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Field Observations on Courtship and Spawning Behavior of the Giant Sea Bass, *Stereolepis gigas*

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Aspects of the reproductive behavior of Giant Sea Bass, *Stereolepis gigas*, were observed and monitored at Goat Harbor, Santa Catalina Island, California from June 2014 to August 2015. The site was visited daily during the summer months (the known spawning season); aggregations were not present during the rest of the year. Numbers of Giant Sea Bass observed at Goat Harbor ranged from 1 to 19 individuals with an average of 6. Giant Sea Bass produced booming sounds (40–80 Hz), which were often associated with aggressive behavior but may also be associated with courtship. Courtship behavior was observed during the late afternoons and was most prominent around dusk (1900–2100 h). Courtship involved sexually dimorphic, temporary color changes and displays such as circling in pairs and the nudging of the abdominal area of the presumed female by the snout of the presumed male. The courtship behaviors observed were similar to those observed for Giant Sea Bass in captivity. Although spawning was not observed directly, the available evidence suggests that spawning occurs just after dusk. Confirmation of spawning at or near the aggregation site was obtained through DNA barcoding with CO1 primers of eggs sampled from Goat Harbor near dusk. This study provides insights into courtship behavior that can be used to identify potential Giant Sea Bass spawning aggregations in the wild that are crucial for management of the species.

COURTSHIP and spawning behavior are critical for reproductive success because they increase the likelihood of gamete release and production of viable offspring. These behaviors allow for conspecifics to identify one another, portray readiness to reproduce, determine mate quality, and synchronize gamete release in aggregating fishes (DeMartini and Sikkel, 2006). Sound production is common behavior observed in soniferous (sound producing) fishes during courtship and reproduction (DeMartini and Sikkel, 2006; Aalbers and Drawbridge, 2008; Mann et al., 2009; Walters et al., 2009). It has been hypothesized that fish sounds and vocalizations are used for development of spawning aggregations (Gilmore, 2003), to signal reproductive readiness (Connaughton and Taylor, 1996), and for gamete release (Lobel, 2002).

Fishes are known to form aggregations that are temporary gatherings for specific purposes (Colin et al., 2003). Fishes aggregate for many different reasons, including feeding (Heyman et al., 2001), protection and shelter to structure (Castro et al., 2002), and spawning (Sadovy and Domeier, 2005). Fish spawning aggregations are the only places many species are able to reproduce (Sadovy and Domeier, 2005). Spawning aggregations tend to form at predictable sites and times of the year, and this predictability allows large quantities of fishes to be harvested with minimal effort (Sadovy de Mitcheson et al., 2008). This, in turn, may lead fishers to believe they are harvesting from a healthy population while actual abundance is declining. This is known as an "illusion of plenty," where fishing continues unrestricted until population collapse, and possibly ending the fishery (Sadovy and Domeier, 2005; Sadovy de Mitcheson et al., 2008; Erisman et al., 2011).

Studying reproductive strategies is important for creating effective management strategies for fisheries (Sadovy and Eklund, 1999) and has led to regulations for harvesting the spawning aggregations of many fishes, such as wreckfish, *Polyprion americanus* (Wakefield et al., 2013), hapuku, *Polyprion oxygeneios* (Wakefield et al., 2010), and goliath grouper, *Epinephelus itajara* (Sadovy and Eklund, 1999). Spawning often occurs when abundances are greatest at aggregation sites. The observation of high densities of fishes at a specific site only during certain times of the year therefore indicates where and when a spawning aggregation is going to form (Mann et al., 2010). Another indicator of spawning aggregation sites is the presence of fish eggs and larvae (Moser and Watson, 2006). Identification of eggs and larvae has generally been done using morphological differences, although many species cannot be identified based on morphology alone (Watson et al., 1999). Therefore, the use of DNA barcoding (mitochondrial COI sequencing) has been used to identify fish species that cannot be identified by morphological traits (Harada et al., 2015).

The Giant Sea Bass, *Stereolepis gigas,* is a part of the northeast Pacific nearshore fish fauna, ranging from the Gulf of California to Humboldt Bay, California (Miller and Lea, 1972) but is most common south of Point Conception (Allen and Andrews, 2012). It is thought to form spawning aggregations during the summer months (July–September; Shane et al., 1996; Love, 2011). Giant Sea Bass are often found in kelp forests on rocky reefs as adults, while juveniles are found at sandy bottom areas (Domeier, 2001). These large, demersal teleosts reach lengths greater than 2 m, weights greater than 200 kg, and ages of up to 76 years (Horn and Ferry-Graham, 2006; Allen and Andrews, 2012; Hawk and Allen, 2014; House et al., 2016).

The Giant Sea Bass is a member of the wreckfish family, Polyprionidae, which is represented by five species within two genera: *Polyprion* (three species) and *Stereolepis* (two species; Nelson, 2006). Wreckfishes grow to large sizes (>150 cm and >70 kg) and are very long lived (>60 years; Peres and Klippel, 2003; Hawk and Allen, 2014). Although no formal studies have been conducted on the reproductive behavior of members of the wreckfish family, research has been conducted on their reproductive development (age at maturity, gonadal development, and fecundity). Wreckfishes mature between 7 and 14 years of age depending on the species (Sedberry et al., 1999; Peres and Klippel, 2003; Wakefield et al., 2010), and Giant Sea Bass are thought to fall within this range (Fitch and Lavenberg, 1971; Domeier, 2001; Allen and Andrews, 2012). These fishes mature very late in life

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compared to most other demersal teleosts (Wakefield et al., 2013). There are no observations of spawning behavior of species of *Polyprion* in the wild, but studies of wreckfish and hapuku reproductive physiology suggested that spawning in wreckfishes occurs during warm water months (Peres and Klippel, 2003). This varies with latitude and is around the Austral winter for both wreckfish (July–October; Peres and Klippel, 2003) and hapuku (June–August; Wakefield et al., 2010). These studies have also shown that males have relatively large testes, which suggests that they engage in group spawning (Sadovy, 1996; Peres and Klippel, 2003; Wakefield et al., 2010).

Although it has been hypothesized that wreckfishes are group spawners based on the large testes of hapuku and wreckfish, courting pairs of Giant Sea Bass have been observed in the wild during summer months (June–September; W. Bushing and W. Marti, pers. obs.), and this suggests that they are pair spawning fish. Observations of pair spawning in captivity have been made, although there were only two individuals present in the aquarium (Hovey, 2001). Spawning was observed on three separate occasions, and environmental conditions in aquaria mimicked that of the summer water conditions in California. This only confirms that they are able to pair spawn, not that pair spawning would be preferred in nature.

The general goal of this study was to investigate the reproductive behaviors of *Stereolepis gigas* in the field and to compare them with behaviors exhibited in the aquarium by Hovey (2001). The specific goals of this project were to: 1) determine when Giant Sea Bass abundances are highest throughout the day at the aggregation site, 2) describe the general reproductive behavior including potential associated sounds and vocalizations, and 3) confirm if Giant Sea Bass eggs occurred at the aggregation site with DNA barcoding to confirm spawning in the area.

MATERIALS AND METHODS

Study site.—We conducted this study at Santa Catalina Island, California (Fig. 1) during June–August in 2014 and 2015. The Long Point Marine Protected Area (MPA) at Santa Catalina Island, California, hosts the best-known aggregation of Giant Sea Bass in southern California (https://www.wildlife.ca.gov/Conservation/Marine/MPAs/Network/). Prior to the start of the present study, eight different sites around Catalina were examined as potential aggregation sites for Giant Sea Bass. During this study (House et al., 2016), observations of large aggregations were made at Goat Harbor, on the front (leeward) side of the island, and at The V's, on the back (windward) side of the island. At both Goat Harbor and The V's, densities of Giant Sea Bass did not significantly vary during the study period (June–August periods of 2014 and 2015, no observations were made at The V's in 2015). Goat Harbor, an east facing cove, was chosen as the primary study site because it was 1) within the Long Point MPA that also includes Italian Gardens, 2) more readily accessible by vessel than the other sites, 3) protected from the elements (wind, wave action), and 4) Giant Sea Bass could be observed there on a daily basis during the summer. While The V's did have a large aggregation, the site was difficult to access and water conditions varied drastically from day to day, making daily observations unfeasible. Giant Sea Bass were not present at Goat Harbor during surveys made during the non-spawning months of October and March 2014, and December 2015 (B. Clark, pers. obs.). Therefore, for the purposes of this study we

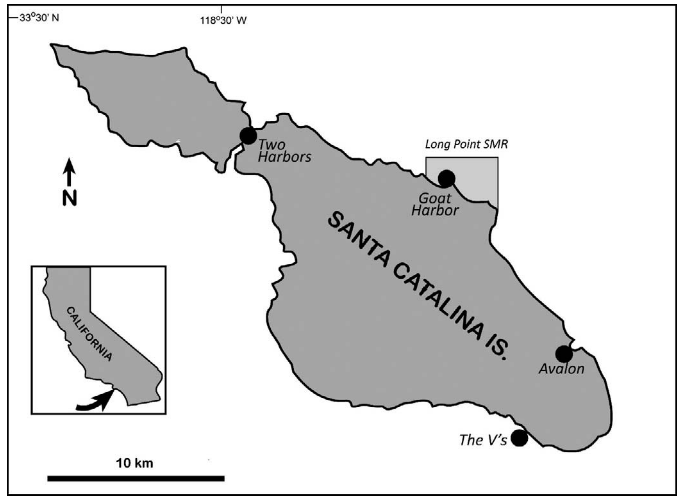

Fig. 1. Map depicting Santa Catalina Island, California. This study was conducted at Goat Harbor within the Long Point State Marine Reserve.

are considering Goat Harbor an aggregation site. Goat Harbor has a steep slope, dropping from 10–30 m in depth in just under 150 m distance from shore.

Residency at aggregation site.—To evaluate residency at the aggregation site, SCUBA divers conducted four, 200-meter transects at four depths (25, 18, 12, and 8 m) per survey. These depths were chosen because they allowed divers to make observations along the entire slope of the study site. Surveys were conducted one to two times per month (5 surveys in 2014, 4 surveys in 2015). Transects were conducted during three different time periods: morning (0800–1200 h), afternoon (1300–1600 h), and evening (1700–2100 h). The number of individuals were recorded on each transect (108 total sightings). Distinct individuals were identified based on spot patterns, scarring, and total lengths. The number of surveys conducted during the different time periods differed between the two years (2014, 2015). In 2014, the same numbers of dives were conducted at each time period (5 dives per period) but no dives could be conducted after 1800 h. In 2015 diving was focused around the afternoon and evening time periods (Dives: Morning = 2, Afternoon = 4, Evening = 4) and evening dives were conducted after 1800 h. As with most Giant Sea Bass transects at Catalina Island (House et al., 2016), zero counts were common (>56%) during transects; therefore, a Kruskal-Wallis non-parametric test of independence was used to test for differences in Giant Sea Bass abundances at the study site by time period (data met the assumptions for this test).

Description of courtship behavior.—Visual observations were carried out on SCUBA and documented on dive slates as well as digital video recordings. A total of 63 dives were conducted at Goat Harbor, totaling over 35 hours of underwater observation of Giant Sea Bass behavior over the two-year period. Dives averaged 33.7 minutes per dive and were partitioned among three time periods: morning (17 dives, 9.1 hrs), afternoon (35 dives, 17.3 hrs), and evening (11 dives, 9 hrs).

Courtship and aggressive behaviors between individuals were recorded following the protocols of Erisman and Allen (2006). Behaviors involving two individuals (a gravid female and a male) similar to those described by Hovey (2001) were considered courtship behaviors. Interactions between males in which one male terminated the interaction was considered aggression. Outside of courtship, no aggressive interactions between males and females were observed; female to female aggression was never observed. Individuals within 0.5 m of each other were considered either paired (two individuals) or grouped (three or more individuals). Behaviors were then cataloged into an ethogram (catalogue of behaviors) describing various reproductive behaviors.

Audio and video were paired using GOPRO® (GoPro, Inc. USA) Hero 3 cameras to ground-truth vocalizations produced by Giant Sea Bass. A digital spectrogram long-term acoustic recorder (Soundtrap 202: Ocean InstrumentsNZ) also was used to capture vocalizations. This device was anchored to the bottom at 20 m depth and passively recorded vocalizations for 1 minute every 5 minutes, 24 hours each day (Rowell et al., 2012) during both the 2014 and 2015 field seasons. Sounds captured by the Soundtrap and Hero 3 then were analyzed using AUDACITY® (AUDACITY Team, 2015: free audio editor and recorder) to identify the frequencies produced (Hz).

Confirmation of Giant Sea Bass eggs at aggregation site.—Spawning was not observed directly at Goat Harbor during our study; therefore, alternative methods to detect spawning were pursued. Fish egg samples were collected from Goat Harbor where courtship activity was observed, to see if spawning had occurred at or near the site. A 1 m diameter zooplankton net (0.505 mm nitex mesh) was towed for 5 min at 3 m from the surface on the evening (2100–2215 h) of 7 July 2015, and eggs were stored immediately in 95% ethanol. Upon removing the eggs from storage one month later, they were rinsed with molecular grade de-ionized water. Fish eggs were sorted from other zooplankton under a dissecting microscope at 50X. The diameter of Giant Sea Bass eggs ranges from 1.5–1.6 mm (Shane et al., 1996), so eggs were separated into two groups, those $<$1 mm diameter and $\geq$1 mm diameter (accounting for eggs that may have decreased in size from the ethanol). Data suggest that Giant Sea Bass hatch around 4.1 mm in length, which is roughly 6 days post spawning (Shane et al., 1996). A subsample of eggs ($n = 20$) was staged and found to be in early developmental stages (between the 64 and 256 cells). Individual eggs were then placed into tubes with a buffer (2/3 AE buffer and 1/3 D. I. water) and crushed using a sterile pipette tip (Harada et al., 2015) to release DNA.

To amplify DNA, we used universal fish COI primers: COI VF1 forward primer (5′–TTCTCAACCAACCACAAAGA-CATTGG–3′) and COI VRI reverse (5′–TA-GACTTCTGGTGGCCAAAGAATCA–3′). PCR reaction used a 10 μl solution: 5 μL Qiagen MasterMix, 0.9 μl BSA, 2.1 μL water, and 1 μL primer mix (2 μL forward, 2 μL reverse primers and 96 μL water). PCRs were performed in a GeneAmp 9700 thermal cycler (Applied Biosystems). The thermocycler profile used was 95°C for 15 min, 35 cycles of 95°C for 30 s, 50°C for 45 s, and 72°C 1 min, and then 72°C for 10 min. PCR samples were run on 10% agarose gel and amplified DNA was detected using ethidium bromide. A subsample was then run on a Quibit 3.0 fluorometer with high sensitivity to see the concentration of DNA for small ($<$1 mm) and large ($\geq$1 mm) eggs.

Samples were sent to Laragen Inc. for purification and sequencing. Sequencing products were validated by eye and aligned in SEQUENCHER (Gene Codes Corporation; Chabot et al., 2015). Ninety-one unknown egg samples were sequenced with five known samples of Giant Sea Bass DNA. After sequencing, results were compared to the NCBI BLAST database (NCBI Resource Coordinators, 2017). All of the eggs identified were at least 99% identical to GenBank sequences.

RESULTS

Description of the Goat Harbor aggregation site.—Giant Sea Bass aggregated adjacent to the rocky reef over large patches of cobble or sandy bottom with little kelp cover within Goat Harbor. Counts at this site varied between 1 to 19 individuals (mean of 6 fish per visit). No patterns in variation were observed (Fig. 2). Giant Sea Bass were observed at depths ranging from 6–30 m, although 80% of the fish observed were between 18 and 25 m in depth. Abundances differed significantly among these depths ($F = 9.140$, df = 3, $P = 0.011$), with greater numbers of fish present within the deepest zone (Fig. 3).

Residency at aggregation site.—During the 2014 and 2015 field seasons, 20 different individuals were observed at Goat Harbor. The number of individuals observed differed by time of day in 2014 (H = 5.99, df = 2, $P = 0.05$), but not in 2015 (H = 4.37, df = 2, $P = 0.11$; Fig. 4). The sampling distributions of data characterizing abundances throughout the day differed between 2014 and 2015 (H = 10.74, df = 1, $P < 0.01$), so 2014 and 2015 data could not be combined.

Reproductive behavior and phenotypes.—Presumed sexual dimorphism was observed within this species while at aggregation sites. Individuals showed distinct color and morphological differences prior to courting and spawning (though spawning was not directly observed, so this may be a supposition). While courting, males presented slim lateral profiles and became much lighter in color (Fig. 5A), while females exhibited much more rotund body shapes, became much darker in color, had a noticeable white patch on their sides above the vent, and had a very dark mask under the eyes (Fig. 5B). For this study, individuals were considered males and females based on these characteristics.

Courtship was observed most often in pairs (Fig. 6), and these behaviors were repeated throughout the afternoon and evening. During surveys, Giant Sea Bass were encountered in pairs 50% of the time, alone or single 31% of the time, and in groups (3 or more) 19% of the time. Females were stationary and in a resting position near the sea floor, unless being courted by a male. On occasion, both a male and female would rest in close proximity (within 1–2 m observed on 2 dives). When not in proximity to a female, males were never observed resting, but were always otherwise observed swimming and approaching resting females. If the female reacted to the approach, the female began to swim with the male, who was "following" closely behind apparently in an attempt to initiate courtship (Fig. 7A). If the female did not return to a resting position after being followed, she began to swim in a circular pattern with the male "following" in a similar fashion. This behavior would continue for up to several minutes (30 sec–10 min; Fig. 7B). During circling, the lead male (if more than one male was "following" the female) would rub and bump the abdominal area of the female near the anal fin with its snout often pushing the female upward into the water column (Fig. 7C). The individuals would occasionally rise up into the water column ($>$3 m from the substrate) and circle much more rapidly (spiral swimming). This behavior would continue until the female swam back to the bottom. The male would then either attempt to court the

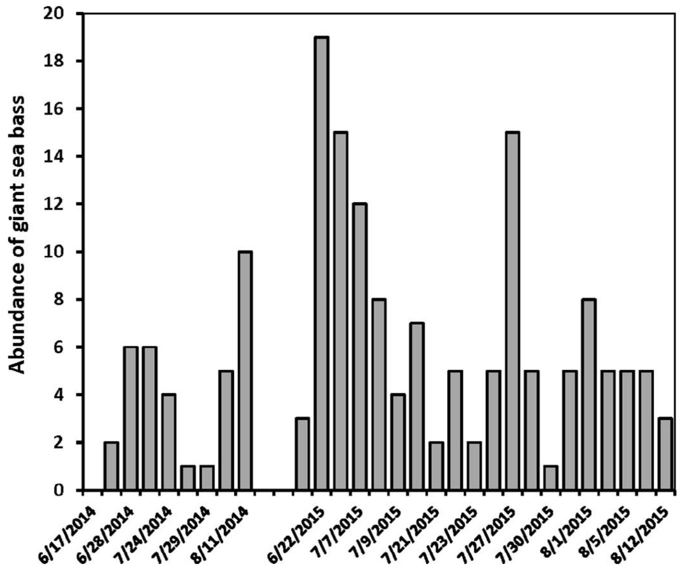

Fig. 2. The counts of Giant Sea Bass observed at Goat Harbor by survey date for 2014 and 2015. Counts at this site varied from 1 to 19 individuals (mean of 6 fish per visit).

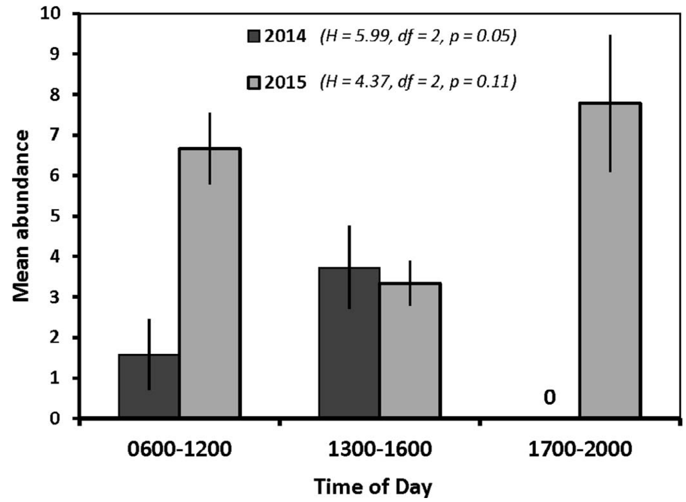

Fig. 4. The mean number of individuals observed by time of day from diver surveys within Goat Harbor for both 2014 and 2015 (±SE).

same female again or move to another female in the area. Observations of three individuals courting were not common (two out of 18 interactions), with one female and two males "following" the same female. If circling behavior commenced, then one of the males would become aggressive toward the other, essentially displacing the other from the act of courting. The aggressive male then would resume courting and nudge the female (Table 1). Although the act of spawning was not directly observed during this study, we have produced a diagrammatic depiction of presumed Giant Sea Bass courtship and possible spawning behavior. The courting behaviors observed in the wild matched those described in aquaria by Hovey (2001), so the actual act of spawning was adapted from his description (Fig. 8).

Courtship behaviors were not observed during the morning period (0600–1200 h) and appeared to be restricted to the afternoon and evening hours (1200–2100 h). However, Giant Sea Bass aggregated at the site throughout the day. First observations of the presumed courtship behavior were at 1200 h and the last observations occurred after dusk (2000 h). All courtship was observed over depths greater than 12 m. Presumed courtships that were observed during the afternoons occurred within a meter of the substratum when more than 50% of the females were observed in a resting position. Only three of 18 interactions proceeded past the pre-courting ("following") behavior. During the evenings (after 1700 h) activity increased, with 10–20% of females observed in a resting position and individuals not only courting near the sea floor, but also up in the water column, 5–15 m above the substrate. Ten of 12 observations of courtship continued past the "following" behavior stage into the more active circling behavior in the evening. Based on the 35 hours of observation of their activity, we hypothesize that spawning in Giant Sea Bass occurs in the evening just after dusk (approximately 2030 h). To test this hypothesis, direct observation of the spawning act using "night vision" or LED technology awaits future study.

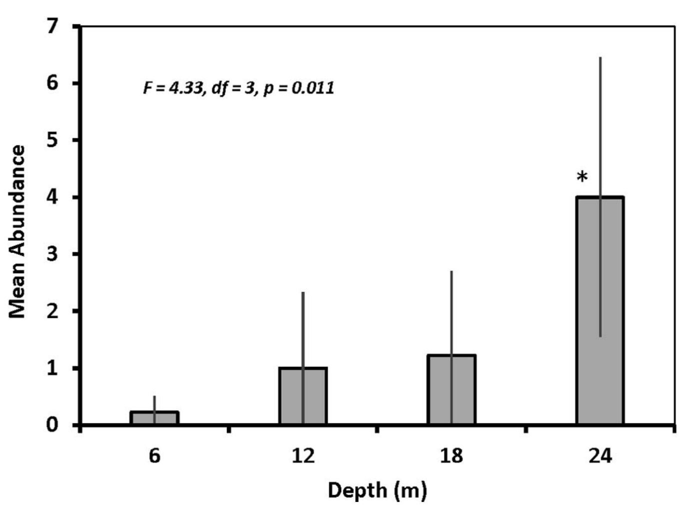

Fig. 3. The average number of Giant Sea Bass per transect ($n = 9$) at four depths (m) at Goat Harbor (±SE) in 2015 (* denotes significantly different mean based on *post hoc* Tukey HSD test).

Sound production and vocalizations.—Giant Sea Bass produced "boom" sounds (single pulses with a low frequency [40–80 Hz with maximum amplitude at 50 Hz]) that were often heard when diving within 1 to 10 m of them. On two separate occasions we were able to verify both visually and audibly capture these "booms" on video cameras. On both

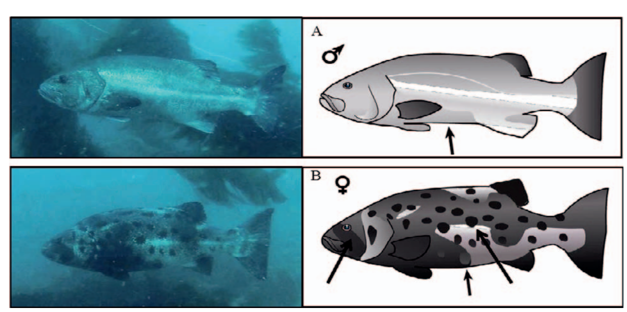

Fig. 5. Sexual dimorphism between male (A) and female (B) Giant Sea Bass in the wild during spawning season, with both *in situ* photographs and drawings of these individuals to show greater contrast. Arrows depict key features in males (A): a slim body profile lighter in color with concave abdominal profile; and in females (B): a very dark mask under the eyes, a more rotund body shape, and a noticeable white patch on the sides above the vent (left to right). (Image Credit: LGA).

Fig. 6. Pair of Giant Sea Bass photographed off Goat Harbor, Catalina Island, California by Parker House, July 2014.

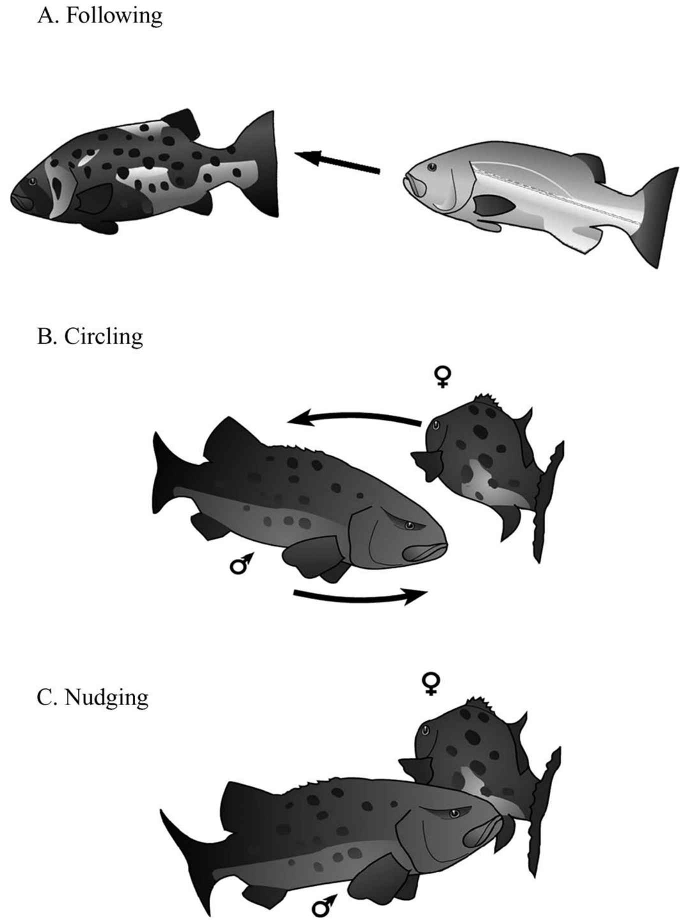

Fig. 7. Diagrammatic depictions of the three common courting behaviors: Following (A), Circling (B), and Nudging (C). (Image Credit: LGA).

occasions the boom was produced because of an interaction between a diver and an individual fish. The individual would boom at the initiation of swimming rapidly away from the diver, causing other fishes in the immediate area to dart away as well.

Confirmation of Giant Sea Bass eggs at aggregation site.—Of the 91 eggs sequenced from the zooplankton collection, 30% of the eggs could not be verified or lined up with SEQUENCHER (Gene Codes Corporation) and contained multiple peaks, leaving 67 samples successfully sequenced (51 large eggs [>1 mm] and 16 small eggs[<1 mm]). Eight different species of eggs were identified. Among the large eggs, eight haplotypes were identified, comprising four local species including *Stereolepis gigas* (3%), *Seriola lalandi* (49%), *Sphyraena argentea* (19%), and *Scomber japonicus* (1%). For small eggs, six haplotypes and four species were identified as *Paralabrax clathratus* (19%), *Semicossyphus pulcher* (3%), *Medialuna californiensis* (3%), and *Trachurus symmetricus* (1%). After Harada et al. (2015), identifications were based on 538 bp of sequence data with greater than 99% identity from the NCBI BLAST database (NCBI Resource Coordinators, 2017).

DISCUSSION

In 2014, we were able to identify Goat Harbor, within the Long Point MPA, as an aggregation site for Giant Sea Bass because individuals encountered on transects there numbered two or more on an almost daily basis. Eighty percent of the Giant Sea Bass observed at this site occurred at between 18 m and 25 m depth, which is consistent with observations made at other locations around Santa Catalina Island (House et al., 2016). In a previous study (House et al., 2016), we concluded that only two aggregations consistently occurred around Santa Catalina Island in the summers of 2014–2015, with Goat Harbor being the only one on the front side of the island. Additional surveys at Goat Harbor conducted during the fall (October 2014, December 2015) and spring (March 2014) encountered no Giant Sea Bass at Goat Harbor (B. Clark, pers. obs.). These observations strongly suggest that Goat Harbor is an aggregation site for Giant Sea Bass.

We hypothesize that densities should be significantly higher around dusk than at other times of the day (morning, afternoon, and evening), similar to the peak spawning time in many fishes (Adreani et al., 2004; Erisman and Allen, 2006; Mann et al., 2010; Rowell et al., 2012). Abundances of Giant Sea Bass in 2014 and 2015 differed significantly; therefore, the data could not be combined over both years. Two factors may have caused this discrepancy. In 2015 dives were not restricted to daylight hours, unlike 2014 (when dives were not conducted after 1830 h). No Giant Sea Bass courting activity was observed during morning periods in 2014, and we therefore subsequently focused our efforts on diving later during the day when more activity was observed. In 2015 a few dives were conducted during the morning period (before 1200 h) to confirm the 2014 pattern of morning inactivity, and all fish then observed were in the resting position. Dives during the morning period were discontinued in 2015. Environmental conditions between the two years might have varied because of the influence of the 2015 El Niño. Namely, in 2014 Goat Harbor had large patches of kelp cover that had disappeared by 2015. Water temperature also varied between years: average temperatures were 20.4°C and 20.8°C in 2014 and 2015, respectively (The Coastal Data Information Program). Although these discrepant temperatures might not differ significantly, temperature can play a central role in the seasonal movement and distribution of fishes (Lowe and Bray, 2006). The depth of the thermocline (boundary layer at which the temperature of the water changes rapidly) increased (at about 18 m and 30 m in 2014 and 2015, respectively; B. Clark, pers. obs.). Giant Sea Bass were mostly observed at 18 to 24 m in both years; therefore, the described change in the thermocline could possibly have affected their behavior.

Table 1. Catalogue of observed behaviors during the spawning season in Giant Sea Bass, *Stereolepis gigas*.

Behavior	Frequency	Description
Aggregating	Common	Individuals loosely grouped over sandy bottom adjacent to kelp forest and rocky reefs in numbers of 5–20 individuals.
Resting	Common	Throughout the entire day, presumed females were commonly observed hovering <1 m above the substratum unless approached by a courting male.
Pairing	Common	A male rests in close proximity to a gravid female, both hovering <1 m above the substrate.
Approach	Common	Male advances towards a presumed gravid female to begin courtship behaviors.
Following	Common	One to three males swim behind a gravid female, no contact with females; common during early courtship.
Circling	Common	Gravid female begins to swim in a circular pattern, while the one to two males follow in the same motion; common during courtship up to several minutes.
Nudging	Common	The lead presumed male following/circling approaches the female from the side or underneath and begins rubbing and bumping his snout against the presumed gravid female's abdominal area near the anal fin pushing the female upward.
Spiral swimming	Rare	Male and female begin to circle rapidly upward into the water column; only observed after dusk.
Spawning	Not observed directly	Male and female continue circling near surface, beating with caudal fins, pair tilts slightly along dorsal-ventral axis with the release of eggs, followed closely by release of sperm (Hovey, 2001).

In 2014, the number of Giant Sea Bass was greatest in the afternoon (1300–1600 h period). In the late afternoon, Giant Sea Bass tended to be most active compared to mornings. Unfortunately, no fish were observed (courting or otherwise) during the evening period in 2014 because no evening surveys were conducted that year. In 2015, the pattern of greater afternoon activity was not repeated, and no difference in abundance was detectable throughout the day. Although the abundances of Giant Sea Bass differed from year to year, their behavior was similar in both years. Marked differences in types of behavior did occur throughout different periods of day though. In the afternoons, interactions between individuals began (50% of all individuals), but activity remained close to the bottom. In the evenings (after 1600 h) through dusk (2000 h), almost all (80–90%) of the individuals were no longer in resting positions, and multiple courtship interactions between males and females were observed throughout the water column. The similarities in behaviors seen in 2014 and 2015 associated with time of day lead us to hypothesize that activity and interactions increase throughout the day with courtship being initiated in the afternoon, intensifying in the evening leading to spawning beginning around dusk. This particular pattern of dusk spawning is common in many different species of temperate and tropical fishes (Colin, 1992; Erisman and Allen, 2006; Aalbers and Drawbridge, 2008).

During this study distinct, presumed sexual dimorphism was observed in the aggregating Giant Sea Bass. Presumed females were very rotund (most likely due to large ovaries filled with hydrated eggs) and would darken in color, with a dark mask under the eyes and a noticeable white patch on their sides. The presumed males that we observed retained a slender body shape but became much lighter in color. Hovey (2001) observed similar color patterns and changes in the aquarium where he was able to verify sex of the individuals via biopsy of gamete tissue that confirmed the sexes of the different morphs. These changes in color and morphology have been seen in many other fishes such as kelp bass and

Fig. 8. Proposed depiction of spawning behavior exhibited by Giant Sea Bass. The final stages of courtship and actual spawning are believed to occur near and just after dusk as represented by the light to dark gradient (left to right) in the illustration. (Image Credit: LGA).

indicate potential readiness to reproduce (Colin, 1992; Erisman and Allen, 2006). Although spawning by Giant Sea Bass was not directly observed during this study, many of the courtship behaviors we observed in the wild were previously described during observations of two separate spawning events of Giant Sea Bass in a large tank at the Aquarium of the Pacific in Long Beach, California (Hovey, 2001). Thus the behaviors described herein are assumed to be courting (or possibly courting/spawning) behaviors based on their similar nature.

The combination of "following," "circling," and "nudging" are behaviors commonly seen in marine fishes (Colin, 1992; Erisman and Allen, 2006; Erisman et al., 2007; Froeschke et al., 2007; Aalbers and Drawbridge, 2008) and were also seen during aquarium observations of spawning Giant Sea Bass (Hovey, 2001). "Following" behavior was commonly observed to involve a single female and one or more males. During these interactions males slowly closed the distance between them and their potential mate, but no actual contact was ever made. "Circling" behavior would sometimes begin with multiple males attempting to court a single female, but shortly after "circling" began, aggression by the lead male directed at the second and third males would invariably lead to a cessation of "circling" by the additional males, thus leaving the pair to continue courting. The subordinate males would then continue courting, but shift their attention to other females in the immediate area. Circles would become tighter and increase in speed as the male nudged at the abdominal area near the anal fin. This nudging behavior is known as a sign of readiness to reproduce by males, as an apparent prelude to starting a spawning rush similar to what has been observed in white seabass (Aalbers and Drawbridge, 2008). On two of these occasions of tighter "circling," single females spiraled upward with a single male, but these ended without the apparent release of any gametes. These apparently "false rushes" involved only two individuals, further supporting our contention that Giant Sea Bass are primarily pair spawning fishes. Observations of pair spawning have been observed for Giant Sea Bass in captivity (Domeier, 2001; Hovey, 2001), which further supports our observations in the wild.

Although the field observations suggest that Giant Sea Bass are primarily pair spawning fishes, the possibility remains that they are group spawners. Many confamilials of similar size and egg storage capacity are group spawners (Roberts, 1989; Peres and Klippel, 2003; Wakefield et al., 2010). However, we observed no such group or aggregate spawning activity in Giant Sea Bass.

The "booms" that the Giant Sea Bass produced were heard frequently but sporadically. In the summer of 2014, we heard constant booming on several dives at the site called the "The V's" on the backside of the Santa Catalina Island in an aggregation of 15–20 individuals (Fig. 1). Booms were not as frequent at Goat Harbor, perhaps because the aggregation at that site was smaller. These sounds were often heard in association with an aggressive behavior: 1) an individual would produce a boom that caused other fishes in the immediate area (including other Giant Sea Bass) to vacate the immediate area or 2) a single Giant Sea Bass would become startled by a diver and boom as it swam away at a rapid pace.

Other large fishes, particularly groupers, produce similar ("boom") sounds during acts of aggression as well as when they are startled, but they are also used as signals for the beginning of a spawning rush (Mann et al., 2009, 2010; Schärer et al., 2012). Giant Sea Bass might be using sound for the same purpose during intraspecific interactions.

Because we were unable to directly observe spawning due to low light levels, we attempted a new approach to verify whether Giant Sea Bass were actually spawning in the study area. This approach, in turn, supported our contention that the behaviors described here were indeed courtship behaviors leading to spawning. Use of the universal fish 600 bp COI primers allowed us to document that multiple species were spawning at this specific site at Santa Catalina Island. Eight species were identified as variably contributing to fertilized fish eggs in the nearfield. Although fish counts made on our transects did not quantitatively estimate the numbers of other fish species present in the study area, on any given day during the summer months high numbers of all eight species were qualitatively noted as present at this site. For example, schools of greater than 100 California yellowtail (*Seriola dorsalis*) commonly swam through the site, and kelp bass (*Paralabrax clathratus*) were the dominant reef fish in the area. These two species contributed the highest percentage of eggs recorded within their respective size class (large: >1 mm; small: <1 mm). Most importantly, Giant Sea Bass eggs were identified and comprised at least 3% (2 of 67 eggs) of our sample. This low percentage might be expected because recently spawned eggs had already begun to disperse throughout the extremely large volume of water, thus reducing our chances of collecting their eggs.

With multiple species of broadcast spawners present around the same time in this open system, the chances of actually finding any Giant Sea Bass eggs was very slim. The presence any fertilized eggs at this site does provide support for Giant Sea Bass using this general area as a spawning aggregation site, however, and provides conclusive evidence that the observed behaviors are for courtship. The staged eggs were all in the 64-cell to 256-cell stage; they therefore were recently fertilized (within 2–3 hours). Hence these Giant Sea Bass eggs most likely came from the immediate or nearby adjacent areas. It is highly likely that these eggs were produced and fertilized by the Giant Sea Bass observed at Goat Harbor because no other consistent aggregations have been recorded on the front side of Santa Catalina Island (House et al., 2016). This egg-method of monitoring spawning behavior (Harada et al., 2015) offers an effective way to monitor spawning activity around the islands and could be a useful way to find potential areas where spawning aggregations of Giant Sea Bass and other aggregating fishes form on the islands and the coast.

The historically low population size of Giant Sea Bass has discouraged researchers from studying them (Pondella and Allen, 2008), in contrast to previous research on the reproductive physiology and general biology of other, more abundant wreckfishes of the family Polyprionidae (Roberts, 1989; Peres and Klippel, 2003; Wakefield et al., 2010). House et al. (2016) document recent temporal increases in Giant Sea Bass numbers around Santa Catalina Island though, and this has facilitated our behavioral research. Recent studies have focused on life history traits as well as the population status and vulnerability of Giant Sea Bass (Allen and Andrews, 2012; Hawk and Allen, 2014; Chabot et al., 2015). The present study attempted to increase knowledge of Giant Sea Bass by examining the different behaviors that they exhibit in the wild that are associated with reproduction, and it offers insights into the reproductive behavior of polyprionids in general. Our observations reported herein match courtship behaviors encountered in nature to those seen in an aquarium, thus providing new methods for detection of Giant Sea Bass spawning aggregations. Further repeated observations of three or more Giant Sea Bass spawning together in the wild are needed to fully evaluate whether it is a pair spawning species.

The annual formation of spawning aggregations is very important to the reproductive success of many fishes, and the overharvesting of these aggregations can cause detrimental effects to populations. For critically endangered species such as the Giant Sea Bass, identifying spawning aggregations is crucial to their recovery. Observations of courting, site fidelity, and our verification of the presence of fertilized Giant Sea Bass eggs at an aggregation site should help establish protocols used to positively identify spawning aggregation sites in the future. Further research identifying additional aggregations are needed for better management of Giant Sea Bass populations in California and Mexico.

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