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THE DIVERSITY AND VARIATION OF THE MICROBIOTA BETWEEN SEA ANEMONE AND CLOWNFISH DURING THE INITIAL STAGES OF SYMBIOSIS.

Kajal Manjhi
Research Scholar
Deptt. of zoology
CMJ University Meghalaya

Shakshi Pushker
Research Supervisor
Deptt.of zoology
CMJ University Meghalaya

ABSTRACT

Clownfish and their symbiotic sea anemone have a fascinating and mysterious connection. The molecular mimicry of clownfish epithelium mucus is crucial to understanding this tolerance mechanism. Invertebrates rely on their epithelial mucus chemical signature, driven by resident bacteria. New research suggests that generalist clown fish undergo a reorganisation of their skin microbiota upon first contact with their symbiotic anemone. The study investigated the epithelial microbiota dynamics in 18 sets of percula clownfish and the symbiotic anemone Heteractis magnifica using metataxonomics over four weeks. The results showed signs of slowly merging epithelial microbiota before any physical contact between couples. The communal structure of the anemone's epthelial community and the fish's community structure retained the characteristics of the relationship. The interaction signature persisted in both the Remote Interaction and Physical Interaction groups, indicating that even when the fish and anemone couples were separated, the skin microbiota was permanently changed by chemical communication via water between the symbiotic partners. The study also found that three strains of Flavobacteriaceae were more associated with the reconfiguration of the microbiota in fish anemones. The findings challenge the long-held belief in one-way chemical concealment and suggest that the epithelial microbiota from both spouses might be crucial in fostering mutual acceptance.

KEYWORDS microbiota, microbiome, clownfish, anemone,

INTRODUCTION

Clownfish, a member of the Pomacanthidae family superclass Pisces, are small fish species found in various oceans, including the Indian and Pacific oceans, the Great Barrier Reef, and the Red Sea. They establish symbiotic, mutualistic relationships with sea anemones, which they feed on their waste products. Clownfish can be found in sheltered environments of coral reefs or lagoons, far below the surface of the ocean.Sea anemones get their nutrition from the waste products of clownfish, which consume 20-25% algae in their diet. They also consume copepods, tiny mollusks, and crustaceans for food. When housed in captivity, they are fed a diet of fish flakes and pellets and eat the sea anemones' undigested food.Clownfish are the only fish species known to be immune to the sea anemone's poison, with theories ranging from the sea anemone not seeing the fish as food sources due to their mucus covering being made of carbohydrates instead of proteins. Another theory suggests that clownfish may have evolved a resistance to the sea anemone's poisons due to their shared evolutionary history.Anemones often live in couples, with the male changing its sex to that of a female once the female dies. Clownfish are protandrous hermaphrodites, born with a male sex, and their lack of completely formed sex organs indicates that they are neuter. Clownfish have a unique bond with sea anemones, protecting

them from their poisons by increasing water circulation around it, which the anemone then uses to its advantage.

The clownfish provide nutrients (ammonia, sulphur, and phosphorus) to the anemone's endosymbiotic zooxanthellae algae while the anemone protects them from predators excreted by the anemone. This dynamic is a captivating example of a mutualistic relationship. Clownfish have two major defense mechanisms: benefiting their skin's natural protective mucus and covering themselves in anemone mucus for chemical camouflage.

Clownfish's immune profile changes to resemble the anemone's during acclimation, and the epithelium mucus of clownfish serves as a "chemical camouflage" to avoid "not-self" detection linked to the release of nematocysts. It has been shown that the clownfish skin mucus begins to undergo chemical change prior to physical contact with the anemone, as the transfer of amino acid from the clownfish's skin mucus to the anemones occurs. Studies into clownfish anemone symbiosis have only been studied in two ways: during contact with the anemone and during testing of remote connection. For example, a study on the Amphiprion clarkii, a generalist clownfish known to possess an inherent defense mechanism against the release of nematocysts, showed that the control group of fish was always exposed to the same water pressure as the control group of fish, resulting in remote interaction between the two species. In conclusion, clownfish play a crucial role in the marine environment and the mutualistic relationships they form with sea anemones. Their unique characteristics and unique defense mechanisms make them fascinating and fascinating to study.

LITERATURE REVIEW

Delgado, Alonso & Benedict, Charlotte & Macrander, Jason & Daly, Marymegan. (2022). Predatory marine creatures known as sea anemones possess a wide array of poisons. They employ venom for defense, eating, and competition; it's an essential part of their existence. We don't yet know how the three different lineages symbiotic interactions between clownfish and sea anemones have an impact on host's venom composition during evolution. The possibility that clown fish's symbiotic association with sea anemones influences their venom characteristics is explored here. Six species of sea anemones, representing the three recognized clades of sea anemones that host clownfish, were compared using transcriptome data for their venom profiles. In every species we looked at, haemolytic and hemorrhagic toxins were the most common and varied, and we found 1,121 transcripts that matched confirmed poisons. With these findings, we may analyse the current hierarchical structures in venomic research, learn more about the venom variety of sea anemone species, and confirm what is already known about their biology.

Herbert, N. & Bröhl, Stefanie & Springer, Karin & Kunzmann, Andreas. (2017). The symbiotic relationship between clownfish and anemones provides nutrition and safety from predators. It's unclear whether clownfish engage in nighttime actions like aeration in response to hypoxia or if both species benefit from restocking oxygen levels. In a study, the sea anemone Entacmaea quadricolor was evaluated under three different light conditions, with and without the presence of the anemonefish Amphiprion frenatus. Under dark conditions, hypoxia was noted at a distance of 0.2 cm from the anemone's surface. A. frenatus eradicated this limited layer of hypoxia, but it has an exceptionally high tolerance for low oxygen levels. Clownfish do not benefit physiologically from aeration behavior, as they cannot breathe water at 0.2 cm and are not oxygen-limited at distances greater than 1.2 cm. Aeration behavior assists the O2-conforming host's metabolism alone.

Miyagawa, Kazuko. (2010). Using innocent young anemonefish, researchers experimentally examined the anemonefish-sea anemone symbiosis, focussing on the first interaction between the two species. When

compared to earlier research that used adult fish, the outcomes were drastically different. Five different species of Amphiprion in Japan's coastal area have an inherent defense mechanism against the symbiotic anemones that they encounter as youngsters. In addition, the young Nearly every one of the four species that were examined managed to avoid being eaten by the predatory anemones on amphibians. The chemical signals generated by annemones allow naive juveniles to identify their species-specific mate, resulting in a relationship peculiar to that species. When meeting for the first time, visual clues are not crucial for partner identification. The authors explore potential explanations for the striking discrepancy between the current study's findings and those of earlier research.

Roux, Natacha & Lecchini, David. (2015). The process by which creatures of marine reefs that undergo a pelagic larval stage grow in offshore seas and then go to reef settings that are scattered over the ocean poses a significant difficulty. This research examined the chemical recognition ability of the clownfish larvae of the genus Amphiprion ocellaris and the sea anemone Heteractis magnifica in a series of tank tests using a choice flume. In addition, the investigation was carried out on young fish to see whether their chemical skills alter following settling. Larvae and juveniles had a strong predilection for their host, according to the data. As a result, our research demonstrated that olfactory signals may be crucial in guiding pelagic larval stage fishes to an appropriate reef for settlement.

Scott, Anna & Malcolm, Hamish & Damiano, Cristiana & Richardson, Darren. (2011). The symbiotic decline of anemonefish and sea anemones as hosts in the Indo-Pacific is causing significant changes in their population trends. A study on four spots on North Solitary Island, Australia, compared data from 2008 with surveys from 1994 and 1995 to examine long-term changes in the abundance of anemones and anemonefish. The most prevalent species were anemonefish (Amphiprion akindynos, A. latezonatus, and A. melanopus) and annemones (Entacmaea quadricolor and Heteractis crispa). The two most prevalent species, A. akindynos and E. quadricolor, were 532% and 133% more prevalent than in 2008, respectively. Densities of A. akindynos were strongly correlated with anemone cover, while A. latezonatus was greater in deeper waters. The species maintained stable densities, with A. melanopus and Heteractis crispa present in small quantities.

RESEARCH METHODOLOGY

Samples of water and host microorganisms

This sampling procedure consisted of seven steps: (T0, after a three-week acclimatization period; T1, following the initial fifteen minutes of physical interaction (PI) between anemones and clownfish; T2, one and a half weeks subsequent to T1; T4, and T5, one and a half weeks subsequent to the separation of fish-anemone pairs from the physical and remote interaction groups, respectively; and T6, immediately following both control groups. Keep in mind that each of the six PI subjects had a long RI (ranging from zero to twenty-four hours) at T1 before coming into direct touch with their anemone. As soon as the fish were caught, a sterile cotton swab was gently rubbed over regarding half of the fish's right side, while it's not submerged, to collect their skin mucus. To ensure consistency, we sampled the same spot on every fish. The same procedure was used to collect annemone epithelium mucus samples: the water level in the aquarium was momentarily lowered, enabling a delicate cotton swab to be rubbed against the tentacles while they were dry. Two liters of tank water were drawn out at each sample interval in clean Nalgene bottles and promptly passed through peristaltic pumps that move membranes as small as 0.22 µm across describe the bacterioplankton population of each group. Each experimental group used four bacterioplankton duplicates.

processes include DNA isolation, library construction, and 16S amplicon sequencing

The DNA from sea anemone and clownfish epithelial mucus, as well as 0.22 µm membranes from water samples, was extracted using the Qiagen® Blood and Tissue Kit following the manufacturer's instructions. The 16S rRNA segment V3-V4 was amplified using a two-step dual-indexed polymerase chain reaction (PCR) technique developed by the Plateforme d'Analyses Génomiques (IBIS, Université Laval, Quebec City, Canada). Illumina instruments were the target of this approach. Primers were 5'-tailed using an Illumina TruSeq adaptor and unique to the 16S region in the first polymerase chain reaction (PCR). Flowcell and library-specific barcode annealing sections make up the rest of the adaptor sequence—was attached using a second PCR. Primers utilized in this study include sequences that are exclusive to Illumina and are so protected by intellectual property laws. Customers of Illumina are only entitled to utilize their derivative works using equipment and goods manufactured or distributed by Illumina. No other usage is allowed under any circumstances. For both rounds of amplification, the oligonucleotide sequences listed below were used:

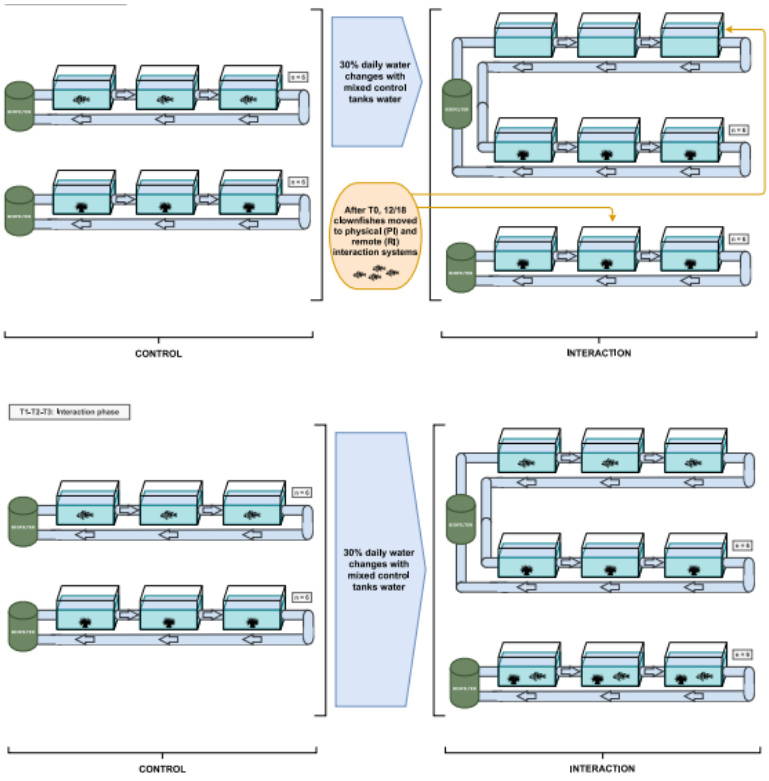
Priority one polymerase chain reaction primer:

ACACTCTTTCCCTACACGACGCTCTTCCGATCT-(347F)GGAGGCAGCAGTRRGGAAT,

Primers 1 and 2: reverse-specific (PCR #1):

GTGACTGGAGTTCAGACGTGTGCTCTTCCGAT; forward-specific (PCR #2):

CCACCRGGGTATCTAATCC.



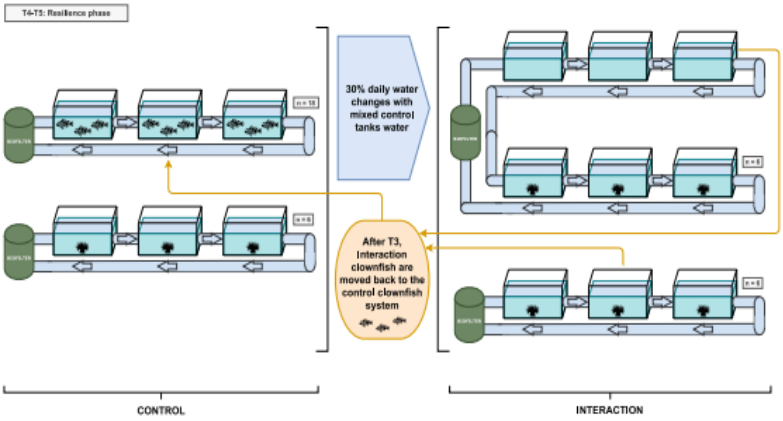


Fig. 3.7 Anemone control (AC), fish control (FC), physical interaction (PI), and remote interaction (RI) were each tested six times according to the experimental design. Three weeks till T0 for acclimation. Two weeks of interaction (from Task 1 to Task 3). c Resilience, two weeks (between T4 and T5)

Here is the first index: C[index1]

the code word is "ACACGAC."

First, the reverse general primer for PCR #2:

CAAGCAGAAGACGGCATACGAGAT [index2].

The fifth question is: Polymerase chain reactions were carried out using High-Fidelity DNA Polymerase, which was supplied by New England Biolabs. (PCRs) in 25-µL reactions. The 35 amplification cycles were as follows: (a) 30 seconds at heat to 98 degrees Celsius, (b) hold for 10 seconds, (c) heat to 64 degrees Celsius, (d) heat to 72 degrees Celsius, and (e) hold for 2 minutes. As instructed by Beckman Coulter Genomics, AMPure beads were used to purify the amplified DNA, removing primers, dimers, proteins, and phenols. We sequenced the amplifying and control libraries using the Illumina MiSeq, which is located in San Diego, CA, USA.

Identifies amplicon sequence variants (ASVs) and reads de-noising

Bioinformatics processing was performed using dada2, as described in. The raw values were reduced from 4,770,388 to 270 base pairs by quality filtering. Prior to proceeding, we ensured the quality of the sequencing data. Coverage of the 16S rRNA gene's V3 region by forward reads improved the taxonomic accuracy. According to recent research, the V3 is the way to go when profiling diverse microbial communities in order to provide more precise and comprehensive assessments of the richness and diversity of these communities. As a result, downstream analyses were conducted using 270 bp reads that covered V3, as shown in. All settings were set to default as per version 1.14.1 in the usual dada2 pipeline, without the "dada" step that aggregates all samples for ASV inference. There was a limit of 2 anticipated errors (maxEE) when the pipeline was executed. From the smallest number of post-dada2 samples (6736) to the largest number of 54,137, a median of 23,762 reads was discovered across all samples.

Taxonomic annotation

Using the blastn matches in the NCBI "16S Microbial" database, amplicon sequence variations (ASVs) were taxonomically annotated. Our needs for comprehensive information on less-known species were met, with minimal unclear annotations, by the National Centre for Biotechnology Information's (16S) database, which receives more updates than any other source. We provided the taxonomic identification of matches that were more than 99% identical. A taxonomy was given to sequences that did not meet the identity criteria using a lowest common ancestor technique that was constructed from the fifty most played blastn matches. This method employs its cues from MEGAN's LCA algorithm.

ANALYSIS

Association Rates and the Sigmoid Factor

The sigmoid association factor was reevaluated to understand the exponential growth and decay of terms associated with association rates. The original equations were reworked to address the erratically growing terms. The units were simple for equations involving clownfish or anemones, but caution was needed when depicting association rates in linked pairs and triples.After much deliberation, the authors decided to view association rates as a subset of the success rate of searches. Clownfish seek vacant anemones to raise their young and ensure their safety. The affiliation factor must also rely on the anemone population size. The units of association rates must be different for each equation, with the rate per anemone denoted as rac.The units of the equations were expressed in anemone units, with rac representing the price for one clownfish and rac denoting the rate per anemone when subtracted from Mc(t). It was essential to see A2(t) and Ma(t) · Mc(t) as a pair.While ideas like rac · Ma(t) · Mc(t) were introduced in the writings of Kostitzin and other scholars, the units themselves were never discussed. The equations were used in biological contexts by assuming certain assumptions:

$$
\begin{aligned}
J_a(t+1) &= \underbrace{(1-d_{ja})\cdot J_a(t)}_{\text{surviving } J_a} + \underbrace{b_{ma}\cdot M_a(t)}_{\text{birth from } M_a} + \underbrace{b_{2a}\cdot A_2(t)}_{\text{birth from } A_2} + \underbrace{b_{3a}\cdot A_3(t)}_{\text{birth from } A_3} - \underbrace{m_{ja}\cdot J_a(t)}_{\text{maturation to } M_a};\\
J_{c1}(t+1) &= \underbrace{b_{3c}\cdot A_3(t)}_{\text{birth from } A_3};\\
J_{c2}(t+1) &= \underbrace{(1-d_{jc})\cdot J_{c1}(t)}_{\text{surviving } J_{c1}};\\
M_a(t+1) &= \underbrace{(1-d_{ma})\cdot M_a(t)}_{\text{surviving } M_a} + \underbrace{m_{ja}\cdot J_a(t)}_{\text{maturation from } J_a} + \underbrace{k_{2a}\cdot A_2(t)}_{\text{dissociation from } A_2} + \underbrace{k_{3a}\cdot A_3(t)}_{\text{dissociation from } A_3}\\
&\quad - \underbrace{r_{ac}\cdot M_a(t)\cdot M_c(t)}_{\text{association to } A_2};\\
M_c(t+1) &= \underbrace{(1-d_{mc})\cdot M_c(t)}_{\text{surviving } M_c} + \underbrace{(1-d_{jc})\cdot J_{c2}(t)}_{\text{surviving } J_{c2}} + \underbrace{k_{2c}\cdot A_2(t)}_{\text{dissociation from } A_2} + \underbrace{2\cdot k_{3c}\cdot A_3(t)}_{\text{dissociation from } A_3}\\
&\quad - \underbrace{r_{ac}\cdot M_a(t)\cdot M_c(t)}_{\text{association to } A_2} - \underbrace{r_{2c}\cdot A_2(t)\cdot M_c(t)}_{\text{association to } A_3};\\
A_2(t+1) &= \underbrace{(1-k_{2a}-k_{2c})\cdot A_2(t)}_{\text{returning } A_2} + \underbrace{r_{ac}\cdot M_a(t)\cdot M_c(t)}_{\text{association from } M_a \text{ and } M_c} + \underbrace{k_{32}\cdot A_3(t)}_{\text{dissociation from } A_3}\\
&\quad - \underbrace{r_{2c}\cdot M_c(t)\cdot A_2(t)}_{\text{association to } A_3};\\
A_3(t+1) &= \underbrace{(1-k_{3a}-k_{32}-2\cdot k_{3c})\cdot A_3(t)}_{\text{returning } A_3} + \underbrace{r_{2c}\cdot A_2(t)\cdot M_c(t)}_{\text{association from } M_c \text{ and } A_2}.
\end{aligned}
$$

Juvenile Consolidation and Carrying Capacity

The first step was to merge Jc1 and Jc2 into a single population, which we called Jc. There is a weak correlation between the number of days from hatching and the morphological variations between immature clownfish, although these differences do not account for much of the mutualism. In order to get to a workable model, we had to make assumptions that may not have been completely correct from a biological standpoint. Populations kept going negative, even though this simplification eliminated certain factors. This caused other population formulae to include these exponentially "negative" populations, which in turn caused the growth to be exponential.

Before we could implement an artificial condition that would stop the model if populations started to go negative, we needed to figure out why they were dropping below zero. So, we investigated adding a stop condition to the model. All populations either raised or fell to numbers on the scale of ±10200 or higher, with the exception of juvenile clownfish, which fell, adult anemones, which rose, adult clownfish, which fell, linked pairs, and linked triples. Little anemones shrank to −1018, while associated clownfish increased and adult anemones decreased. The model was run for two years with each population starting at 50 and the matrices given in Section 3.2 for birth rates. These values are the consequence of such settings.

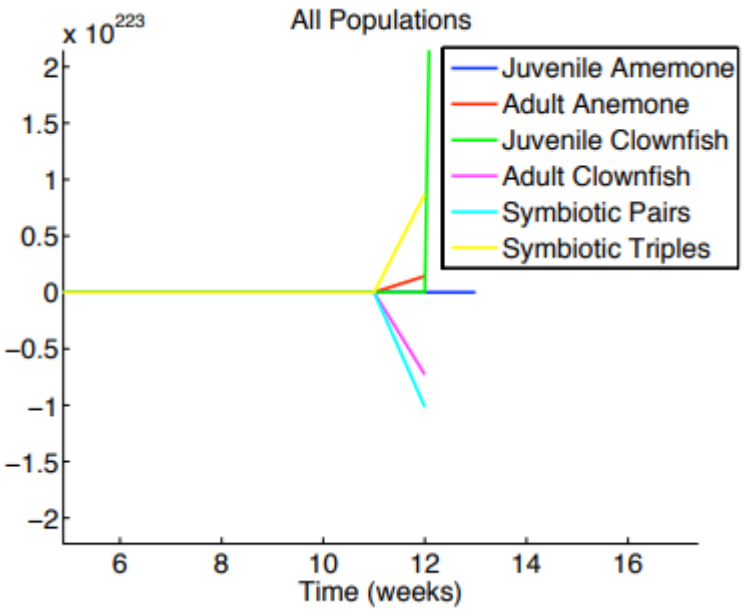


Figure 4.1 Exponentiated growth and decay populations are shown.

Ratios of Births and Complete Model

The birth rate matrices were removed from our model for the sake of additional simplification. Our premise was that these seasonal rates follow a normal distribution every year and may be approximated using average values, even though clownfish and sea anemone reproductive cycles are lunar-dependent and vary with the phases of the moon and the times of each lunar month. After we had a good grasp we intended to re-integrate the time-dependent birth rates into the whole system, and this simplicity would make it easy to manipulate other aspects.

Their comparable values also allowed us to boost our faith in our birth rates. Holbrook and Schmitt have out that the quantity of fish living in an environment is positively correlated with certain host anemone traits. The researchers found that anemones with fish had three times the rate of individual development compared to anemones without fish. Additionally, two-fish anemones had the greatest fission rates were lowest in areas devoid of anemonefish. There was a higher-than-expected death rate for anemones that were not protected by anemonefish. The anemones that had two anemonefishes were the ones with the greatest net gain in surface area, without any had almost no increase. The anemonefish community improved anemone survival rates, growth rates, and the frequency of asexual reproduction. Our model upheld the result determined by these findings that $bma < b2a < b3a$ and $k3c < k2c << 1$.

We can see our simplified model in Figure 4.6.

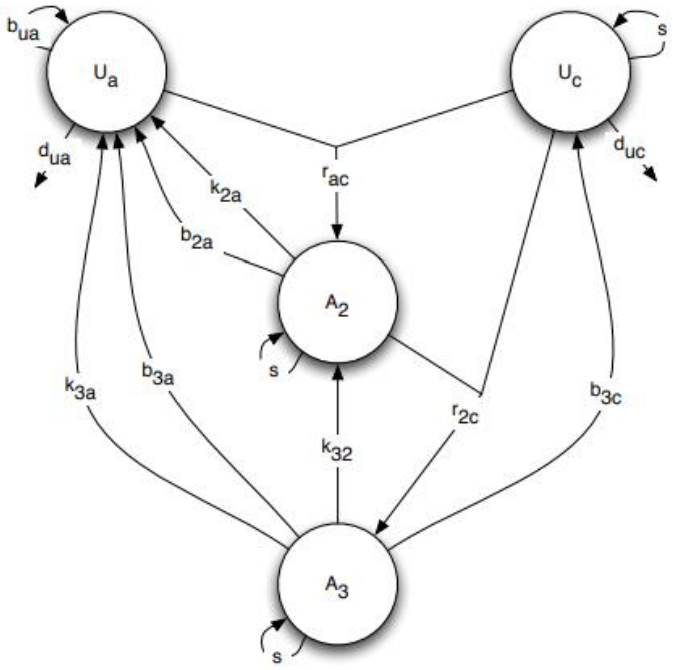


Figure 4.6: Visualization of a simplified model

4.6 SNOPT: Sparse Nonlinear Optimizer

One program that can handle optimization issues on a grand scale is SNOPT, which uses sequential quadratic programming. This method determines the minimum of a nonlinear smooth function that is constrained in both the linear and nonlinear directions. Before we could find the unknown parameters' initial values and in order to minimize the objective function, we have to choose a standard of constraint functions and a vector of starting values for the parameters. For the purpose of determining which parameter values would provide the intended result, this program basically works backwards from the outcomes that we specify.

Since we know that populations act erratically at association rates close to.01, we turned our attention to these rates and set out to determine the lowest possible value for the association rates that could still be considered mutualistic. We observed that at a certain level, populations start to expand at an exponential pace, which leads us to conclude that a low rate of mutualism is necessary to control populations since related individuals gain so much. Some fish may not even be able to locate an anemone to live in because of how severe the competition is.

An objective function When rac = r2c does not permit differentiation, was characterised as t = max (rac, r2c) so that we could minimize rac and r2c. Following the specifications in an extra file, x is a column vector with the initial values of the unknowns; F is a vector with the goal and constraint functions; and we built the following vectors:

$$x = \begin{bmatrix} r_{ac} \\ r_{2c} \\ b_{ua} \\ b_{2a} \\ b_{3a} \\ b_{3c} \\ t \end{bmatrix}; \quad x_0 = \begin{bmatrix} .005 \\ .005 \\ .010 \\ .015 \\ .020 \\ .035 \\ 1 \end{bmatrix}; \quad F = \begin{bmatrix} t \\ t - r_{ac} \\ t - r_{2c} \\ b_{3a} - b_{2a} \\ b_{2a} - b_{ua} \\ U_a \\ U_c \\ A_2 \\ A_3 \end{bmatrix}.$$

Vectors representing Ua, Uc, A2, and A3 are the population values for the t variables that are important. The next step was to determine the maximum and minimum values for x and F. We allowed all x-values to decrease to.0001, rac and r2c to rise to.0075, and all other parameters to reach 1. At the outset, we allow t to range from 0 to 1 for F, whereas all other numbers fall between 0 and infinity. Given that rac and r2c were likewise constrained to values between 0 and 1, it follows that (t − rac) and (t − r2c) would undergo similar limitations.

Our next step was to see whether either exponential growth or decay was being shown by the system which may lead to SNOPT numerical challenges; nevertheless, this did not fix the problem. We next attempted restricting the populations to 1000. We opted should exclude the birth rates from x and maintain them at constant levels rather than allowing SNOPT to vary them because estimating the association rates was the rationale for employing this strategy. We got decent outcomes where r2c=.0000099 and rac=.000388 after making this modification. You can see the Figures 4.7 and 4.8 show the populations' short-term and long-term trajectories with these association rates, respectively.

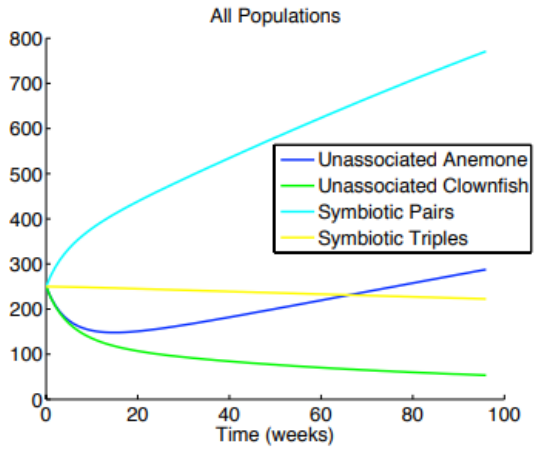


Figure 4.7: Societies where the r2c value is.0000099 and the rac value is.000388.

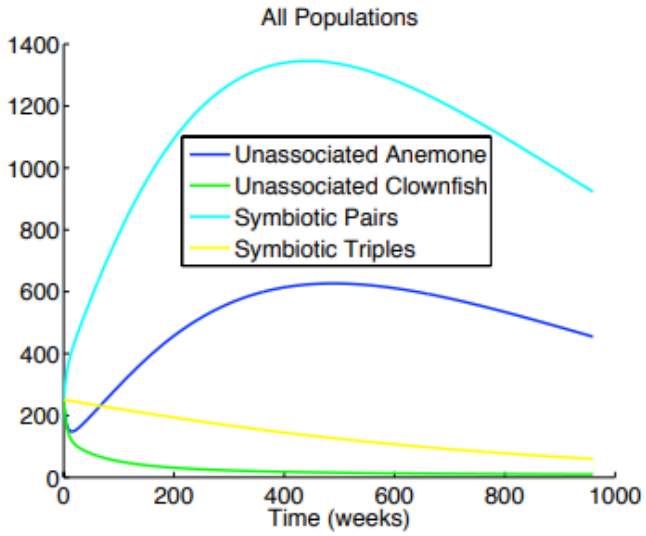


Figure 4.8: Longitudinal populations with rac =.000388 and r2c =.0000099.

It seems that this conduct is acceptable. Since SNOPT found rac to be around 40 times higher than r2c, we may anticipate that linked pairs will flourish, unlinked anemones will expand, linked clownfish will collapse, and linked triples will stay stable at first but gradually approach zero. While adjusting rac and r2c, SNOPT minimized t, the goal function. The algorithm improves the objective function and constraints by a quadratic and a linear approximation, respectively, at each major iteration. We were essentially with this optimization, we need $t \geq$ rac and $t \geq$ r2c, therefore we minimised the largest of the two association rates. In order to discover the best potential results, SNOPT adjusts rac and r2c. If the algorithm had started perturbing one association rate instead of the other, it may have returned rac =.0000099 and r2c =.000388. We wondered whether the values might have been obtained for the opposite parameters. Here are some graphs showing how the populations with these affiliation rates have behaved both temporarily and over the long term.

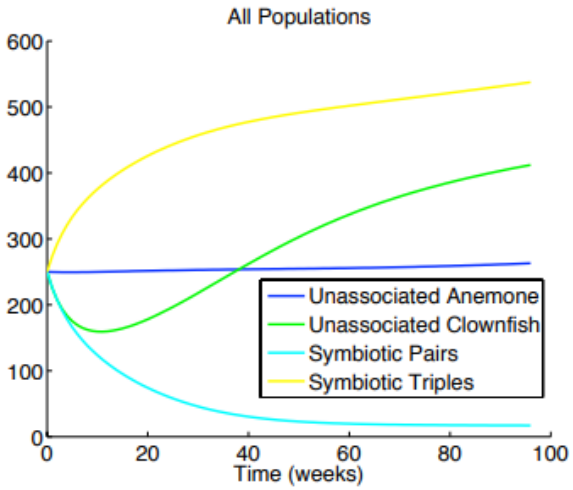


Figure 4.9: Populations where the ratio of rac to r2c is 0.00388.

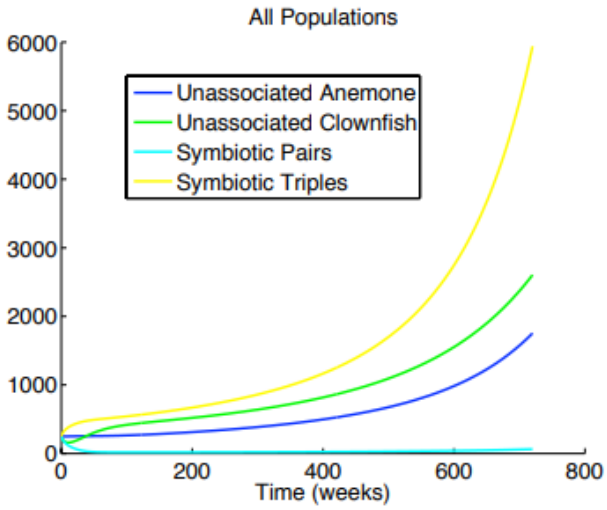


Figure 4.10: Communities with a 15-year rac =.0000099 and r2c =.000388.

CONCLUSION

The study aimed to investigate the growth plasticity in vertebrates, specifically A. percula and E. quadricolor, when there is a change in mutualistic environment. Juvenile A. percula development was found to be positively correlated with anemone area, suggesting a mutualistic relationship between the two species. Anemone size in nature can be a proxy for environmental conditions and resource accessibility, leading to adaptive phenotypic responses. A. percula can maximize reproduction, defend its host, and encourage anemone development, but it also prevents individuals from expanding to unsustainable sizes. The study found that the plastic response to anemone size occurred independently of food availability, suggesting that larger anemones have more room to spread out or more egesta to work with. The connection between anemone size, group size, and female size may be better understood with more research on this mutualistic interaction. The study also supports the growing body of evidence that clown fish's epithelial mucus immunological profile mimics that of the anemone before first contact. The chemical dialogue may also induce a restructuring of the sea anemone's epithelial mucus, casting doubt on the long-standing theory of unidirectional chemical camouflage. In conclusion, the study lays the groundwork for future mechanistic investigations into the complex web of interactions between clownfish, anemones, and bacteria in their symbionts.

REFERENCES

1. with Sea Anemones. Genome biology and evolution. 11. 10.1093/gbe/evz042. Delgado, Alonso & Benedict, Charlotte & Macrander, Jason & Daly, Marymegan. (2022). Never, Ever Make an Enemy… Out of an Anemone: Transcriptomic Comparison of Clownfish Hosting Sea Anemone Venoms. Marine Drugs. 20. 730. 10.3390/md20120730.

2. Herbert, N. & Bröhl, Stefanie & Springer, Karin & Kunzmann, Andreas. (2017). Clownfish in hypoxic anemones replenish host O2 at only localised scales. Scientific Reports. 7. 10.1038/s41598-017-06695-x.

3. Miyagawa, Kazuko. (2010). Experimental Analysis of the Symbiosis between Anemonefish and Sea Anemones. Ethology. 80. 19 - 46. 10.1111/j.1439-0310.1989.tb00727.x.

4. Roux, Natacha & Lecchini, David. (2015). Clownfish chemically recognized their sea-anemone host at settlement. Vie et Milieu. 65. 17-20.

5. Scott, Anna & Malcolm, Hamish & Damiano, Cristiana & Richardson, Darren. (2011). Long-term increases in abundance of anemonefish and their host sea anemones in an Australian marine protected area. Marine and Freshwater Research. 62. 10.1071/MF10323.

6. Nguyen, Hai-Thanh T & Zhao, Min & Wang, Tianfang & Dang, Binh Thuy & Geffen, Audrey & Cummins, Scott. (2024). Sea anemone–anemonefish symbiosis: Behavior and mucous protein profiling. Journal of Fish Biology. 105. n/a-n/a. 10.1111/jfb.15772.

7. Meron, Dalit & Chadwick, Nanette. (2004). Effects of anemonefish on giant sea anemones: expansion behavior, growth, and survival. Hydrobiologia. 530-531. 513-520. 10.1007/s10750-004-2688-y.

8. Mebs, Dietrich. (2009). Chemical biology of the mutualistic relationships of sea anemones with fish and crustaceans. Toxicon : official journal of the International Society on Toxinology. 54. 1071-4. 10.1016/j.toxicon.2009.02.027.

9. Pratte, Zoe & Patin, Nastassia & McWhirt, Mary & Caughman, Alicia & Parris, Darren & Stewart, Frank. (2018). Association with a sea anemone alters the skin microbiome of clownfish. Coral Reefs. 37. 10.1007/s00338-018-01750-z.

10. Marcionetti, Anna & Rossier, Victor & Roux, Natacha & Salis, Pauline & Laudet, Vincent & Salamin, Nicolas. (2019). Insights into the Genomics of Clownfish Adaptive Radiation: Genetic Basis of the Mutualism