MATING SUCCESS OF THE SIX-BAR WRASSE (*THALASSOMA HARDWICKE*, LABRIDAE) UTILIZING TWO MATING STRATEGIES

BY

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Abstract

Spawning aggregations are a critical aspect of population resilience and fisheries sustainability for many fish species. Spawning aggregations are found in all five oceans and are utilized by over 44 fish families; however, little is known about these aggregations. What is known is that spawning aggregations may be the sole means of reproduction and can represent the entire annual reproductive output for a population of a given species. Many species of wrasse and parrotfish (family Labridae) will spawn within an aggregation, but the dynamics of these aggregations varies throughout the family. This study used a combination of methods to investigate the spawning behavior of *Thalassoma hardwicke* in a resident spawning aggregation at Finger Reef, Apra Harbor, Guam. *Thalassoma hardwicke* spawning occurred daily, beginning in the morning, and ending in the midafternoon. Group spawning was the more successful spawning method within this aggregation. The abundance of *T. hardwicke* did not affect spawning success or method of spawning. Spawning success and method were not influenced by seasons or tides, and lunar phase did not affect spawning success. Pair spawns, however, occurred more often during the full moon, and group spawns occurred more often during the new moon. The abundance of egg predators at the spawning aggregation site did not affect spawning success or influence spawning method. Egg predation was higher in group spawns compared to pair spawns, however. Finally, there was some correlation between egg predator abundance and egg predation, however, egg predator abundance was not a good predictor of egg predation. These observations increased our knowledge of labrid spawning behavior and will provide a model study to compare spawning behavior and success, environmental influences, and egg predator influences at other spawning aggregation sites.

Keywords: *Spawning aggregations, Thalassoma hardwicke, mating behavior*

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Introduction

Spawning Aggregations

Many animals, both terrestrial and marine, participate in migrations to aggregate at specific locations and predictable times (Colin, 2012; Erisman et al., 2017). For many marine species, the purpose of this migration is spawning, and these spawning aggregations are critical for the resilience of marine fish populations and the sustainability of fisheries. These aggregations are usually the sole means of reproduction for many marine fishes, often representing the entire annual reproductive output for those species (Domeier & Colin, 1997; Robinson et al., 2004; Nemeth & Kadison. 2013; Gruss et al., 2014; Sadovy de Mitcheson, 2016; Erisman et al., 2017). Spawning aggregations are highly predictable, in timing and location, and for many species, these events mark the start of the fishing season, which makes them targets for fisheries (Sadovy de Mitcheson et al., 2008; Heyman et al.,2010; Gruss et al., 2014; Sadovy de Mitcheson, 2016; Erisman et al., 2017; Ohta & Ebisawa, 2017). Spawning aggregations support commercial, recreational, and subsistence fisheries, and benefit fisherman due to the predictability, often high availability and availability, and efficiency of catch rates of aggregating fish (Domeier & Colin, 1997; Sadovy de Mitcheson et al., 2008; Gruss & Robinson, 2015; Sadovy de Mitcheson, 2016; Erisman et al., 2017). Marine fishes are the last animal resource still taken from the wild in large quantities, provide approximately one fifth of the global protein supply, and are important for both livelihoods and food security (Sadovy de Mitcheson, 2016). The predictability of these spawning aggregations has led to negative consequences globally for populations of species that form them, most importantly through overexploitation (Robinson et al., 2004; Sadovy de Mitcheson et al., 2008; Gruss et al., 2014; Sadovy de Mitcheson, 2016; Erisman et al., 2017; Farmer et al. 2017; Roff et al., 2017).

Targeted fishing upon a spawning aggregation can remove a large proportion of the population during the reproductive season resulting in lower abundance levels and a complete breakdown in aggregation structure, as well as the abandonment or loss of aggregation sites (Erisman et al., 2017; Farmer et al. 2017; Ohta & Ebisawa, 2017). Spawning aggregations require a minimum number of adults for them to function, and as overharvesting occurs the minimum number of adults necessary for reproduction may not be met (Colin, 1996). Many of the aggregating fish species that have declined due to overfishing are threatened or endangered (Erisman et al., 2017). Hundreds of fish species spawn in aggregations, and of the 20 known major fish species that supply global fisheries, many aggregate to spawn and are exploited during their aggregations (Nemeth & Kadison, 2013; Sadovy de Mitcheson, 2016; Erisman et al., 2017). In the Indo-Pacific, 44% of known aggregations are in decline or have been eliminated, while in the tropical western Atlantic, and 54% of known aggregations are in decline or have been eliminated (Sadovy de Mitcheson, 2016). Some well-known examples of overexploited species targeted at spawning aggregations sites are the Nassau grouper (*Epinephelus striatus*), yellow croaker (*Larimichthys polyactis*), and the Atlantic bluefin tuna (*Thunnus* thynnus) (Sadovy de Mitcheson et al., 2008; Sadovy de Mitcheson, 2016; Erisman et al., 2017). The Nassau grouper is a commercially important fish that was targeted at its spawning aggregation sites, but many of the aggregations do not form anymore and fisheries have collapsed throughout much of its range and it is now listed as an endangered species (Sadovy de Mitcheson et al., 2008; Erisman et al., 2017). Atlantic bluefin tuna stocks have declined since the 1970's after being targeted at its spawning sites, thus causing their numbers to drop from billions of fish to just a few million (Sadovy de Mitcheson, 2016). The yellow croaker and other croaker species (*Sciaenidae*) have also been fished on aggregation sites and many of the species are threatened and have not recovered (Sadovy de Mitcheson, 2016).

Many spawning aggregation sites have not been well documented and many have only anecdotal evidence to describe them (Colin, 1996; Colin, 2012; Erisman et al., 2017). Long-term detailed site usage is not available for most spawning sites, and there is a variety of conflicting and unconfirmed hypotheses about the reasons behind the location and timing of reef fish spawning aggregations (Colin, 1996; Domeier & Colin, 1997; Colin, 2012; Heyman & Kjerfve, 2008). The tropical western Atlantic has had more scientific research conducted on aggregating species and aggregation sites than most parts of the world; spawning aggregations in the western Indo-Pacific are not as well studied and discovery, and both geospatial and biological characterization is needed throughout much of the region (Galzin, 1987; Robinson et al., 2004; Sadovy de Mitcheson et al., 2008). In the Indo-Pacific, fisher interviews are often used as the main source of information because knowledge of the timing and locations of fish aggregations that has been developed over generations often serves as a reliable source of information for locating spawning aggregations previously unknown to scientists (Sala et al., 2003; Robinson et al., 2004; Sadovy de Mitcheson et al., 2008; Colin, 2012; Erisman et al., 2017). These interviews also provide a baseline to compare changes in aggregations and populations where formal data does not exist (Sadovy de Mitcheson et al., 2008). Currently, most data on fish spawning aggregations comes from coral reef species but a greater representation of species from higher latitudes is needed to provide a more complete understanding of how fish spawning aggregations function (Erisman et al., 2017). The lack of long-term data sets on aggregating species, along with documented declines, compromises the ability of managers to make scientific, socioeconomic, and political cases for effective management of aggregating species and has led to inaction (Sadovy de Mitcheson et al., 2008). This lack of knowledge may have led to the disappearance of spawning aggregations of large reef fishes without fisheries biologists and managers noticing their loss (Sala et al., 2003).

A spawning aggregation is a group of conspecific fish gathered for spawning with fish densities or numbers significantly higher than those found in the area of aggregation during non-reproductive periods (Domeier & Colin, 1997; Sala et al., 2003; Heyman & Kjerfve, 2008; Sadovy et al., 2008; Ohta & Ebisawa, 2017). A three-fold increased density of spawning fish at a specific location has been suggested as a benchmark indicative of a spawning aggregation; some species, however, will spawn at densities that are similar to those seen during nonreproductive periods (Sala et al., 2003; Robinson et al., 2004). Spawning aggregation dynamics may differ across geographic area, and spawning species (Domeier & Colin, 1997). Fish spawning aggregations have been documented in all five oceans in 44 fish families and over 300 species (Domeier & Colin, 1997; Colin, 2012; Erisman et al., 2017). Spawning aggregations are most studied on coral reefs, but they have been found in nearly all marine habitat types and ecoregions including: rocky reefs, temperate offshore banks, deep ocean seamounts, estuaries, and in pelagic waters (Sadovy de Mitcheson, 2016; Erisman et al., 2017). Fish families with species that aggregate include those from all trophic levels including detritivores, predators, corallivores, herbivores, spongivores, planktivores, and invertivores (Nemeth & Kadison, 2013). There are two different types of spawning aggregations: *resident spawning aggregations*, consisting of individuals that spawn within a small area adjacent to or including their home range, and *transient spawning aggregations*, consisting of individuals that migrate outside of their home range, and frequently over relatively great distances to a specific site to spawn before returning to their home range (Domeier & Colin, 1997). These sites often host multiple species concurrently (Colin, 2012; Claydon et al., 2014). Resident spawning aggregation species often spawn more frequently, with many spawning daily, and over longer reproductive periods, near their resident areas (Domeier & Colin, 1997; Robinson et al., 2004; Heyman & Kjerfve, 2008; Colin, 2012; Nemeth & Kadison, 2013). Resident spawning aggregation species are often, but not exclusively, smaller omnivores, planktivores, and herbivores, while transient spawners are generally large, predatory fish (Colin, 2012; Domeier, 2012; Nemeth, 2012). Transient spawning aggregation species often spawn only once or a few times annually and concentrate their total annual reproductive output during one or a few migrations; transient aggregation sites can also host multiple spawning species (Domeier & Colin, 1997; Robinson et al., 2004; Heyman & Kjerfve, 2008; Sadovy de Mitcheson et al., 2008; Colin, 2012; Nemeth & Kadison, 2013; Gruss et al., 2014). Transient spawning aggregations are common in socioeconomically and commercially important species, including members of the families Acanthuridae, Epinephelidae, Lutjanidae, and Siganidae (Domeier & Colin, 1997; Robinson et al., 2004; Sadovy de Mitcheson et al., 2008; Gruss et al., 2014).

Spawning Methods

Within both types of spawning aggregation, there are two common types of spawning methods: group and pair spawning (Domeier & Colin, 1997; Nemeth & Kadison, 2013). Group spawning consists of multiple individuals participating in a spawning rush; it often consists of one female and many, two to over one hundred, primary males, with the males competing to fertilize the eggs; it can also occur with an even ratio of males to females participating in the spawning rush (Warner, 1984; van de Berghe & Warner, 1989; Domeier & Colin, 1997; Habrun & Sancho, 2012; Nemeth & Kadison, 2013). Pair spawning is between one male and one female; the male will defend a temporary courtship territory against other males which increases access to females and thus paternity rates; pair spawning can occur in both transient and resident spawning species (Domeier & Colin, 1997; Adreani & Allen, 2008; Habrun & Sancho, 2012; Nemeth & Kadison, 2013; Roff et al., 2017). An alternate spawning method that some primary males participate in is streaking, which is when a non-courting or competing male rushes up and releases sperm close to the main spawning pair (Domeier & Colin, 1997; Habrun & Sancho, 2012). Both group and pair spawning contain a spawning rush that occurs when the fish ascend rapidly (inclined or vertically) into the water column and release gametes at the apex of the ascent. The release results in a gamete cloud; a suspended concentration of gametes in the water column left by spawning fishes (van de Berghe & Warner, 1989; Domeier & Colin, 1997; Heyman et al. 2005; Sara et al., 2005; Adreani & Allen, 2008; Heyman & Kjerfve, 2008; Habrun & Sancho, 2012).

Effects of Spawning Aggregations

Spawning aggregations cause temporary peaks in fish biomass that can affect the food web and energy transfer through, feeding, reproduction, and predation (Nemeth, 2012). Some predation by piscivores on spawning adults has been observed, but the predation rates are low compared to the rates of predation on freshly released eggs (Colin, 2012; Nemeth, 2012). Several quantitative studies and direct observations have provided evidence of egg predation, with most coming from the Pacific (Nemeth, 2012). Many of these oophagous predators or egg predators have been observed feeding on freshly released eggs in gamete clouds (Heyman & Kjerfve, 2008; Colin, 2012; Nemeth, 2012). At least 86 fish species in 17 families, such as damselfishes (Pomacentridae), butterflyfishes (Chaetodontidae), goatfishes (Mullidae), seabreams (Sparidae), manta rays (Mobulidae), whale sharks (Rhincodontidae), and wrasses (Labridae), throughout the Pacific alone have been observed preying upon eggs, but fish eggs do not comprise more than

25% of their diets (Heyman et al., 2001; Hirayama et al. 2005; Colin, 2012; Hartup et al., 2013). There have been no described obligate fish egg predators among reef fishes, but fish eggs have been confirmed in the diets of omnivorous and planktivorous fishes, making them facultative fish-egg eaters (Hirayama et al. 2005). Group spawns more so than pair spawns are likely targets for egg predation due to the large amounts of eggs and sperm being released that makes the gamete cloud visible longer (Sancho et al., 2000). However, multiple group and pair spawns can occur at the same time producing a large amount of eggs into the water column which can reduce the per capita egg predation rates (Sadovy de Mitcheson, 2016). Repeatable spawning aggregation formation within a given site can frequently lead to feeding aggregations that will target gametes released by that aggregation; this is seen with whale sharks and manta rays gathering at known aggregation sites (Heyman et al., 2001; Domeier, 2012; Hartup et al., 2013).

Reproductive Strategies

Many fishes are hermaphroditic of which there are two principal forms: simultaneous or sequential (Warner, 1984; Munday et al., 2006; Smith & Wootton, 2016). Simultaneous hermaphrodites have both male and female sexual organs at the same time and may reproduce either as males or females in turn depending upon the circumstances. Sequential hermaphrodites begin as either males or females, then change to the opposite sex at some point in life. Both forms of sex change are mediated by either age-dependent or social mechanisms (Warner, 1984; Smith & Wootton, 2016). Sequential hermaphrodites are either protandrous, start as a male then switch to a female, protogynous, start as female then switch to male, or serial (bidirectional), can switch back and forth between male and female (Warner & Hoffman, 1980; Warner, 1984; Kuwamura & Nakashima, 1998; Adreani & Allen, 2008; Smith & Wootton, 2016). Hermaphroditism occurs in fishes for several reasons and may be explained by different hypotheses. Ghiselin's (1969) "size-advantage model," proposed that if the expected number of offspring produced differs between the sexes in relation to adult body size, then an individual that changes sex at the right age or size will have more offspring than an individual that does not change sex (Warner, 1984). Sex change can also be mediated by social control (Robertson, 1972). Protandrous fish species, such as the anemonefishes (Pomacentridae), will change sex from male to female if the dominant female is removed from the mating group. Then, the largest male in the mating group will change to female and another smaller male will become the functional male in the pair (Warner, 1984). In protogynous fish species, the removal of the dominant male from the mating group usually results in the dominant female adopting male behaviors, then changing into a male (Robertson, 1972; Warner & Hoffman, 1980; Warner, 1984). Protogyny occurs in at least 14 fish families including the Labridae (the wrasses but also including the parrotfishes of the subfamily Scarinae), Epinephilidae, Serranidae, Cirrhitidae, Gobiidae, and some genera of Pomacanthidae, to name a few (Robertson, 1972; Thresher, 1982; Warner, 1984; Cole & Shapiro, 1992; Sadovy and Donaldson, 1995; Sakai & Kohda, 1997). For some families, such as the Labridae, both TP or secondary dominant males exist along with initial phase (IP) or primary males. The TP males are protogynous and larger in body size than both females and the IP males, and their color patterns and morphology usually differ significantly from the IP males. TP males are often brightly or distinctively colored, usually hold a temporary mating territory, and defend it against other males while attempting to attract and pair spawn with females (Robertson, 1972; Warner & Hoffman, 1980; Warner, 1984; Sara et al., 2005; Adreani & Allen, 2008; Nemeth & Kadison, 2013; Ochi et al., 2017; Roff et al., 2017). This system may resemble that of a lek, is often termed "lek-like" and may be found in protogynous and gonochoristic species (Loiselle and Barlow, 1978; Donaldson, 1990; Gladstone, 1994). The coexistence of gonochoristic males and protogynous hermaphrodites has been seen in several wrasse species including the congeners *Thalassoma bifasciatum* and *T. lucasanum* (Warner & Hoffman, 1980).

The coexistence of sex-changed males (terminal phase males, TP) and gonochoristic, primary males (initial phase, IP) that matured directly without passing through female maturation stages is called diandry and can be shown by the presence of small males (Ochi et al., 2017). Diandry can been seen in a variety of reef fishes, including species in the family Labridae (Warner & Hoffman, 1980; Ochi et al., 2017). Primary males in this mating system which are also called initial phase males, are small and nonterritorial (Warner & Hoffman, 1980). Primary males appear in higher proportions on larger and denser reefs and are more fit than TP males on these larger reefs because the territorial males often are swamped by the IP males leading to the breakup of the territorial structure of TP males (Warner & Hoffman, 1980; Warner, 1984; Domeier & Colin, 1997; Adreani & Allen, 2008).

Family Labridae

The Labridae are a large and highly diverse family of fishes that includes 82 genera and over 600 species that vary in coloration, size, body shape, and habitat. Most species occur mainly in found tropical and subtropical waters (Myers, 1999; Parenti & Randall, 2010). The genus *Thalassoma* is the fourth largest genus in the family and has at least 28 species (Parenti & Randall, 2010). *Thalassoma* spp. are common in circumtropical and subtropical coral and rocky substrates worldwide, and they can be found to depths of around 25m; they vary in color pattern and hues both intra- and interspecifically (Heiser, 1981; Randall & Edwards, 1984; Myers, 1999; Bernardi et al., 2004). *Thalassoma* spp. are globally successful and occur in a range of habitat types because they display a range of traits, such as trophic versatility, prey-switching, locomotor abilities, and generalized foraging (Fulton et al., 2017). These traits allow them to outcompete and outnumber sister taxa requiring similar habitats and trophic needs (Fulton et al., 2017). The widespread geographical distribution of species of this genus provides an ideal system to study biogeographic patterns (Fulton et al., 2017). As with many other labrids, *Thalassoma* spp. are considered protogynous hermaphrodites (Robertson, 1972; Adreani & Allen, 2008). In many diandrouns species the IP males will be smaller than TP males and will often resemble females in appearance (Warner & Robertson, 1978; Myers, 1999; Ochi et al., 2017).

Spawning Characteristics of *Thalassoma hardwicke*

Thalassoma hardwicke is distributed throughout much of the Indo-Pacific in shallow lagoon and seaward reefs up to around 15m in depth (Myers, 1999; Lecchinni et al., 2007; Geange, 2010). They are moderately common in clear water areas with sand, rubble, and mixed corals, particularly on the upper edges of protected reef slopes (Myers, 1999). *Thalassoma hardwicke* spawn pelagic eggs that develop into pelagic larvae that settle after ~47 days in the plankton (Shima, 2001). Successful larvae may settle into complex microhabitats that provide structural refuges and maintains a tight association with the favored habitat immediately after settlement (Chen et al., 2004; Lecchinni et al., 2007; Geange, 2010). They are opportunistic omnivores that feed upon benthic and planktonic crustaceans, small fishes, foraminiferans, and pelagic fish eggs (Myers, 1999; Lecchinni et al., 2007; Pasko, 2010; Gill, 2018). *Thalassoma hardwicke* can be identified by six vertical black bars on a light green body and pink band radiating from the eye (Sadovy and Cornish, 2000). TP males are similar in color, although slightly flashier than primary males, with more blue on the body (Myers, 1999).

Many of the most important food fish that aggregate to spawn cannot always be found and studied sufficiently; therefore, widely distributed, noncommercial, and reef-associated fishes such as *T. hardwicke* provide an opportunity to study spawning aggregations easily in detail (Chen et al., 2004; Colin, 2012). Characterizing the spawning behavior of *T. hardwicke* can potentially provide a greater understanding of spawning behavior of other reef fishes in the region and can be used to compare results found between exploited and unexploited species (Chen et al., 2004). Learning from more easily accessible spawning aggregations can lead to greater insight on their dynamics which can be used to inform fisheries and conservation biologists and managers so that they can better protect and manage other spawning aggregations before they disappear. The purpose of this research is to develop a better understanding of the spawning behavior of *T. hardwicke* by measuring spawning success of individuals of this species that participate in group and pair spawns. To meet the goals of this study, four hypotheses will be evaluated through the duration of this study.

Objectives

The purpose of this research is to develop a better understanding of the spawning behavior of *T. hardwicke* by measuring spawning success of individuals of this species that participate in group and pair spawns. I used a combination of approaches to examine questions regarding spawning behavior of *T. hardwicke* within a resident spawning aggregation. A variety of in-field observations were used to answer following questions about the spawning behavior of *T. hardwicke*: 1) which spawning method is most successful?, 2) does the abundance of *T. hardwicke* affect the spawning success or spawning method used?, 3) is the spawning success or method influenced by lunar phases, seasons, or tidal states?, 4) does egg predator abundance affect the spawning success or spawning method?, and 5) are egg predation events influenced by egg predator abundance, and is there a relationship between predation and spawning method?

Thalassoma hardwicke, being a protogynous hermaphrodite, may have few secondary dominant males and many IP males and females within the same population. This species has a dualistic mating strategy seen in other diandrous spawning aggregation species: small and medium sized IP individuals will group spawn while large TP males defend temporary territories and attempt to pair spawn with IP females (Domeier & Colin, 1997; Adreani & Allen, 2008). At the spawning aggregation site, IP males tend to outnumber TP males, and the former generally mix with IP females. Because of their greater abundance, it is predicted that group spawning will be more successful than pair spawning. Success is defined as the visible release of a gamete cloud following an upward rush into the water column by groups or pairs.

Many studied wrasse species use a dualistic mating strategy, but there have been many studies that show the ratios of TP to IP individuals changes depending on population densities. One example is *Halichoeres semicinctus*, a temperate wrasse found to group spawn regardless of local densities but will pair spawn only at high densities (Adreani & Allen, 2008). However, other studies have shown that pair spawning will only occur at lower population densities because as densities increase the number of small, non-territorial IP males increases compared to TP males and can lower the ability of the larger TP males to successfully defend their territories and spawn. This outcome is common in labrid fishes (Warner, 1984; Sara et al., 2005; Roff et al., 2017). Increased population densities can be seen by the presence of many smaller males; lower populations densities at spawning sites often have few to no smaller males (Warner, 1984). Warner & Hoffman (1980) showed that the mating success of territorial *T. hardwicke* males decreased as density increased in the population. Based upon the results of these studies, it is predicted that the density of *T. hardwicke* will affect the spawning success of wrasses using the two spawning strategies seen on Finger Reef.

Marine fish spawning behavior is influenced by many different factors (Adreani & Allen, 2008). Many tropical coral reef fish species, including important commercial species, aggregate and spawn in correlation with the lunar and seasonal cycles (Domeier & Colin, 1997; Adreani & Allen, 2008; Heyman & Kjerfve, 2008; Colin, 2012; Nemeth & Kadison, 2013; Sadovy de Mitcheson, 2016; Erisman et al., 2017; Ohta & Ebisawa, 2017). Both resident and 17 confirmed, transient species, from multiple families, have been found to aggregate and spawn in synchrony with the lunar cycle (Heyman & Kjerfve, 2008; Ohta & Ebisawa, 2017). Also, previous studies have found that spawning of *Thalassoma* spp., and other wrasse species, will usually occur after high tide during an outgoing tide (Colin & Bell, 1991). Based on these previous studies, it is predicted that *T. hardwicke* spawning will be influenced by the lunar phase, tides, and seasons, and will spawn more often and successfully in correlation to certain lunar phases, tides, and seasons.

The presence of a repeated spawning aggregation within a given site will often lead to feeding aggregations of oophagous and piscivorous species that will feed on freshly released gametes and spawning adults (Heyman et al., 2001; Heyman & Kjerfve, 2008; Domeier, 2012; Hartup et al., 2013). Multiple studies have found facultative fish-egg predators in at 17 families, and have found that these egg predators can have both direct and indirect negative impacts on fish populations (Domeier & Colin, 1997; Sancho et al., 2000; Heyman et al., 2001; Hirayama et al. 2005; Colin, 2012; Hartup et al., 2013).These fish egg predators are more likely to target group spawns due to the larger, more visible amount of gametes released (Sancho et al., 2000). Based upon previous studies, it is predicted that increased abundance of egg predators will lower the success of the two spawning methods. It is also predicted that egg predation events will occur more often as the abundances of egg predators increases, and that egg predation will occur more frequently on group spawning events.

Methods

Site Description

This study was conducted at one site at Finger Reef (13.26.5N, 144.38.4E), located inside Apra Harbor, Guam (Figure 1). Apra Harbor is located on the western coast of Guam, Marianas Islands, in the Western Pacific. Finger Reef is a finger-like extension of Gab Gab Reef that extends perpendicularly out into the southwest side of Apra Harbor. This reef ranges in depth from 5m on the reef flat to 27m down the slope of the reef. Finger Reef is a popular site for divers and snorkelers brought to it in boats and has a history of feeding fish by both there.

Data Collection

For this study, a total of 956 spawning observations were recorded during a 24-month period between January 2018 and December 2019 to account for differences in season and lunar period. Spawning observations was collected while snorkeling, and data were recorded using waterproof paper and underwater video. Data variables included: the time of the spawning event, the spawning method (group or pair spawning), whether the spawning event was successful, (TRUE or FALSE, determined by the release of a gamete cloud), and observations of egg predation by resident planktivorous fishes. Observations also included *T. hardwicke* and egg predator counts. Observations occurred over more than 70 hours with spawning events occurring on 21 of 32 observation days. Observations for each day occurred between 0900-1600H, with varying start and ends times for each day. Of the 21 observation days where spawning occurred, nine were during a full moon, five were during a half moon, and seven were a new moon. There were 13 observation days occurring during an incoming tide, and eight during an outgoing tide.

There were two observation days where the tide switched from an incoming to outgoing tide, and one day where the tides switched from outgoing to incoming while observations were still being taken. Finally, 14 of the observation days were during dry season, and seven during wet season. This uneven number of observation days comes from cancelled field days being more common during wet season when thunderstorms made conditions unsafe.

Environmental data were collected for each observation day and included lunar phase, tides, and seasons. Lunar phases and tides for each collection day were determined by using NOAA's astronomical data set (Tidesandcurrents.noaa.gov, 2018). Lunar phases were binned into three phases: full, half, and new, based on the moon illumination percentage (new-moon = 0-33%, half-moon = 34-66%, full-moon = 67-100%). Lastly, two seasons were defined as wet (January- June) and dry (July-December).

The abundance of *T. hardwicke* and egg predators were recorded hourly for each field day within a 5m radius stationary point count (SPC) "cylinder" following NOAA's Rapid Ecological Assessment (REA) survey methodology for fish surveys (Bohnsack & Bannerot 1986; Pifsc.noaa.gov, 2016). Two visual point count "cylinders" surveys were made every hour for three minutes at a time. Observations during the first SPC included a count of egg predators that swam through the "cylinder." The second SPC would be done immediately after the first and included a count of *T. hardwicke*. To ensure abundances were not influenced by the presence of observers entering, or previously in the water, counts were not started until at least 30 minutes after entering the water. This prevented over-sampling of *T. hardwicke* or egg predators in the cylinder by allowing an adjustment period between entering the water and the start of the first count.

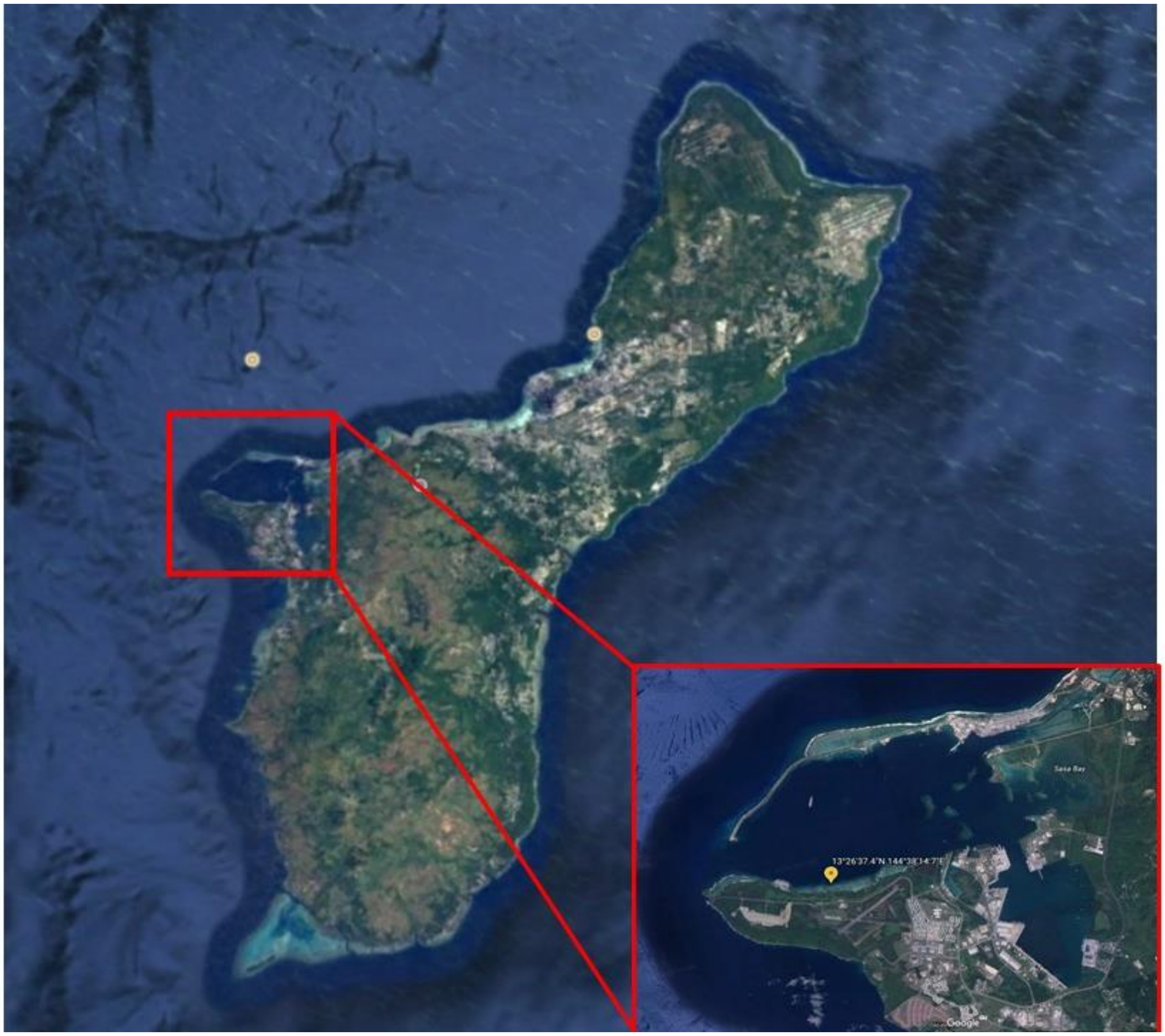

Figure 1: Map of the study site, Finger Reef (indicated by yellow point), located on the southwest side of Apra Harbor, Guam Marianas Islands, Western Pacific. (GoogleEarth)

Statistical Analysis

All data were analyzed using Rstudio. Testing for the independence between the two spawning methods and spawning success was done using the chi-sq (X^2) test. If the expected values were found to be less than 5, then a Fisher's exact test was also run. A logistic regression was then run to analyze the effect of *T. hardwicke* abundance on spawning success. A second linear regression was then run to examine the effect of *T. hardwicke* abundance on the spawning method observed. The effects of environmental data (lunar phases, season, and tides) on spawning success, and spawning method, was examined using the chi-sq (X^2) test. Three chi-sq (X^2) tests were run for each environmental factor; pair spawn success, group spawn success, and between methods using only TRUE (successful) spawns. Again, if the expected values were found to be less than 5, then a Fisher's exact test was also run. The effects of egg predator abundance on spawning success and spawning method were examined using logistic regression. A chi-sq (X^2) test was run to determine a relationship between egg predation on spawning method. Finally, a logistic regression was also run to examine the effect of egg predator abundance on the occurrence of egg predation.

If a chi-sq (X^2) test returned a significant p-value, the standardized residuals were calculated for each factor (i.e. pair spawning/success) to determine which one was contributing most to the significant chi-sq (X^2) test. A standardized residual above ±2 showed that the factor was a significant contributor.

Results

Spawning Success

A total of 956 spawning events were recorded. Of these, 923 were group spawns, with 7.5% of these being FALSE spawning attempts (groups rising into the water column but aborting the spawn attempt). Paired spawning was observed 33 times, with 18% being FALSE spawns. A significant relationship was found between the spawning method and spawning success, (X^2 = 5.05 (1, N = 956, p = .04)). After determining that expected frequencies were less than 5 for more than 20% of the cells, a significant relationship was confirmed between spawning success and spawning method (Fisher's Exact, p = .04). Pair spawning events were more likely to be FALSE than group spawns, as indicated by a chi-sq standardized residual above 2, meaning that the number of FALSE pairs spawns was higher than expected (Figure 2).

Thalassoma hardwicke Abundance

Thalassoma hardwicke abundance did not predict spawning success (logistic regression, R^2 = .001, p = .8) (Figure 3). Similarly, abundance was not a significant predictor of spawning method for successful spawns only (logistic regression, R^2 = .002, p = .46) (Figure 4).

Thalassoma hardwicke Spawning

Tides

There was no significant relationship between tidal state and spawning success for each spawning method, or between tidal state and the spawning method. Pair spawning success and tidal state had no significant relationship (X^2 = .67, (1, N = 33, p = .41)). After finding low expected values, it was confirmed there was no significant relationship between tidal state and pair spawning (Fisher's Exact, p = .66). Group spawning success also showed no significant relationship to tides (X^2 = .06, (1, N = 923, p = .81)). Finally, after determining there was no significant relationship between tidal state and spawning success, the FALSE spawns were removed and no significant relationship was found between tidal state and spawning method (X^2 = .57, (1, N = 881, p = .45)).

Seasons

There was no significant relationship between season and spawning success for each spawning method, and between season and the spawning method. Pair spawning success showed no significant relationship (X^2 = .011, (1, N = 33, p = .91)). After finding low expected values, it was confirmed there was no significant relationship between season and pair spawn success (Fisher's Exact, p = 1). Group spawning success also showed no significant relationship (X^2 = .00049, (1, N = 923, p = .98)). Finally, after determining there was no significant relationship between season and spawning success, the FALSE spawns were removed and found no significant relationship between season and spawning method (X^2 = .003, (1, N = 881, p = .96)).

Lunar Phase

There was no significant relationship between lunar phase and spawning success of each spawning method. Pair spawning success showed no significant relationship (X^2 = .17, (2, N = 33, p = .92)). After finding low expected values, it was confirmed there was no significant relationship between lunar phase and pair spawning success (Fisher's Exact Test, p = 1.0). Group spawning success also showed no significant relationship (X^2 = 5.14, (2, N = 923, p = .077)).

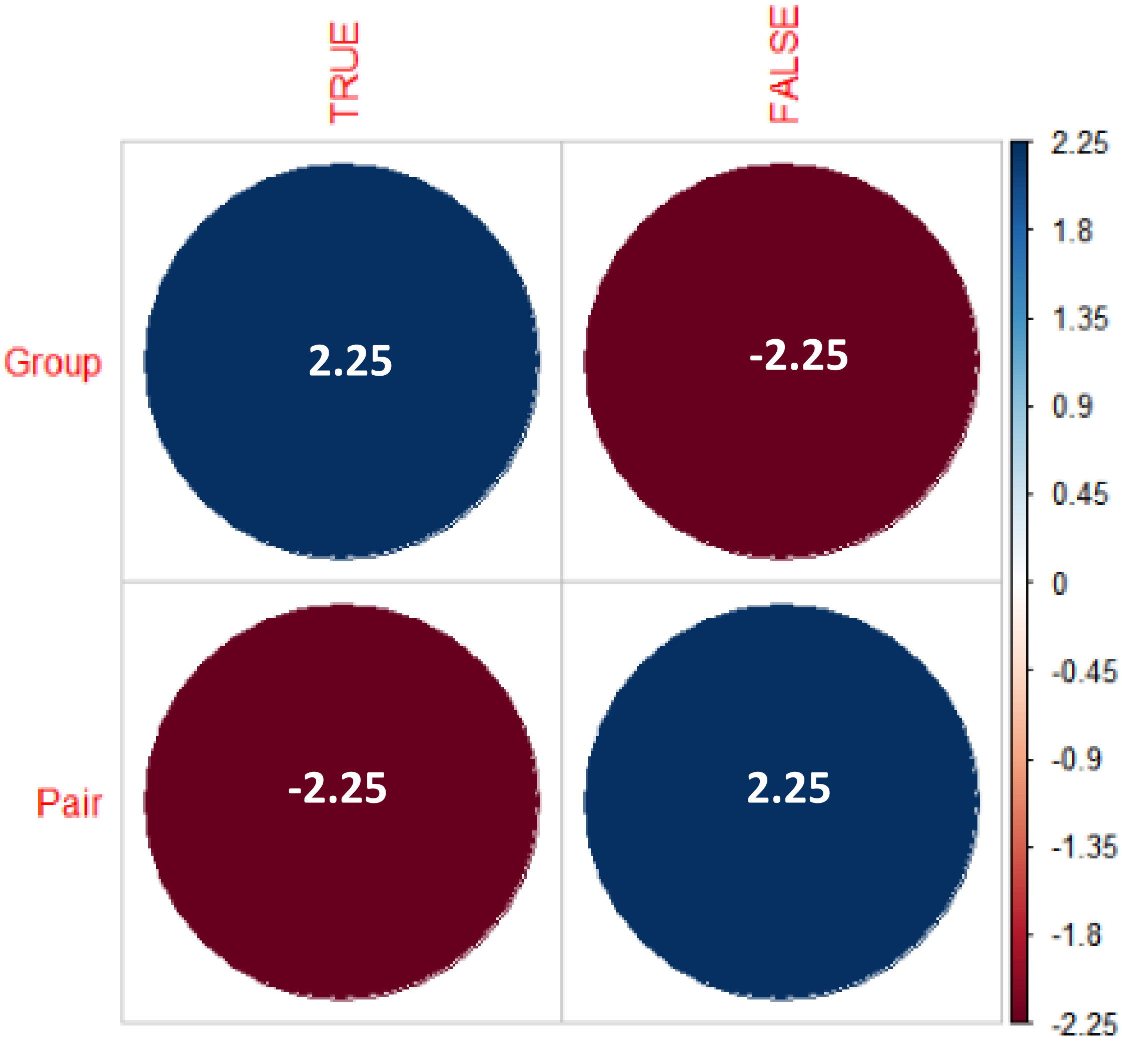

Figure 2: Corrplot of standardized residuals from chi-sq test of spawning success between the two spawning methods of *Thalassoma hardwicke*. Positive standardized residuals (2.248) are shown by the large blue circles for TRUE group spawns and FALSE pair spawns. Successful (TRUE) groups spawns and unsuccessful (FALSE) pair spawns were observed at higher frequencies than expected.

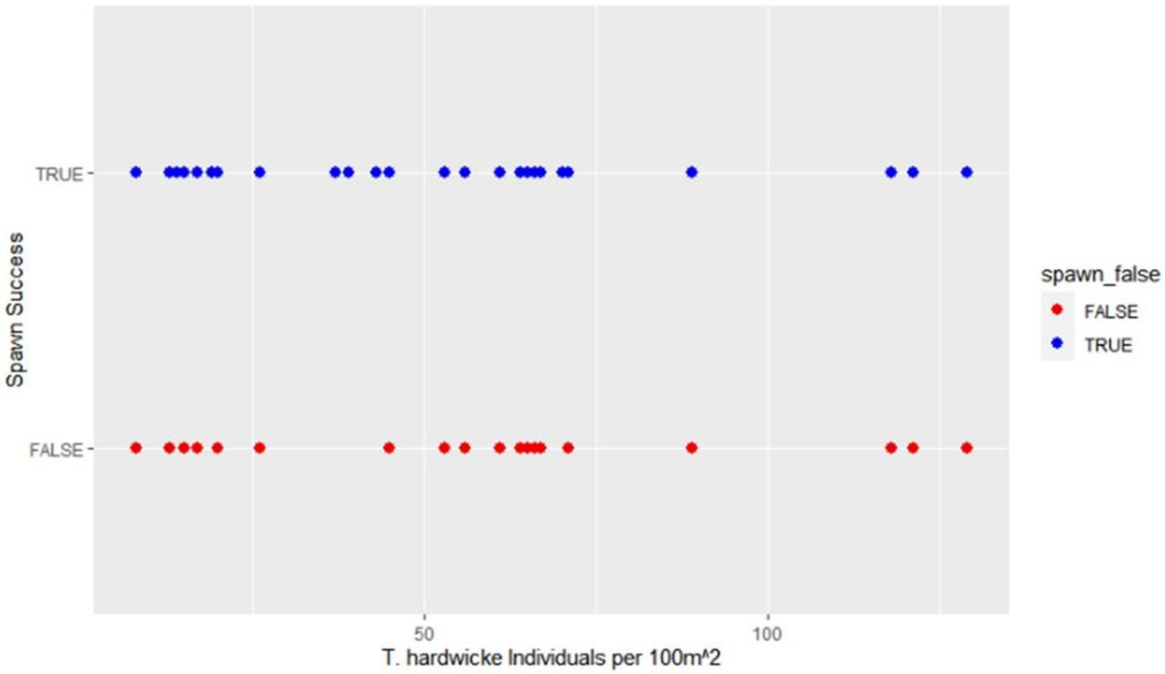

Figure 3: Logistic regression between *Thalassoma hardwicke* abundance and spawning success.

Spawning success is not related to the abundance of *Thalassoma hardwicke* (R^2 = .001, p = .801).

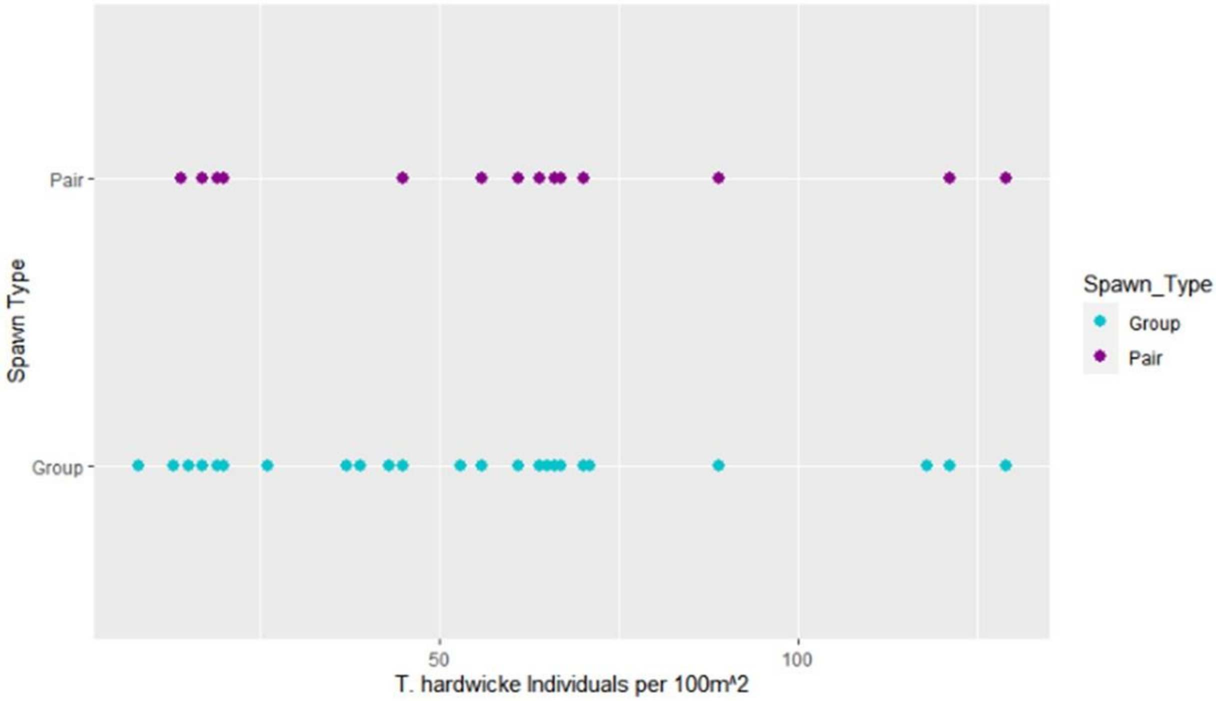

Figure 4: Logistic regression between *Thalassoma hardwicke* abundance and spawning method. Spawning method is not related to the abundance of *Thalassoma hardwicke* (R^2 = .002, p = .463).

After determining there was no significant relationship between lunar phase and spawning success, FALSE spawns were removed. There was a significant relationship found between spawning method and lunar phase ($X^2 = 10.24$, (2, N = 881, p = .0065)) (Figure 5). After finding low expected values, it was confirmed there was a significant relationship between lunar phase and spawning method (Fisher's Exact Test, p = .0054). The standardized residuals for pair spawns during full moon phases was 3.141 and for group spawns on a new moon phase was 2.709. This showed that more pair spawns occurred during a full moon phase, and more group spawns occurred during a new moon phase.

Egg Predator Abundance

There was no significant relationship found in the ability of egg predator abundance to predict spawning success (logistic regression, $R^2 = .001$, p = .8) (Figure 6). Similarly, abundance was not a significant predictor of spawning methods for successful spawns only (logistic regression, $R^2 = .002$, p = .46) (Figure 7).

Egg Predation

Egg predation occurred in 55% of group spawns (n = 472), and 18.5% of pair spawns (n = 5). A significant relationship was found between spawning method and egg predation ($X^2 = 14.237$, (1, N = 881, p = .0005)). The standardized residuals for pair spawns without predation, and group spawns with predation was 3.7 (Figure 8). Pair spawns had fewer predation event occur than expected, and group spawns had more predation events occur than expected. There was also a significant relationship found between the abundance of egg predators and egg predation (logistic regression, $R^2 = .032$, p = 1.86e-09) (Figure 9).

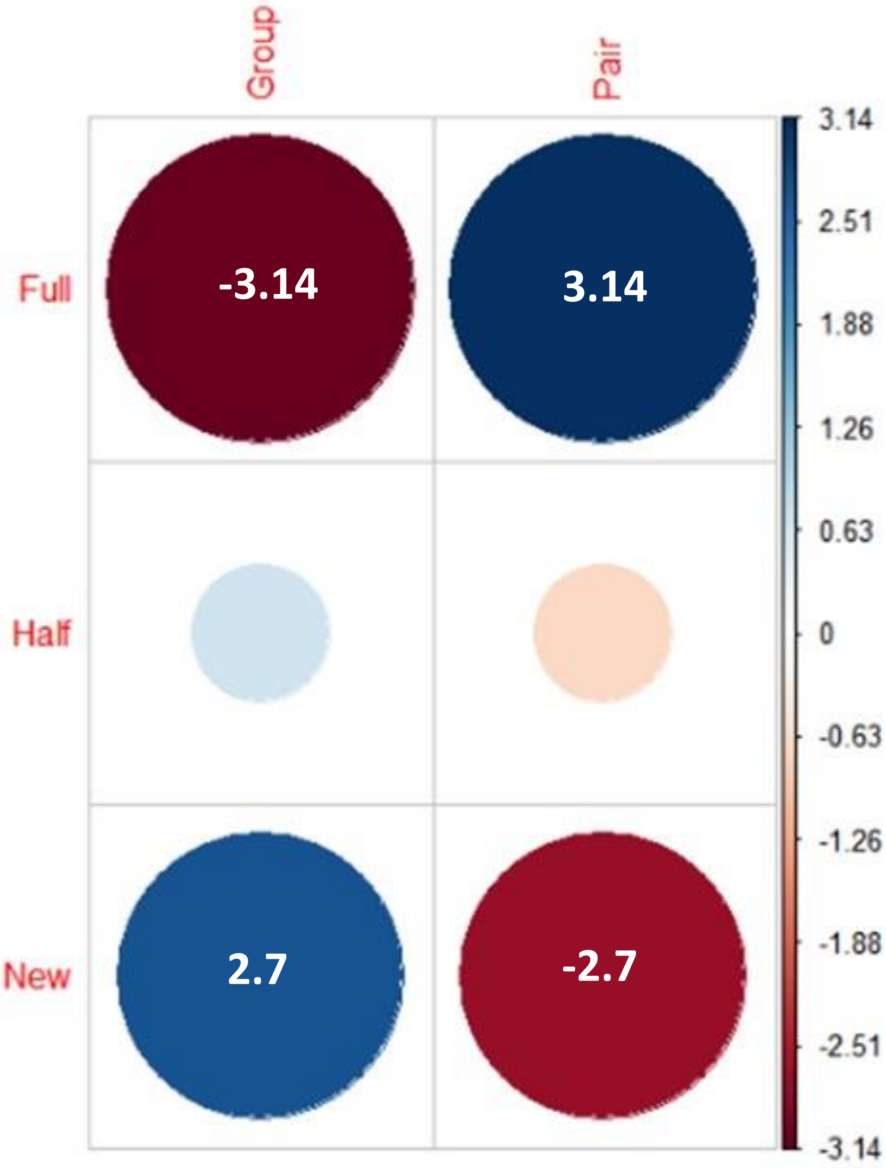

Figure 5: Corrplot of standardized residuals from chi-sq test of spawning method of *Thalassoma hardwicke* by lunar phases. Positive standardized residuals for pair spawns on a full moon lunar phase (3.141), and group spawns on a new moon phase (2.709). Pair spawns occurred more frequently than expected during full moon phases, and groups spawns occurred more frequently than expected during new moon phases.

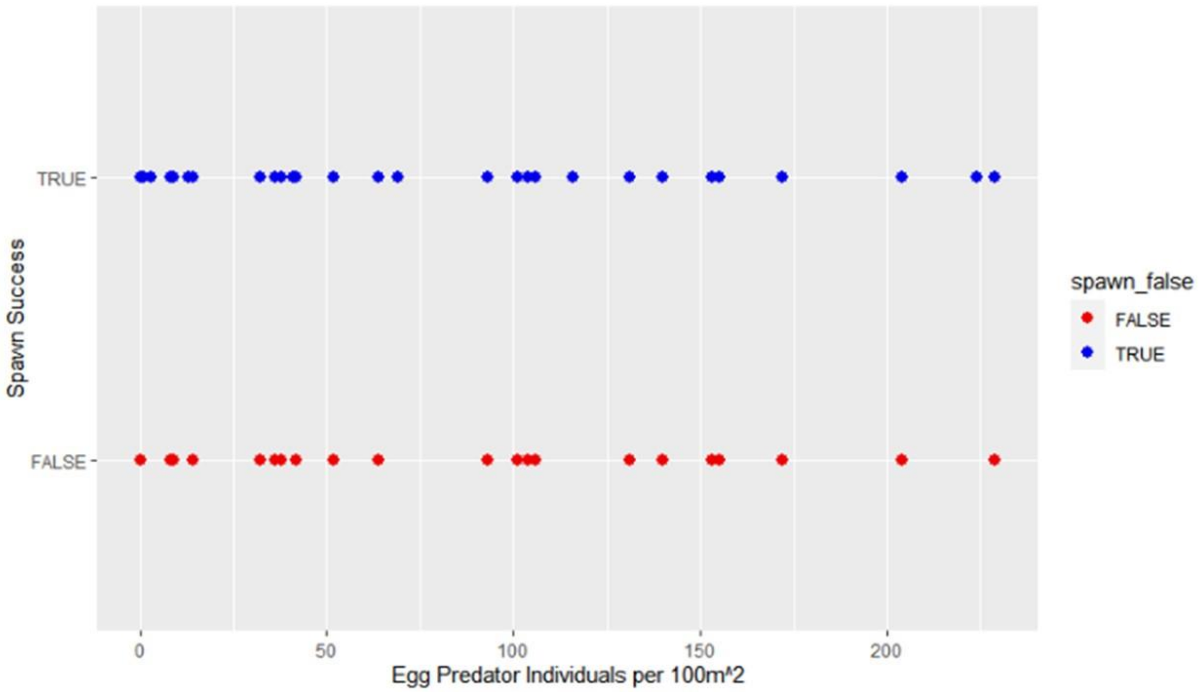

Figure 6: Logistic regression between egg predator abundance and spawning success. The abundance of egg predators does not influence the spawning success (R^2 = .0065, p = .215).

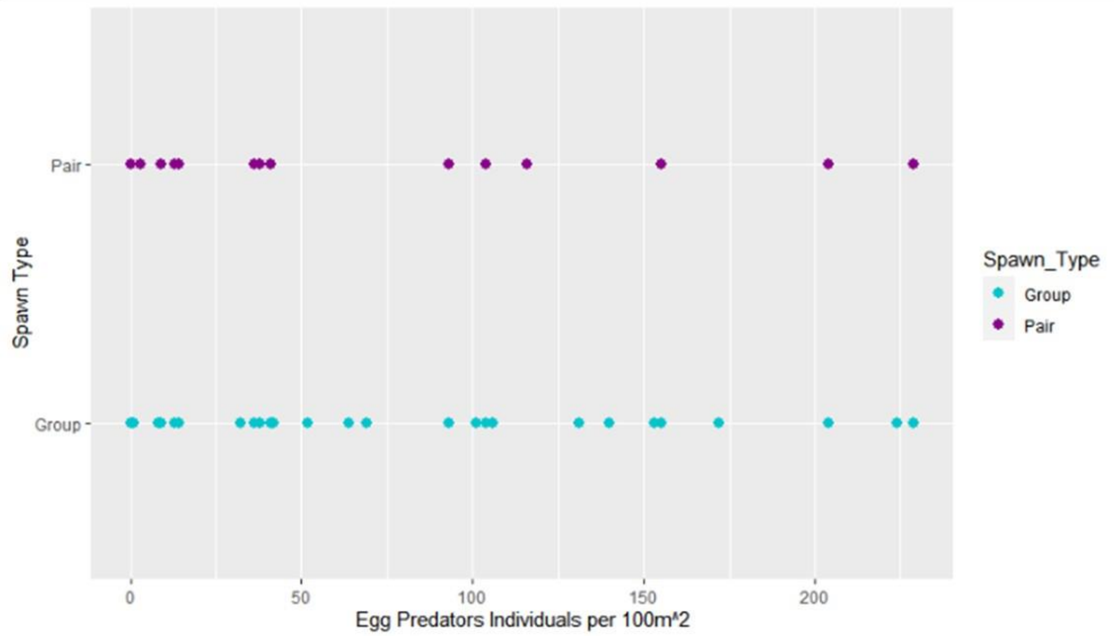

Figure 7: Logistic regression between egg predator abundance and spawning method. Egg predator abundance does not influence the type of spawning method used ($R^2 = .0033$, $p = .194$).

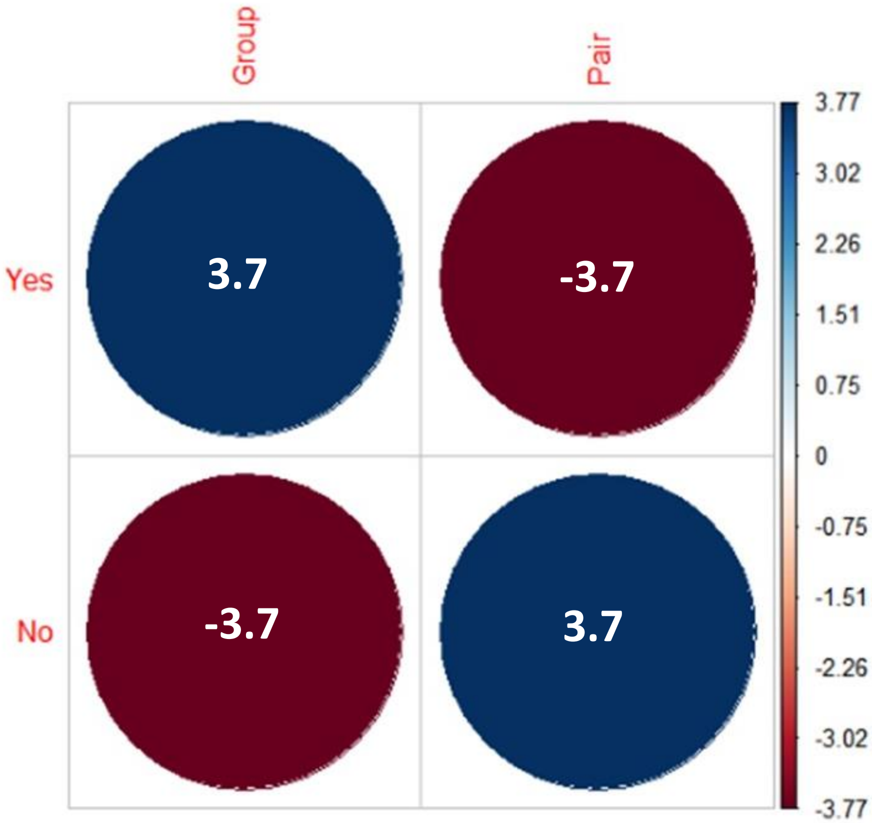

Figure 8: Corrplot of standardized residuals from chi-sq test of spawning method of *Thalassoma hardwicke* and egg predation. Positive standardized residuals for groups spawns with predation and pair spawns without predation (3.773). Groups spawns had egg predation occur more frequently than expected, while egg predation occurred less frequently than expected on pair spawns.

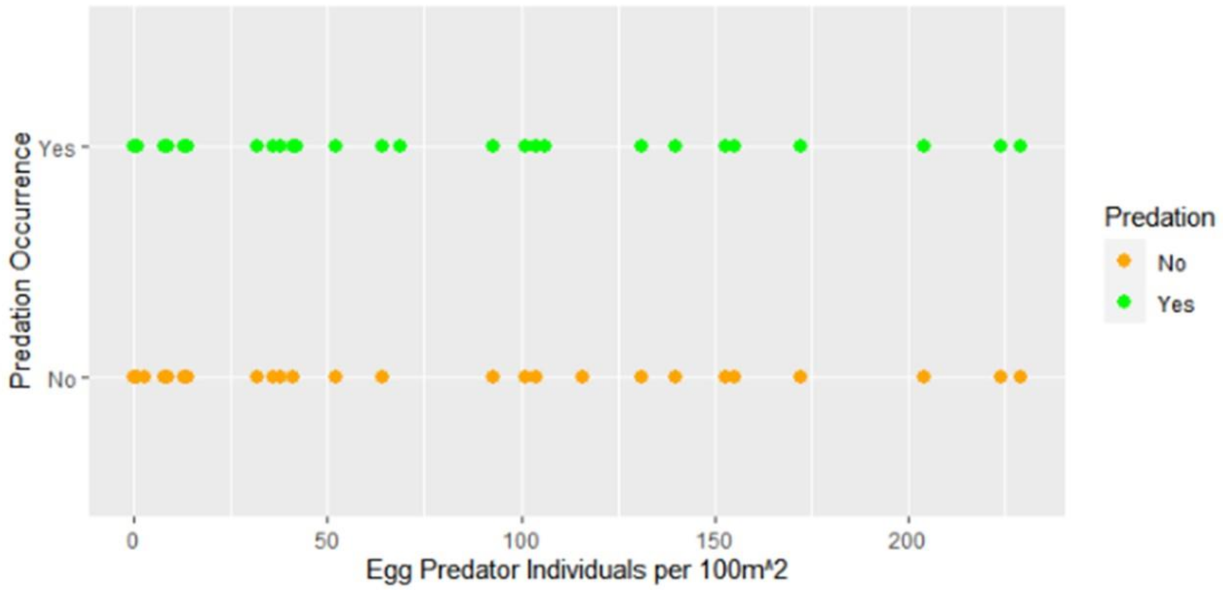

Figure 9: Logistic regression between egg predator abundance and egg predation. The occurrence of egg predation is related to the egg predator abundance, but egg predator abundance is not a good predictor of egg predation occurrence. ($R^2 = .003$, $p < .05$).

Discussion

Spawning Success

Spawning success was predicted to be different between the two spawning methods, with group spawns likely to be more successful. The results given here support this prediction. Few spawning events observed were pair spawns, as previous studies only saw pair spawning occur with very few to no group spawning events observed (Robertson & Choat, 1974; Colin & Bell, 1991). Also, of interest was that group spawns were more successful and frequent because other *Thalassoma* spp., i.e., *T. amblycephalum, T. quinquevittatum,* and *T. lutescens,* seen on Finger Reef were only observed pair spawning, although their abundances were much lower. Pair spawning is likely the dominant spawning method of the congeneric species due to lower abundances and absence of IP males (Warner, 1984; Sara et al., 2005; Roff et al., 2017).

Group spawning events occurred on each observation day that had spawning events, however pair spawns did not. *Thalassoma hardwicke* is a diandrous species with a dualistic mating strategy, had group spawning for small and medium sized IP males and pair spawning for large TP males (Warner & Hoffman, 1980; Adreani & Allen, 2008; Ochi et al., 2017). With this mating strategy, and the higher observed number of IP males at the site, it was not unexpected that group spawns were more successful than pair spawns.

IP males will appear in higher proportions on larger and denser reefs and have higher spawning opportunities than TP males on these larger reefs because the territorial males often are swamped by the IP males leading to the breakup of the territorial structure of TP males (Warner & Hoffman, 1980; Warner, 1984; Domeier & Colin, 1997; Adreani & Allen, 2008). More studies are necessary to determine what the driving factor is behind the difference in the success of the spawning method. It would be useful to identify and study other *T. hardwicke* spawning aggregations to determine if the difference in success is due to the size of the reef, or the density of *T. hardwicke* located on the reef. The presence of other actively spawning species concurrently at this spawning aggregation should be examined quantitatively to see if this affects spawning success.

Thalassoma hardwicke **Abundance**

The abundance of *T. hardwicke* was predicted to affect the spawning success of each spawning method, as well as which spawning method is expected to occur. These predictions do not appear to be supported. Adreani and Allen (2008), found that rock wrasses (*Halichoeres semicinctus*) were likely to group spawn regardless of local densities but would pair spawn only at high densities. Interestingly, studies by Warner (1984), Sara et al. (2005) and Roff et al. (2017) found that at higher population densities, group spawning may be the dominant spawning method because the greater number of IP males would swamp TP males and lower their ability to defend territories; they also stated that this outcome is common in labrid fishes. With multiple studies showing that densities of wrasses may influence spawning success and method used in aggregations, it was unexpected that there did not appear to be a relationship between spawning success or spawning method. Observed abundances recorded here did not separate TP and IP males, so it is possible that partitioning the abundances could have an impact upon measures of spawning success and spawning method. More observational data from Finger Reef and other spawning aggregation sites of the species could verify these findings.

***Thalassoma hardwicke* Spawning**

Tides

Tides were predicted to have an impact on *T. hardwicke* spawning; however, no significance was found between spawning success and tidal state, or spawning method and tidal state. Initially, the spawning success of each method was tested to determine if there were differences in the success between the two spawning methods. When no significance was found, the recorded FALSE spawns were removed, and the data were rerun to determine if tides influenced the spawning method (using successful spawns only). No significant relationship between the spawning method and tidal state was found. This result was slightly unexpected because some studies have found that spawning of *Thalassoma* spp. and other wrasse species will usually occur after high tide, and on an outgoing tide (Colin & Bell, 1991). A relationship between spawning and tidal state would have been expected because spawning during an outgoing tide would pull the fertilized gametes off the reef and away from potential predators.

Seasons

Seasonal influences were predicted to have an effect upon spawning of *T. hardwicke,* but no significance was found between spawning success or spawning method, and season. This was unexpected because many studies of fish spawning aggregations have found relationships between spawning success and seasonal cycles (Adreani & Allen, 2008; Heyman & Kjerfve, 2008; Colin, 2012; Nemeth & Kadison, 2013; Sadovy de Mitcheson, 2016; Erisman et al., 2017; Ohta & Ebisawa, 2017). Considering these previous studies, it was surprising to find no significant differences between seasons. Guam, unlike some other locations, has only two seasons, wet and dry. While the two seasons do not have extreme differences in temperature, which can cause the same species at a higher latitude to stop spawning during cooler seasonal temperatures, they do vary greatly in rainfall, which was expected to have an impact on spawning success and method. As rainfall increases, more sediment may be washed onto reefs, thus increasing turbidity, and greatly reducing visibility. Such conditions could cause *T. hardwicke* to limit or abandon spawning efforts. Reduced visibility could decrease spawning success because of the inability of *T. hardwicke* to court using visual cues and reduce their ability to see potential predators. Further studies are needed to determine the impact of turbidity upon spawning success in the species.

Lunar Phase

Lunar phase was predicted to affect spawning, but no significant relationship between spawning success and lunar phase was found. A significant relationship was found between spawning method and lunar phase, however. Pair spawns were found to occur more than expected during a full moon phase while group spawns occurred more frequently during a new moon phase. The half-moon lunar phases did not influence spawning for either spawning method.

Some species have been studied spawning only during certain lunar phases, but many resident and transient species aggregate and spawn in synchrony with lunar phases (Adreani & Allen, 2008; Heyman & Kjerfve, 2008; Colin, 2012; Ohta & Ebisawa, 2017). As a diurnal spawning species, it was interesting to find that there was significance found with *T. hardwicke* spawning between the spawning methods but not the spawning success. Since lunar phase influences tidal state, it was not unexpected that it influenced spawning behavior, as outgoing tides are likely to disperse gametes. Since spawning did not show a significant relationship to tidal state tides there is likely another factor causing this relationship. Further studies are needed to determine what other factors related to the lunar phase may be causing this relationship.

Egg Predator Abundance

Increased egg predator abundances were predicted to affect spawning success and spawning method. There was no significant relationship found between egg predator abundance and spawning success. There was also no significant relationship found between egg predator abundance and spawning method. This too was unexpected because there have been studies that showed that facultative fish-egg predators can have direct negative impacts upon fish populations by consuming gametes, and indirect negative impacts by interrupting and influencing reproductive behavior of fishes (Domeier & Colin, 1997; Sancho et al., 2000; Colin, 2012). At Finger Reef, I observed six species of egg predators; three were damselfishes *Abudefduf sexfasciatus, Abudefduf vaigiensis,* and *Chromis viridis* (Pomacentridae), and three were halfbeaks *Hemiramphus lutkei, Hemiramphus far,* and *Hyporhamphus acutus* (Hemirhamphidae). Egg-preying damselfishes were the most common and observed consuming gametes followed by halfbeaks. The latter were not observed at every egg predation event but were seen more than a third of the time. A comparison of other spawning aggregation sites and species would provide an opportunity to see if this pattern among egg predators is common.

Egg Predation

While egg predator abundance did not significantly relate to spawning method, there was a significant relationship between spawning method and egg predation. Egg predation occurred more frequently than expected on group spawns than it did on pair spawns, whereas pair spawns had fewer predation occurrences than expected. The higher occurrence of predation on group spawns is not surprising as group spawning fish produce a larger gamete cloud. This was seen in previous studies where the presence of facultative egg predators had an indirect negative impact on spawning by interrupting and influencing spawning behavior (Domeier & Colin, 1997;

Sancho et al., 2000; Colin, 2012). This impact was seen in this study with FALSE pair spawning being more likely than FALSE group spawns. This is potentially due to the abandoning of courtship by the pair spawners in the presence of potential egg predators, however, this will require more research. A significant relationship was found showing there was some correlation between egg predator abundance and egg predation, it did not explain the variation found between the two factors. A future study could investigate how the predation occurrences may differ between Finger Reef and a less popular dive and snorkel site, and a site with fewer to no instances of fish feeding. Piscivore predation impacts were not studied for this project as only one predation attempt was observed but examining the relationship between piscivore abundance and spawning success would be an intriguing study given that the threat of predation during spawning could be a significant deterrent.

Conclusions

This study was done to provide insight into the spawning behavior of *T. hardwicke* at a resident spawning aggregation site located on Finger Reef, Apra Harbor, Guam. As a common, widespread, and reef-associated species, *T. hardwicke* is a great model species that can be used to develop a better understanding of spawning aggregations and reproductive behavior of labrid species, and also provide inferences towards how larger, commercially-important spawning aggregation species reproduce.

Thalassoma hardwicke spawning events began in the morning and continued until midafternoon. Between the two spawning methods, pair spawns had a higher occurrence of FALSE spawns compared to groups spawns. The abundance of *T. hardwicke* individuals did not affect spawning success, or the spawning method that was used. Spawning success and method were not influenced by season or tides, and lunar phase did not affect spawning success. Pair spawns, however, occurred more often during the full moon, and group spawns occurred more often during the new moon. The abundance of egg predators at the spawning aggregation site did not affect spawning success or influence spawning method. Egg predation was higher in group spawns compared to pair spawns. Finally, the significant result between the abundance of egg predators and egg predation suggests that the occurrence of egg predation is related to egg predator abundance. However, the R^2 value shows that while there is a relationship between the two factors, egg predator abundance is not a good predictor of egg predation occurrence. Further study is necessary to determine the causes behind this relationship.

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