had begun before the eggs could be aliquoted into the treatment vials and consequently were not tested. The OFAV eggs passed this quality control check and were used in the nutrient assay. For the September spawning, eggs for CNAT, PSTR and OFRA were all found to be unfertilized prior to the start of the assay and were assayed. Gametes from CNAT and PSTR were also collected during October spawning and are referred to as CNAT2 and PSTR2 to differentiation them of the samples collected in September. Both the CNAT2 and PSTR2 eggs passed the visual quality control checks and were assayed. Gametes for DLAB were collected during May 2026 and passed the visual quality control check.

***Table 2**: List of the nutrient treatments used for the fertilization and settlement assays. The treatment IDs are used throughout the report as shorthand to easily identify the nutrient toxicity being assessed and the range of concentrations used.*

Treatment ID	Nutrient Regime	Target Nutrient Concentrations (µmol L^{1})
NP	Variable **N**itrate, Constant **P**hosphate	Phosphate 0.3; Nitrate 0.05, 0.1, 0.5, 1, 5, 10, 50, 250
AP	Variable **A**mmonium, Constant **P**hosphate	Phosphate 0.3; Ammonium 0.05, 0.1, 0.5, 1, 5, 10, 50, 250
UP	Variable **U**rea, Constant **P**hosphate	Phosphate 0.3; Urea 0.05, 0.1, 0.5, 1, 5, 10, 50, 250
PN	Variable **P**hosphate, Constant **N**itrate	Nitrate 1; Phosphate 0.01, 0.03, 0.05, 0.1, 0.3, 0.6, 1, 6
PA	Variable **P**hosphate, Constant **A**mmonium	Ammonium 1; Phosphate 0.01, 0.03, 0.05, 0.1, 0.3, 0.6, 1, 6
PU	Variable **P**hosphate, Constant **U**rea	Urea 1; Phosphate 0.01, 0.03, 0.05, 0.1, 0.3, 0.6, 1, 6

***Table 3**: List of the controls used for the fertilization and settlement assays. The control IDs are used throughout the report as shorthand to readily identify the control being used. The full name and origin of the control material are also given.*

Control ID	Control Name	Description
AS	Artificial Seawater	Synthetic seawater used in the coral nursery aquaria at the HML.
SW	SeaWorld seawater	Synthetic seawater used in the aquaria at the Florida Coral Rescue Center.
ES	Enriched Seawater Artificial Water	Synthetic seawater used as the base media for the nutrient amendments in this project (also referred to as ESAW; Berges *et al*. 2001).
KL	Key Largo seawater	Surface seawater collected at Horseshoe Reef (Key Largo, Florida Keys).
SS	Sargasso Sea seawater	Surface seawater collected from the Sargasso Sea (approx. 31.72°N, 76.62°W; used as base media for oligotrophic phytoplankton cultures at the HML).

2.5. Settlement Assays

All larvae from the August, September and October spawning events were prepared by the FCRC and were transported back to the HML to conduct the nutrient-settlement assays. In August, at the end of the 7-day incubation, the OFAV settlement tiles were preserved for subsequent enumeration by microscopy, the number of swimming larvae counted, and the media preserved for nutrient analysis. In the case of the MCAV experiment, the number of settled and swimming were counted and the media preserved for nutrient analysis. As with the August spawning, FCRC prepared CNAT, PSTR and OFRA larvae that were brought back to the HML to conduct the nutrient-settlement assays. While preparing for the nutrient-settlement assays at the HML, it was found that insufficient larvae had survived the transfer to conduct an assay for PSTR. In the case of OFRA, there was only sufficient larvae to test two nutrients (AP and UP Series per Table 1). For CNAT, sufficient larvae survived to allow for a full test of all six nutrient combinations. At the end of the 7-day incubation, the OFRA settlement tiles were preserved for subsequent enumeration, the surviving swimming larvae counted, and the media preserved for nutrient analysis. Larvae for settlement assays for CNAT2 and PSTR2 were prepared at FCRC for transfer back to the HML for the settlement assays. However, both CNAT2 and PSTR2 appear to have suffered crashes within 48 hours of arriving at the HML and insufficient larvae had survived. Consequently, the settlement assays were not conducted. Similarly, DLAB larvae prepared at FCRC during the May 2026 spawning event were transferred back to the HML but did not survive.

For each species tested, larvae (~10-20 competent individuals) were transferred to an acid-washed 60 mL glass jar containing 40 mL of media and a small limestone block that provided a surface for settlement (Figure 1). The limestone block (~20 mm x 20 mm x 12 mm) was cut from unglazed limestone tiles, triple rinsed with tap water, and triple rinsed with deionized water. The cleaned blocks were then preconditioned in a separate coral aquarium for ~14 days prior to their placement in the assay jars to allow the growth of a crustose coralline algae (CCA) biofilm that provided additional settlement cues. The larvae were allowed to develop and settle for ~7 days at 26.5°C. For each incubation, 50% of the nutrient-amended media was replaced every three days. 20 mL of the media from each incubation jar was drawn off by pipette through a 40 µm cell sieve and replaced with 20 mL of fresh nutrient-amended media. At the end of the incubation, the number of larvae left swimming in the glass jar were counted and the limestone scanned for settled polyps. Survivorship was calculated as the total number of swimming larvae and the number of settled polyps.

2.6. Limestone Control Tests

To test the effect of the presence of both the limestone settlement blocks and crustose coralline algae (CCA) present on the conditioned blocks on the nutrient levels in the incubation media, a series of experiments was carried out to assess the likely impact of the blocks (Shrimali & Singh 2001; Price et al. 2010; Li *et al*. 2023). A preliminary experiment was conducted to determine if a standard rapid fluorometric chlorophyll *a* method using a 90% acetone extraction (Welschmeyer 1994) could be used as a proxy for CCA present on the blocks. Since the findings of this experiment

showed that the Welschmeyer method could be used to measure chlorophyll *a*, two additional more comprehensive experiments were conducted. Both experiments were conducted at the same incubation temperature has the settlement assys. The first involved placing limestone blocks in NP and PN series media for ~5 weeks to ensure the development of substantial CCA biofilm (referred to as the CCA test). The second experiment involved placing limestone blocks in NP, PN, PA and PA series media for 7 days, which is the typical length of the settlement incubation (referred to as the NUTS test). At the end of each incubation, the media collected for nutrient analyses and the limestone blocks were extracted with 10 mL of 90% acetone. Chlorophyll *a* measurements were conducted using a Turner Designs 10AU Fluorometer against a spinach standard (Welschmeyer 1994; Ritchie 2008).

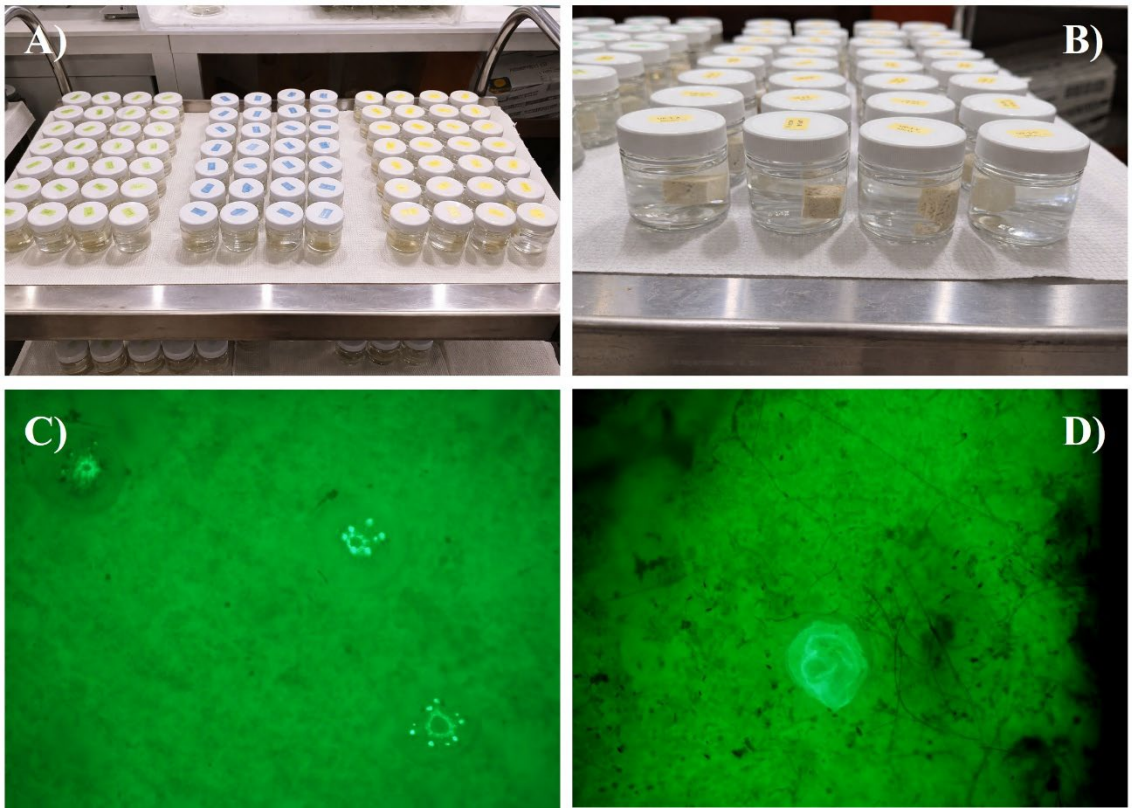

Figure 1: A) *Sets of nutrient assays in a temperature-controlled walk-in incubation chamber used for the tests,* ***B)*** *Close-up of the incubation jars showing the CCA-conditioned limestone settlement blocks,* ***C)*** *Exemplar microscope image of settled* Colpophyllia natans *on a limestone block, and* ***D)*** *Exemplar microscope image of settled* Pseudodiploria strigosa *on a limestone block. Ultraviolet light is used to excite green fluorescent protein (GFP)-like molecules in the organisms.*

2.7 Statistical Analyses

As a preliminary triage step, the slope of the linear regression for the results of each fertilization or settlement assay was examined to determine if the slope was significantly different to zero (Linear Regression t-test, $p<0.05$). The data for any result

whose slope was found to be significantly different to zero was then fitted with a 4-Parameter Logistic Curve to determine the EC_{50} for that nutrient. All statistics were conducted using SigmaPlot 13.0 (Systat Software/Grafiti LLC).

3. RESULTS

3.1. Impact of Crustose Coralline Algae on Nutrients.

The chlorophyll *a* results from both the CCA and NUTS experiments show considerable amounts of biomass were associated with the limestone blocks used for larval settlement (see Figure 2 for the CCA experiment results). By comparison, the chlorophyll *a* levels in the media were consistently less than 20 mg L^{-1}.

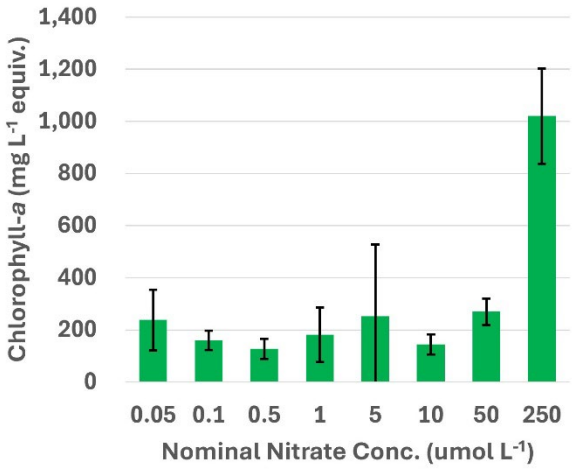

Figure 2: *Results of the chlorophyll a analyses from the CCA incubation for the NP series treatments. The chlorophyll* a *concentrations are given as equivalents since the blocks were extracted directly in 90% acetone and the CCA community was not filtered and pre-concentrated on to a glass-fiber filter as is typical for seawater samples. Error bars, where present, represent the 90% confidence interval.*

While CCA primarily describes the red macroalgae component of the biofilm, there is a considerable and variable microbiome associated with the macroalgae (van der Heijden & Kamenos 2015; Sneed et al. 2015; Hochart et al. 2024). Given that the CCA biofilm was allowed to develop on the limestone blocks for 14 days prior to their use, this community will therefore likely have an impact on the nutrients present in the media. Moreover, these impacts would be separate from the effects of the limestone blocks themselves blocks (Shrimali & Singh 2001; Price et al. 2010; Li *et al*. 2023).

The results from the nutrient analyses for the CCA experiment (NP-series treatments are shown in Figure 3) and NUTS experiment (PN-series treatments are shown in Figure 4) show that there are considerable variation and differences. Similar results were observed with the PN-series treatments during the CCA experiment and with the NP, PA and PU-series treatment during the NUTS experiment (data not shown). The CCA experiment results show elevated levels of inorganic nitrogen in excess of that added to the treatments. Furthermore, the results show the presence of nitrite and ammonium when none was added to the media. Together, these findings

point to active cycling of inorganic nitrogen including possible nitrogen fixation as the source of increased inorganic nitrogen in the system.

The results for the phosphate analyses of the CCA PN-series (data not shown) and NUTS PN-series (Figure 4) also show consistently higher phosphate levels than would be expected based on the dosage regime of the treatments. Similar results were seen for the NUTS PA and PU-series treatments. These findings are concordant with the results of Shrimali & Singh (2001) who suggested that phosphate is released from limestone when it comes into contact with full strength seawater such as the 36 ppt ESAW used for this work. On the basis of the findings from the CCA and NUTS experiments, it was decided that all results from the fertilization and settlement assay would be plotted against the initial (nominal) nutrient concentration for each treatment.

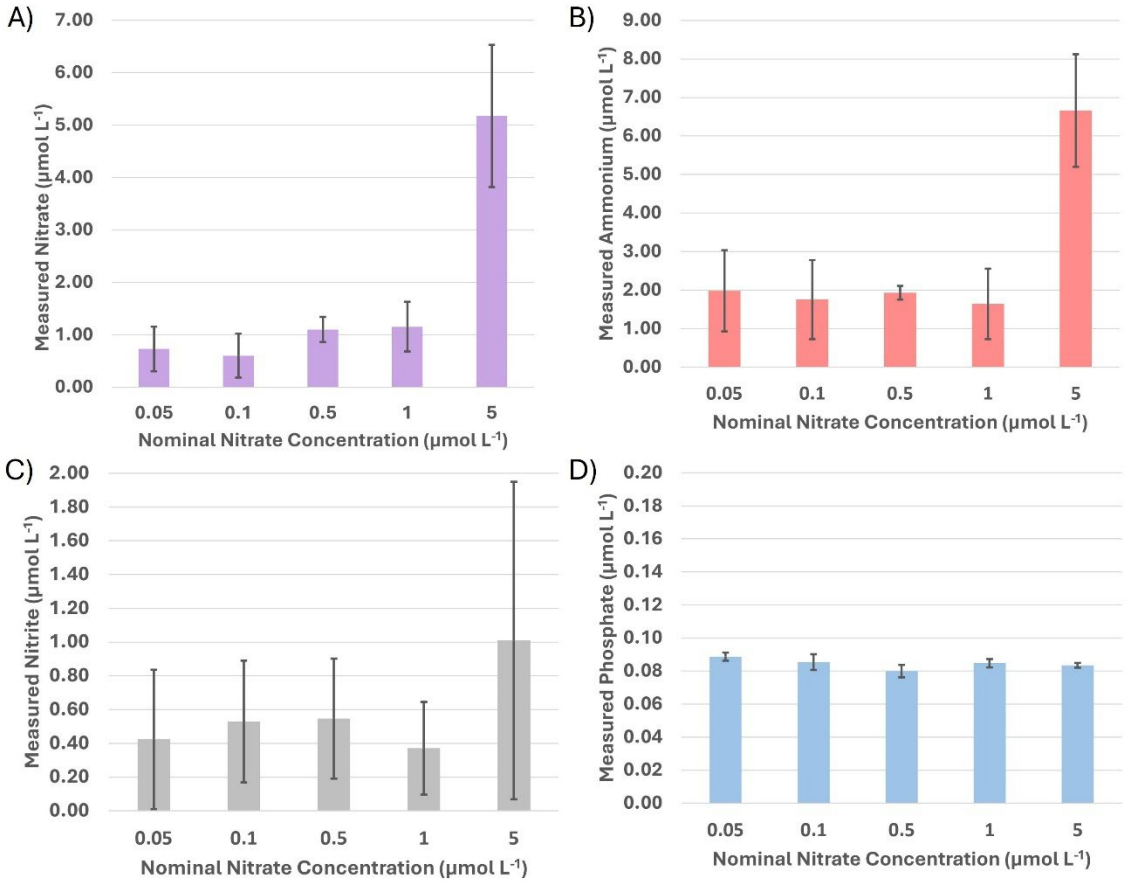

Figure 3: *Results of the nutrient analyses from the CCA incubation for the NP series treatments.* ***A)*** *Measured final nitrate level versus the initial (nominal) nitrate concentration,* ***B)*** *Measured final ammonium level versus the initial (nominal) nitrate concentration,* ***C)*** *Measured final nitrite level versus the initial (nominal) nitrate concentration, and* ***D)*** *Measured final phosphate level versus the initial (nominal) nitrate concentration. Only the five lowest nominal concentrations are shown. Error bars, where present, represent the 90% confidence interval.*

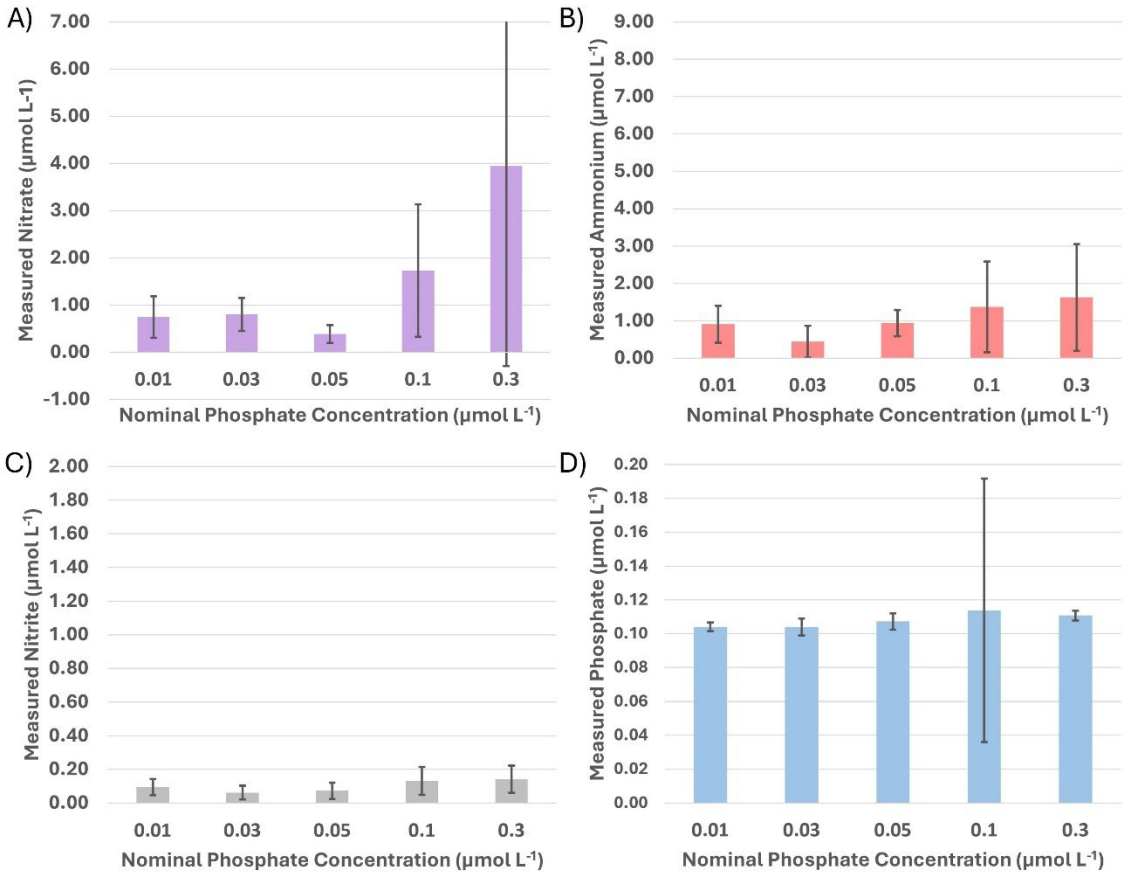

Figure 4: *Results of the nutrient analyses from the NUTS incubation for the PN series treatments.* ***A)*** *Measured final nitrate level versus the initial (nominal) phosphate concentration,* ***B)*** *Measured final ammonium level versus the initial (nominal) phosphate concentration,* ***C)*** *Measured final nitrite level versus the initial (nominal) phosphate concentration, and* ***D)*** *Measured final phosphate level versus the initial (nominal) phosphate concentration. Only the five lowest nominal concentrations are shown. Error bars, where present, represent the 90% confidence interval.*

3.2. Impact of Nutrient Levels on Coral Fertilization

Fertilization assays were conducted for OFAV, CNAT (and CNAT2), PSTR (and PSTR2), OFRA and DLAB. During a routine check of the *p*H levels of the media for the OFAV experiment, discrepancies were discovered between the *p*H levels of the stock solutions prepared for the experiment and the media transferred to the fertilization chambers. Since the discrepancies could not be resolved after additional tests, the results from the OFAV fertilization assay were discarded. Data from the remaining fertilization assays are plotted in Appendix 1 (Section 6.1, Figures 7-37). The results show that none of the nutrient regimes tested affected the percentage of total fertilized eggs. This finding may result, in part, from the testing protocol which calls for the assay to be stopped by fixing the samples at a predetermined time after the initiation of the fertilization process based on the visually confirmed presence of fertilized eggs. As

such, the rate of fertilization was not monitored. However, the counts of eggs at various stages of cell division (i.e. 2-cell stage vs 4-cell stage vs. 8 or more cell stage) were counted as part of the assay.

Interestingly, for several species under varying nutrient regimes (OFRA-UP, PSTR-PA, DLAB-PN/PA/PU, CNAT2-NP), these results show that high levels of nutrients appeared impact the number of eggs advancing past a 2-cell stage. Except for DLAB-PN, the other species/nutrient regimes showed this general trend, but the decline was not significant. In the case of DLAB-PN, the decline was large enough to allow an assessment of the toxicity of phosphate towards DLAB fertilization (Figure 5).

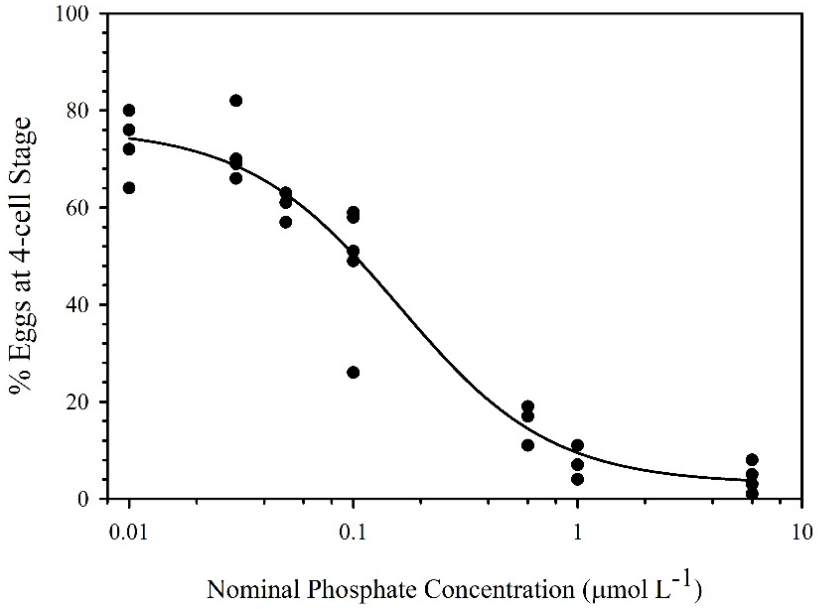

Figure 5: *Plot of the % Eggs at 4-cell stage for Diploria labyrinthiformis (DLAB; all replicates plotted) versus nominal phosphate (with nitrate) concentration with a 4-Paramater Logistic Curve fitted.*

The Half Maximal Effective Concentration (EC_{50}) or the phosphate concentration at which the number of 4-cell DLAB eggs has fallen to 50% of the initial numbers is calculated as 0.33 µmol L^{-1}.

3.3. Impact of Nutrient Levels on Coral Settlement.

Settlement assays were conducted for OFAV, OFRA, MCAV and CNAT. Data for all settlement assays conducted are plotted in Appendix 2 (Section 6.2, Figures 38-53). In general, the results of the settlement assays show that elevated nutrient levels appear to have little effect with three exceptions. For OFAV-PA and OFAV-PN, slight declines were observed in survivorship but in both cases, the decline was not significant. For MCAV-PN, the decline was significant and allowed an assessment of the toxicity of nitrate towards the survivorship of MCAV larvae (Figure 6).

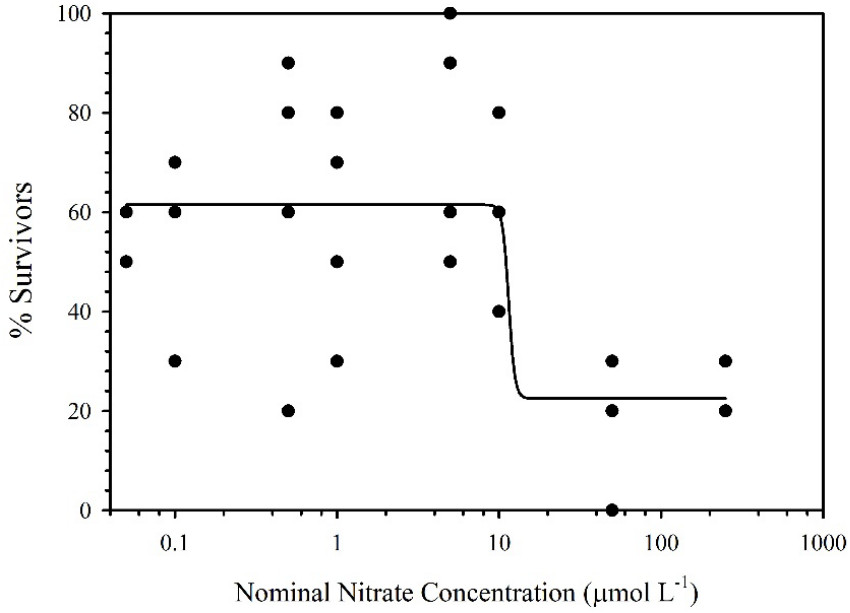

Figure 6: *Plot of the % Survivors (all replicates) versus nominal nitrate concentration for Montastraea cavernosa (MCAV) with a 4-Paramater Logistic Curve fitted.*

The Half Maximal Effective Concentration (EC_{50}) or the nitrate concentration at which the number of surviving MCAV larvae (either swimming or settled) has fallen to 50% of the initial numbers is calculated as 11.5 µmol L^{-1}.

4. DISCUSSION AND MANAGEMENT RECOMMENDATIONS.

The results also show that for at least two coral species, *Diploria labyrinthiformis* and *Montastraea cavernosa*, elevated nutrient levels have a detrimental impact on the early life stages of these species. With regard to *Diploria labyrinthiformis,* an EC_{50} of 0.33 µmol L^{-1} towards phosphate suggests that *Diploria labyrinthiformis* is highly susceptible to fertilization failures in polluted waters of the Florida Keys National Marine Sanctuary. Several authors report maximal phosphate levels well in excess of 0.33 µmol L^{-1} for the Florida Keys (1.19-2.49 µmol L^{-1} Table 1; Briceño et al. 2013; Briceño 2022; Lipp et al. 2020). Phosphate levels below 0.33 µmol L^{-1} tend to only occur in pristine coastal environments (Tomascik & Sander 1987) or open ocean locations away from coastal environments (Levitus et al. 1993; Conkright et al. 2000). This finding also presents a challenge for the mitigation of elevated phosphate levels as it would require lowering phosphate levels in polluted areas to that of a pristine environment. Nonetheless, any phosphate reduction initiatives should utilize 0.33 µmol L^{-1} as the target for post-reduction assessments. This result also has practical implications for the *ex-situ* rearing of *Diploria*

labyrinthiformis. Tight control of the phosphate levels in the fertilization media will be required to ensure that fertilization is not impeded in any way. It should also be noted that that assay showed an apparent slowing or halting of the fertilized eggs between the first and second cell divisions. Additional work is required to determine if the affected eggs were able to continue dividing albeit at a slower rate and if those eggs developed into competent larvae capable of settling and growing into polyps.

In the case of *Montastraea cavernosa*, an EC_{50} of 11.5 µmol L^{-1} towards nitrate indicates that *Montastraea cavernosa* larvae are unlikely to experience any negative impacts during settlement from these levels of nitrate in the waters of Florida's Coral Reef Tract. In the studies cited in Table 1, nitrate levels were less than 3 µmol L^{-1} (Szmant & Forrester 1996; Leichter et al. 2003; Briceño et al. 2013; Briceño 2022; Lipp et al. 2020). Exceptions to this finding could include circumstances where there are nitrate hotspots resulting from substantial coastal runoff or possibly associated with oxidation of ammonia/ammonium (>150µmol L^{-1}; Szmant & Forrester 1996). Interestingly, *Montastraea cavernosa* is thought to be highly susceptible to recruitment issues such as poor larval supply and post-settlement survival (Jones & Gillam 2024). The results of this study suggest that elevated nitrate does not contribute to those issues as it pertains to larval settlement. While current nitrate levels may not pose a problem, results from future surveys can be compared to this benchmark to determine if intervention is required.

Overall, the results of the assays suggest that several of the corals tested can withstand relatively high levels of certain nutrients during the fertilization and settlement phases of their life cycles. However, the EC_{50} values determined by this study provide tangible benchmarks for the mitigation of phosphate and nitrate pollution along Florida's Coral Reef Tract. Lowering the nutrient concentrations to below the EC_{50} values will likely have a positive effect on the fertilization rate of *Diploria labyrinthiformis* and the settlement rate for *Montastraea cavernosa*. Even for those species where non-significant declines were observed (*Orbicella faveolata*, *Pseudodiploria strigosa* with phosphate and *Colpophyllia* natans for nitrate), a reduction in these nutrients would also be beneficial as it would increase the number of eggs being fertilized and subsequently undergoing cell division.

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6. APPENDICES

6.1. Appendix 1. Results from the Fertilization Assays.

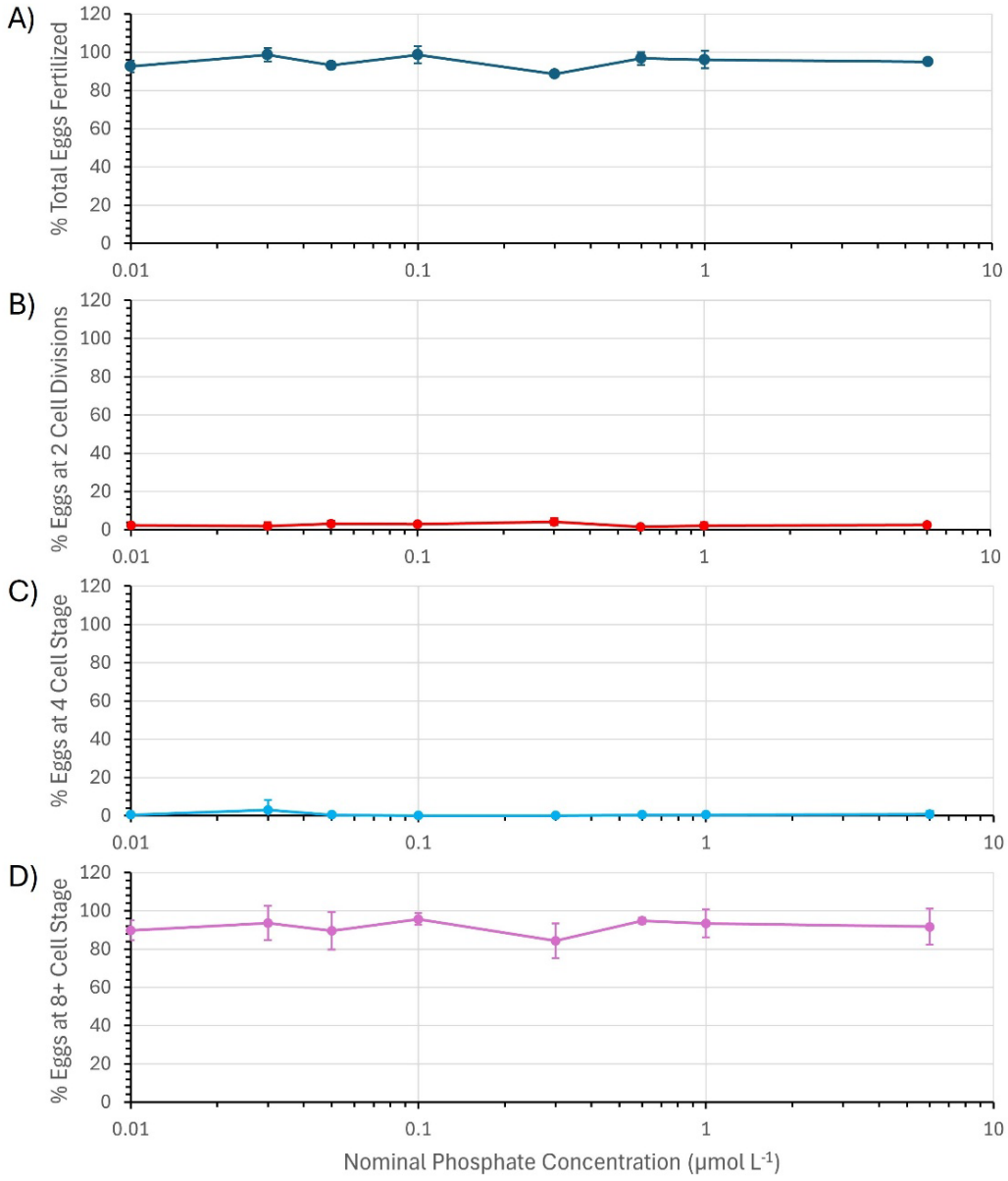

Figure 7: *Impact of Phosphate (with Nitrate as the nitrogen source) on the fertilization of* Colpophyllia natans *gametes (**CNAT**, spawned 09/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

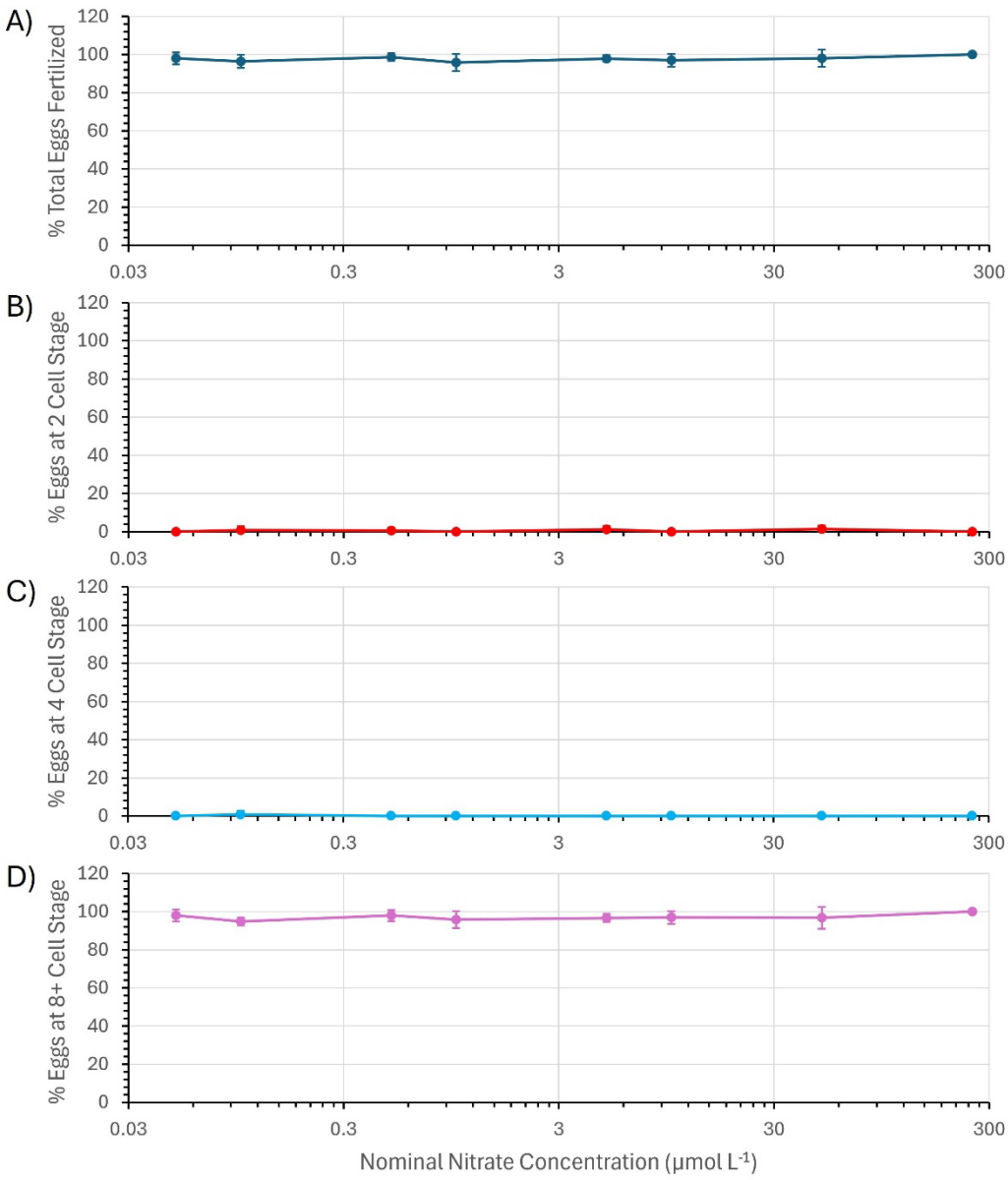

Figure 8: *Impact of Nitrate on the fertilization of* Colpophyllia natans *gametes (**CNAT**, spawned 09/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

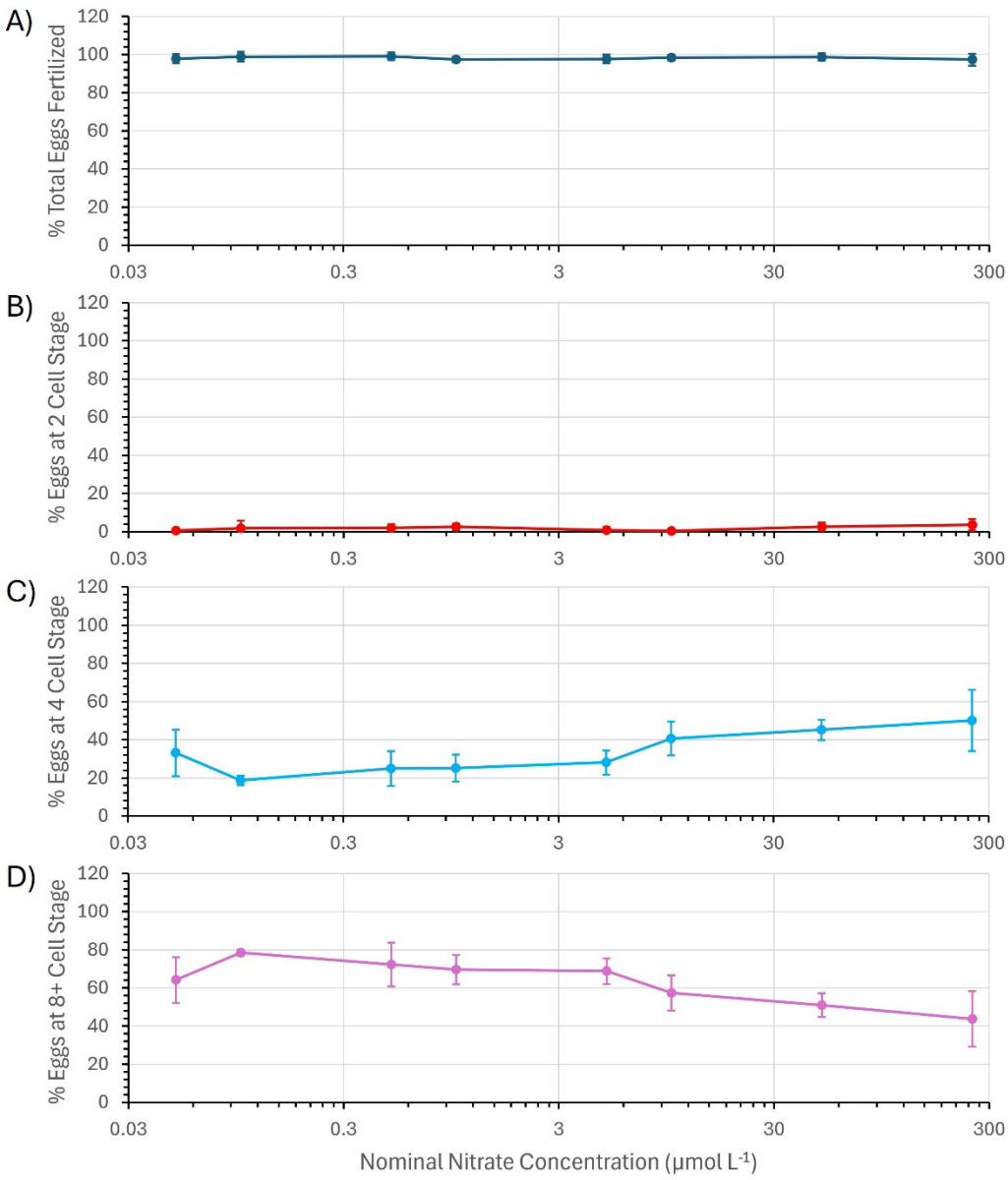

Figure 9: *Impact of Nitrate on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

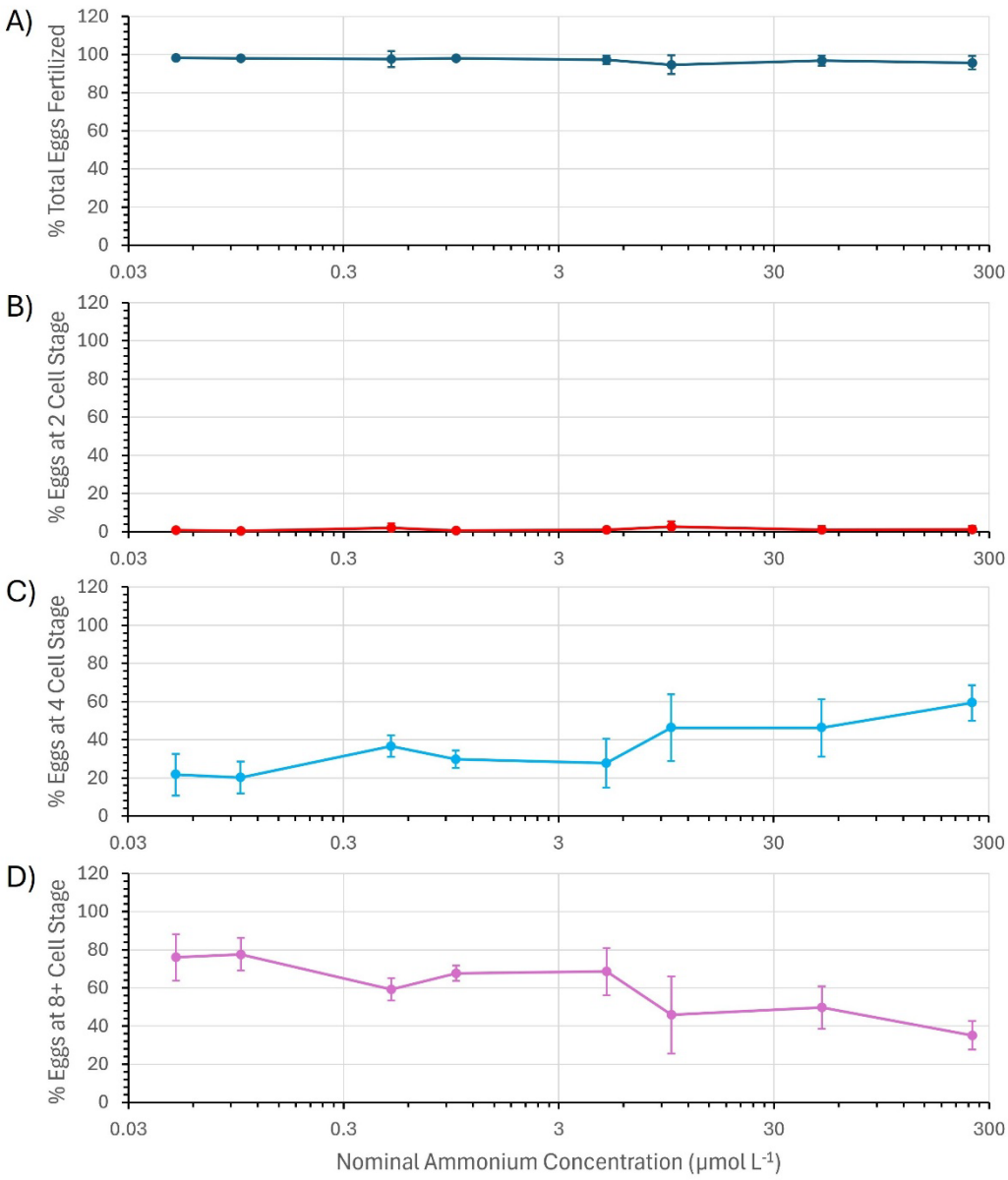

Figure 10: *Impact of Ammonium on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

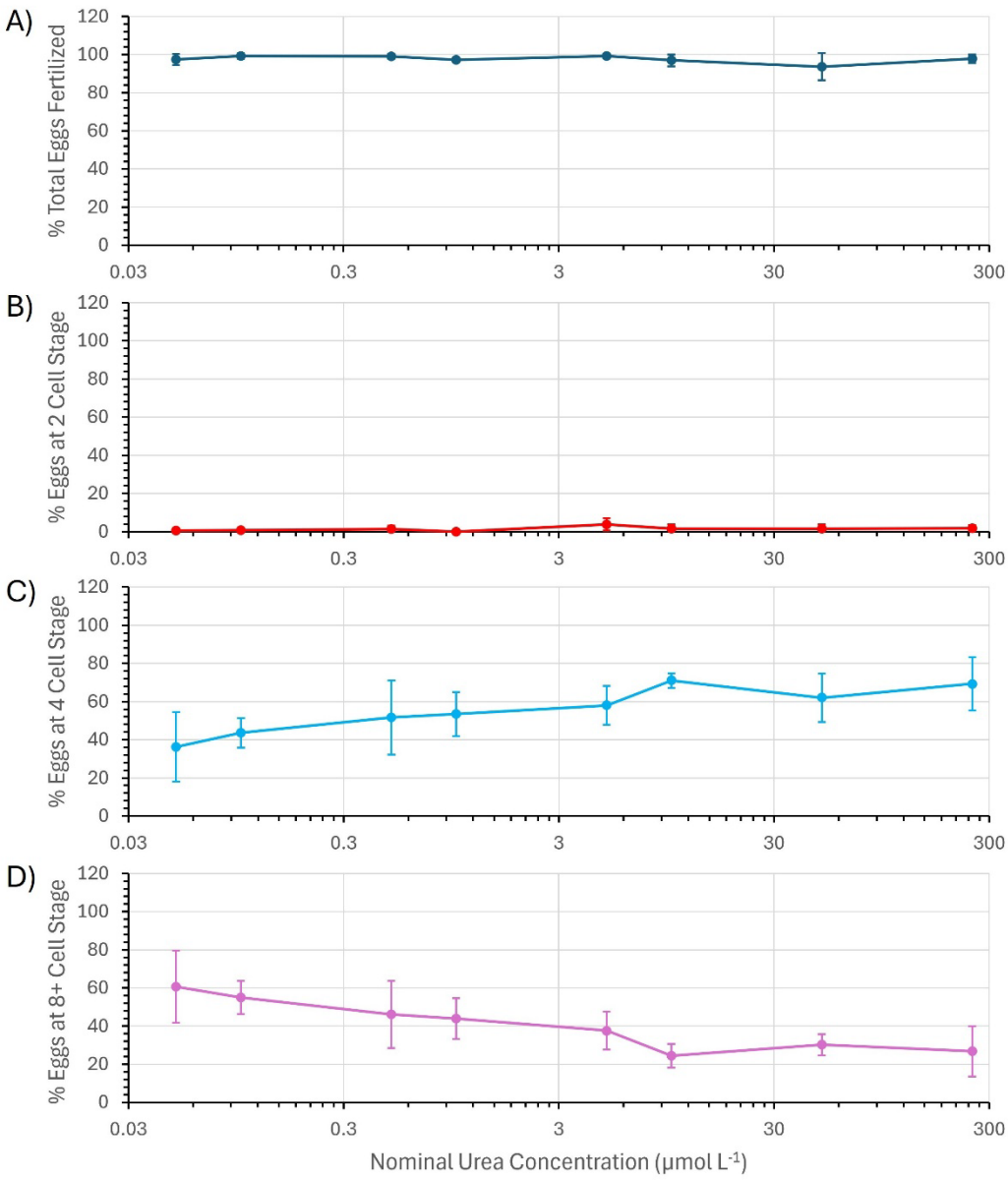

Figure 11: *Impact of Urea on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

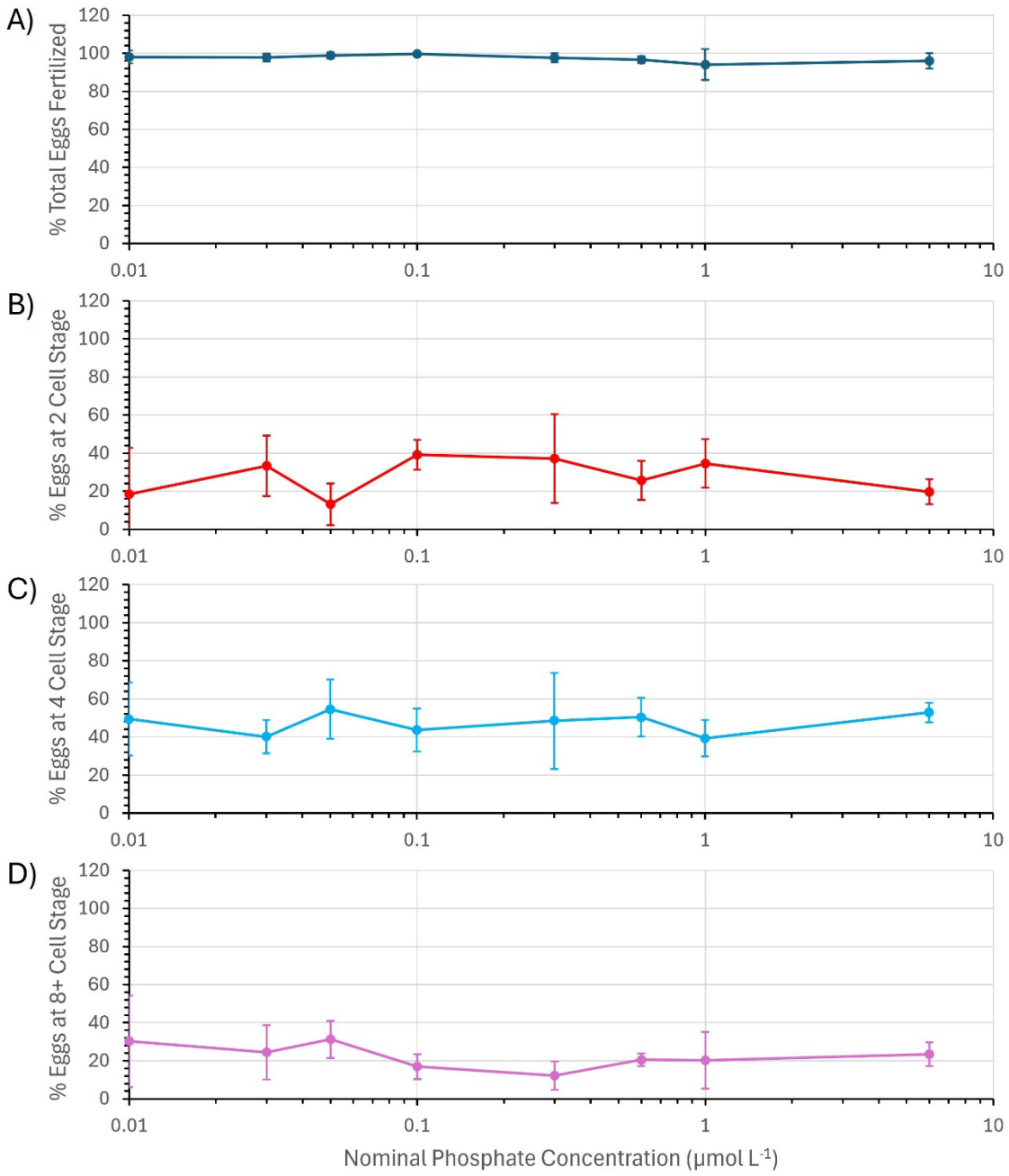

Figure 12: *Impact of Phosphate (with Nitrate as the nitrogen source) on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

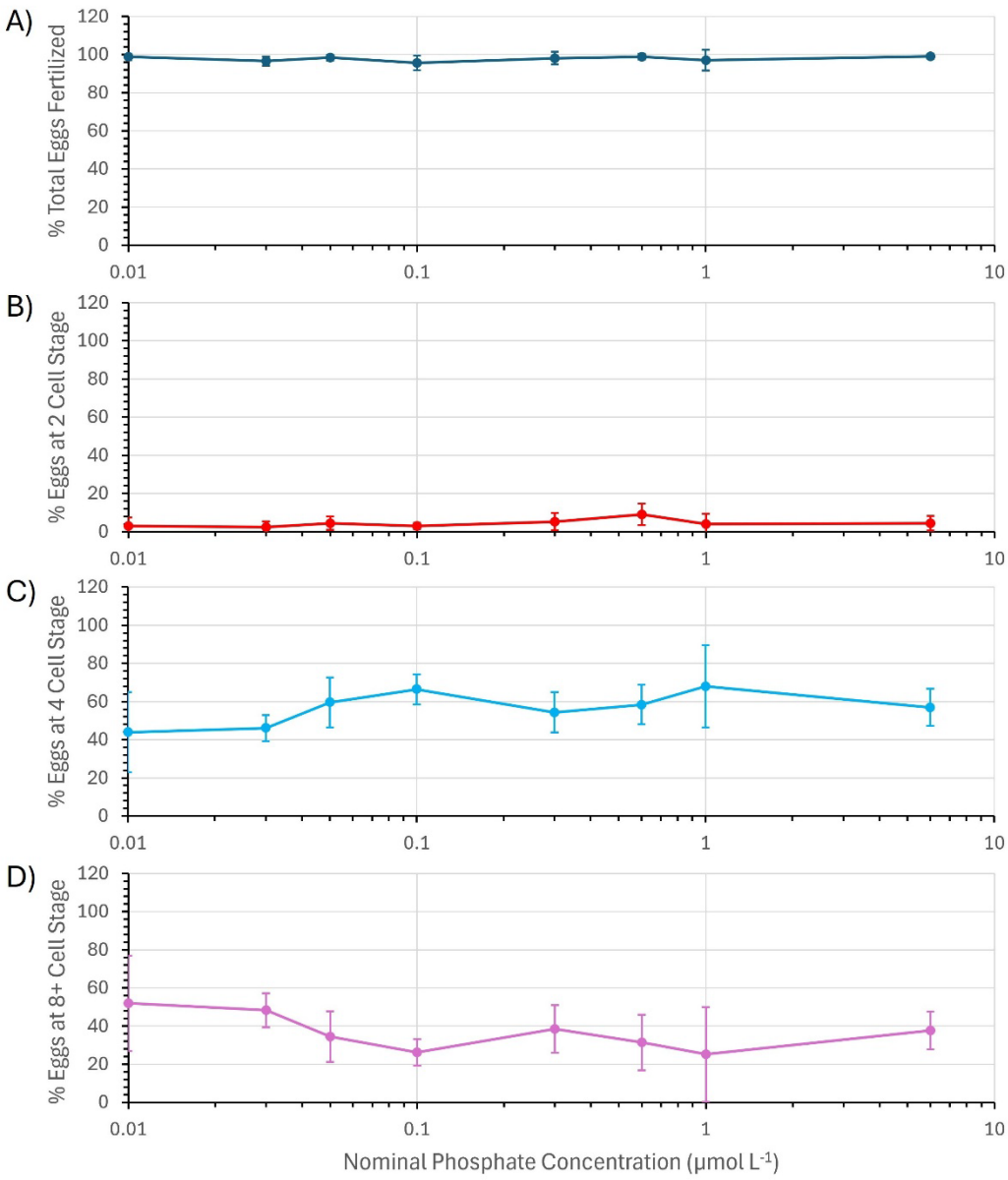

Figure 13: *Impact of Phosphate (with Ammonium as the nitrogen source) on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

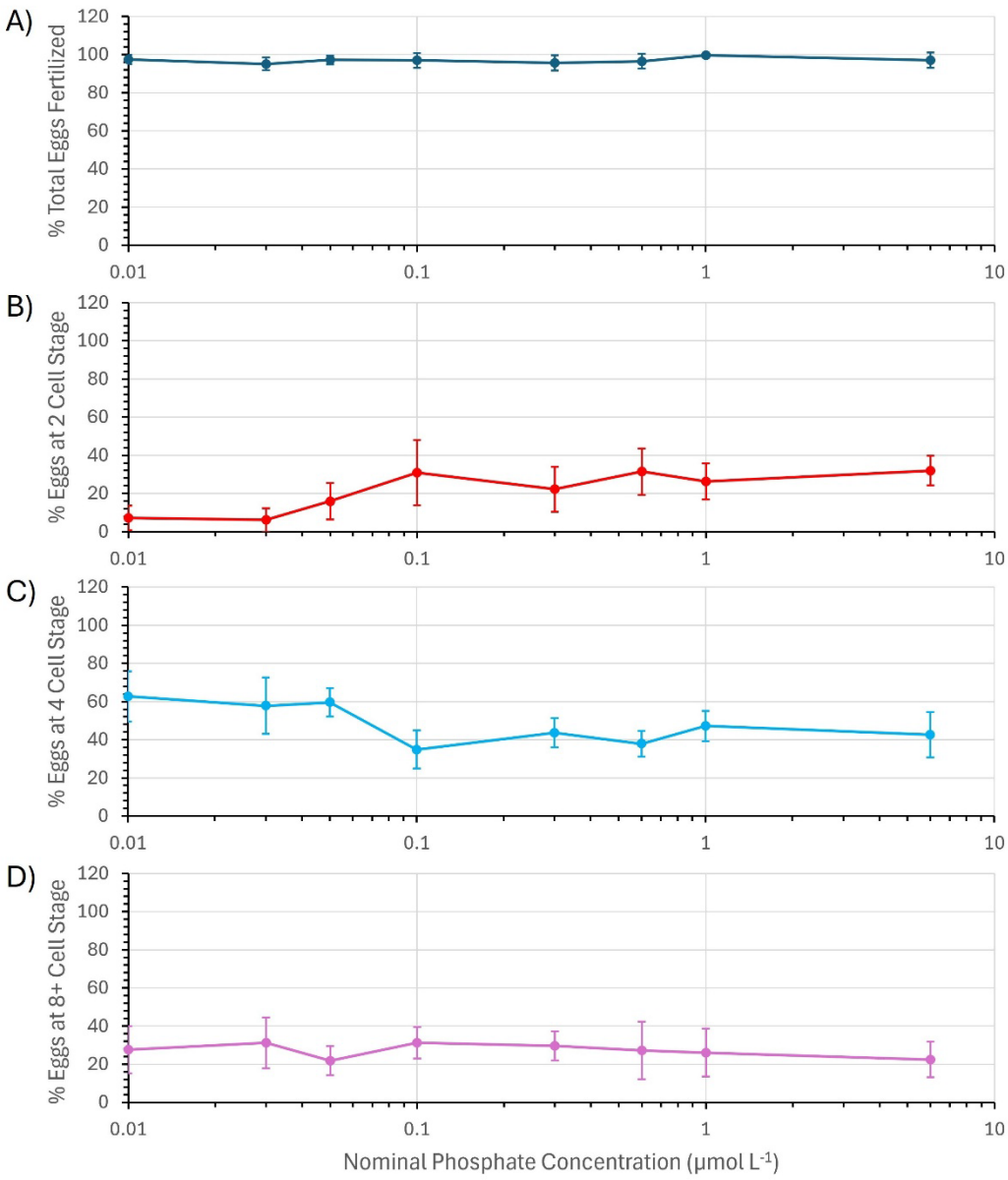

Figure 14: *Impact of Phosphate (with Urea as the nitrogen source) on the fertilization of* Colpophyllia natans *gametes (**CNAT2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

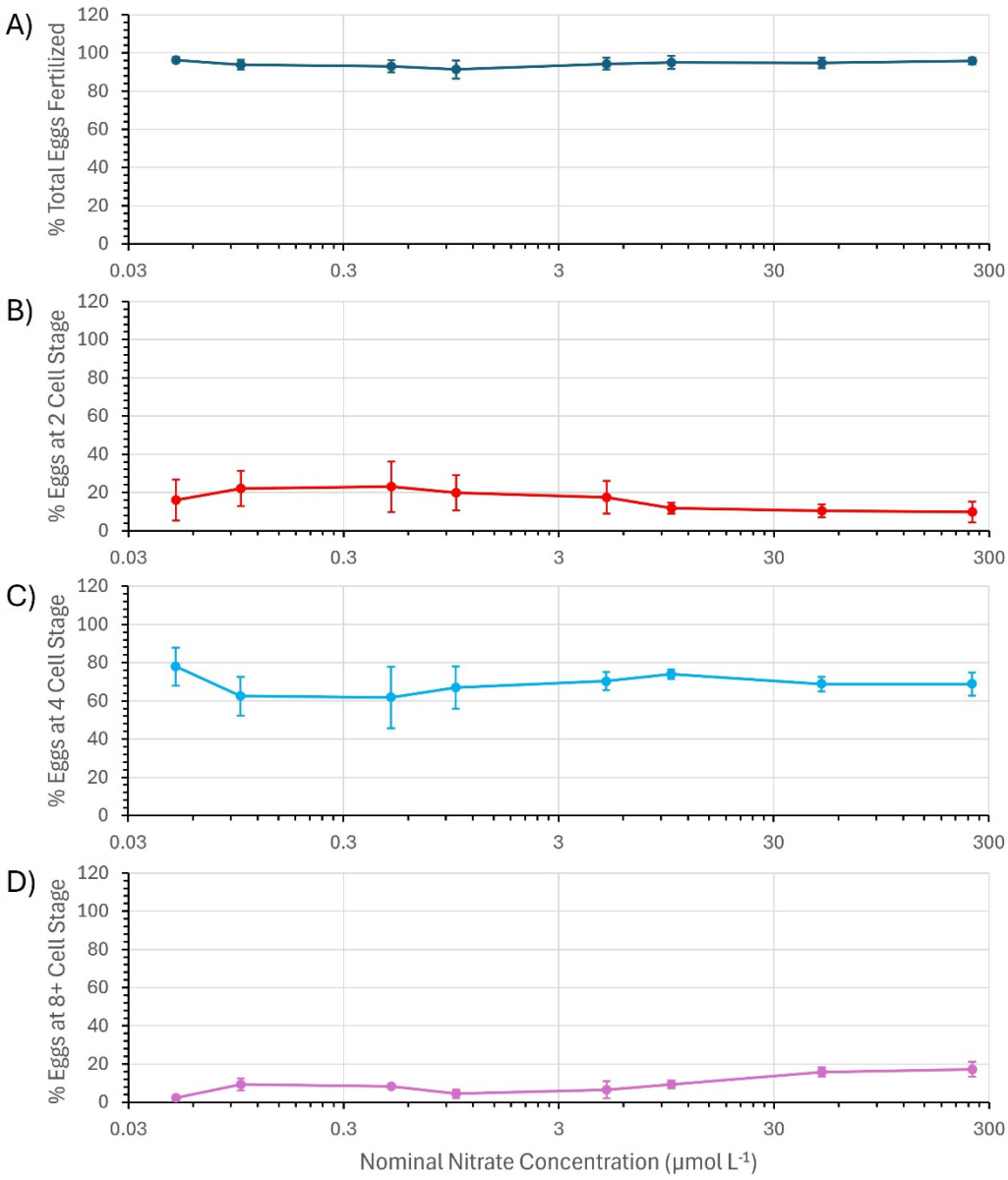

Figure 15: *Impact of Nitrate on the fertilization of* Diploria labyrinthiformis *gametes (**DLAB**, spawned 5/26). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

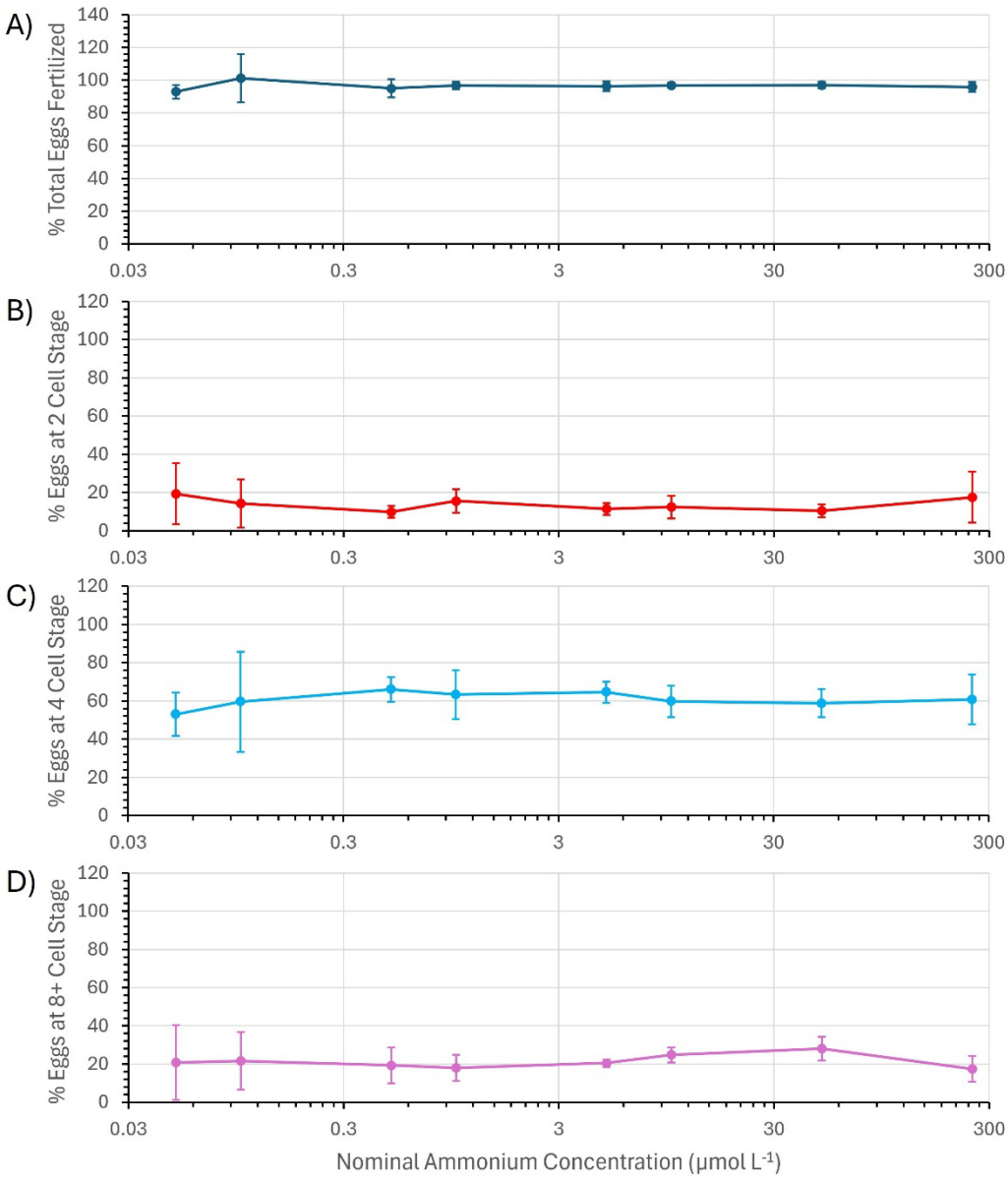

Figure 16: *Impact of Ammonium on the fertilization of* Diploria labyrinthiformis *gametes (**DLAB**, spawned 5/26). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

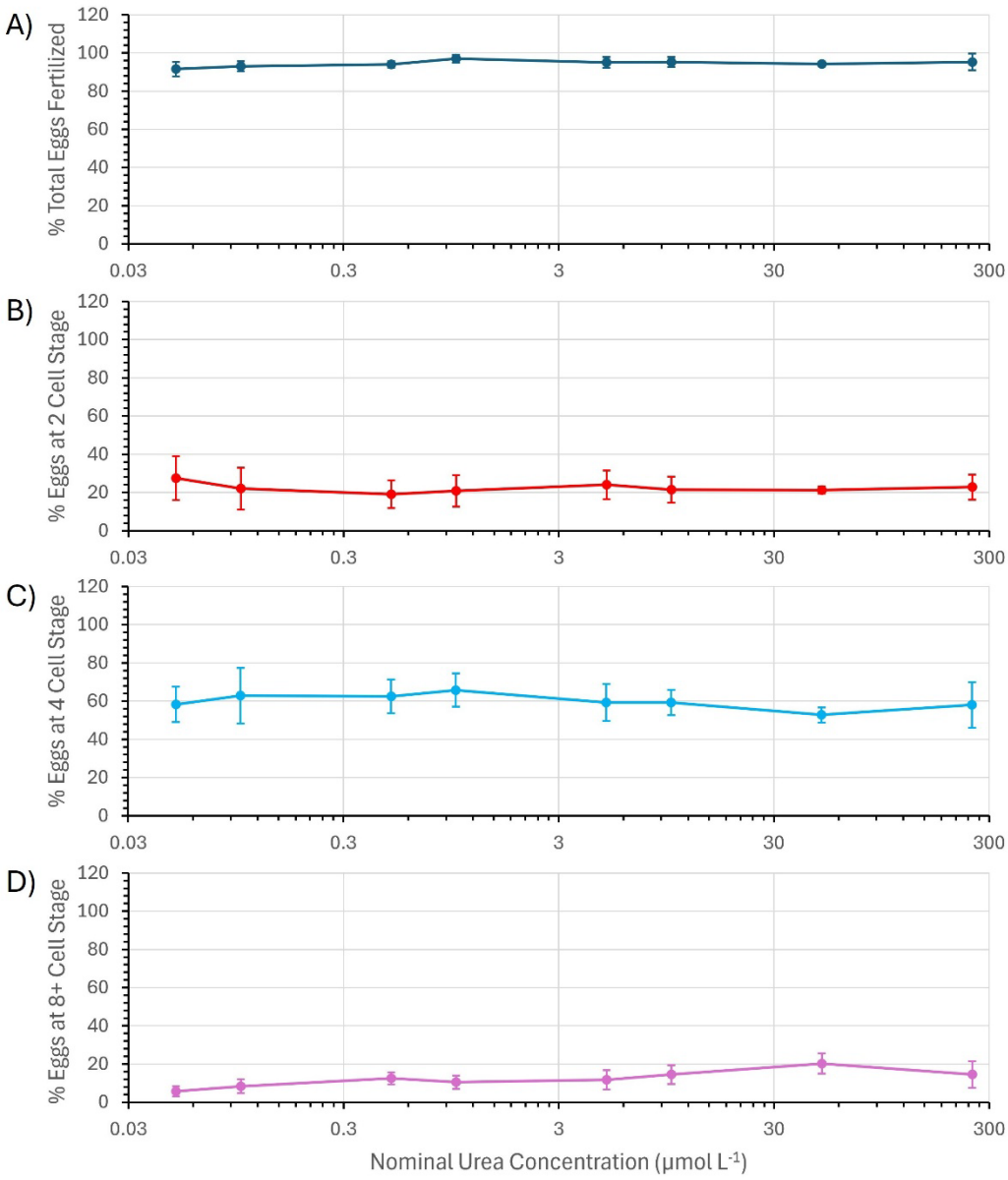

Figure 17: *Impact of Urea on the fertilization of* Diploria labyrinthiformis *gametes (**DLAB**, spawned 5/26). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

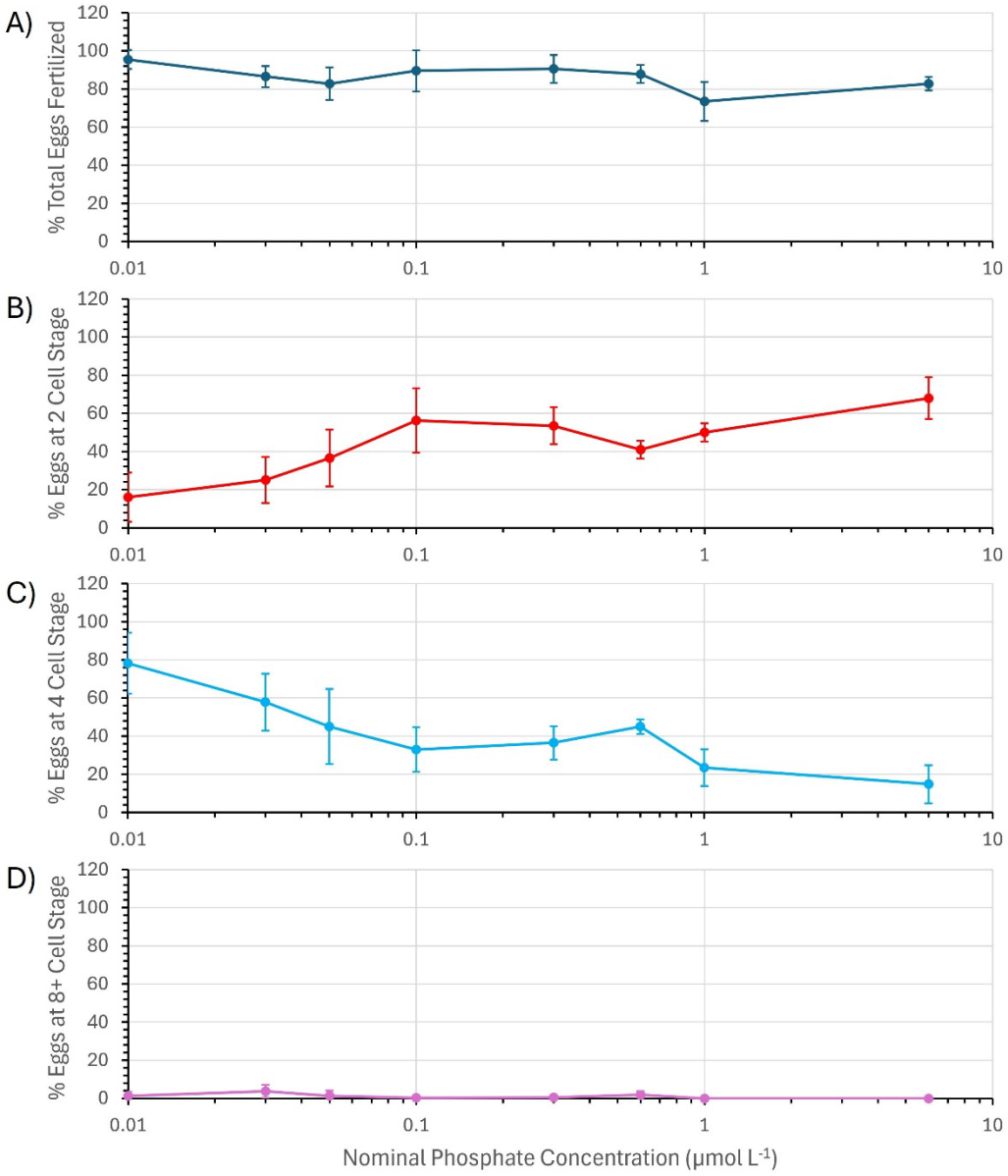

Figure 18: *Impact of Phosphate (with Ammonium as the nitrogen source) on the fertilization of* Diploria labyrinthiformis *gametes (**DLAB**, spawned 5/26). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

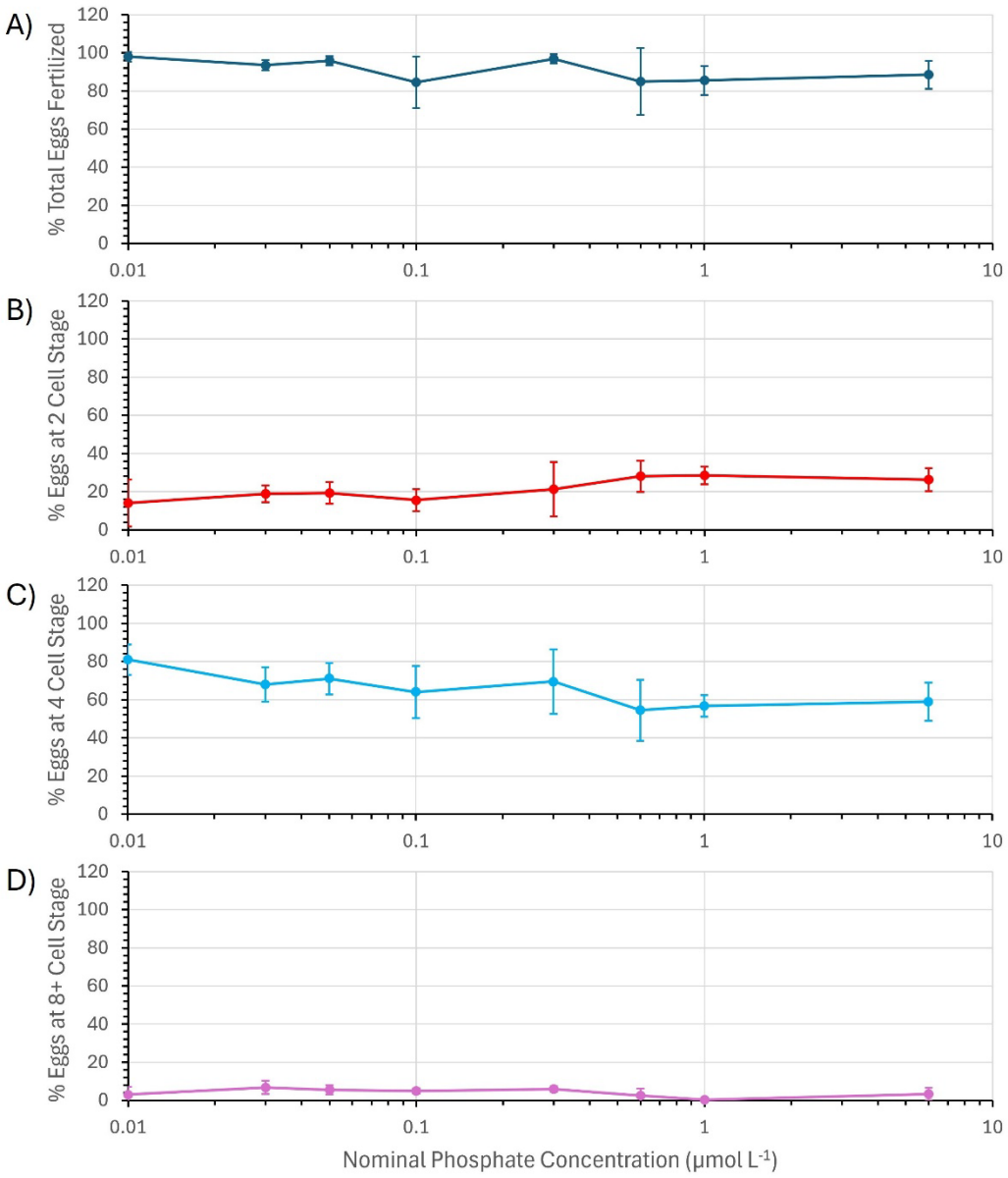

Figure 19: *Impact of Phosphate (with Urea as the nitrogen source) on the fertilization of* Diploria labyrinthiformis *gametes (**DLAB**, spawned 5/26). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

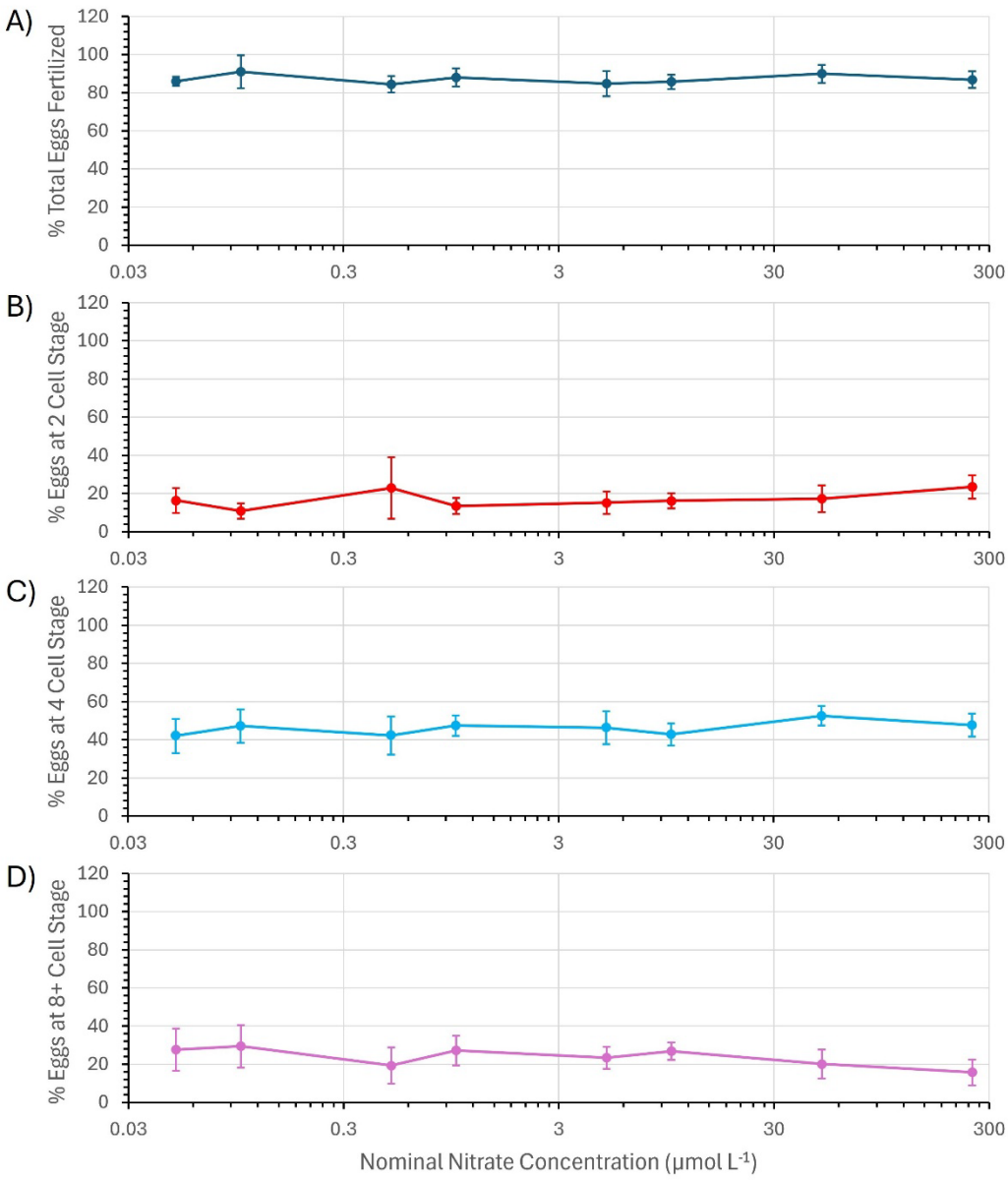

Figure 20: *Impact of Nitrate on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

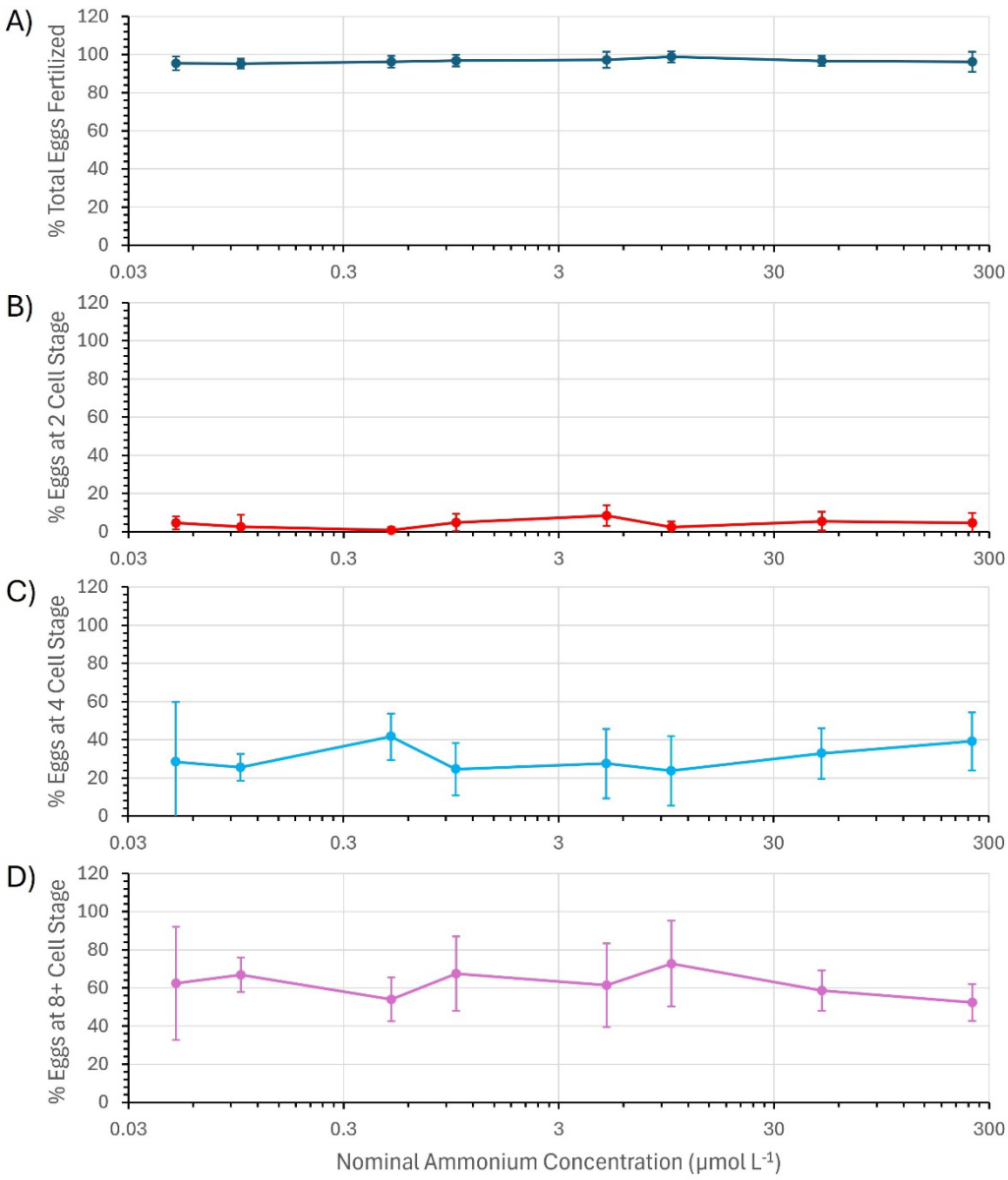

Figure 21: *Impact of Ammonium on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

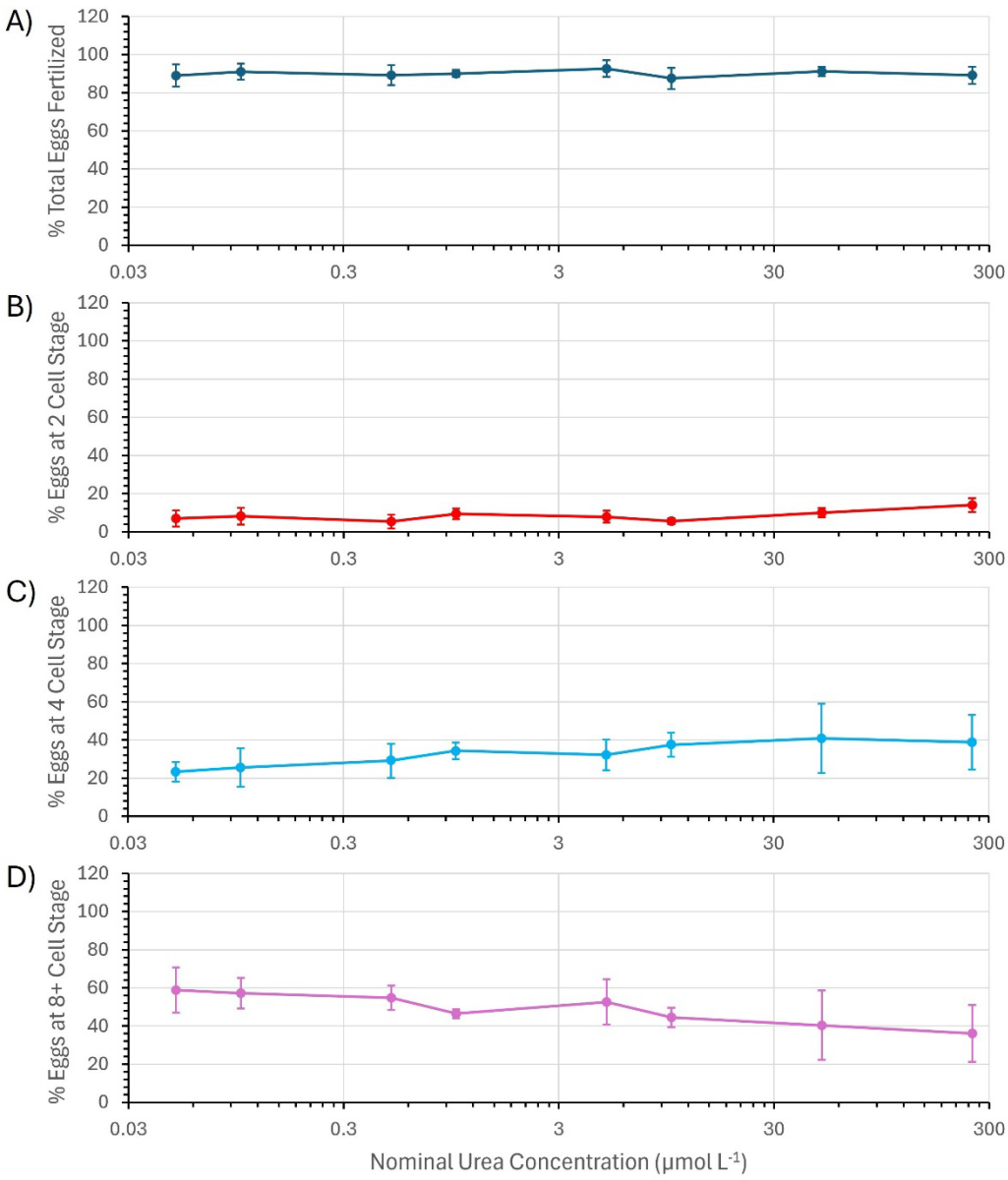

Figure 22: *Impact of Urea on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

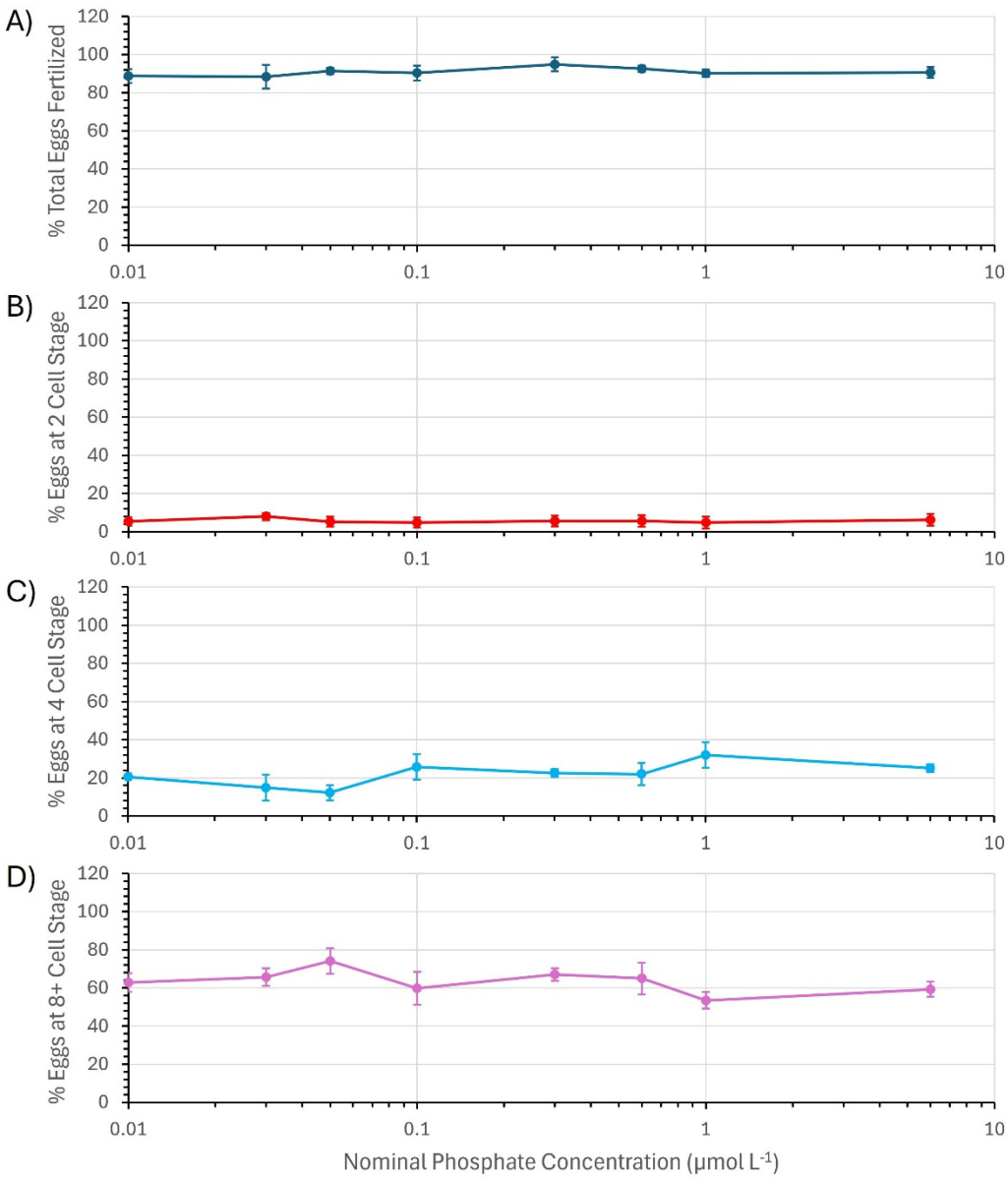

Figure 23: *Impact of Phosphate (with Nitrate as the nitrogen source) on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

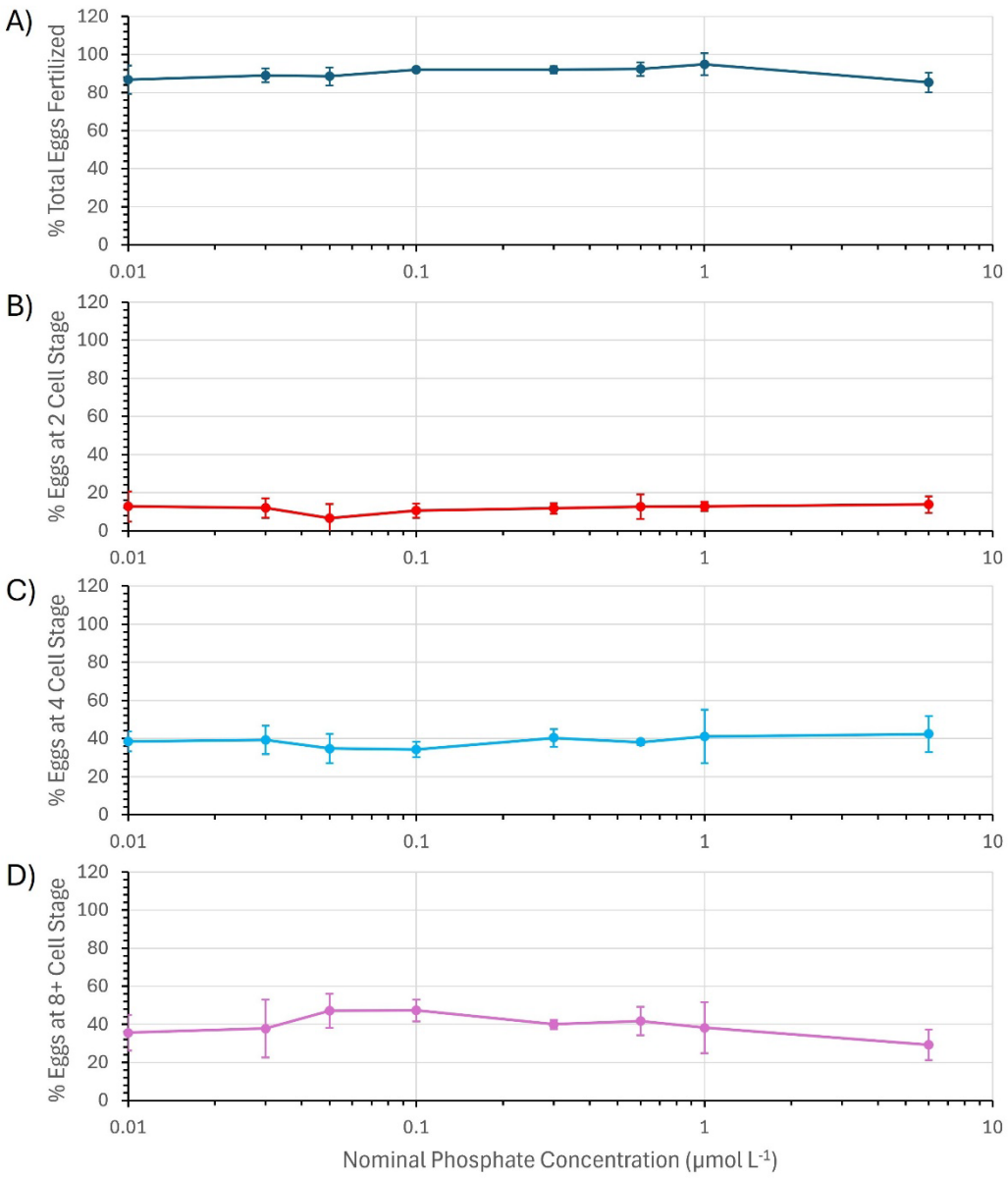

Figure 24: *Impact of Phosphate (with Ammonium as the nitrogen source) on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

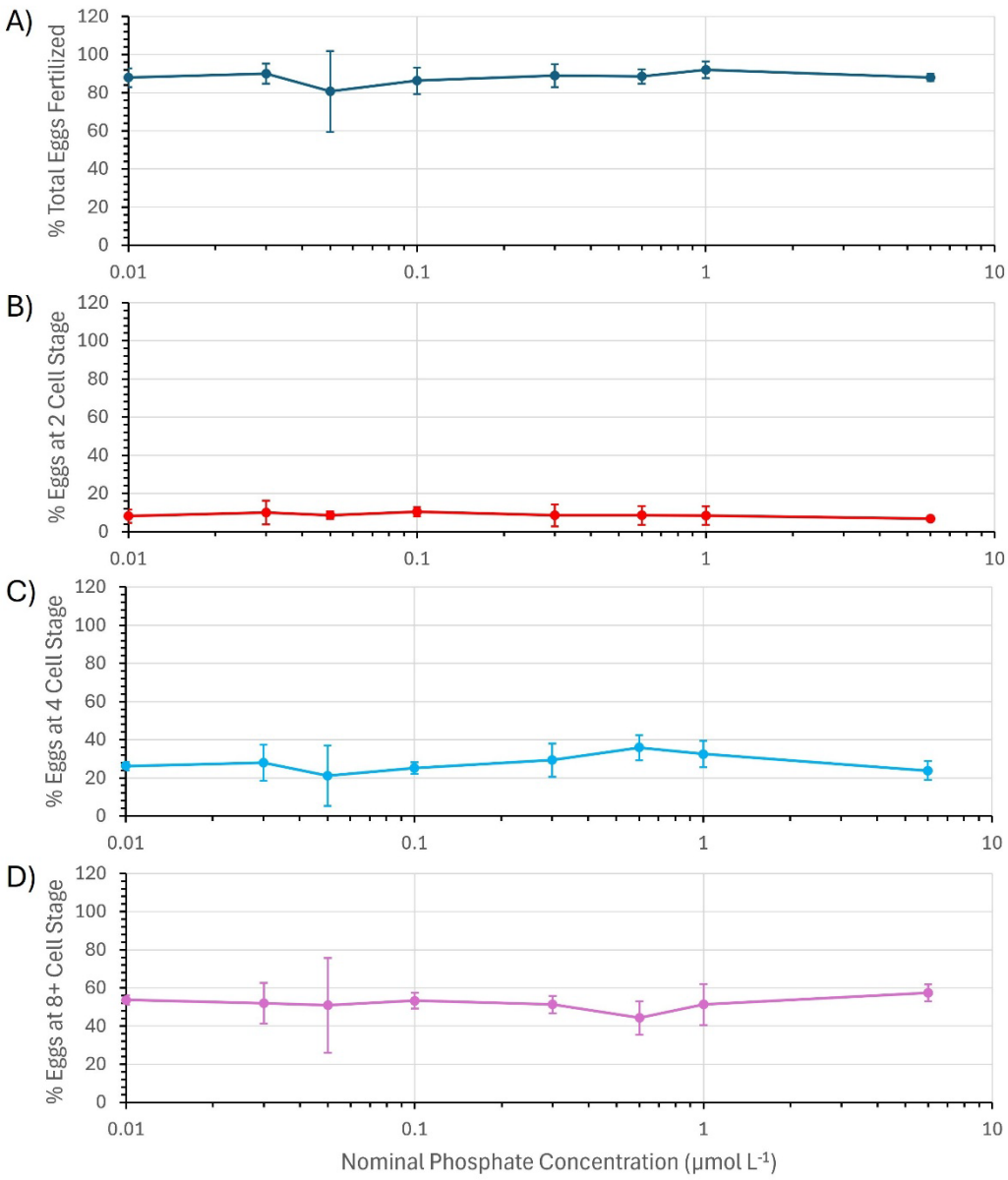

Figure 25: *Impact of Phosphate (with Urea as the nitrogen source) on the fertilization of* Orbicella franksi *gametes (**OFRA**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

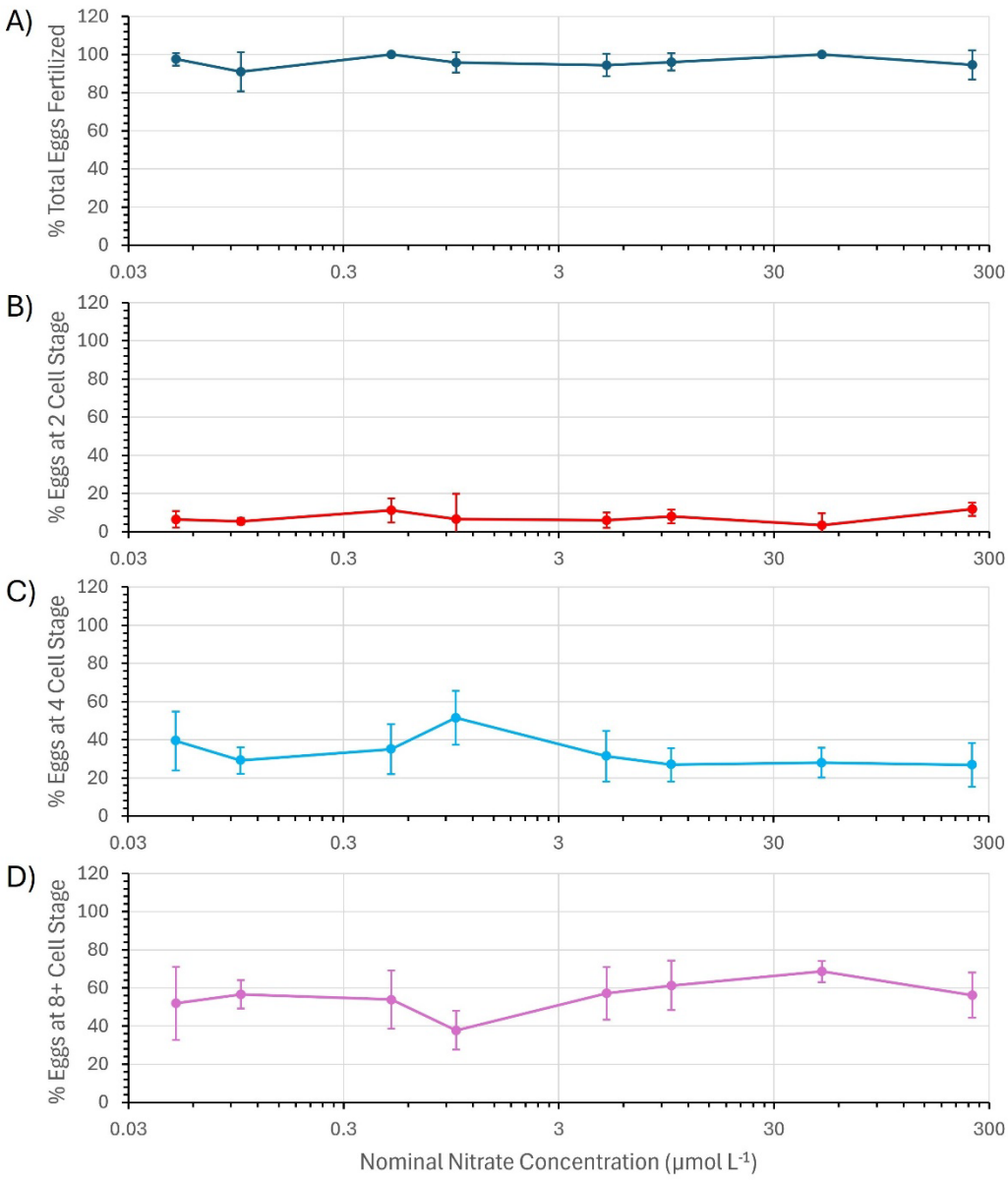

Figure 26: *Impact of Nitrate on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

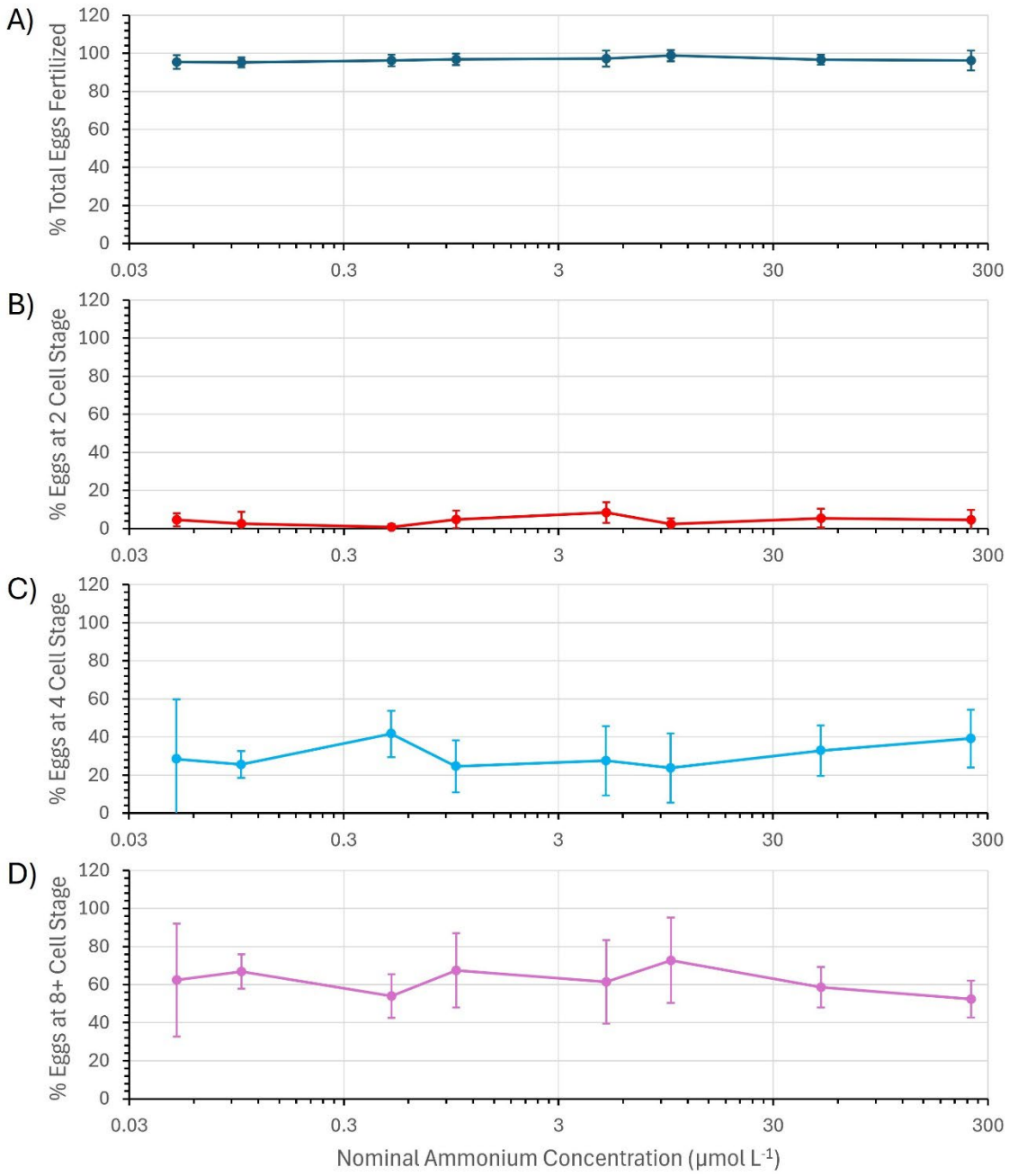

Figure 27: *Impact of Ammonium on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

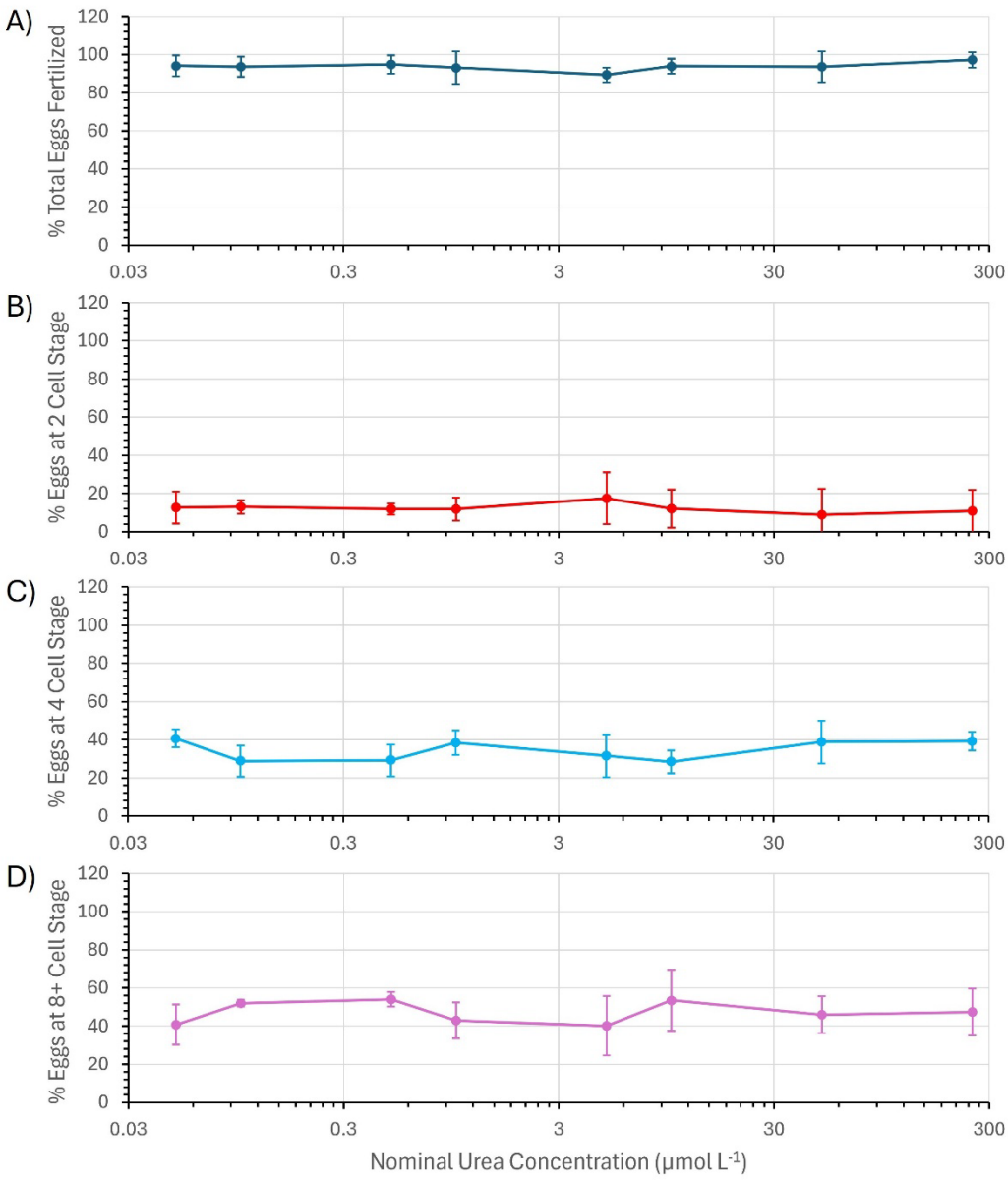

Figure 28: *Impact of Urea on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

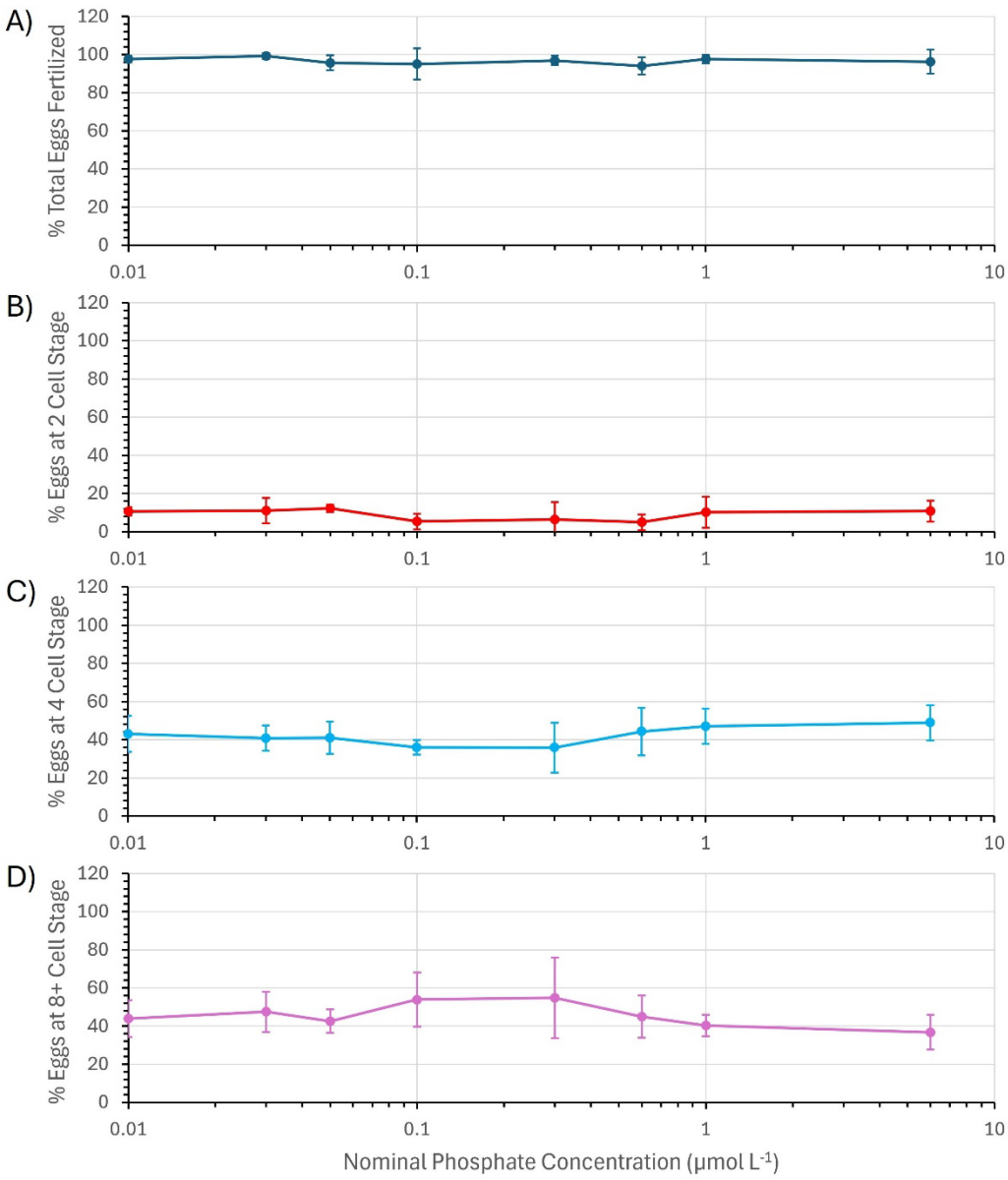

Figure 29: *Impact of Phosphate (with Nitrate as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

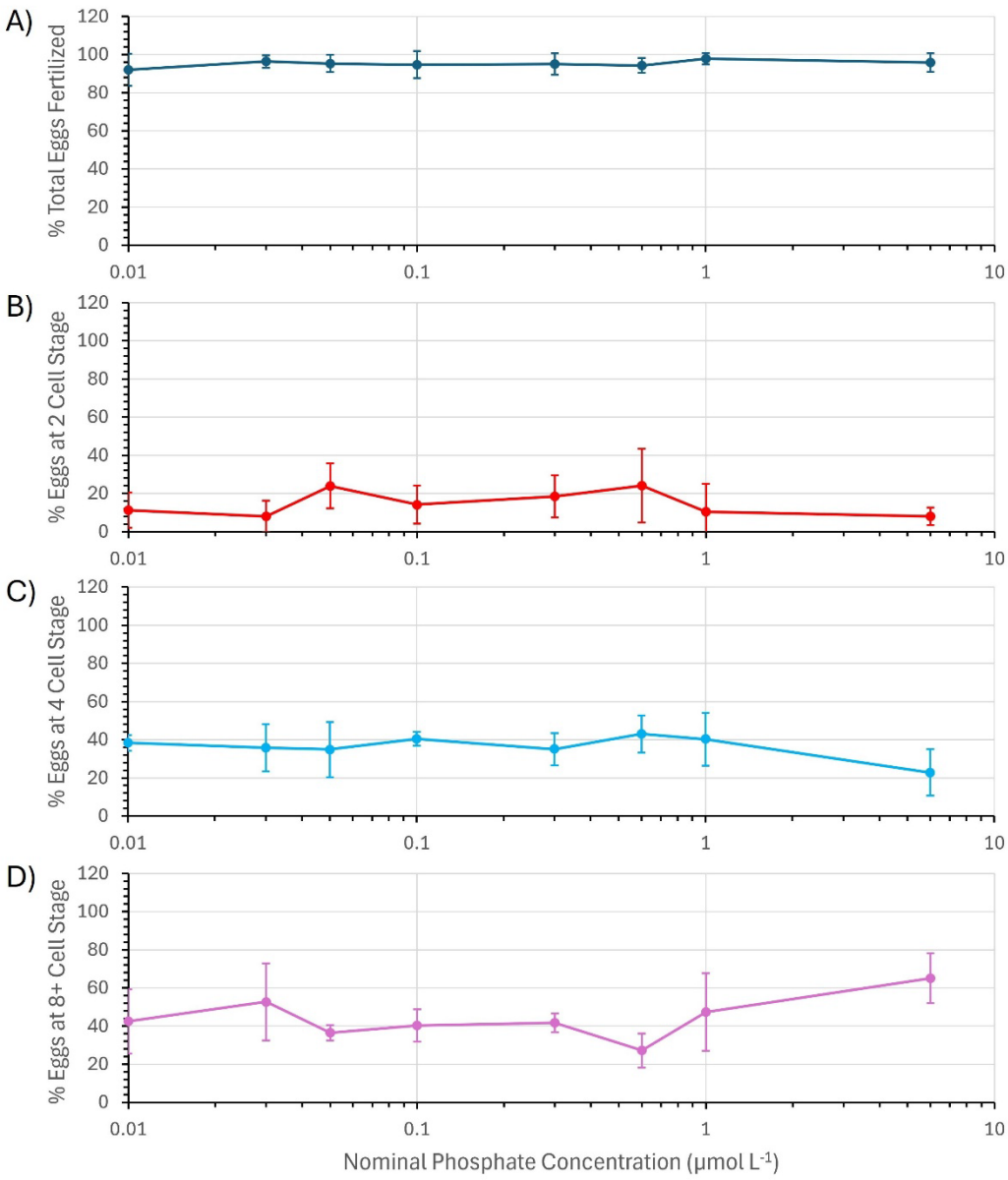

Figure 30: *Impact of Phosphate (with Ammonium as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

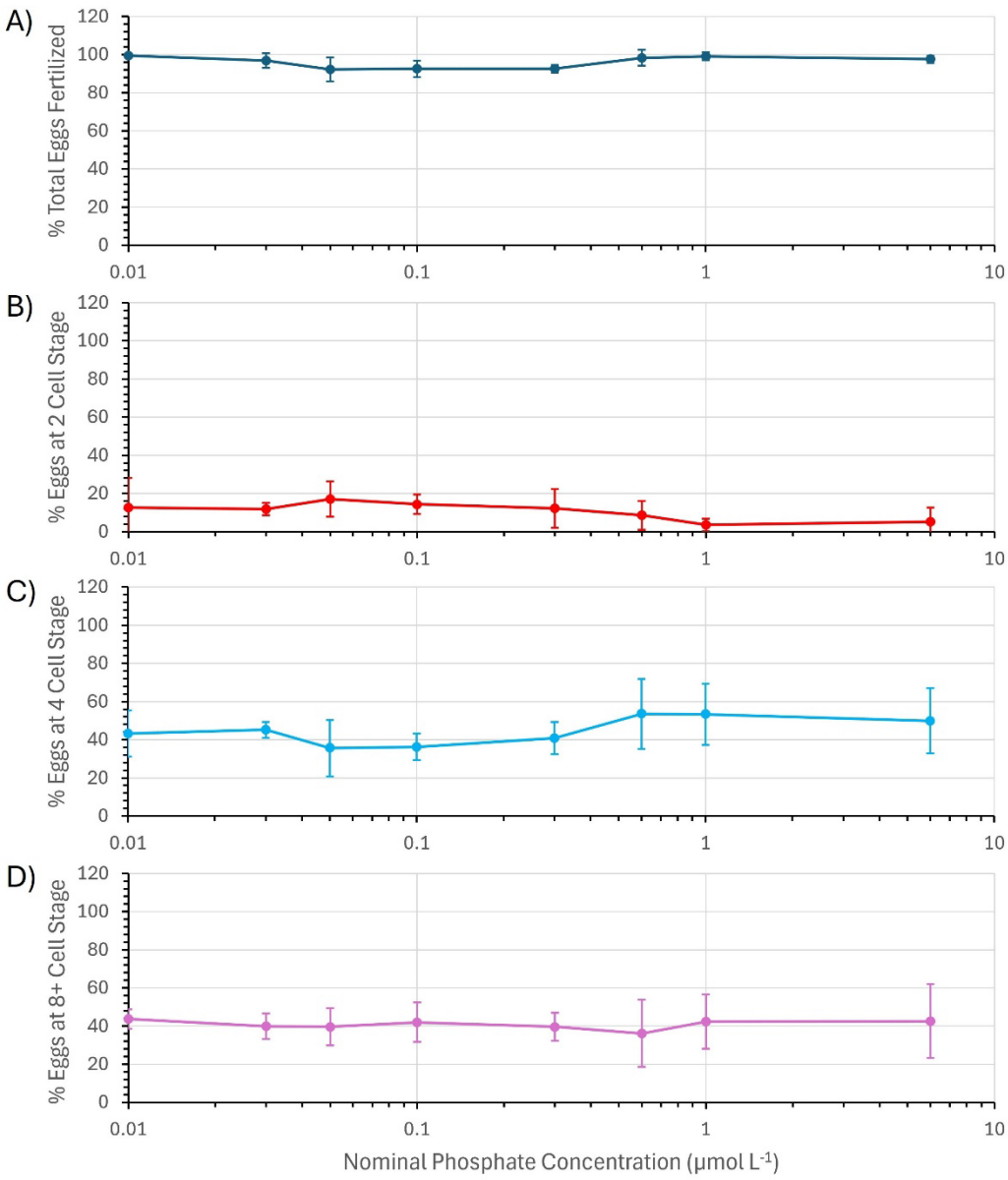

Figure 31: *Impact of Phosphate (with Urea as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR**, spawned 9/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

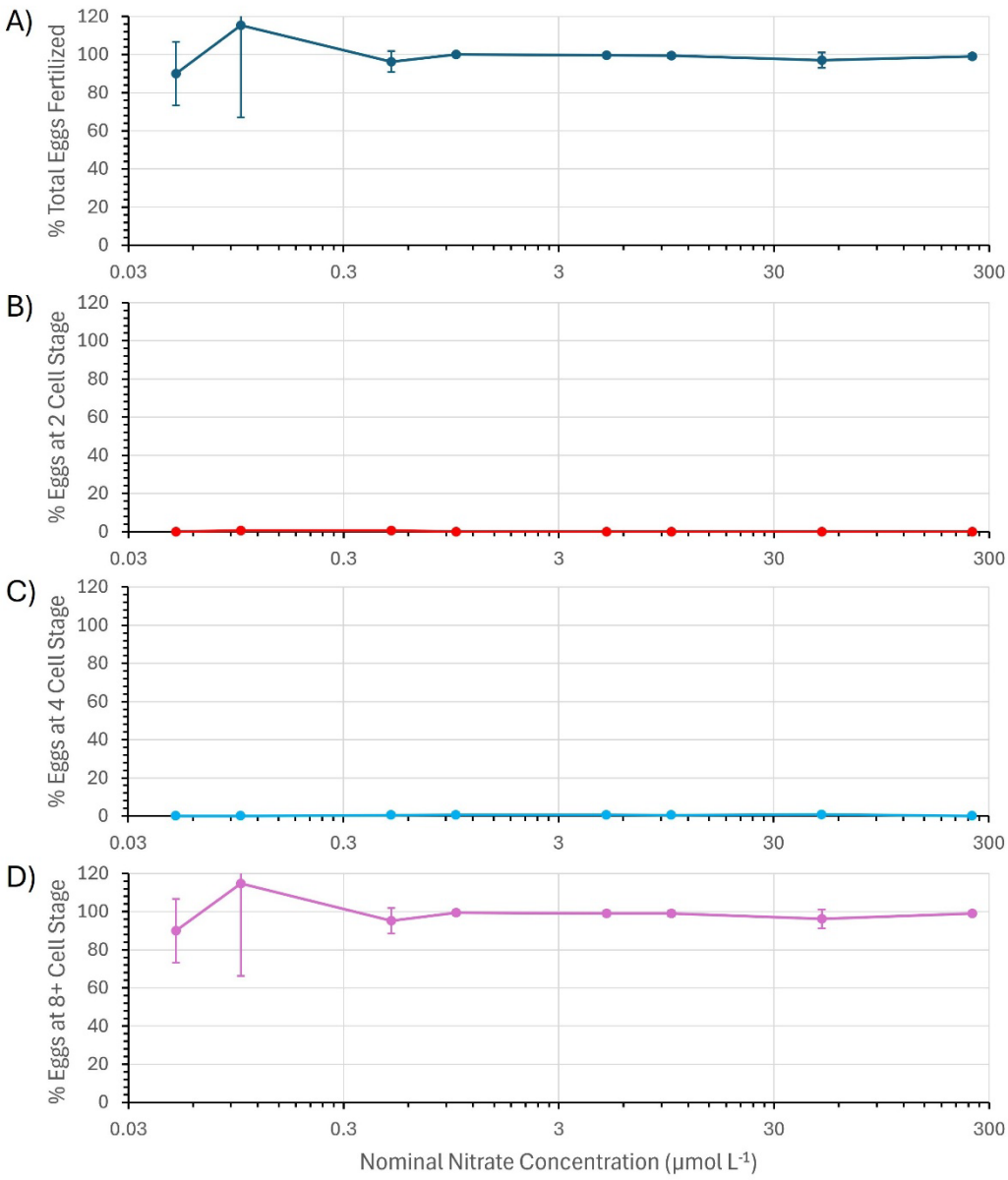

Figure 32: *Impact of Nitrate on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

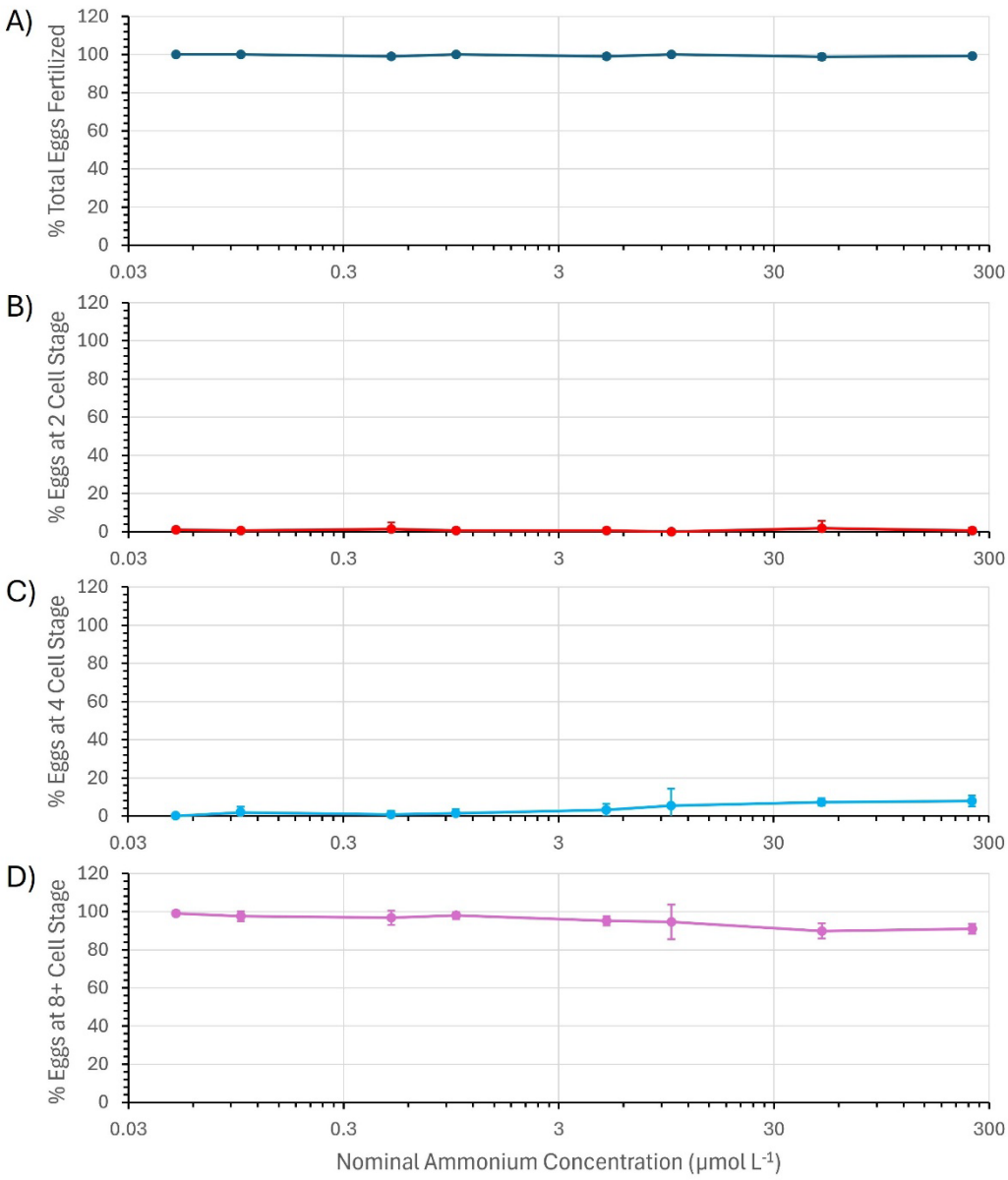

Figure 33: *Impact of Ammonium on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

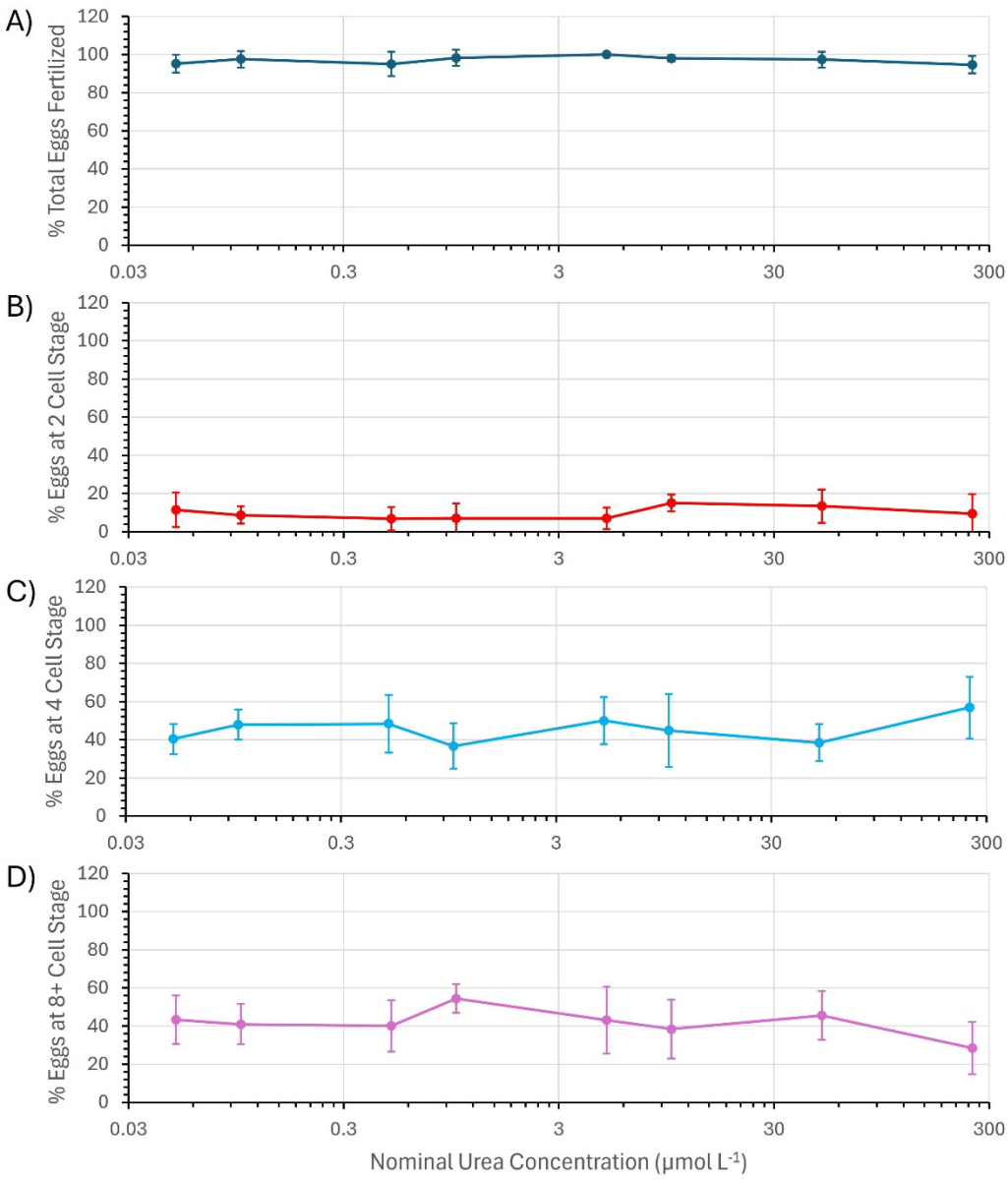

Figure 34: *Impact of Urea on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

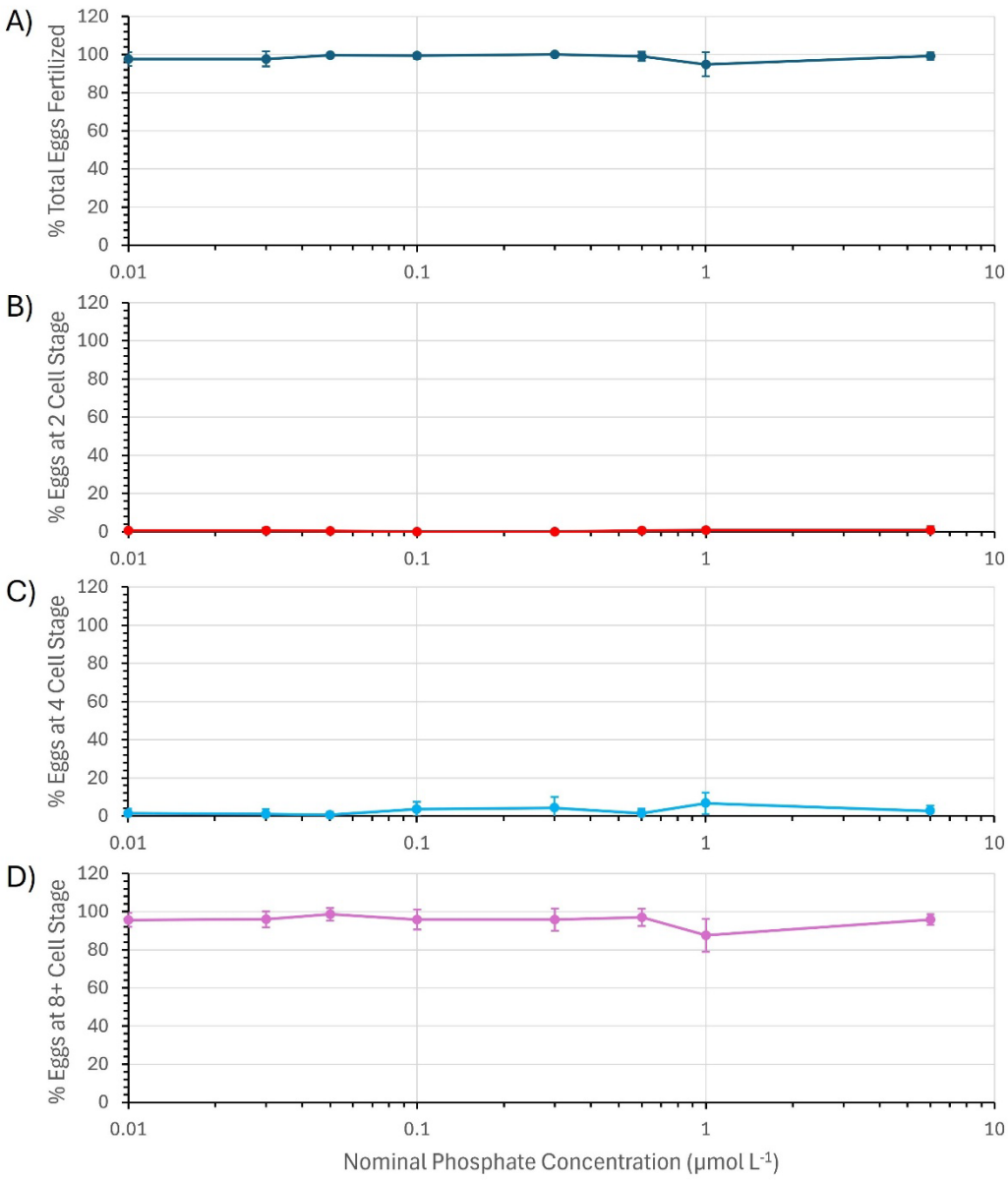

Figure 35: *Impact of Phosphate (with Nitrate as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

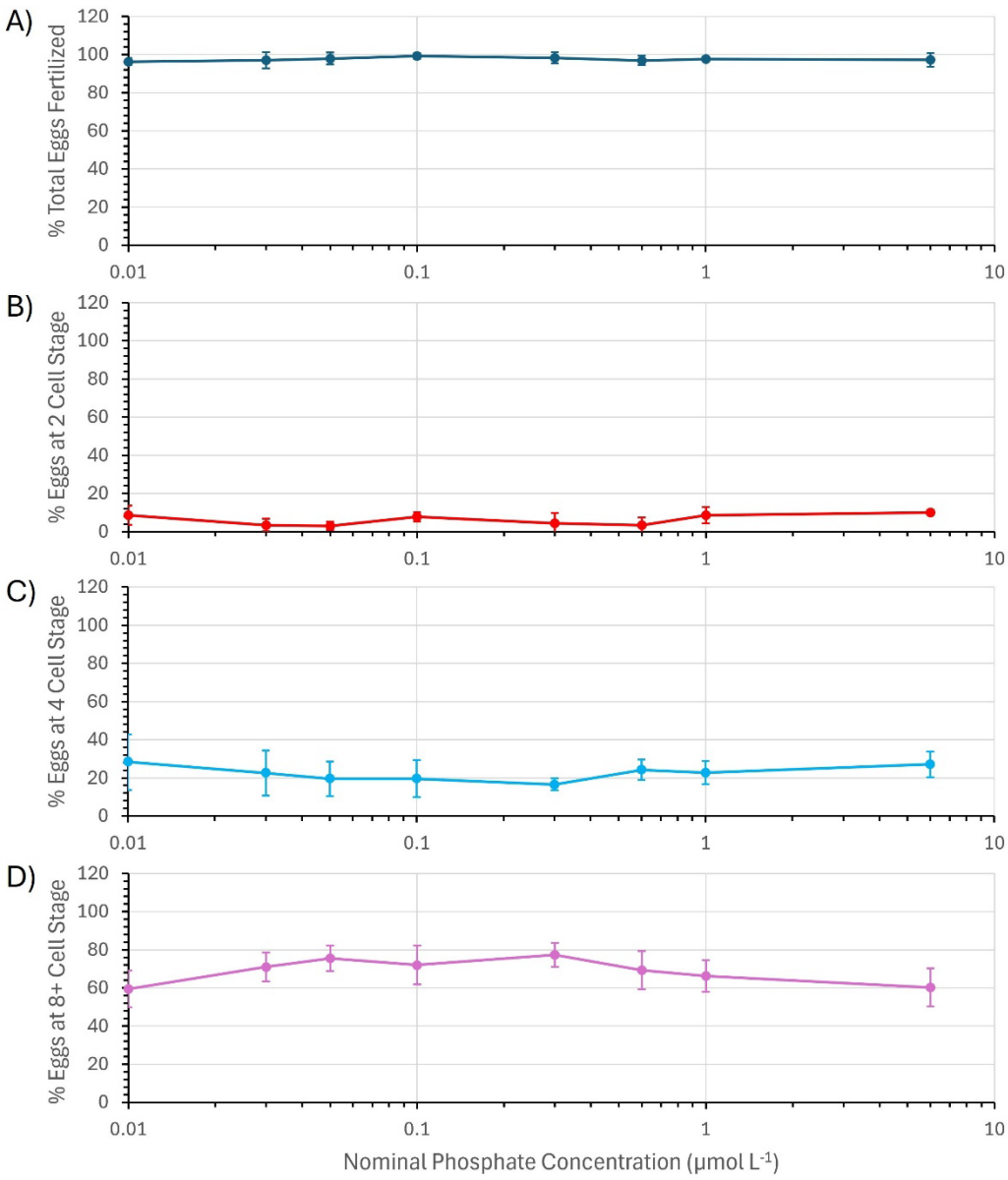

Figure 36: *Impact of Phosphate (with Ammonium as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

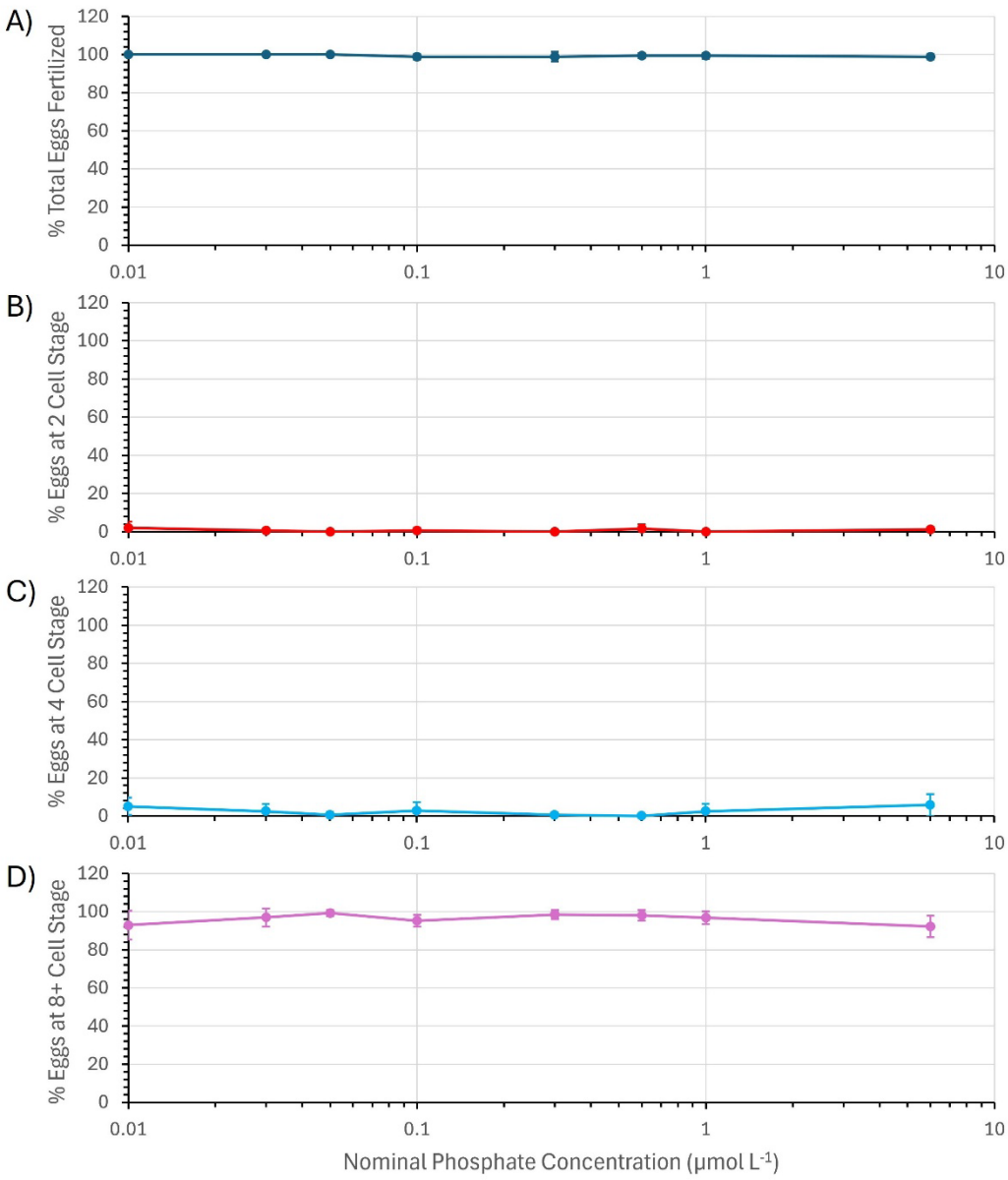

Figure 37: *Impact of Phosphate (with Urea as the nitrogen source) on the fertilization of* Pseudodiploria strigosa *gametes (**PSTR2**, spawned 10/25). **A)** Percentage of Total Eggs Fertilized, **B)** Percentage of Eggs at the 2-cell stage (undergone 1 division), **C)** Percentage of Eggs at the 4-cell stage (undergone 2 divisions), **D)** Percentage of Eggs at the 8+-cell stage (undergone 3 or more divisions). Error bars, where present, represent the 90% confidence interval.*

6.2. Appendix 2. Results from the Settlement Assays.

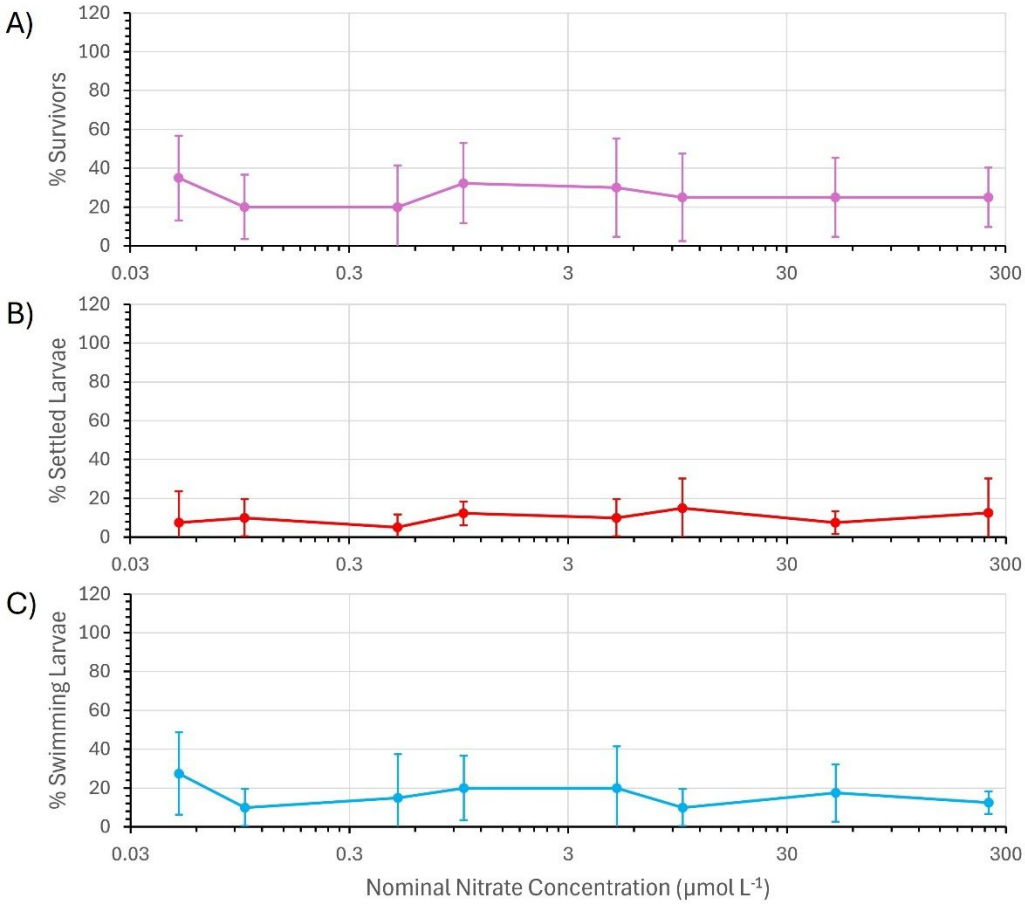

Figure 38: *Impact of Nitrate on the settlement of* Colpophyllia natans *larvae (**CNAT**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

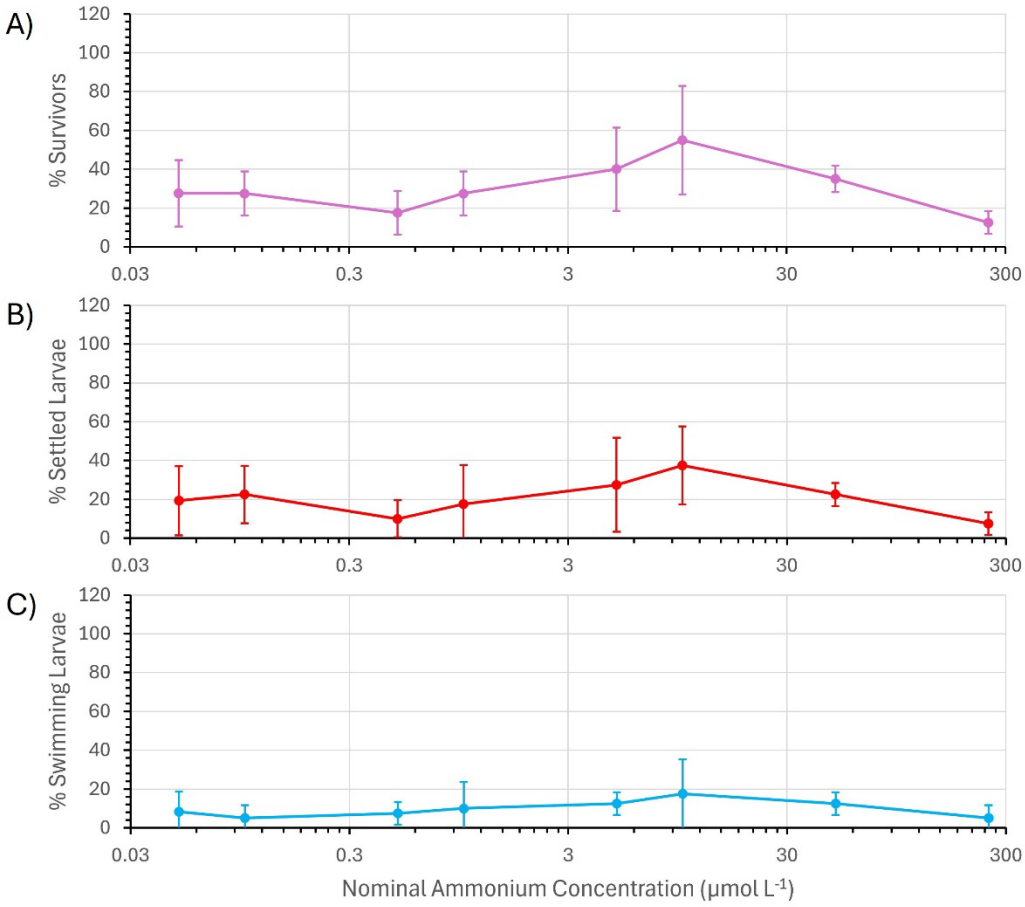

Figure 39: *Impact of Ammonium on the settlement of* Colpophyllia natans *larvae (**CNAT**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

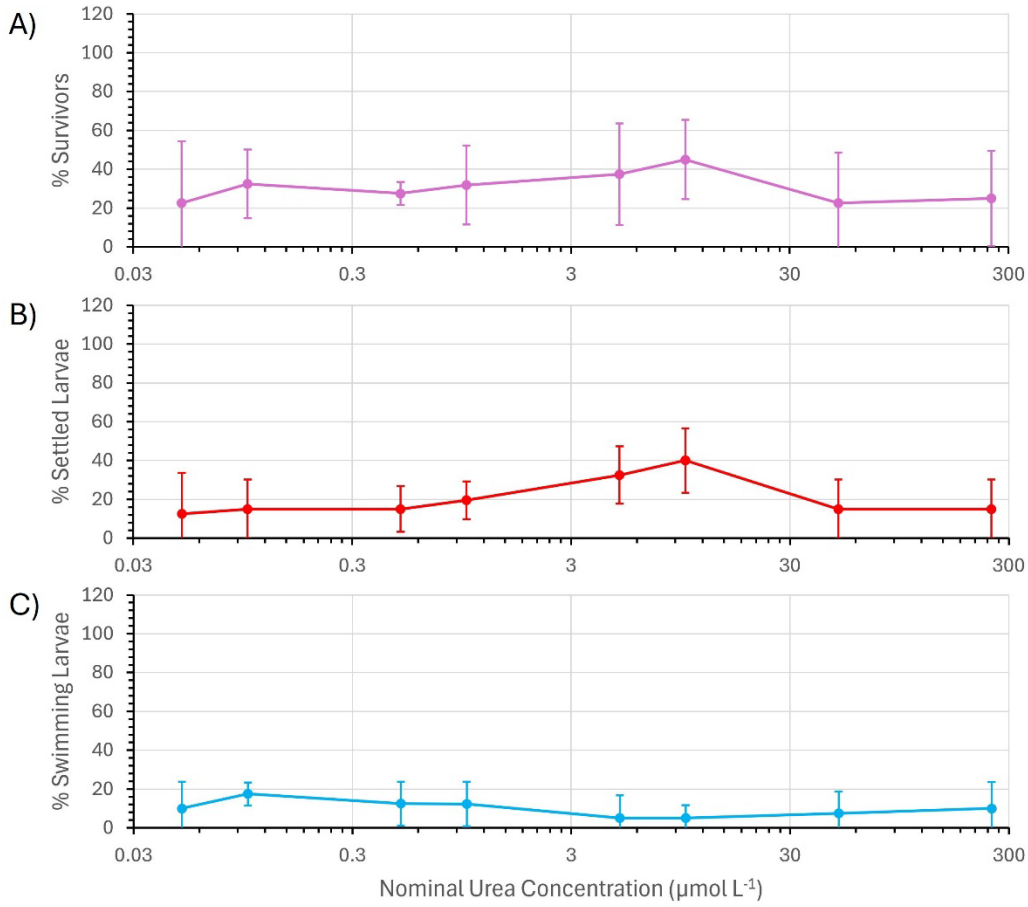

Figure 40: *Impact of Urea on the settlement of* Colpophyllia natans *larvae (**CNAT**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

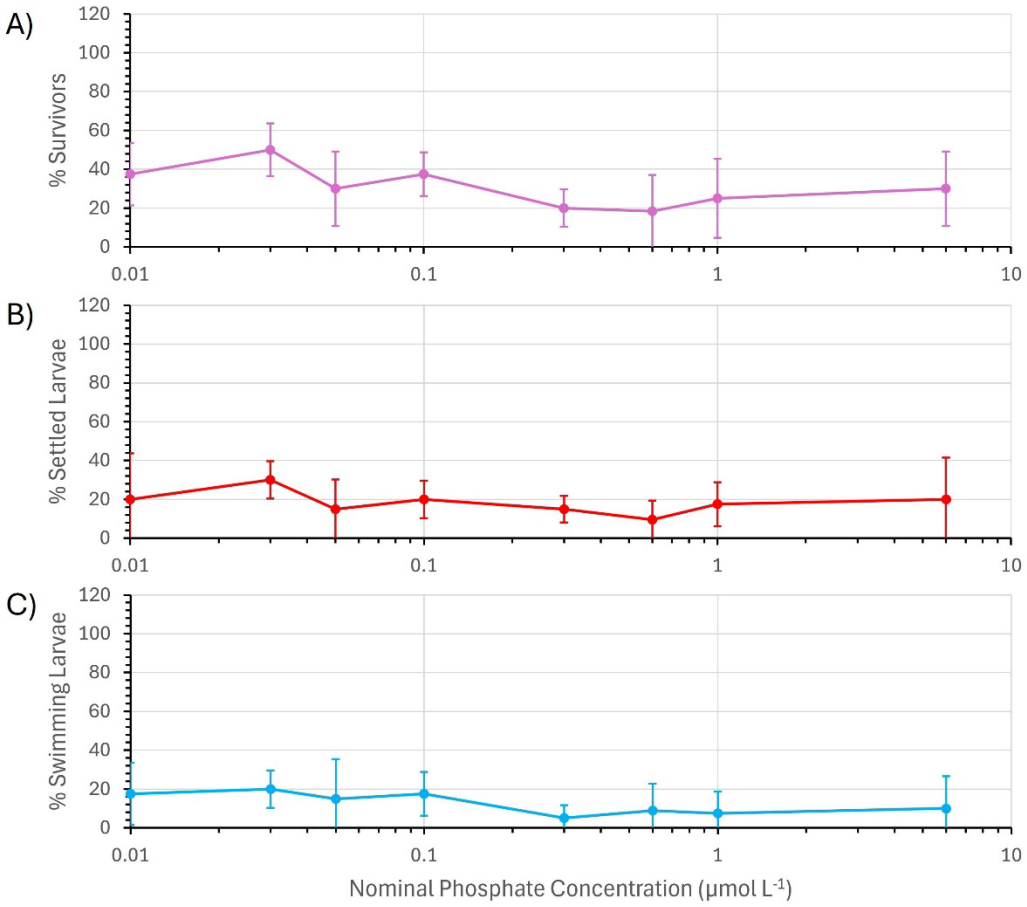

Figure 41: *Impact of Phosphate (with Nitrate as the nitrogen source) on the settlement of* Colpophyllia natans *larvae (**CNAT**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

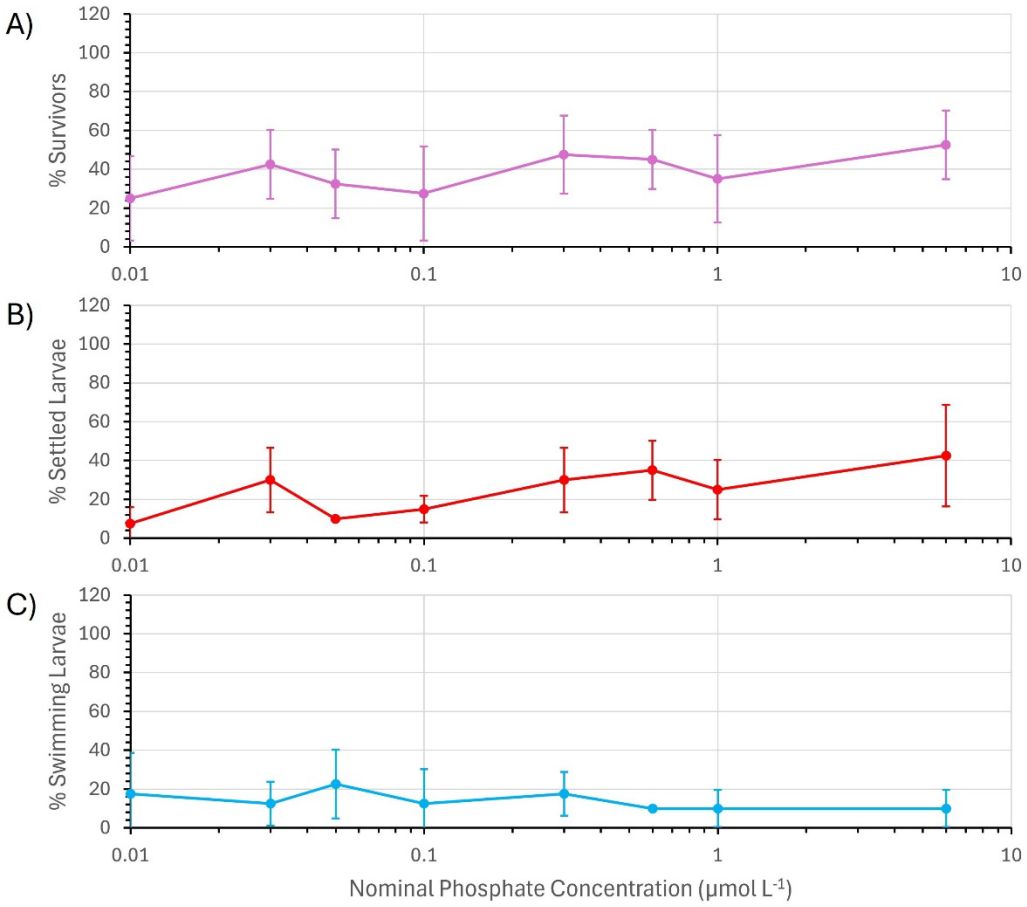

Figure 42: *Impact of Phosphate (with Ammonium as the nitrogen source) on the settlement of* Colpophyllia natans *larvae* ***(CNAT****, spawned 9/25).* ***A)*** *Percentage of Survivors,* ***B)*** *Percentage of Settled Larvae,* ***C)*** *Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

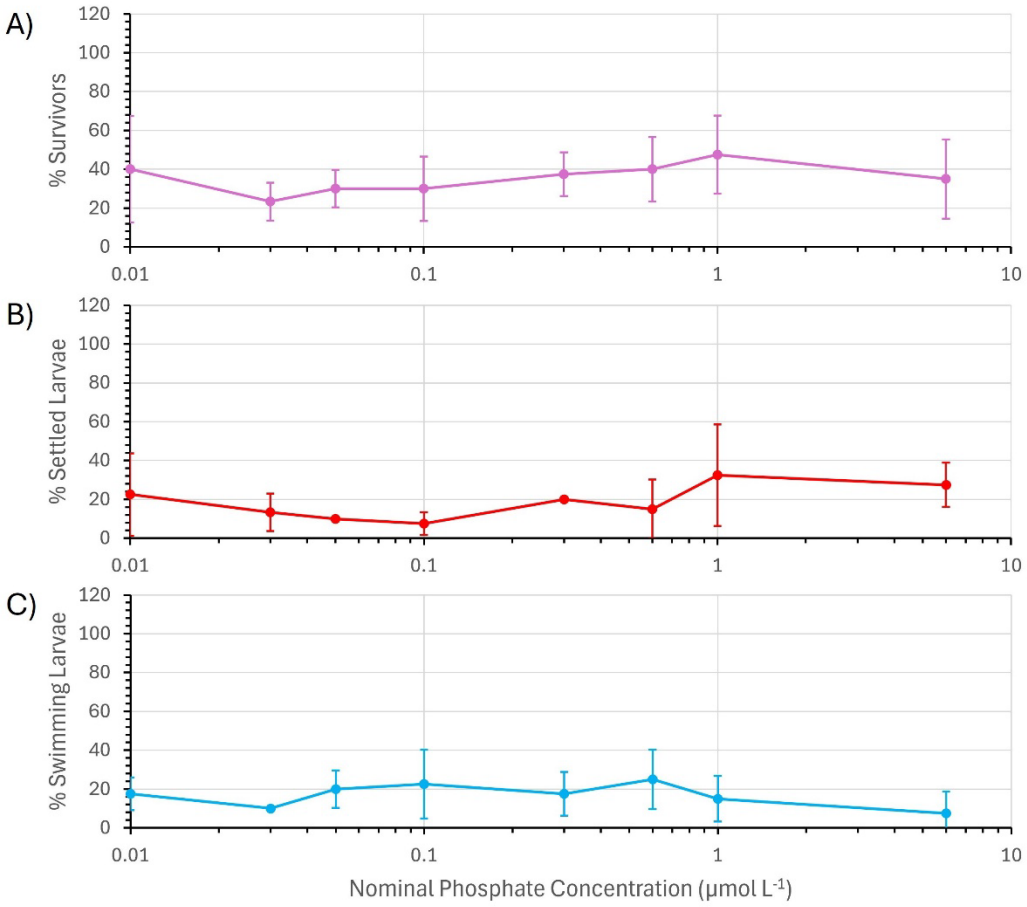

Figure 43: *Impact of Phosphate (with Urea as the nitrogen source) on the settlement of* Colpophyllia natans *larvae (**CNAT**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

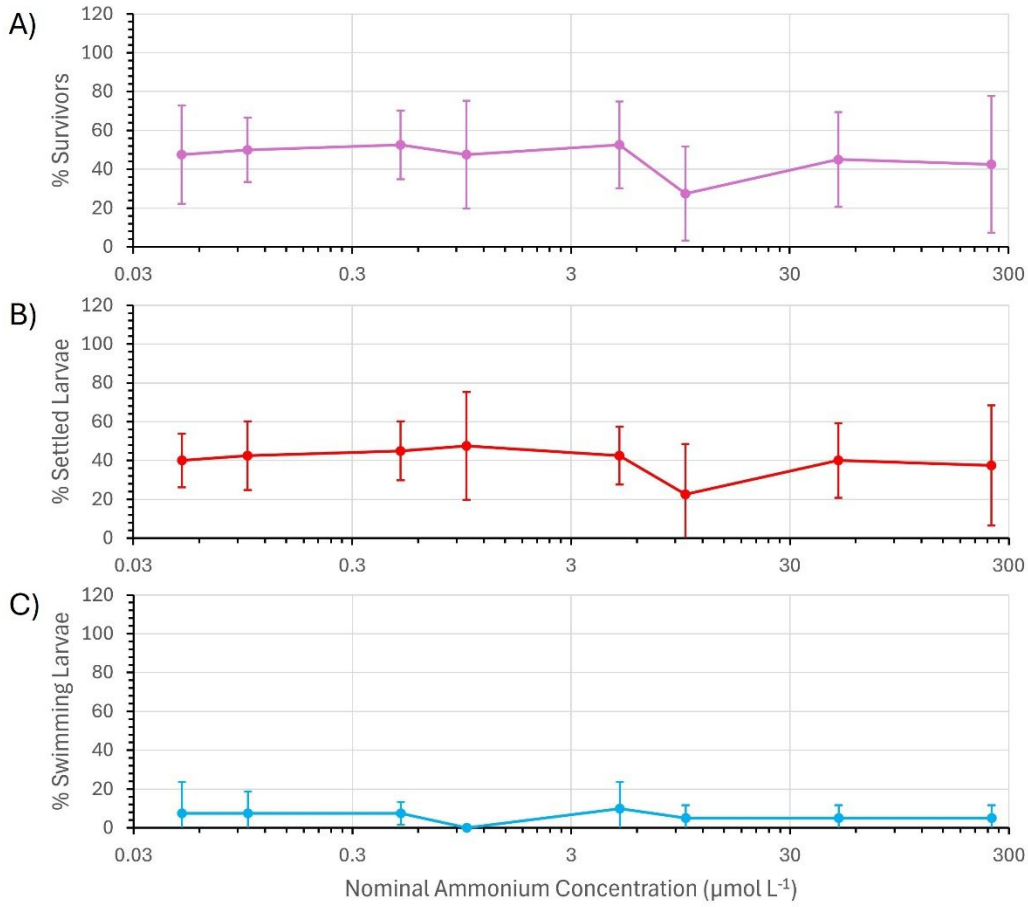

Figure 44: *Impact of Ammonium on the settlement of* Montastraea cavernosa *larvae (**MCAV**, spawned 8/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

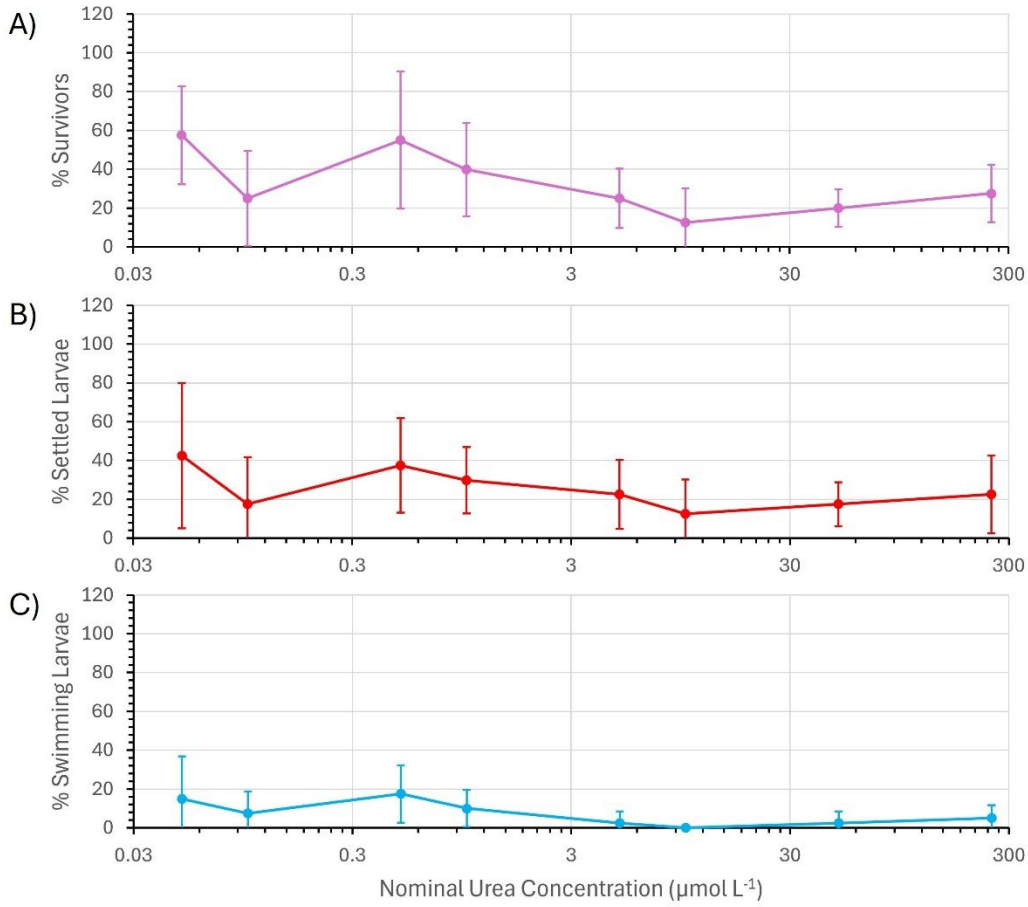

Figure 45: *Impact of Urea on the settlement of* Montastraea cavernosa *larvae (**MCAV**, spawned 8/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

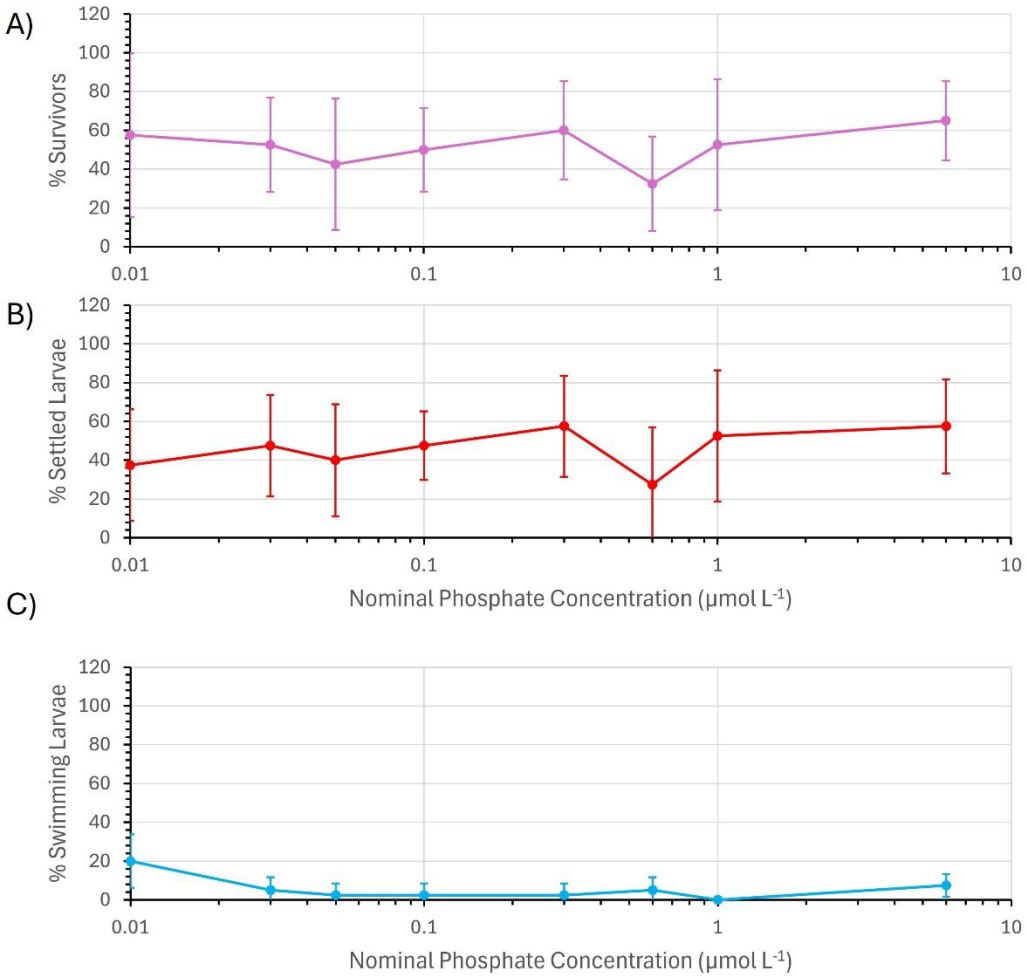

Figure 46: *Impact of Phosphate (with Nitrate as the nitrogen source) on the settlement of* Montastraea cavernosa *larvae (**MCAV**, spawned 8/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

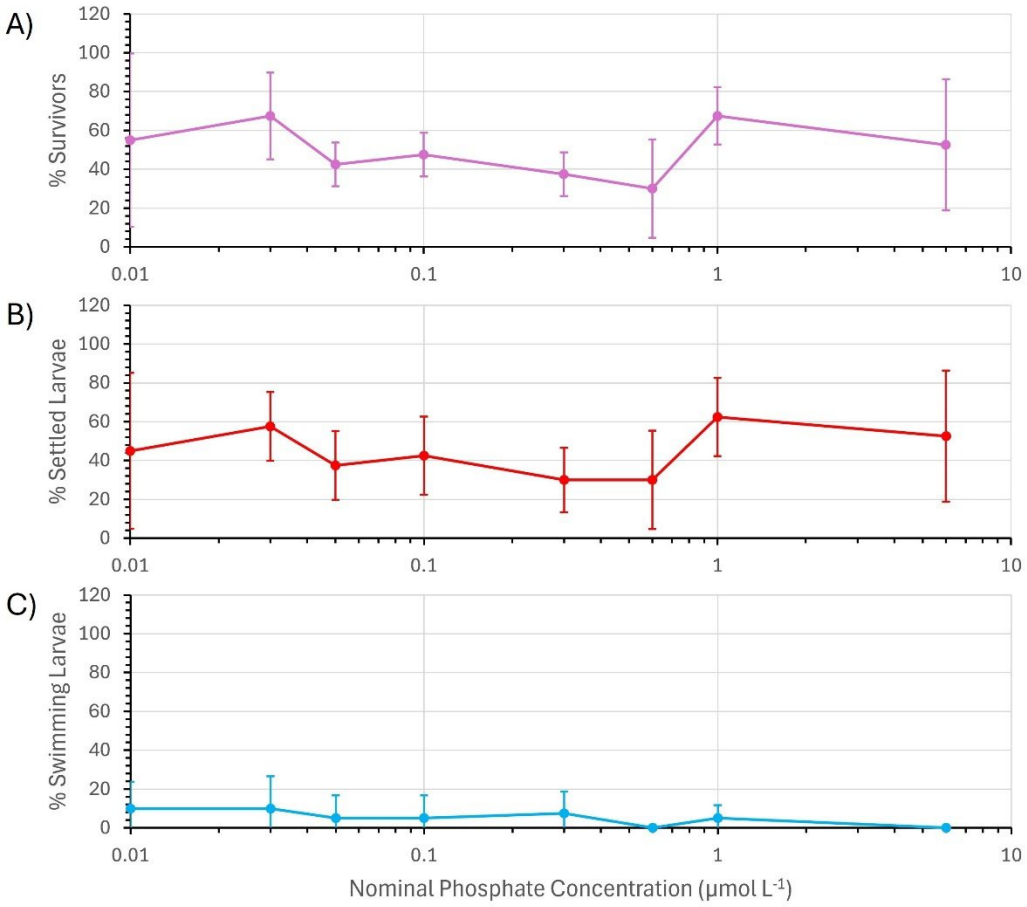

Figure 46: *Impact of Phosphate (with Ammonium as the nitrogen source) on the settlement of* Montastraea cavernosa *larvae (**MCAV**, spawned 8/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

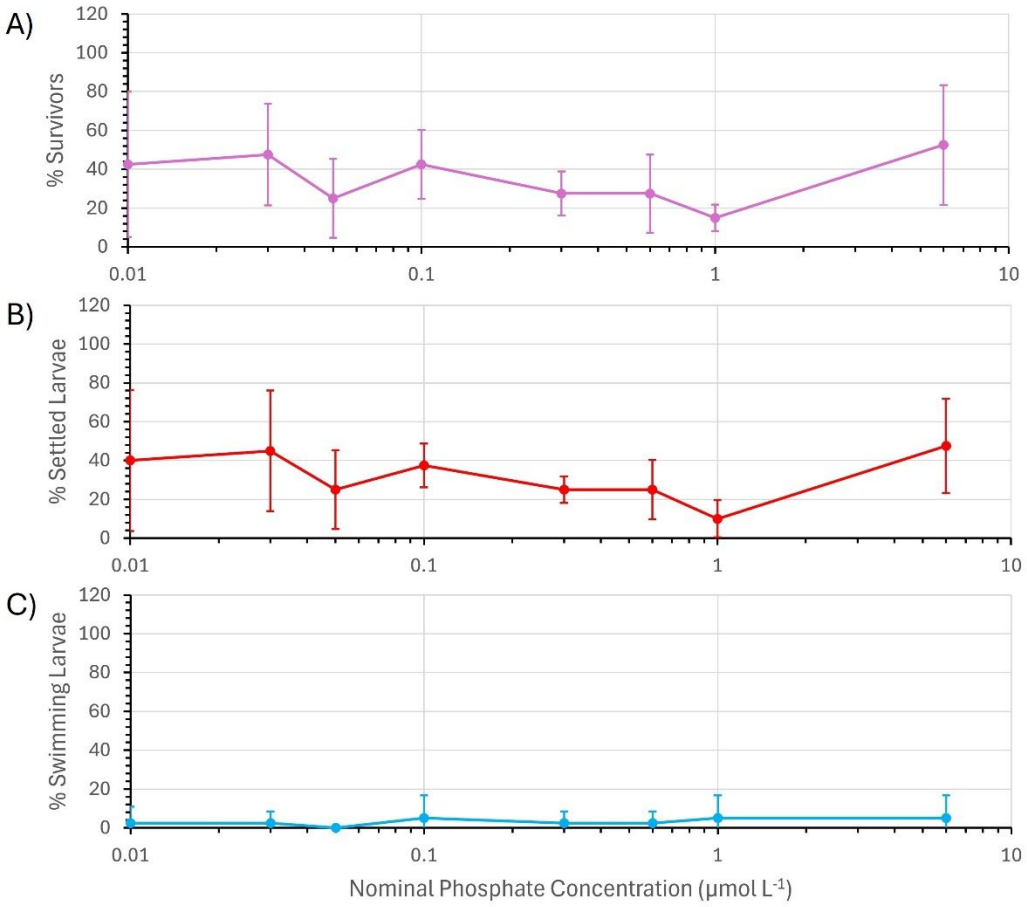

Figure 47: *Impact of Phosphate (with Urea as the nitrogen source) on the settlement of* Montastraea cavernosa *larvae (**MCAV**, spawned 8/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

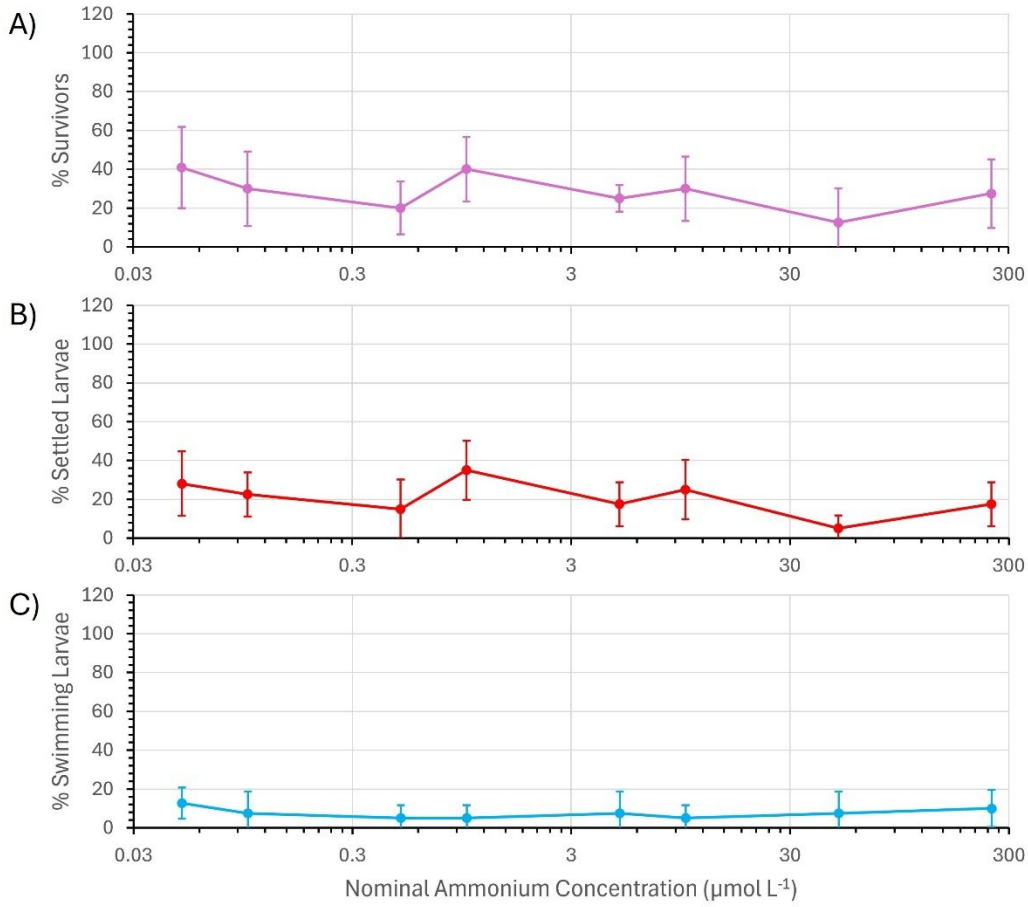

Figure 48: *Impact of Ammonium on the settlement of* Orbicella faveolata *larvae (**OFAV**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

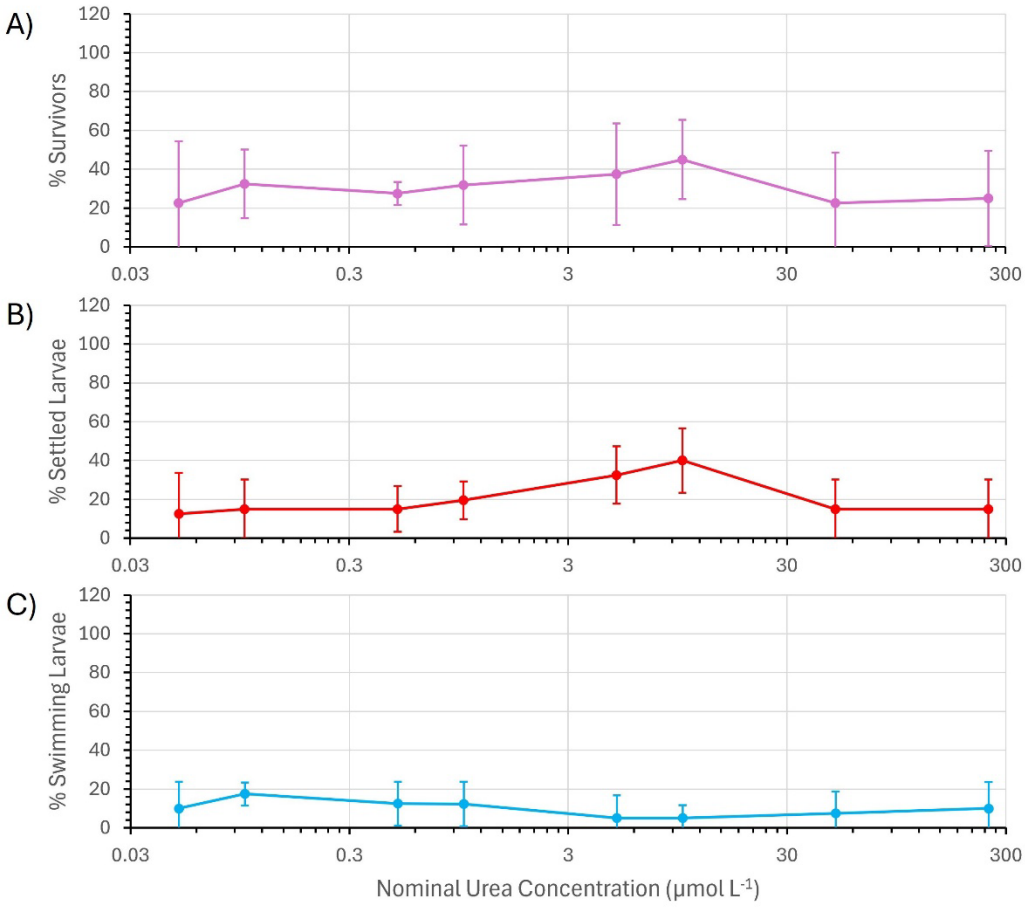

Figure 49: *Impact of Urea on the settlement of* Orbicella faveolata *larvae (**OFAV**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

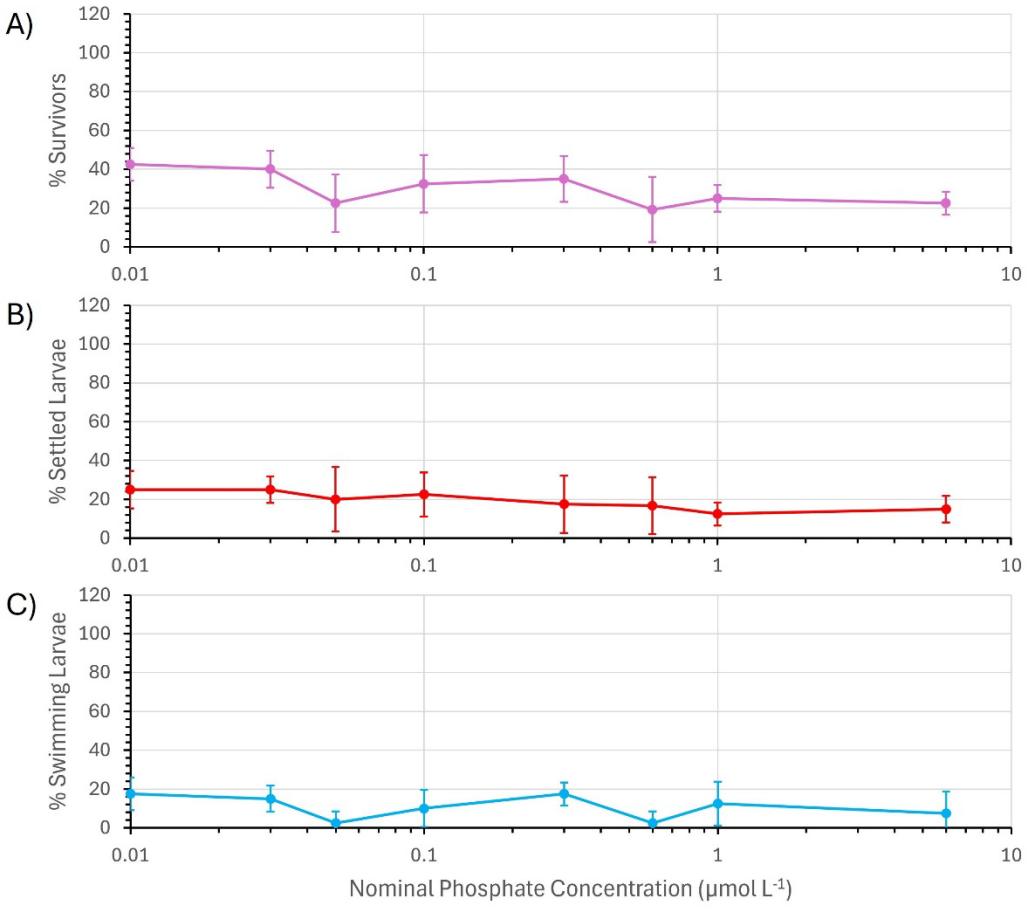

Figure 50: *Impact of Phosphate (with Nitrate as the nitrogen source) on the settlement of* Orbicella faveolata *larvae (**OFAV**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

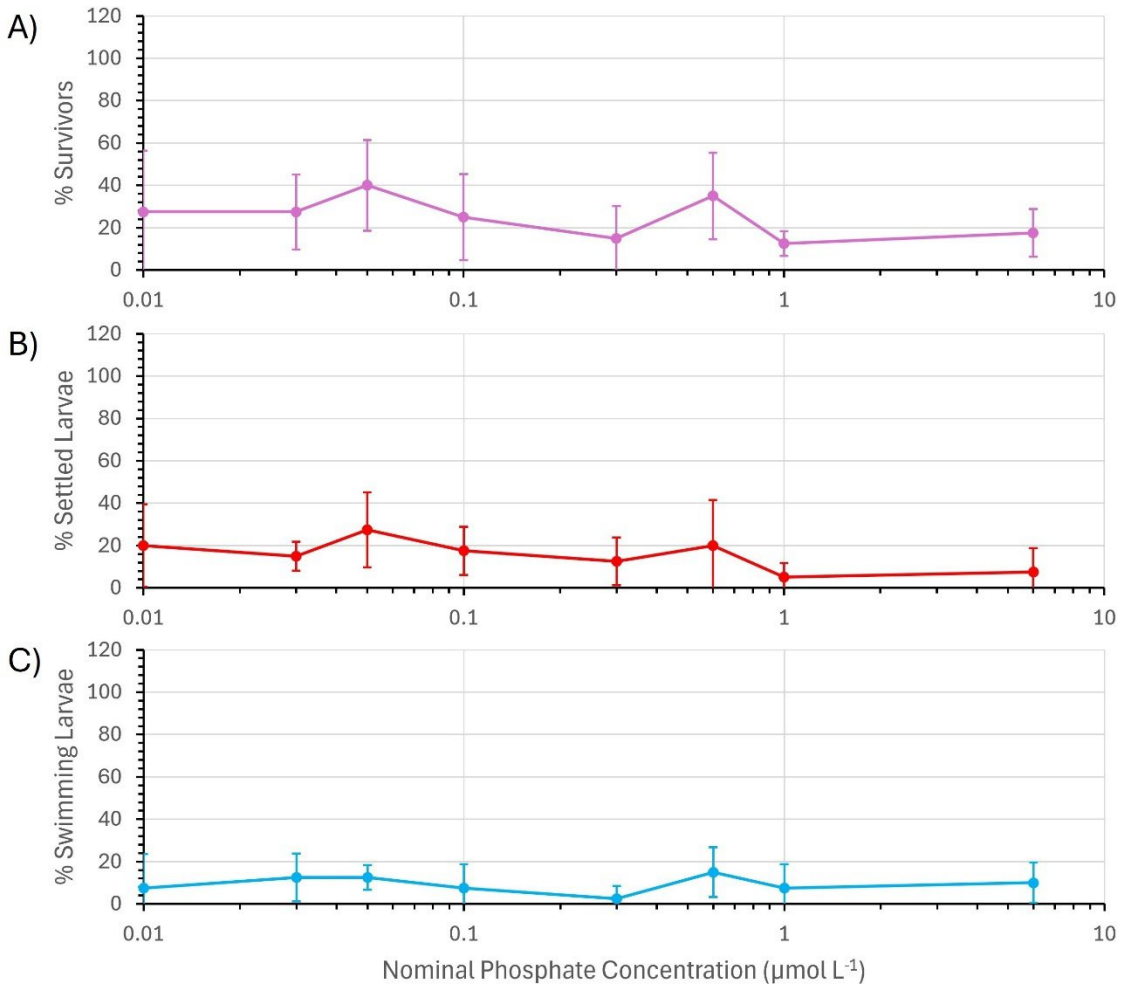

Figure 51: *Impact of Phosphate (with Urea as the nitrogen source) on the settlement of* Orbicella faveolata *larvae (**OFAV**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

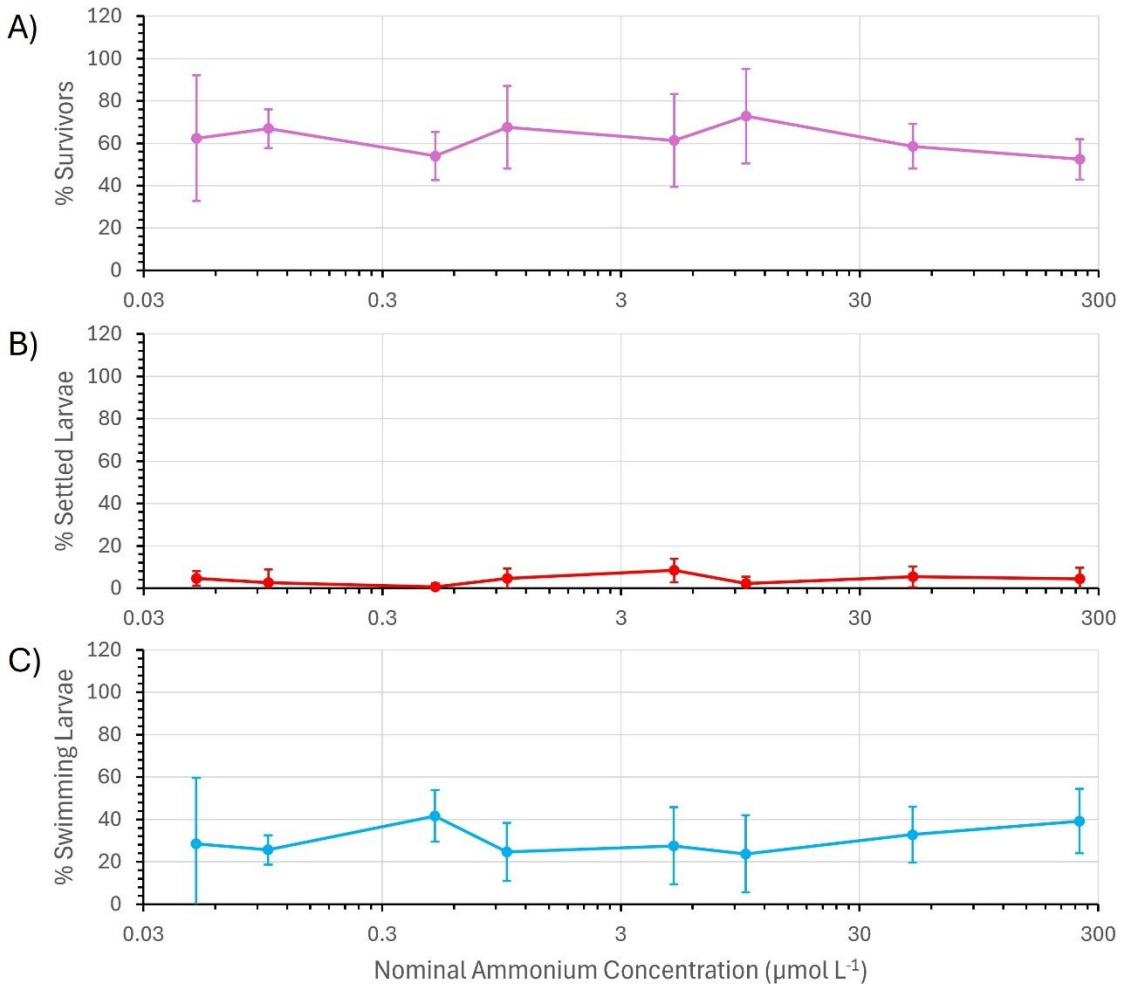

Figure 52: *Impact of Ammonium on the settlement of* Orbicella franksi *larvae (**OFRA**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

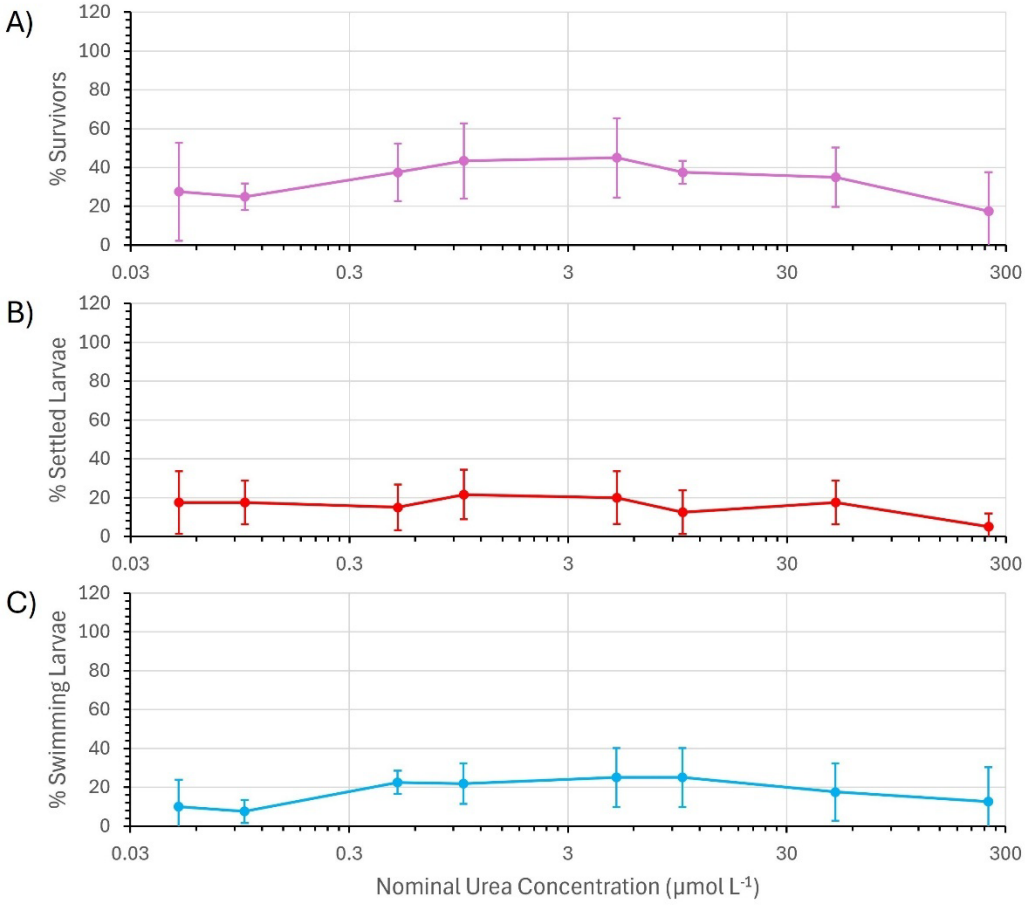

Figure 53: *Impact of Urea on the settlement of* Orbicella franksi *larvae (**OFRA**, spawned 9/25). **A)** Percentage of Survivors, **B)** Percentage of Settled Larvae, **C)** Percentage of Swimming Larvae. "Survivors" is defined as the total of the swimming larvae and settled larvae at the end of the incubation. Error bars, where present, represent the 90% confidence interval.*

6.3. Appendix 3. Genotype/Colony Identifications for all corals used during the fertilization assays.

Table 4*: Identities of the individual genotypes/colonies that were used to generate the gamete batch sample for the fertilization assays conducted during the August 2025 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Orbicella faveolata	OFAV	8/16/25	6_OFAV_21
			NC003_OFAV_005
			6_OFAV_001A
			OFAV_001
			38_OFAV_002

***Table 5**: Identities of the individual genotypes/colonies that were used to generate the gamete batch sample for the fertilization assays conducted during the September 2025 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Montastraea cavernosa	MCAV	9/13/25	4_MCAV_003
			6_MCAV_20
			80_MCAV_002
Orbicella franksi	OFRA	9/15/25	UNK_OFRA_4A
			43_OFRA_008
			PEPS_OFRA_008
			NC001_OFRA_003
			SARA_OFRA_005
Pseudodiploria strigosa	PSTR	9/15/25	BW16_PSTR_002
			CAKE_PSTR_008
			MIDDLE_UNK_PSTR
			BW5_PSTR_008A
			LUCY_PSTR_005
Colpophyllia natans	CNAT	9/15/25	GREENCAN_CNAT_003
			MQ10_CNAT_007
			BW5_CNAT_011

Table 6: *Identities of the individual genotypes/colonies that were used to generate the gamete batch sample for the fertilization assays conducted during the May 2026 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Diploria labyrinthiformis	DLAB	5/15/26	BDAY_DLAB_005
			CAKE_DLAB_001
			5753_DLAB_001
			OG_DLAB_001
			BW24B_DLAB_004

6.4. Appendix 4. Genotype/Colony Identifications for all corals used during the settlement assays.

Table 7*: Identities of the individual genotypes/colonies that were used to generate the batch larval sample for the settlement assays conducted for the August 2025 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Montastraea cavernosa (Eggs)	MCAV	8/16/25	OUTPLANT_MCAV_001
			LAURIES_MCAV_001
			UNK_MCAV
Montastraea cavernosa (Sperm)	MCAV	8/16/25	6_MCAV_020
			CS_MCAV_018
			39_MCAV_036
			4967_MCAV_002
			4_MCAV_003
			R2_MCAV_006
Orbicella franksi	OFRA	8/16/25	NC0001_OFRA_010
			PEPS_OFRA_008
			SARA_OFRA_005
Orbicella faveolata	OFAV	8/16/25	6_OFAV_001A
			6_OFAV_020
			ABE_OFAV_002

***Table 8**: Identities of the individual genotypes/colonies that were used to generate the batch larval sample for the settlement assays conducted for the September 2025 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Orbicella faveolata	OFAV	9/14/25	6_OFAV_001A
			6_OFAV_020
			ABE_OFAV_002
Orbicella franksi	OFRA	9/14/25	NC0001_OFRA_010
			PEPS_OFRA_008
			SARA_OFRA_005
Pseudodiploria strigosa	PSTR	9/15/25	4_PSTR_008
			LAURIES_PSTR_004
Colpophyllia natans	CNAT	9/15/25	OUTPLANT_CNAT_002
			MQ_CNAT_007

***Table 9**: Identities of the individual genotypes/colonies that were used to generate the batch larval sample for the settlement assays conducted for the October 2025 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Pseudodiploria strigosa	PSTR2	10/15/25	R2_PSTR_005
			BDAY_PSTR_004
			BW16_PSTR_006
			BW5_PSTR_006
			MQ10_PCLI_007***
Colpophyllia natans	CNAT2	10/15/25	LAURIES_CNAT_003
			GREENCAN_CNAT_007
			NC0002_CNAT_007
			BW5_CNAT_004
			BW5_CNAT_005
			4_CNAT_004
			NC0001_CNAT_013
			CS_CNAT_011

*** is PSTR but is mislabeled and requires a new ID tag per FCRC Staff

***Table 10**: Identities of the individual genotypes/colonies that were used to generate the batch larval sample for the settlement assays conducted for the May 2026 spawning event at FCRC. The table contains the species name, the sample ID used to identify the samples collected for analysis for this project, the spawning date and the FCRC identifier for the individual colonies.*

Species	Sample ID	Spawning Date	Genotype/Colony ID
Diploria labyrinthiformis	DLAB	5/15/26	BDAY_DLAB_005
			CAKE_DLAB_001
			5753_DLAB_001
			OG_DLAB_001
			BW24B_DLAB_004