Yellowtail blue damselfish (*Chrysiptera parasema)* response to climate change: Impact of temperature on behavior

By

Rebecca Danielle Caldwell

Department of Environmental Science and Sustainability

Allegheny College

Meadville, Pennsylvania

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Dr. Casey BradshawWilson	**Date**	**Dr. Mark Kirk**	**Date**

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Rising ocean temperatures are a major indicator of climate change. Changes in ocean temperature can alter marine ecosystems, such as coral reefs, and the organisms that depend on them. Reef fish such as *Chrysiptera parasema* (yellowtail-blue damselfish) can act as an indicator species for reef health. Understanding how yellowtail damselfish respond to an increase in water temperature is key to examining how climate change can affect marine ecosystems. The purpose of this study was to test yellowtail damselfish aggressiveness in response to an increase in temperature. This study draws on the methods of da Silva Pinto et al. (2020). 7-8 yellowtail damselfish were divided into groups of 2-3 among three tanks. These tanks were subject to temperature change (22℃-26℃) to determine how the fish's behavior changed throughout the experimental process. Temperature was not found to be a significant factor in yellowtail behavior ($p<0.66$). However, we did find significance in the type of behavior as temperature increases ($p<0.026$) as well as a difference between behavior totals ($p<0.018$). Our analysis emphasizes the importance of studying these interspecies interactions to understand reef fish survivability, thermal tolerance, and ecosystem importance.

1 | INTRODUCTION

Humans are primarily responsible for contributing greenhouse gases (GHG) into the atmosphere, acting as a driving force for climate change (Rosa et al., 2015; Zanna et al., 2019). These gases are trapped in the atmosphere, most of which is absorbed by the oceans leading to a rise in water temperature (Kweku et al., 2018). Between 1971 and 2010, the ocean has absorbed 90% of this heat energy, while the rest warms the atmosphere and land surface (Cheng et al., 2017; da Silva-Pinto et al., 2020). Over the past century, the average surface temperature of ocean water has increased by 1°C (Hoegh-Guldberg, 1999; Scripps Institution of Oceanography, 2014). Slight changes in sea surface temperature have altered sensitive marine ecosystems (US EPA, 2023).

Understanding increasing ocean temperatures can lead to the conceptualization of effects that climate change has on marine ecosystems. Identifying these impacts allows us to recognize critical areas that are susceptible to rising ocean temperatures. Surface temperature of the ocean varies with latitude, with warm tropical regions around the equator experiencing average temperatures of 30°C (Scripps Institution of Oceanography, 2014; US EPA, 2023).

As a complex and diverse habitat, coral reefs are increasingly subject to thermal-stress events caused by climate change (Hoegh-Guldberg, 1999; Pratchett et al., 2011). Corals are sensitive to "bleaching" from temperature changes as slight as 1.0°C and with the increase of tropical ocean waters, results of reef systems include mass bleaching and mortality. With these disturbances comes corresponding changes in habitat, causing disruption in abundance and diversity of fishes (Pratchett et al., 2011).

As ectotherms, coral reef fishes are reliant on static temperature to maintain physiological levels of performance (Alfonso et al., 2021; Munday et al., 2008; Johansen & Jones, 2011). Even slight fluctuations in environmental conditions can trigger behavioral responses, especially in sensitive species of reef fishes. Rapid temperature increases related to climate change are known to impact reef fishes by inducing stress. In addition to their regular physiological responses to stressors, additional thermal stress may inhibit a fish's ability to cope with stressors such as habitat loss, which has effects on individual performance, population dynamics, and fitness (Alfonso et al.,

2021; Munday et al., 2008). Subsequently, increased water temperature has a direct impact on fishes' metabolism, increasing their respiration rate, nutritional requirements, and influences their reproduction and immunological defenses (da Silva-Pinto et al., 2020). The most immediate impact of these fine-scale, biological impacts will be the loss of fish diversity and abundance in reef ecosystems (Hoegh-Guldberg et al., 2007; da Silva-Pinto et al., 2020; Munday et al., 2008).

As reef species, damselfish (family Pomacentridae) are a keystone species whose role is the maintenance of coral structures due to their cultivation of algae mats, controlling algae diversity (Ceccarelli et al., 2005; Ceccarelli, 2007; Fishelson, 1998). Although damselfish are pivotal to reefs and maintaining a diverse ecosystem, mass bleaching events and warming waters are affecting damselfish populations and physiology (Alfonso et al., 2021; Keith et al., 2018). As a diverse family of reef fishes, damselfish are one of the most abundant groups of fish worldwide. They're known for their territorial behavior and aggressively defend territory against conspecifics and grazers. These territories include individuals "algae farms", while also using them as refuge and reproduction sites. Algae farms help sustain a greater diversity of invertebrates and algae, increasing overall biodiversity to a reef (Souza et al., 2011).

Previous studies on damselfish have analyzed how metabolic rate and cognitive function are compromised in response to rising ocean temperatures (Johansen & Jones, 2011; Silveira et al., 2023). However, there is a current lack of knowledge of damselfishes' behavioral changes in response to rising ocean temperatures. One study examined damselfish behavior, conducted by da Silva-Pinto et al. (2020). Their data on the impact of temperature and habitat structure complexity supported how damselfish behavior changed in association with environmental conditions. The results showed that at lower temperatures, damselfish were more aggressive than at higher temperatures. Thermal stress likely decreased attacks towards threats and changed the way individuals interact with an intruder. This trade-off of aggression for passive behavior in the presence of a potential threat indicates that with an increase in temperature, damselfish become an easier prey item for fs (da Silva-Pinto et al., 2020). However, this trade-off would limit predator/threat recognition that provides animals with the ability to adapt to the risk of predation (Mitchell et al., 2013). This limitation may be one of many that affects the survivorship of damselfish and makes them a more favorable prey species.

Chrysiptera parasema (yellowtail blue damselfish) is one member of the Pomacentridae family. Damselfishes inhabit near-shore tropical regions and are descendants of major reef fish assemblages (McCord et al., 2021). Yellowtails are one of the most common of the marine fishes and are often sought out in the aquarium trade. The anterior of the fish's body is a bright blue color, which extends to the caudal fin where it gradually changes to a yellow-orange color (Olivotto et al., 2003). As a coral-dependent reef fish, damselfish are particularly susceptible to declines in coral due to bleaching events. *C. parasema* is highly vulnerable because they use branching coral as habitat and only associate themselves with a few coral species. This is of particular concern because branching corals are dominant in reef structures (Bonin, 2012).

The primary objective of this study is to determine changes in behavior of yellowtail-blue damselfish in response to an increase in water temperature in a lab environment. This study focused particularly on behavior exhibited during feeding time. Specifically, observing and classifying behavior compared to natural and increased water temperatures. My hypothesis is that yellowtail damselfish will become less aggressive as water temperature increases. Since the long-term effects of climate change on reef species has yet to be fully outlined, damselfish can be used as an indicator species for ecosystem and reef health in tropical oceans. Changes in behavior are one component of the impacts of climate change that are affecting marine ecosystems. In general, reef fishes are a critical component in determining how agonistic behavior can be a tool in measuring the effects climate change has on reef ecosystems. Given their global importance, reef ecosystems must be at the center of our understanding of the effects of rising sea temperatures and the steps that must be taken to mitigate these impacts.

2 | METHODS

2.1 METHODOLOGY & SPECIMENS

This study draws on methods conducted by da Silva-Pinto et al. (2020). Eight yellow-tail blue damselfish were imported from Sea Cave in Erie, PA. This specific species of damselfish are wild-caught, however, the location for capture of these damselfish was available upon request. The size of the damselfish are relatively the same, life stage, sex, and age are unknown.

2.2 TANK SET-UP

All fish were kept in three, separate, 10 gallon tanks. Due to their territorial and potentially aggressive behavior, 2-3 damselfish were kept in each tank successfully (Yellowtail, n.d.). Tank A had two fish, Tank B had 2 (the third fish died due to unknown causes half way through experimentation), and Tank C had 3. These tanks were set up in Steffee Hall at 72-78°F (22-26°C) with a pH of 8.2-8.4 and salinity of 35-36 ppt (Damsel, n.d.; da Silva-Pinto et al., 2020; Yellowtail, n.d.). The tanks were set on a 12:12 hour light:dark cycle with appropriate filters and heaters common across all tanks. Damselfish were fed once a day with commercial saltwater fish food within the same timeframe.

The process of tank cycling occurred over the course of 4 weeks to ensure all tanks were suitable for the addition of live fish. In the first week, tanks were set up with distilled water, salt, and were controlled for temperature. The tanks were monitored for salinity and temperature, then seeded with water from an established saltwater tank. The tanks were monitored for another two weeks for water quality and nutrient levels before the addition of live fish during the fourth week.

2.3 BEHAVIORAL TESTING & DATA COLLECTION

After the completion of the first round of testing, two tanks were warmed between 24-26°C. These tanks are the experimental groups. One tank, kept at the same conditions (22°C), represented the control group. Over the course of 4 days, the second tank was increased to 24°C, while the third tank was approximately 26°C. The same procedure was repeated to conduct behavioral testing on the damselfish to reflect the second round of testing. Although the projected maximum temperature of tropical oceans by the end of this century is between 30-34°C, representing the worst possible conditions, preventing the fish from getting stressed with a quick temperature transition was a priority (IPCC, 2013; Rummer et al., 2014).

The fish's health and vitality was monitored daily. In the event a fish had died, it was immediately removed from the tank and stored in Steffee Hall's freezer space. After the conclusion of the adjustment period, the damselfish were recorded using an iPhone 15 from the front of the tank at nine minute intervals during feeding time for every trial. Each recording

captured three minutes before feeding time, three minutes during the time the fish were fed, and for an additional three minutes after being fed. Each tank was recorded on three separate occasions, for a total of six recordings per tank over the course of six days, pre and post temperature changes.

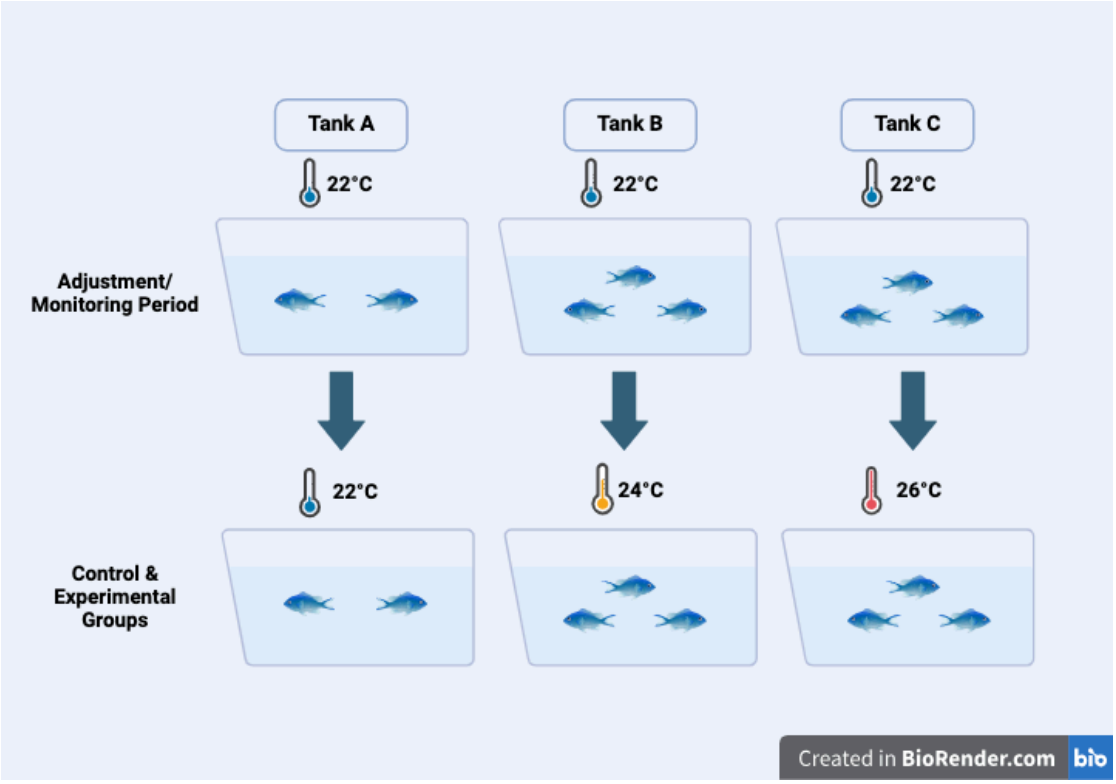


Figure 1: Visual representation of experimental tank design and control vs. experimental groups. Tank A represents the control group, maintaining 22°C throughout the experiment. Tank B was warmed to 24°C from the first round of testing and Tank C was warmed to 26°C.

2.4 CLASSIFYING BEHAVIOR

Using the recorded footage, videos were analyzed for behavioral responses under normal and experimental tank conditions. An ethogram was kept to classify damselfish behavior during behavioral testing. Behaviors include nipping, charging, chasing, advancing, and coexisting (Table 1).

Table 1. Descriptions of *C. parasema* behaviors being investigated.

Behavior	Description
Nipping	A direct attack on another fish through physical contact.
Charging	A quick lunge towards another fish.
Chasing	The continued pursuit of another fish by the attack, often for a longer duration than a charge.
Coexisting	Is in the presence of another fish without any further interaction.
Advancing	Slower than a charge, a deliberate movement towards another fish.

2.5 DATA ANALYSIS

The sum of total occurrences (pre and post temperature changes) of each behavior was tested for standard error to determine the confidence intervals and accuracy of the estimations. Using Google Sheets, a two-way ANOVA ($\alpha=0.05$) was used to test significance between tanks and behaviors. One-way ANOVAS ($\alpha=0.05$) were also used to test significance between tanks and between types of interactions. The Tukey test was used to identify which behaviors were significant.

2.6 POST-BEHAVIORAL TESTING

After the completion of this study, the damselfish were donated to Sea Cave. All animal procedures were authorized by the Animal Research Committee (ARC) before the conduction of this study.

3 | RESULTS

3.1 INTERACTIONS AND BEHAVIORAL DIFFERENCES

In total, there were 242 intraspecies interactions between all tanks. Overall, charge had the highest total behavior, followed by advance, nip, coexist, and chase. Between tanks, Tank C yielded the most interactions, followed by Tank B and Tank A (in decreasing order). Across all

tanks, charge was the highest interaction observed (tied with advance for Tank A), while chase was the lowest (nip was lower for Tank A, Table 2).

Table 2. Number of interactions between tanks and behavior, yielding total number of interactions.

	Tank A	**Tank B**	**Tank C**	
	22℃	**24℃**	**26℃**	**TOTAL**
Nip	3	18	12	33
Charge	19	34	61	114
Chase	5	3	3	11
Advance	19	14	20	53
Coexist	13	9	9	31
TOTAL	49	78	105	**242**

Overall, we found there was a significant difference in the type of behavior as temperature increased ($p<0.026$). However, there was no significant difference between the interactions per tank and temperature ($p<0.40$).

Table 3. A Tukey test compared all possible pairs of interactions to determine which are significant (N=Nip, CH=Charge, CA=Chase, A=Advance, CO=Coexist).

Pair	|x1-x2|	Critical Value	Sig at ɑ = 0.05?
N+CH	27	4.6	Yes
N+CA	7.33	4.6	Yes
N+A	6.67	4.6	Yes
N+CO	0.67	4.6	No
CH+CA	34.33	4.6	Yes
CH+A	20.33	4.6	Yes
CH+CO	27.67	4.6	Yes
CA+A	14	4.6	Yes
CA+CO	6.67	4.6	Yes
A+CO	7.33	4.6	Yes

Comparing these variables independently revealed that there was no significance in interactions between tanks ($p<0.66$). However, there was a significant difference between behavior totals ($p<0.018$). To determine which behaviors were significant, a Tukey test was conducted, which found that all possible behavior combinations were significant except for nipping and coexisting (Table 3).

3.2 CHANGE IN TEMPERATURE

Comparing the average number of interactions to the change in temperature, charge had the highest average across all tanks. The number of charge behaviors also appeared to increase as temperature increases. Chasing had the least average across all tanks, while advancing and coexisting remained relatively the same. Nipping increased from Tank A to B, then decreased from B to C (Figure 2).

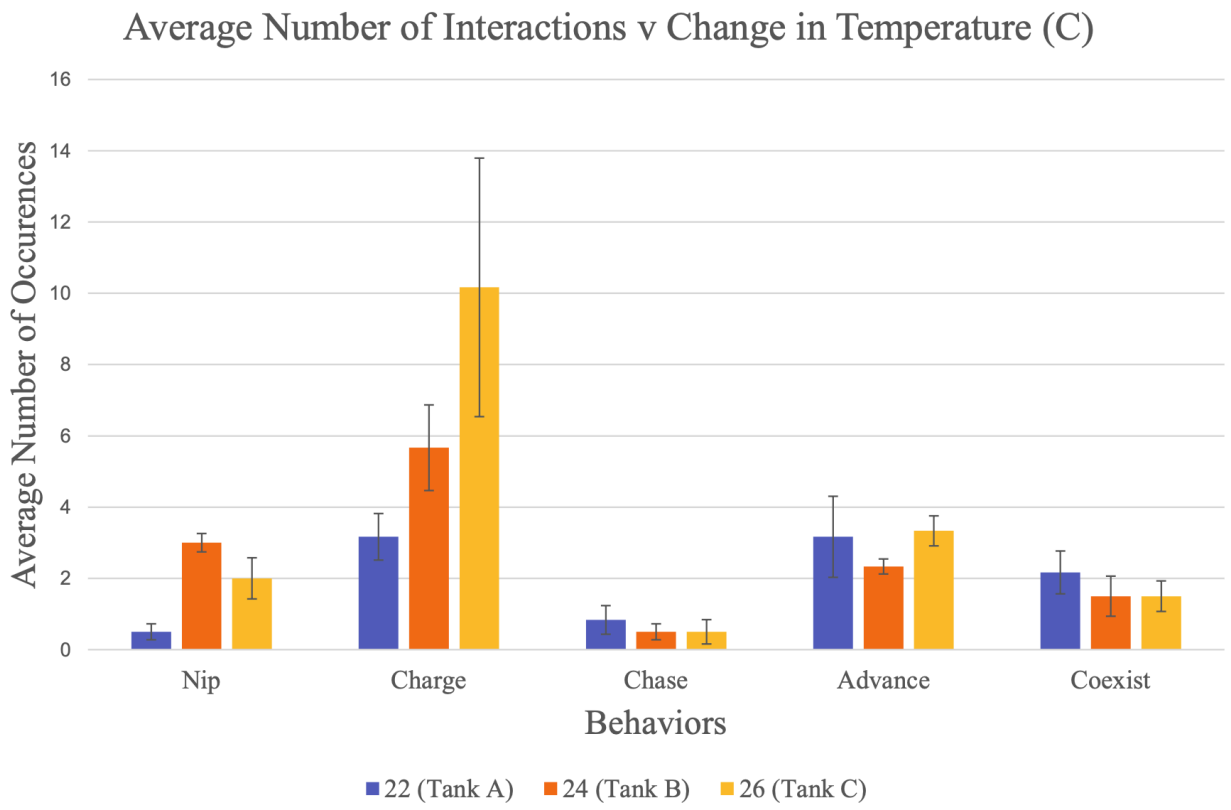


Figure 2. The average number of interactions per behavior compared to the change in temperature (℃). Charge had the highest average for every temperature change. There appears to be no correlation between temperature and nip, chase, advance, or coexist.

4 | DISCUSSION

4.1 LIMITATIONS

Identifying the boundaries of our research will contextualize our findings and frame our conclusions within a very specific scope. First is the limited quantity and confined geographical location of our research. Since we focused on a marine species of fish, we had to consider the space and resources available to make this research a possibility within the means of our institution. We simulated a small-scale version of an in-situ environment for yellowtail damselfish in a lab setting as an alternative to observing these fish in their natural habitat and in a larger ratio. We also chose to focus on one particular species of damselfish. In reality, we would want to take a holistic approach to observe the effects temperature has on multiple species or families of reef fish.

Regarding the limited sample size of our study, we encountered several complications with the mortality of fish. Our sample size was cut in half from the start and fish were shuffled between tanks to avoid an uneven distribution. Half way through the experimentation process, the fish were redistributed due to a death. This unevenness may have skewed the results due to a decline in the number of potential interactions to be had between multiple fish. A sample size of anywhere between 35-200 individuals may have provided more accurate results to the way in which damselfish interact with one another in response to elevated temperatures. These numbers are based on other studies analyzing damselfish as well as from previous experiments (Cleveland, 1999; Warren & McCormick, 2019).

The range of temperature looked at in this experiment was rather conservative due to the apprehension of thermal stress and further reducing our already small sample size. Pushing the thermal tolerance of damselfish to reflect projected ocean temperatures may have resulted in more significant data. Lastly, differentiating behavior during observations was a challenge due to the similarity in feeding behavior and swimming pattern versus an aggressive attack. Individual fish may also react differently depending on their individual “personalities” (Eriksson et al., 2010).

4.2 AGGRESSIVE BEHAVIOR: PREDATION AND TERRITORY

The differences observed between tank temperature and behavior helps us answer our primary research question. Although our hypothesis and logic is derived from the findings of da Silva-Pinto et al. (2020), that state how rising temperature decreases damselfish behavior, we did not find a clear relationship between number of interactions and temperature (Table 2). Instead, we can shift our focus to the type of interaction and how that relates to damselfish behavior. Specifically, charge was the only behavior that increased alongside temperature (Figure 2). This form of aggressive behavior is likely similar to the interaction that damselfish exhibit between conspecifics when defending their territory, acting like a warning or scare-tactic. It's possible that this behavior is the most effective in deterring competition, such as conspecifics, grazers (who would feed on the damselfish's algae farms) and predators. Other behaviors observed in this study, such as chase and nip, are aggressive behaviors that may exude more energy compared to charge and also risk the possibility of putting the damselfish in danger. The cost of energy expenditure used in territorial defense includes increased predation, increased risk of injury, and decreased time for foraging and mating (Cleveland, 1999). Meanwhile, more passive behaviors such as advance and coexistence are not aggressive enough to deter competition, leaving them exposed to predation and losing territory. The rationale behind this would explain why we don't see these behaviors exhibited as often as charge, since none are as effective in their endeavor of protecting their territory.

Despite our study not finding significance between elevated temperature and increased behavior, we can analyze literature to inform our findings of an increase in defensive (in our case charging) behavior with temperature. A study conducted by Warren and McCormik (2019) analyzed intraspecific interactions between two species of reef damselfish, the blue damselfish *Pomacentrus nagasakiensis*, and the whitetail damselfish *Pomacentrus chrysurus*. Their results revealed that there was a four-fold increase of aggressive behavior of *P. nagasakiensis* with increasing temperature (Warren & McCormick, 2019). In addition, Warren et al. (2017) reported that exposure to elevated temperatures affects the escape response of reef fishes. They looked at the lemon damsel *Pomacentrus moluccensis* and the Ambon damselfish *Pomacentrus amboinensisi.* These results are similar to Allen et al. (2015) and da Silva-Pinto et al. (2020). Both papers suggest that fish exposed to increased temperature affect predator-prey interactions

and performance. In particular, predators exposed to similar elevated temperatures increased their maximal attack speeds, while the escape speeds and distances of prey decreased, leading to higher predation rates (Allen et al., 2015). Although our results focus on conspecifics, interspecies interactions (specifically damselfish and their predators) may be inhibited by elevated temperatures. As a result, the thermal tolerance of damselfish may influence the selection of performance and the network of reef ecosystems.

Looking at the range of behaviors exhibited by damselfish, we can conceptualize the relationship that damselfish have with their environment. Specifically, how damselfish rely on reef structures and vice versa. In Chase et al. (2020), 5 species of damselfish were assessed for behavioral trade-offs that determine how they interact with their host coral colonies. It is well known that damselfish heavily rely on coral reefs for shelter. This study demonstrated that damselfish use their host corals in very different ways, suggesting a range of observable behaviors. Some species sustained interactions around corals, while others had weaker associations with their host colonies. These variations in coral use correlates to an increase or decrease in aggressive behavior. Frequent colony visits aligned with more aggressive behavior towards other fish, while fewer visits were associated with low aggression (Chase et al., 2020). This rationale may explain why yellow-tail damsels exhibit such a high level of aggressive behavior compared to passive or low levels of aggression.

To speak on how damselfish use their coral colonies, Little et al. (2012) tested interspecific interactions between two species of damselfish. The longfin damselfish (*Stegastes diencaeous*) and the dusky damselfish (*S. adustus*) share similar habitat, but react very differently to perceived threats. They hypothesized that this difference is due to the fact that the longfin's territory in which they spawn eggs is more exposed to that of duskies. Our results of the Tukey test that compared significance of behaviors found that almost all behavior pairs were significant, suggesting that within the range of behaviors that damselfish exhibit, they may lean toward aggression due to the level of reliance they have on the corals they associate themselves with and how they utilize them (Table 3).

4.3 NEXT STEPS

Based on the conclusions we've made in this study, further investigation into how rising water temperatures affect reef species, damselfish in particular, may emphasize the need to push for climate action. Damselfish are essential to the overall health of reef ecosystems, specifically the cultivation of algae and primary production. Algae communities cultivated by damselfish were found to be 1-3x more productive than algae growing outside of this range (Klumpp et al., 1987). However, the growing threat of climate change challenges this species' habitat through coral bleaching and a decrease in coral cover. Therefore, limiting the spread of algae cultivation and creating concentrated areas of habitat. A study by Boström-Einarsson et al., (2013) focuses on our specific species of damselfish, *Chrysiptera parasema*, and density-dependent mortality. Their results have demonstrated a strong relationship between yellowtail damsels and *Acropora* coral. *C. parasema* were found to be highly specialized to this species of coral, making them extremely vulnerable to habitat loss during this time of rapid decline in coral cover (Boström-Einarsson et al., 2013).

With increasing temperatures, damselfish may need to adjust to multiple factors including predation avoidance, changes to level of aggression and territoriality, and habitat specialization. To further understand this relationship between damselfish specialization and their susceptibility to climate change, studies should consider reef fishes dependency on their habitat and behavioral plasticity in conversation with rising ocean temperatures. These research questions offer major implications for how temperature plays a fundamental role in marine ecosystems. Based on behavior, we can use this information to broaden our understanding about damselfish interactions, survivability, and ecosystem importance.

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