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Demography, movement patterns, and mating system of leopard sharks (*Triakis semifasciata*) aggregating along the open coast of southern California, USA

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Publication Date

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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Demography, movement patterns, and mating system of leopard sharks (*Triakis semifasciata*) aggregating along the open coast of southern California, USA

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Marine Biology

by

Andrew Phillip Nosal

Committee in charge:

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2013

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Chair

University of California, San Diego

2013

DEDICATION

This dissertation is dedicated to the memory of Dr. Jeffrey B. Graham, who accepted me into his laboratory in 2007 and sadly passed away on December 8, 2011. Jeff taught me how to think and write like a scientist. He gave me the freedom and confidence to explore my interests in shark ecology and encouraged me to be an independent researcher. I am forever grateful for his friendship, mentoring, constructive feedback, and for never allowing me to sell myself short.

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ACKNOWLEDGEMENTS

I am forever grateful for the guidance and friendship of Jeff Graham, who was my dissertation advisor from July 2007 until he passed away in December 2011. I am equally grateful for the support and mentorship of my current advisor, Phil Hastings, who unreservedly accepted me into his lab in January 2012 and made the transition as seamless and with as little disruption to my dissertation research as possible. I would like to thank the members of the Graham and Hastings labs and Scripps Institution of Oceanography support staff, particularly Nick Wegner, Dan Cartamil, and Eddie Kisfaludy, who taught me how to fish, drive a boat, tie knots, and track using acoustic telemetry, in addition to assisting me in the field and providing constructive feedback on presentations and manuscripts. I would also like to thank Ron Burton and the members of his laboratory for their attentive support in completing the genetics portion of this dissertation (Chapter 3). Next, I would like to thank my dissertation committee for their valuable feedback on various aspects of my dissertation research. Finally, I want to thank the many undergraduate volunteers who dedicated their summers to assisting me in the field. Completing this dissertation research would not have been possible without the help of these individuals. This is by no means an exhaustive list, and more detailed acknowledgements are provided at the end of each of the three chapters.

Chapter 1, in full, was submitted and accepted for publication as: Nosal AP, Cartamil DC, Long JW, Lührmann M, Wegner NC, Graham, JB (*in press*). Demography and movement patterns of leopard sharks (*Triakis semifasciata*) aggregating near the head of a submarine canyon along the open coast of southern California, USA.

Environmental Biology of Fishes, DOI: 10.1007/s10641-012-0083-5. The dissertation author is the primary investigator and author of this manuscript.

Chapter 3, in full, was submitted for publication as: Nosal AP, Lewallen EA, Burton RS (*in review*). Multiple paternity in leopard shark (*Triakis semifasciata*) litters sampled from a female-dominated aggregation in La Jolla, CA, USA. *Journal of Experimental Marine Biology and Ecology*. The dissertation author is the primary investigator and author of this manuscript.

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Nosal AP, Cartamil DC, Long JW, Lührmann M, Wegner NC, Graham JB (*in press*).
Demography and movement patterns of leopard sharks (*Triakis semifasciata*) aggregating
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Environmental Biology of Fishes. DOI: 10.1007/s10641-012-0083-5

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Nosal AP, et al. (2012). Low incidence of multiple paternity in leopard sharks (*Triakis semifasciata*) sampled from a predominantly female aggregation. Oral paper presented at the 30th Annual Meeting of the American Elasmobranch Society, World Congress of Herpetology, Vancouver, BC, Canada.

Nosal AP, et al. (2011). Aggregation behavior of the leopard shark (*Triakis semifasciata*) at the head of La Jolla Submarine Canyon, La Jolla, California, USA. Oral paper presented at the 29th Annual Meeting of the American Elasmobranch Society, Joint Meeting of Ichthyologists and Herpetologists, Minneapolis, MN, USA. ***Samuel H. Gruber Award for Best Student Oral Paper**

Nosal AP, et al. (2011). Aggregation behavior of the leopard shark (*Triakis semifasciata*) associated with the head of La Jolla Submarine Canyon, La Jolla, California, USA. Oral paper presented at the *First International Conference on Fish Telemetry, Sapporo, Japan*. ***Award for Best Student Oral Paper**

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FIELDS OF STUDY

Behavioral ecology of elasmobranch fishes
Reproductive and population genetics of elasmobranch fishes
Marine conservation

ABSTRACT OF THE DISSERTATION

Demography, movement patterns, and mating system of leopard sharks (*Triakis semifasciata*) aggregating along the open coast of southern California, USA

by

Andrew Phillip Nosal

Doctor of Philosophy in Marine Biology

University of California, San Diego, 2013

Philip A. Hastings, Chair

This dissertation describes the aggregation behavior of the leopard shark (*Triakis semifasciata*) along the open coasts of La Jolla (LJ; primary focus) and Del Mar (DM), California, which are 12 km apart. The male-to-female ratio was 0.0299 in LJ ($n = 140$) and 1.0450 in DM ($n = 45$). Twenty females and 13 males were surgically fitted with coded acoustic transmitters and passively tracked for 1,148 days by an array of seven underwater acoustic receivers spanning 120 km of coastline from San Clemente, California to the Mexican border, including LJ and DM. Female abundance at the LJ receiver was highest in late June – early December and was directly related to sea surface temperature and inversely related to swell height; seasonal arrival to and departure from

LJ was highly synchronous and coincided with the summer and winter solstices, respectively. Eight additional sharks in LJ were externally fitted with continuous acoustic transmitters and actively tracked for up to 48 hours. These sharks were generally confined to a divergence zone of low wave energy, caused by wave refraction by an offshore submarine canyon. Sharks spent 80.0% of the daytime in shallow water (≤ 2 m), with some dispersing at night to deeper (max: 53.9 m) and cooler (min: 12.7°C) water, likely to forage. Male abundance at the DM receiver was highest in late April – early October and was directly related to photoperiod and sea surface temperature. Lastly, sharks were seasonally philopatric; 50.0% of females and 60.0% of males returned annually to LJ and DM, respectively, throughout the three-year study period. These findings suggest the anomalously low water turbulence at the LJ site facilitates the invasion of warm shallow water by pregnant females to increase their body temperatures and accelerate gestation. The proximity of the LJ aggregation site to feeding grounds (e.g., submarine canyon) may further benefit females by increasing foraging efficiency. Finally, multiple paternity was detected in only 36.4% of genotyped litters sampled from mothers collected in LJ ($n = 22$). Thus, the predominance of singly fathered litters (63.6%) and scarcity of males at the LJ site suggest this aggregation may also function as a refuge from males by reducing harassment in the form of excessive mating attempts.

INTRODUCTION TO THE DISSERTATION

Coastal sharks are vulnerable to overfishing and habitat degradation due to their proximity to human populations; recovery from population declines is inherently difficult for sharks because of their slow growth rates, late maturation, and low fecundity (Smith et al. 1998, Musick et al. 2000). Understanding the movement patterns of coastal sharks is essential for designing and implementing effective management strategies (Speed et al. 2010). Although sharks vary greatly in size, morphology, and life history, some general movement patterns are shared among species (Castro 2011). These include diel activity patterns (McKibben and Nelson 1986, Sims et al. 2001, Whitney et al. 2007), daily and seasonal site fidelity (also known as philopatry; Holland et al. 1993, Hueter et al. 2005), sexual and size segregation (Wearmouth and Sims 2008), and seasonal migrations (Bres 1993, Bruce et al. 2006). Encompassing all of these movement patterns is the tendency for many species to form site-specific aggregations comprised of a particular demographic group (e.g., predominantly juveniles or females); these aggregations are often seasonal and occur during the daytime (Klimley et al. 1988, Economakis and Lobel 1998, Hight and Lowe 2007). Aggregation behavior exacerbates the susceptibility of sharks to fishing pressure because of the risk of being captured *en masse* (Jacoby et al. 2011). Elucidating the spatiotemporal dynamics and identifying the proximate causes of shark aggregations (the environmental and physiological factors that directly elicit the behavior) may allow managers to target conservation efforts to specific locations and times as well as predict changes in movement patterns in response to future threats, such as climate change. Management decisions are further reinforced by knowledge of the

ultimate causes of shark aggregations (the function, benefits, or adaptive significance of the behavior), which provide tangible reasons to enact conservation measures, such as protecting foraging, mating, or nursery grounds with no-take reserves.

Predator avoidance may be an important ultimate cause of shark aggregations, as in the case of juveniles aggregating in structurally complex environments (e.g., mangrove prop roots), or turbid, shallow regions of lagoons, bays, and estuaries (Holland et al. 1993, Heupel and Simpfendorfer 2005, Duncan and Holland 2006). Increased foraging efficiency may benefit large planktivorous species that aggregate in areas of zooplankton blooms and fish spawn (Sims and Quayle 1998, Heyman et al. 2001, Rowat et al. 2006, Dewar et al. 2008), as well as species that aggregate near pinniped haulout sites (Ainley et al. 1985, Strong et al. 1992, Klimley et al. 1996). Mixed-sex aggregations may facilitate courtship, allow mate selection for females, and increase copulatory success for males (Carrier et al. 1994, Economakis and Lobel 1998, Sims et al. 2000, Pratt and Carrier 2001, Whitney et al. 2004). Mature females may aggregate in warm shallow water to accelerate embryonic development and avoid harassment from males in the form of excessive mating attempts (Economakis and Lobel 1998, Wallman and Bennett 2006, Hight and Lowe 2007). Sharks may also aggregate to rest or to remain central to foraging grounds (Klimley and Nelson 1981, Klimley and Nelson 1984, Klimley et al. 1988, Hearn et al. 2010, Bessudo et al. 2011). Lastly, group living, regardless of location, may confer the classical benefits of dilution (“selfish herd”; Hamilton 1971), heightened vigilance (“early warning”; Lazarus 1979), and predator confusion (Landeau and Terborgh 1986). Proposed proximate causes of shark aggregations include mutual attraction to resources (e.g., food, favorable habitat; see above), conspecifics (for review,

see Jacoby et al. 2011), or recognizable landmarks (e.g., seamounts; Klimley and Nelson 1984). Changing light levels between day and night or endogenous circadian clocks may drive diel patterns of aggregation behavior (Nelson and Johnson 1970, Nixon and Gruber 1988), while seasonal patterns of aggregation behavior may be driven by changes in water temperature, salinity, or dissolved oxygen (Hight and Lowe 2007, Carlisle and Starr 2009).

This dissertation investigates the movement patterns and causes of an aggregation of leopard sharks (*Triakis semifasciata*) that forms seasonally at the southern end of La Jolla Shores beach in San Diego County, California. *T. semifasciata* is a nearshore benthic species, endemic to the western coast of North America and occurring from Samish Bay, Washington, USA to Baja California, Mexico (Ebert 2003, Farrer 2009, Castro 2011). The leopard shark reproduces annually, and gives birth in April – June after a 10 – 11 month gestation period (Ebert 2003, Castro 2011). Leopard sharks are both commercially and recreationally fished and underwent a population decline in southern California during the 1980s and 1990s due to overfishing; the population appears to have since rebounded as a result of the 1994 ban on nearshore (< 3 miles from shore) bottom-set gill nets in southern California (Pondella and Allen 2008). Leopard sharks are known to aggregate in bays and estuaries throughout northern California (Smith and Abramson 1990, Webber and Cech 1998, Hopkins and Cech 2003, Ebert and Ebert 2005) and sheltered coves around the Channel Islands in southern California (Manley 1995, Hight and Lowe 2007). This dissertation is unique in that it describes aggregation behavior of *T. semifasciata* along the open coast in relation to distinct bathymetric (e.g., submarine canyon) and hydrographic features (e.g., alongshore

variation in wave height). The presence of leopard sharks at the aggregation site was first documented in the collection field notes of Carl Leavitt Hubbs in 1950 (Marine Vertebrate Collection, Scripps Institution of Oceanography), although longtime La Jolla residents report seeing “dozens of leopard sharks” at this site as early as 1943 (J. Garfield, pers. comm.). The aggregation site was protected in 1971 by the establishment of the no-take San Diego – La Jolla Ecological Reserve (Parnell et al. 2005). Since then, a thriving ecotourism industry has developed around this reserve offering snorkel and kayak tours and gear rentals to see “Leopard Shark City,” as the aggregation is known and marketed to visitors.

The overarching goals of this dissertation were to resolve the ultimate and proximate causes of this aggregation and assess the effectiveness of the no-take reserve in protecting these sharks. The objectives of Chapter 1 were to determine the demographic composition of the aggregating sharks and to relate their short-term, fine-scale movement patterns (ascertained by active acoustic telemetry) to various environmental characteristics of the site. The objectives of Chapter 2 were to assess the long-term usage patterns of the aggregation site by leopard sharks (ascertained by passive acoustic telemetry) and to relate these to seasonally fluctuating environmental variables, which could potentially cue the behavior. Finally, the objectives of Chapter 3 were to determine the percentage of leopard shark litters from mothers collected from the La Jolla aggregation that were fathered by multiple males and to relate this to observed movement patterns of males and females in the area.

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CHAPTER 1: DEMOGRAPHY AND FINE-SCALE MOVEMENT PATTERNS OF LEOPARD SHARKS (*TRIAKIS SEMIFASCIATA*) AGGREGATING NEAR THE HEAD OF A SUBMARINE CANYON IN SOUTHERN CALIFORNIA, USA

ABSTRACT

The demography, spatial distribution, and movement patterns of leopard sharks (*Triakis semifasciata*) aggregating near the head of a submarine canyon in La Jolla, California, USA, were investigated to resolve the causal explanations for this and similar shark aggregations. All sharks sampled from the aggregation site ($n = 140$) were sexually mature and 97.1% were female. Aerial photographs taken during tethered balloon surveys revealed high densities of milling sharks of up to 5,470 sharks ha^{-1} . Eight sharks were each tagged with a continuous acoustic transmitter and manually tracked without interruption for up to 48 h. Sharks exhibited strong site-fidelity and were generally confined to a divergence (shadow) zone of low wave energy, which results from wave refraction over the steep bathymetric contours of the submarine canyon. Within this divergence zone, the movements of sharks were strongly localized over the seismically active Rose Canyon Fault. Tracked sharks spent most of their time in shallow water (≤ 2 m for 71.0% and ≤ 10 m for 95.9% of time), with some dispersing to deeper (max: 53.9 m) and cooler (min: 12.7°C) water after sunset, subsequently returning by sunrise. These findings suggest multiple functions of this aggregation and that the mechanism controlling its formation, maintenance, and dissolution is complex and rooted in the sharks' variable response to numerous confounding environmental factors.

INTRODUCTION

Elasmobranch fishes are vulnerable to overexploitation because of their slow growth rates, late sexual maturation, and low fecundity (Musick et al. 2000). This vulnerability is exacerbated by the aggregative tendencies of many species and the related risk of being captured *en masse* (Jacoby et al. 2011). The leopard shark (*Triakis semifasciata*) is no exception; it is known to aggregate close to shore throughout California, USA (Smith and Abramson 1990, Ebert and Ebert 2005, Hight and Lowe 2007, Carlisle and Starr 2009), and underwent a population decline in the 1980s and 1990s because of widespread use of nearshore (within three miles) bottom-set gillnets (Pondella and Allen 2008). Although the ban of these nets in 1994 appears to have allowed the population to recover, elucidating the causes of these aggregations remains a priority to ensure this vulnerable species is managed effectively in the future (Pondella and Allen 2008).

Aggregation behavior in elasmobranch fishes can be explained evolutionarily (ultimate causes) by addressing the adaptive significance or function of the behavior, as well as mechanistically (proximate causes) by addressing the immediate environmental and physiological factors that drive the behavior. For example, aggregations of sharks and rays may result from mutual attraction to conspecifics, favorable habitat, or prey (proximate causes); however, the behavior is maintained by natural selection because individuals garner related benefits such as mating opportunities, shelter, and increased foraging efficiency (ultimate causes) (Jacoby et al. 2011). Previous studies on leopard sharks inhabiting bays and estuaries suggest aggregations may form in areas where prey is abundant (Russo 1975, Talent 1976, Webber and Cech 1998, Carlisle and Starr 2009)

and water temperature is high, thus accelerating gestation in pregnant females (Manley 1995, Hight and Lowe 2007). Bays and estuaries are also believed to function as nursery and pupping grounds for leopard sharks (Ackerman 1971, Talent 198, Carlisle et al. 2007, Carlisle and Starr 2009).

In contrast to previous work conducted in bays and estuaries, the present study is the first to investigate a leopard shark aggregation occurring along the open coast, which shows no obvious source of food, shelter, or mating benefits, and thus the ultimate and proximate causes of the aggregation are not readily apparent. To elucidate the causes of this aggregation, which forms annually off a sandy beach in the spring, summer, and autumn, the demographic composition and movement patterns of these sharks were investigated and related to potentially attractive biotic and abiotic features of the site. This provided the opportunity to evaluate the potential influences of unique bathymetric, geologic, and hydrographic features of the open-coast site in comparison to those of bays and estuaries, where most leopard shark aggregations occur, and thus refine our understanding of the ultimate and proximate factors causing leopard sharks to aggregate. In addition, because this aggregation site has been protected since 1971 by the small (2.16 km²) no-take San Diego – La Jolla Ecological Reserve, and thus was not subjected to destructive gillnet fishing in the 1980s and 1990s, it also serves as a model for evaluating the effectiveness of small no-take reserves in protecting these and similar aggregations of sharks.

METHODS

The leopard shark aggregation occurs at the southern end of La Jolla Shores beach (32.8525°, -117.2623°) in San Diego County, California, near the head of La Jolla Submarine Canyon and over the seismically active Rose Canyon Fault. The fault approximately separates two distinct habitats present at the aggregation site: rocky reef and sand flat (Fig. 1.1).

Demographic Survey

To determine the demographic composition of the aggregating leopard sharks, 140 individuals were captured by hook and line from a 5 m skiff, measured for total length (TL), sexed, and tagged with a spaghetti identification tag (Floy Tag FIM-96). Sharks were released < 5 min after being hooked. Seventeen female sharks were transported to Scripps Institution of Oceanography (SIO) to determine pregnancy by allowing the shark to give birth naturally, conducting an ultrasound (Philips Sonos 5500), or dissection. Five sharks that were found dead on the beach were salvaged, dissected, and sampled as per above. Sampling occurred in June – November of 2007 – 2011, when the leopard sharks were present at the aggregation site, and conducted over several years to ensure findings were representative of the aggregation and not the result of sampling during an atypical year.

Aerial Photography

To observe directly the spatial distribution, abundance, and orientation of leopard sharks at the aggregation site, aerial photographs were taken automatically every 1.25 s

using a digital camera (Canon Powershot SD 780 IS), mounted beneath a 1.8 m diameter helium-filled balloon (Arizona Balloon Company) tethered 45 m above the water surface. Photographs were corrected for color, contrast, and exposure in Adobe Photoshop CS2 and imported into ArcMap 10 (ESRI) for scaling and spatial analysis. The geographical position of each shark was defined as the midpoint between the tips of the pectoral fins. Photographs were scaled using the shadow of the balloon on the water surface; its diameter was measured beforehand over the beach by marking the shadow's edges in the sand. Circular statistics were conducted in Oriana v. 3 (Kovach Computing Services). Approximately 5,000 photographs were taken over three days of aerial surveys on 18 and 19 August and 27 September 2010.

Tagging and Tracking

Eight leopard sharks (7 females and 1 male) were each externally fitted with a continuous acoustic transmitter with temperature and pressure sensors (VEMCO V16TP, 51-78 kHz, 1000 ms period) using a nylon dart (Floy Tag FIM-96) inserted into the musculature and through the radials at the base of the first dorsal fin. Active tracking commenced immediately following release from the 5 m skiff using a gunnel-mounted rotatable directional hydrophone (VEMCO VH110) coupled to an onboard acoustic receiver (VEMCO VR100), which continuously decoded and displayed water temperature and depth readings from the shark-borne transmitter. These readings, along with determinations of geographical position (Garmin GPSmap76) of the tracking vessel, which by convention were taken to be the position of the shark, were manually recorded at 5 min intervals during the tracking period. Tracked sharks 1 – 7 were tagged at the

aggregation site and shark 8 was tagged near the head of Scripps Canyon (Fig. 1.1D). Tracks occurred between 4 August 2008 and 13 October 2010.

Data Analyses

Positional fixes from tracked sharks were used to calculate fixed kernel utilization distributions (KUDs) (Worton 1989) in Biotas v 2.0 (Ecological Software Solutions) using the least squares cross validation (LSCV) option for the smoothing parameter, h . The 95% probability contours (total activity space, 95% KUD) and 50% probability contours (core area, 50% KUD) were calculated for each tracked shark and imported as shape files into ArcMap 10 (ESRI) for spatial analysis and map assembly. Daytime and nighttime 95% and 50% KUDs were calculated for each shark and the mean areas were compared between day and night (delineated by local times of sunrise and sunset, United States Naval Observatory Data Services) using a Mann-Whitney U Test. A total 95% and 50% KUD (KUD_{TOT}) was constructed for sharks tagged at the aggregation site by pooling the positional fixes from sharks 1 – 7. Rate of movement (ROM) was calculated for each shark at 5 min intervals and compared between periods of day and night using a Mann-Whitney U Test. The inherent autocorrelation of spatial observations was retained; destructive subsampling of tracking data has been shown to be an ineffective means of reducing autocorrelation and serial independence of observations is not necessary for KUD calculations (de Solla et al. 1999).

Habitat preference within the 95% KUD_{TOT} was determined for sharks 1 – 7 (rocky reef vs. sand flat; habitat map layer accessed on 11 January 2011 from <http://seafloor.csUMB.edu>). The total number of observed positional fixes over rocky reef

and sand flat was compared to the expected number based on their respective areas within the available space (defined as the 95% KUD_{TOT}) using a Chi-Squared (χ^2) Test. This analysis was repeated for positional fixes pooled hourly and by tidal height (0.2 m bins, relative to Mean Lower Low Water, MLLW) to determine whether habitat preference was affected by time of day or tide.

To relate the general alongshore distribution of positional fixes to the location of the Rose Canyon Fault (location taken from the Quaternary Faults and Folds Database of the United States Geological Survey), a best-fit line was calculated for the positional fixes inside the 95% KUD_{TOT}. The positional fixes were then projected onto the best-fit line in 10 m bins to construct a density histogram.

The locations of the total 50% and 95% KUDs were also related to local wave height. The wave climate at the aggregation site and surrounding area was modeled using the SWAN (*Simulating Waves Nearshore*) wave model (Booij et al. 1999), which solves a spectral wave action balance equation to compute wave transformation from deeper water depths into the nearshore region, and produces values of significant wave height (H_s) at a resolution of 15 m alongshore and 10 m cross-shore. The effect of wave shoaling, refraction, and depth-induced wave breaking via the parameterization of Battjes (1978) with constant breaking coefficient of 0.73 were included but not the effect of non-linear wave-wave interactions. Bathymetry for the model domain was derived from multiple sources (Long and Özkan-Haller 2005) and merged using a scale-controlled interpolation routine (Plant et al. 2002). The hourly tidal elevations were obtained by the NOAA tide gauge at the SIO pier and included in each simulation. The offshore directional wave spectra used to initialize the model were taken from the Outer Torrey

Pines directional wave buoy (maintained by the Coastal Data Information Program at SIO) and computed using the Maximum Entropy Method (Lygre and Krogstad 1986). Homogeneous spectra were prescribed along the western and northern model boundaries. The cross-shore variation of the incident spectra along the southern boundary of the domain is approximated assuming refraction over straight and parallel contours, which was computationally efficient and, when compared with data from previous field experiments, provided more accurate nearshore wave conditions near the aggregation site when offshore wave environments were dominated by a southerly swell. The SWAN model has been used in previous studies to estimate wave characteristics along this section of coast with favorable agreement (e.g., Gorrell et al. 2011).

For tracks 1 – 7, the modeled hourly wave conditions were averaged to generate track-specific mean wave climates, which were then averaged to generate a mean wave climate for all tracks. The 95% KUD_{TOT} and 50% KUD_{TOT} were georeferenced and superimposed over this overall mean wave climate. Modeled H_S values were isolated and averaged in each of the following areas: 1) inside the 50% KUD_{TOT} , 2) inside the 95% KUD_{TOT} but outside the 50% KUD_{TOT} , and 3) in the “vicinity” surrounding the 95% KUD_{TOT} bounded by the 10 m isobath between Point La Jolla and SIO pier.

RESULTS

Demographic Survey

Of the 140 sharks sampled, 97.1% were female and 2.9% were male. Mean $TL \pm SD$ was 138.9 ± 10.6 cm for females (range: 110 – 164 cm) and 130.7 ± 15.6 cm for males (range: 116 – 147 cm); all sharks were sexually mature according to Kusher et al.

(1992). When pregnancy was ascertained ($n = 22$), all females were found to be pregnant.

Aerial Survey

Aerial photographs showed that the aggregation typically consisted of distinct high-density and low-density areas (Fig. 1.2A), with the former often occurring near or inshore of the surf line (Fig. 1.2B). Using Figure 1.2A as an example, the minimum convex polygon (MCV) comprising the high-density area contained 92 sharks at 5,470 sharks ha^{-1} , of which 87.0% were < 1 mean body length ($L = 138.9$ m) from their nearest neighbor. In contrast, the MCV comprising the low-density area contained only 20 sharks at 581 sharks ha^{-1} , of which none were < 1 L from their nearest neighbor. The largest shark count in any single photograph was 125, however, additional sharks were likely outside the field of view. Sharks were in constant motion and exhibited very few conspicuous formations or behaviors except for brief bouts (a few seconds, visible in no more than 5 – 10 sequential photographs) of circling, following, and occasionally turning over to expose their white ventral surfaces (see Klimley 2003, Smith 2005; Fig. 1.2C). The sharks' movements are best described as milling, lacking any obvious unimodal polarization (i.e., sharks were not oriented in the same direction). However, the distribution of shark headings was bimodal and significantly oriented to the axis parallel to the swell (Rao's Spacing Test, $p < 0.05$).

Active Tracking

In total, leopard sharks were tracked for 272.4 h and 223.3 km (Table 1.1). Sharks 1 and 8 traveled north along the coast to Flat Rock; shark 1 later returned to the aggregation site (Figs. 1.3A – B). Sharks 2, 3, 6, and 7 remained near the aggregation site for the entire track (Figs. 1.3C – F). Sharks 4 and 5 dispersed from the aggregation site at sunset (Figs. 1.3G – H), traveling along the bottom to the deeper and cooler water of the submarine canyons (Figs. 1.4A – B), before returning to the aggregation site by sunrise. Mean 5-min ROM pooled for all sharks was significantly higher at night ($0.86 \pm 0.61 \text{ km h}^{-1}$) than during the day ($0.77 \pm 0.46 \text{ km h}^{-1}$) (Mann-Whitney *U* Test, $p < 0.05$), however, the mean areas of individual 95% and 50% KUDs did not differ significantly between day and night (Mann-Whitney *U* Test, $p > 0.05$). The sharks spent most of their time in shallow water ($71.0\% \leq 2 \text{ m}$, $95.9\% \leq 10 \text{ m}$), more so during the day ($80.0\% \leq 2 \text{ m}$, $99.8\% \leq 10 \text{ m}$) than at night ($61.4\% \leq 2 \text{ m}$, $91.7\% \leq 10 \text{ m}$), and were located significantly deeper in the water column at night (mean $\pm$ SD = $4.24 \pm 8.14 \text{ m}$) than during the day (mean $\pm$ SD = $1.55 \pm 1.62 \text{ m}$) (Mann-Whitney *U* Test, $p < 0.05$).

The areas of the total 95% and 50% KUDs (KUD_{TOT}), calculated from positions pooled from sharks 1 – 7 (shark 8 was excluded because it was not tagged at the aggregation site), were 0.347 km^2 (28.7% located over rocky reef and 71.3% over sand flat) and 0.038 km^2 (17.0% over rocky reef and 83.0% over sand flat), respectively (Fig. 1.5A). The times at which positions were recorded over rocky reef and sand flat were significantly oriented (Rayleigh Test, $p < 0.05$); mean circular time $\pm$ SD was $0900 \pm 0519 \text{ hrs}$ for rocky reef and $1826 \pm 0823 \text{ hrs}$ for sand flat (Fig. 1.5B), approximately coinciding with the times of mean daily minimum (0900 hrs) and maximum (1700 hrs)

sea surface temperature measured at SIO pier for the months of August – October from 2008 – 2010 (archived data from NOAA Tides and Currents). Sharks were significantly biased toward the rocky reef between 0700 and 1200 hrs and toward the sand flat between 1600 and 2100 hrs (Chi-Squared Test, $p < 0.05$; Fig. 1.5C). Sharks were also significantly biased toward the rocky reef at intermediate tides (1.0 – 1.4 m above MLLW) and toward the sand flat at high (1.6 – 2.0 m above MLLW) and low tides (0.0 – 0.6 m above MLLW) (Chi-Squared Test, $p < 0.05$).

The 95% KUD_{TOT} was bounded in the cross-shore direction by the shoreline and the rim of La Jolla Canyon and alongshore by the divergence (shadow) zone of smaller wave height caused by bathymetric refraction of wave rays away from the canyon head (Fig. 1.6). Mean significant wave height (H_s) $\pm$ SD was 41.5 ± 2.6 cm (range: 33.7 – 47.0 cm) inside the 50% KUD_{TOT}, 47.1 ± 12.2 cm (range: 22.5 – 86.7 cm) inside the 95% KUD_{TOT} but outside the 50% KUD_{TOT}, and 66.9 ± 14.0 cm (range: 11.6 – 113.9 cm) in the vicinity (from Point La Jolla to the SIO pier, inshore of the 10 m isobath) surrounding the 95% KUD_{TOT}, indicating a bias toward the areas with the lowest wave height.

The best-fit line through the 2,596 acoustic tracking positional fixes within the 95% KUD_{TOT} was oriented 41.3° from north and had a coefficient of determination (R^2) of 0.753 (Fig. 1.5A). Figure 7 shows the largest peak of the density histogram along this line occurred at a distance of 510.0 m from the southwestern endpoint, < 1 m from where the Rose Canyon Fault intersected the line at 510.6 m at a nearly perpendicular angle of 88.8° .

DISCUSSION

This study suggests various ultimate and proximate causes for the La Jolla open-coast leopard shark aggregation, some of which resemble previously proposed causes for aggregations in bays and estuaries, while others appear unique to this site. These putative explanations for the leopard shark aggregation, taken together with the movement patterns of individuals in relation to the boundaries of the San Diego – La Jolla Ecological Reserve, provide compelling evidence for the effectiveness of small reserves in protecting leopard sharks and other aggregating species.

Ultimate Causes

The aggregation is site-specific and appears to function as a “home base” to which leopard sharks return regularly to exploit unique environmental features of the site and its proximity to foraging grounds. The head of La Jolla Canyon and the adjacent sandy shelves are known spawning grounds for market squid (*Loligo opalescens*) (McGowan 1954), which dominated the diet of leopard sharks sampled non-destructively from the aggregation site during the course of the study (M. Royer unpubl. data). Tracking data for sharks 4 and 5 (Fig. 1.3G – H) showed nocturnal excursions into deeper water presumably to feed on this abundant prey item. This concept of returning to a “home base” near foraging grounds has been suggested for other elasmobranch species, including scalloped hammerheads (*Sphyrna lewini*) that aggregate at seamounts and oceanic islands (Klimley et al. 1988, Hearn et al. 2010, Bessudo et al. 2011).

In addition, the aggregation site is located in a wave divergence, or “shadow” zone, due to wave refraction over the La Jolla Canyon (Munk and Taylor 1947, Magne et

al. 2007; Fig. 1.6). The locally reduced wave action and mixing results in a higher water temperature than other areas along the coast (e.g., the aggregation site is on average ~ 0.5 °C warmer than 1.8 km north at the SIO pier; Kobayashi 1979). The exceptionally warm water and predominance of pregnant females at this site are consistent with the “incubation hypothesis,” that female elasmobranch fishes behaviorally thermoregulate to accelerate embryonic development (Economakis and Lobel 1998, Hight and Lowe 2007, Mull et al. 2010, Jirik and Lowe 2012). Aggregating in calm surf might also allow the sharks to conserve energy, requiring fewer locomotory adjustments in response to breaking waves and turbulence. The sharks’ bias toward the rocky reef when overall water temperature was coldest (~ 0900 hrs) and toward the sand flat when overall water temperature was warmest (~ 1700 hrs) suggests they may be exploiting the thermal heterogeneity of this area to maintain an optimal body temperature. Preliminary water temperature data indicate the rocky reef habitat is consistently warmer (> 0.2 °C) than the sand flat due to the topographical trapping of warm surface water by prevailing onshore winds, decreased mixing because of the complex rocky bathymetry, and increased absorption of light and radiation of heat by the darker substrata.

The scarcity of males at the site suggests the aggregation could function as a refuge for females by reducing physically costly superfluous mating attempts (Pratt and Carrier 2001, Sims et al. 2001, Ebert and Ebert 2005, Hight and Lowe 2007). Thus, mating-related functions proposed for other shark aggregations (i.e., facilitating courtship, allowing mate selection for females, and increasing copulatory success for males) do not seem to apply (Carrier et al. 1994, Economakis and Lobel 1998, Pratt and Carrier 2001, Whitney et al. 2004). The absence of juvenile leopard sharks in the

demographic survey is consistent with aerial surveys and *in situ* snorkeling observations that individuals under 1 m TL are uncommon at the aggregation site. However, neonate sharks could be highly cryptic and able to hide amongst the rocks and surf grass of the rocky reef. Thus, although other leopard shark aggregations have been hypothesized to serve as pupping grounds (Ackerman 1971, Russo 1975, Talent 1985, Carlisle and Starr 2009), the extent to which the La Jolla site serves as such remains unknown, requiring more extensive sampling and exploration of the rocky reef.

Historically, a sheltered estuarine lagoon existed at the aggregation site, which likely provided additional benefits of shelter, warmth, and food for the sharks. Evidence for this lagoon is a ~10 m thick deposit of estuarine material throughout the head of La Jolla Canyon, containing finely laminated mud, silt, clay, and gypsum, mingled with driftwood, reeds, fibrous root material, and fossils of the brackish water ostracod (*Perissocytheridea meyerabichi*) (Shepard and Dill 1966, Holden 1968, Judy 1987, Le Dantec et al. 2010). Historical photographs from the early 1900s show this lagoon (Moriarty 1964), which has since been reduced to a small duck pond (Fig. 1.1A). Although this location once resembled the sheltered bays and estuaries where leopard shark aggregations usually occur (Smith and Abramson 1990, Ebert and Ebert 2005, Hight and Lowe 2007, Carlisle and Starr 2009), it is unlikely that the current aggregation is merely a vestige of sharks still “searching” for an ancient lagoon; rather, the present-day open-coast site continues to attract sharks because it provides the aforementioned benefits to those aggregating there.

For aggregation behavior at this site to be maintained, the benefits should outweigh the costs, namely predation risk. Hight and Lowe (2007) suggested that

aggregating might make leopard sharks more susceptible to predation by male California sea lions (*Zalophus californicus*), which are known to capture and eviscerate sharks to consume their fatty visceral organs. We observed three such mortalities at the La Jolla aggregation site in November 2009. Nevertheless, the yearly occurrence of this aggregation suggests the cost of predation does not outweigh the benefits, and certain features of this site may actually mitigate predation risk (e.g., access to hiding places in the rocky reef and surf zone).

Proximate Causes

The mechanism governing the formation, maintenance, and dispersion of the leopard shark aggregation is rooted in the interaction between environmental stimuli that elicit the behavior and the responsiveness of the sharks to those stimuli. For example, sunlight appears to be an important extrinsic stimulus, particularly sunset, which elicits some leopard sharks to disperse from the aggregation site (Fig. 1.3G – H). The aggregation could also form and be maintained through mutual attraction to favorable environmental conditions and avoidance of unfavorable conditions. For example, rip currents forced by wave divergence over La Jolla Canyon are generated at the approximate northern boundary of the 95% KUD_{TOT} (Shepard and Inman 1950, see Fig. 8-6 in Komar 1998). Thus, the greater water turbulence adjacent to the aggregation may have a confining effect contributing to the location and size of the home range.

However, being attracted to warm, calm water is inadequate to explain the highest concentration of shark movements directly above the Rose Canyon Fault (Fig. 1.7), which may bear an attractive feature, such as a magnetic anomaly or groundwater

discharge. Cross-strike anomalies in total magnetic intensity have been reported for other intrasedimentary faults, attributable to tectonic juxtaposition of sedimentary layers with differing magnetic properties (Gunn 1997, Grauch et al. 2001, Grauch et al. 2007), and sharks are known to detect magnetic fields (Kalmijn 1982) and have long been hypothesized to use geomagnetic cues as referential landmarks (Klimley 1993). Sharks could also cue into groundwater discharge, which is visible throughout the study area as a blurry mixing of fresh and saltwater (E. Parnell, pers. comm.), and can likely locally depress salinity. However, the extent of submarine groundwater discharge and geomagnetic anomalies in this area has not been quantified.

Summary of Ultimate and Proximate Causes of Leopard Shark Aggregations

Previous studies of leopard shark aggregations reveal their formation in calm, warm water near productive foraging grounds in sheltered bays and estuaries (Smith and Abramson 1990, Ebert and Ebert 2005, Hight and Lowe 2007, Carlisle and Starr 2009). The La Jolla aggregation site is thus unique in being located on the open coast; however, it is similarly sheltered by wave divergence in the lee of a submarine canyon, which results in the calmest and warmest water along the immediate coastline. The demographic composition and movement patterns of leopard sharks aggregating in La Jolla closely resemble those of another aggregation that forms in a sheltered cove on Santa Catalina Island, California (Manley 1995, Hight and Lowe 2007), where mature females also spend the day in warm, shallow water and often disperse at night, presumably to forage. Similar to the present study, Hight and Lowe (2007) observed that leopard sharks at their site selectively occupy the warmest water in the cove, suggesting

these females might also be regulating their body temperature to accelerate gestation. Other leopard shark aggregations, such as in Elkhorn Slough and San Francisco Bay, California, likely also function as nursery and mating areas, where both males and females occur along with immature individuals (Smith and Abramson 1990, Carlisle et al. 2007, Carlisle and Starr 2009). The congregation of these sharks on intertidal mudflats also suggests their potential exploitation of rich on-site foraging grounds (Carlisle and Starr 2009), consistent with the proposed function of the La Jolla site serving as a central location close to abundant food sources (e.g., the submarine canyon).

In conclusion, ultimate causes of leopard shark aggregations appear related to 1) behavioral thermoregulation, 2) maintaining a “home base” close to nearby foraging grounds, and 3) reproduction (avoiding harassment from males in female-dominated aggregations, finding mates in mixed-sex aggregations, or pupping). In addition to sunlight, which may govern the daily formation and dissolution of some leopard shark aggregations, the most likely proximate cause is mutual attraction to 1) low water turbulence, 2) warm water temperatures, 3) food, and, possibly, 4) other unique stimuli associated with local bathymetry or other site-specific features (e.g., magnetic anomalies, salinity gradients).

Conservation Implications

This study demonstrates that leopard shark aggregations can be densely populated and occur very close to shore, making them extremely vulnerable to overexploitation. Given the demographic composition of mostly pregnant females at sites such as the one described in this manuscript, local, concentrated extirpation could negatively affect the

wider population. The individual and total 95% KUDs of all tracked sharks tagged at the La Jolla open-coast aggregation site fell within the boundaries of the no-take San Diego – La Jolla Ecological Reserve, suggesting this small (2.16 km²) sanctuary is effective at protecting the aggregation, at least in the short term, and that other leopard shark aggregations throughout California and Baja California could similarly benefit from small, strategically placed no-take reserves.

Table 1.1: Summary information for eight leopard sharks (*Triakis semifasciata*) actively tracked using acoustic telemetry.

Note:

Abbreviations: FL, fork length; TL, total length; KUD, kernel utilization distribution

Shark ID	Sex (M/F)	Length		Tagging Latitude (N)	Tagging Longitude (W)	Tracking Period (mo/d/yr 24h UTC-7)	Total Contact Time (hh:mm)	Longest Continuous Track (hh:mm)	Mean Depth $\pm$ SD (RANGE) (m)	Mean Temp $\pm$ SD (RANGE) (°C)	Area		Total Distance Traveled (km)
		FL (cm)	TL (cm)								50% KUD (km ²)	95% KUD (km ²)	
1	F	140	157	32.85323	-117.26234	08/25/08 0625 - 08/30/08 1550	41:35	20:45	2.6 $\pm$ 2.4 (0.0 – 13.4)	21.8 $\pm$ 1.0 (17.5 – 23.5)	0.073	0.366	43.2
2	F	124	142	32.8527	-117.26246	09/09/08 0810 - 09/11/08 0940	49:30	49:30	1.2 $\pm$ 0.4 (0.8 – 4.2)	22.7 $\pm$ 0.5 (20.5 – 23.9)	0.009	0.073	34.2
3	F	116	132	32.85297	-117.26299	09/16/08 1000 - 09/17/08 1005	24:05	24:05	0.8 $\pm$ 0.5 (0.0 – 3.9)	19.1 $\pm$ 0.6 (17.9 – 19.9)	0.025	0.120	16.8
4	F	128	147	32.85258	-117.26182	09/29/09 0910 - 10/01/09 0910	48:00	48:00	6.5 $\pm$ 11.7 (0.4 – 53.9)	20.3 $\pm$ 2.5 (12.7 – 22.4)	0.005	0.242	41.9
5	F	122	140	32.85283	-117.26182	10/06/09 1005 - 10/08/09 1005	48:00	48:00	3.4 $\pm$ 7.3 (0.1 – 41.3)	18.7 $\pm$ 1.1 (12.9 – 20.2)	0.021	0.218	40.2
6	M	131	147	32.85388	-117.26182	09/26/10 1355 - 09/27/10 2225	32:30	32:30	1.4 $\pm$ 0.7 (0.0 – 5.5)	17.4 $\pm$ 0.6 (15.7 – 19.3)	0.016	0.087	23.2
7	F	128	147	32.85296	-117.26182	10/13/10 1015 - 09/27/10 2225	03:05	03:05	0.9 $\pm$ 0.5 (0.1 – 1.9)	19.3 $\pm$ 0.2 (18.8 – 19.9)	0.015	0.077	2.1
8	F	95	112	32.87335	-117.25323	08/04/08 1100 - 08/07/08 1200	25:40	24:30	3.8 $\pm$ 2.0 (0.0 – 7.7)	22.2 $\pm$ 0.9 (19.9 – 23.7)	0.035	0.330	21.7

Figure 1.1: Study area. **A:** The western United States of America with the state of California (CA) darkened. **B:** Enlarged view of the white box in A showing the coastline of San Diego County, California. **C:** Enlarged view of the white box in B showing the coastline of La Jolla, California extending northward. **D:** Enlarged view of the white box in C showing the study site at the southern end of La Jolla Shores Beach, La Jolla, California along with the San Diego – La Jolla Ecological Reserve boundaries and the Rose Canyon Fault.

Notes:

C and D are shown with the 10, 20, 40, and 80 m isobaths; bathymetry data were acquired, processed, and distributed by the Seafloor Mapping Laboratory of California State University Monterey Bay

Aerial views in C and D are from Google Earth Pro (Imagery Date: 1 February 2008)

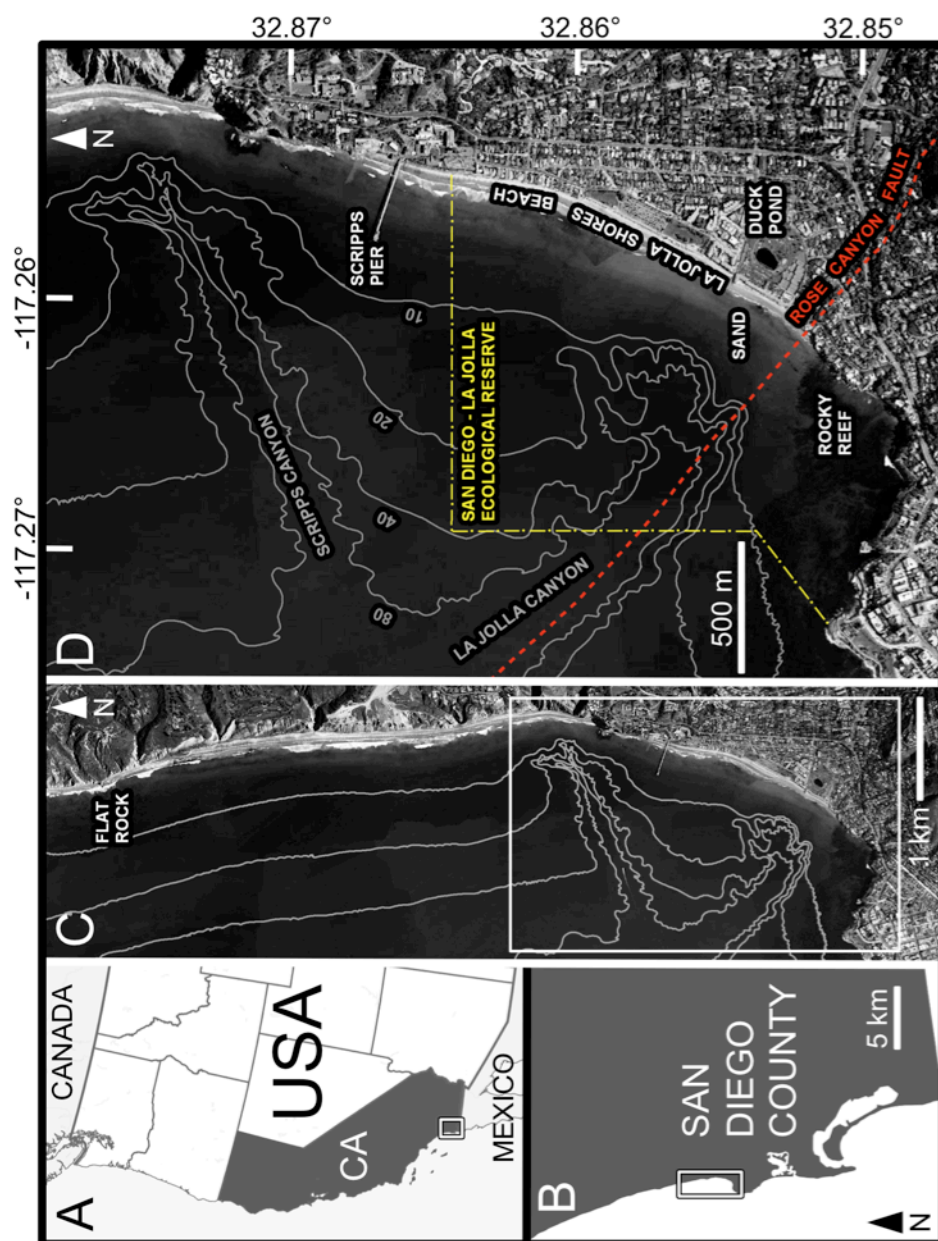


Figure 1.2: Aerial photographs of the leopard shark (*Triakis semifasciata*) aggregation in La Jolla, California. **A:** High- and low-density areas of the aggregation, approximately separated by the incoming wave crest, each bounded by minimum convex polygons. Visible are the tether line and the shadow of the balloon, which was used to scale the photograph. **B:** Aerial photograph showing a wave about to break over the leopard shark aggregation. **C:** Aerial photograph showing a shark ventral-side-up, enclosed by a white box.

Notes:

White arrows in A, B, and C indicate the direction of the shoreline

The scale bar in B is applicable to the entire figure

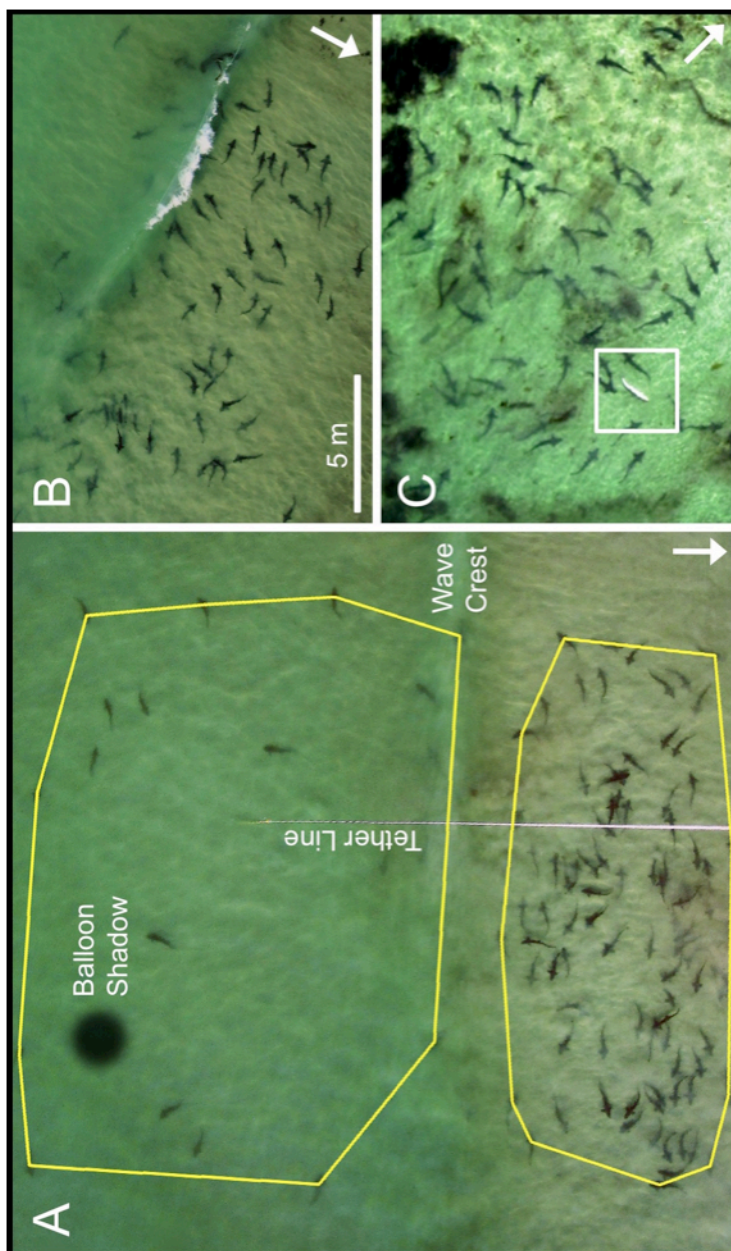


Figure 1.3: Movements of eight leopard sharks (*Triakis semifasciata*) in relation to the aggregation site in La Jolla, CA as determined through active acoustic telemetry. **A:** Shark 1. **B:** Shark 8. **C:** Shark 2. **D:** Shark 3. **E:** Shark 6. **F:** Shark 7. **G:** Shark 4. **H:** Shark 5.

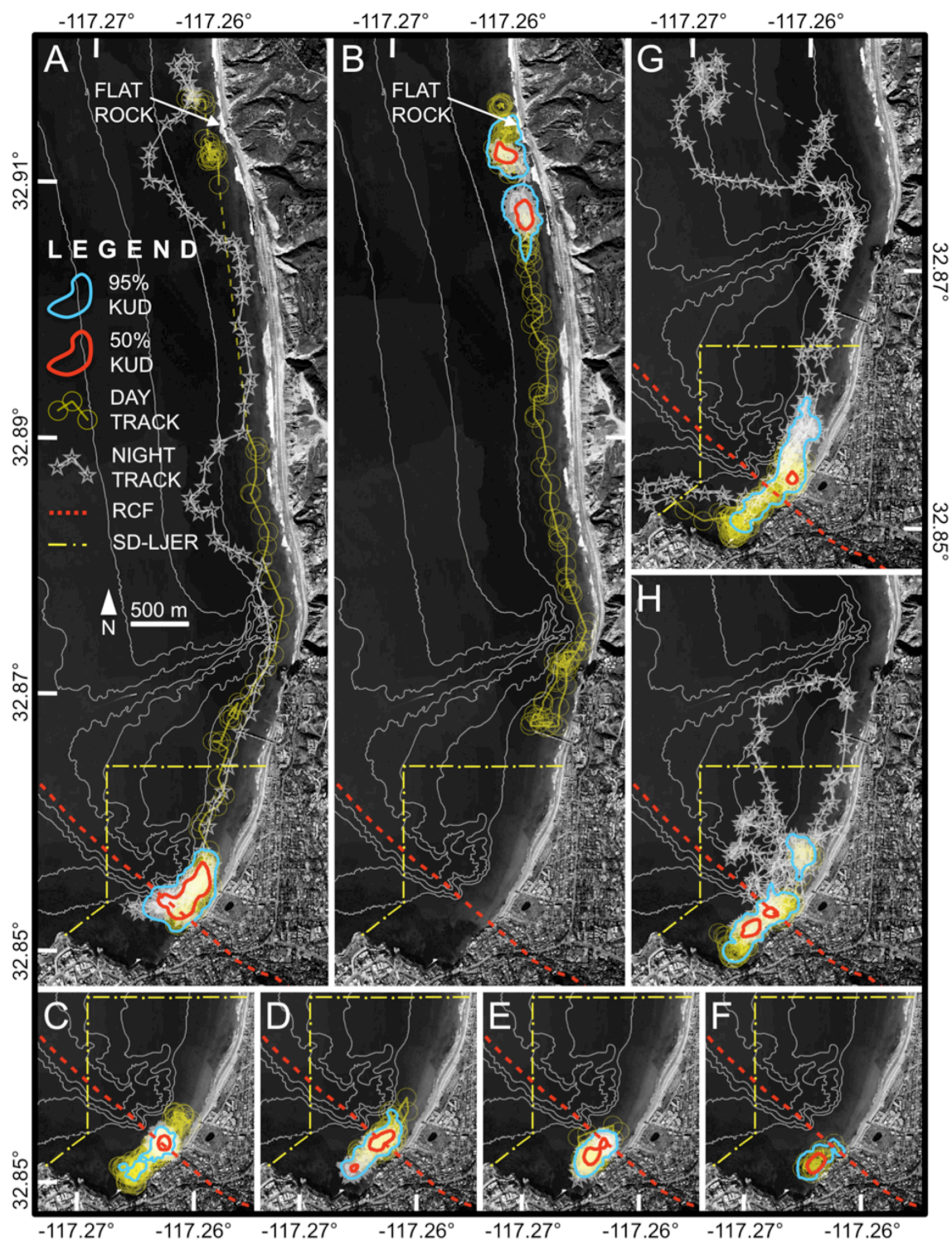
Notes:

See Table 1 for a summary of tracking details for each shark

Abbreviations: RCF, Rose Canyon Fault; SD-LJER, San Diego – La Jolla Ecological Reserve

Semi-transparent dashed lines indicate temporary signal loss with subsequent reacquisition

Isobaths shown are 10, 20, 40, and 80 m; bathymetry data were acquired, processed, and distributed by the Seafloor Mapping Laboratory of California State University Monterey Bay



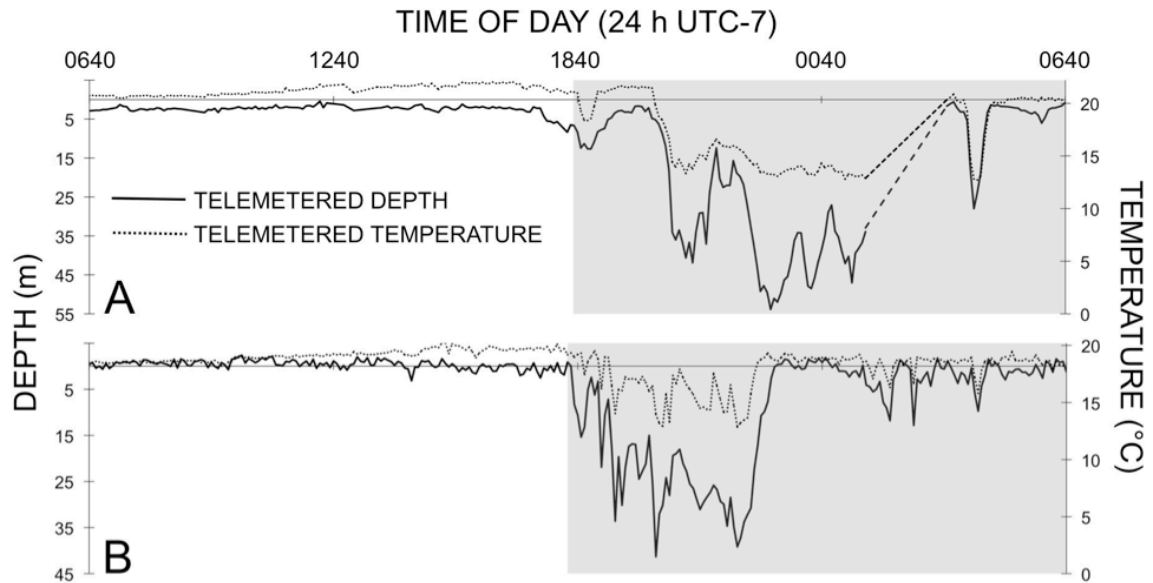


Figure 1.4: Telemetered depth and temperature profiles from a representative 24 h tracking period for leopard sharks (*Triakis semifasciata*) 4 (A) and 5 (B).

Notes:

The dashed line in A corresponds to a temporary loss of signal between 0145 – 0345 hrs

The night period is indicated in gray

Figure 1.5: **A:** Best-fit line through the 2,596 positional fixes (white dots) that occurred inside the total 95% kernel utilization distribution (KUD_{TOT}). **B:** Circular histograms showing the distribution of times of positional fixes that occurred over sand (above) and over rocky reef (below). Circular mean time of positions over each habitat is shown as a red line. **C:** Hourly changes in habitat preference throughout the day as shown by the observed ratio of positions over sand to positions over rocky reef (solid gray line).

Notes:

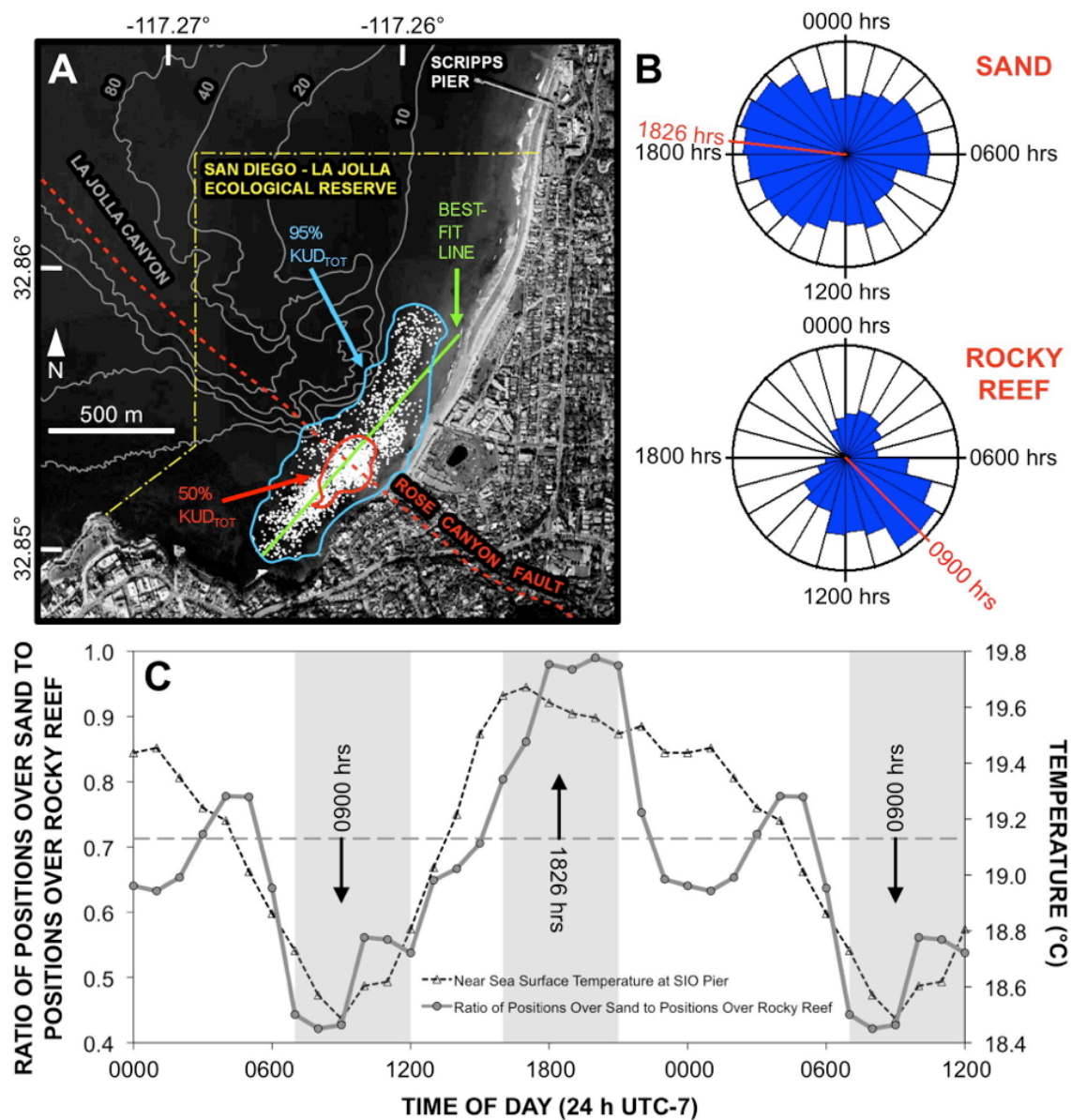
The expected ratio of positions over sand to positions over rocky reef based on the areas of these habitats within the total 95% KUD (sand: 71.3%, rocky reef: 28.7%) is shown by the horizontal dashed gray line

Gray areas indicate the hours when the observed ratio is significantly different from the expected ratio ($\chi^2, p < 0.05$)

Mean circular times of positions over sand (0900 hrs) and rocky reef (1826 hrs) are shown

Mean hourly sea surface temperature from SIO pier, averaged for the months of August – October 2009 – 2010 is shown as a dashed black line

Isobaths shown in A are 10, 20, 40, and 80 m; bathymetry data were acquired, processed, and distributed by the Seafloor Mapping Laboratory of California State University Monterey Bay



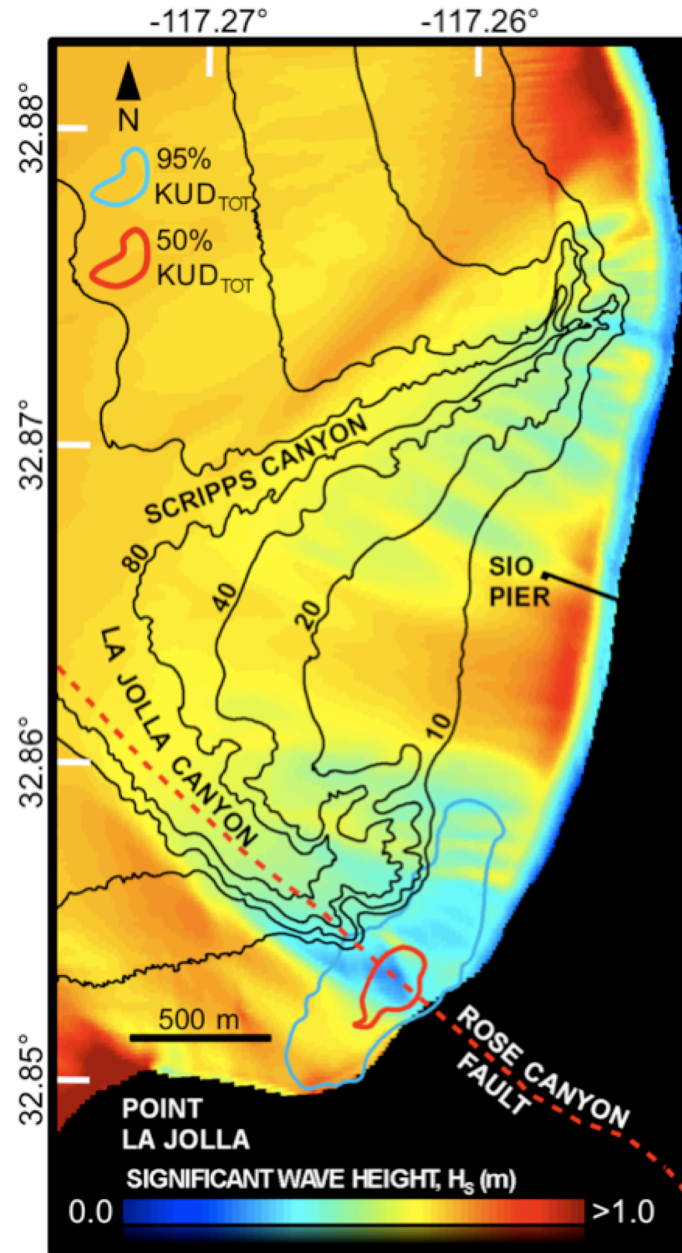


Figure 1.6: Total leopard shark 50% and 95% kernel utilization distributions (KUD_{TOT}) overlaid on the modeled overall mean wave climate for all tracking periods.

Note:

Isobaths shown are 10, 20, 40, and 80 m; bathymetry data were acquired, processed, and distributed by the Seafloor Mapping Laboratory of California State University Monterey Bay

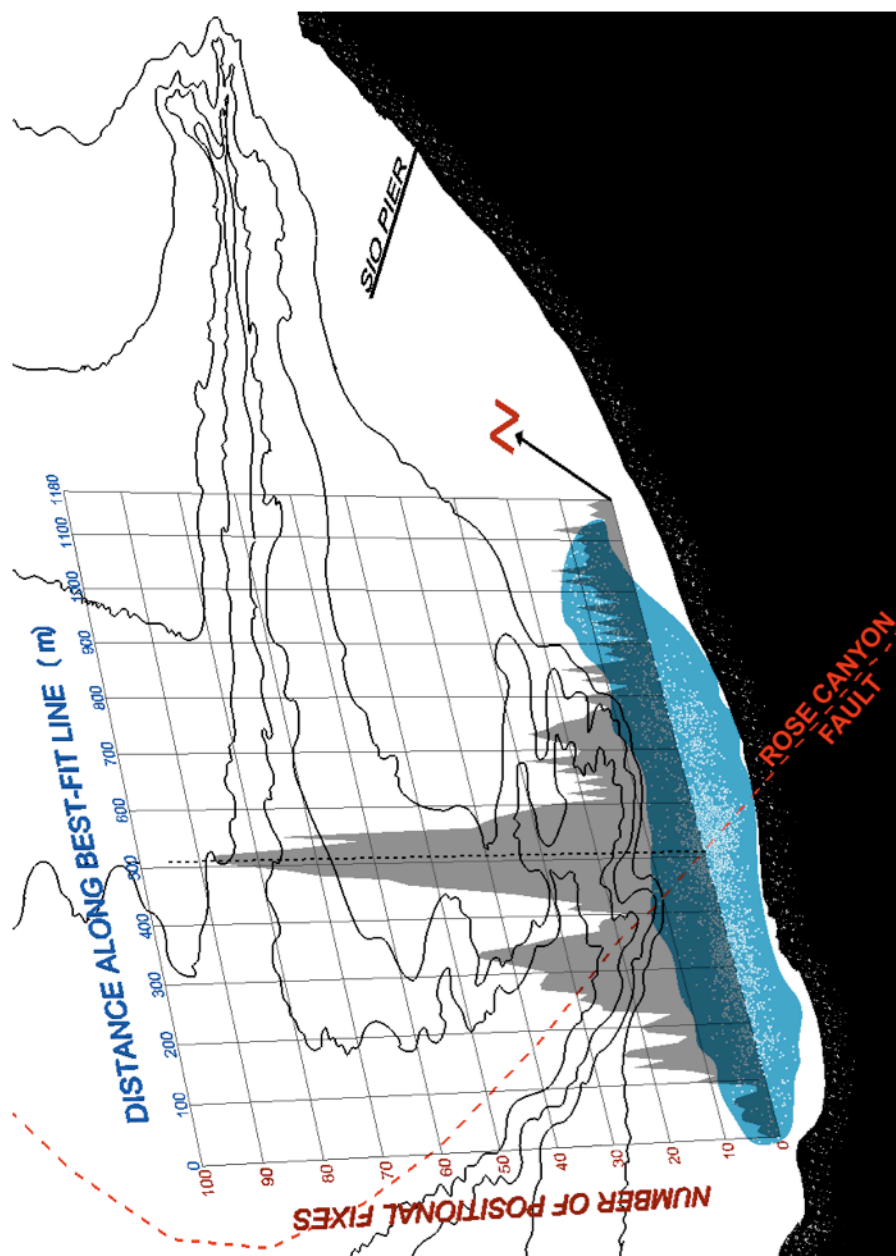
Figure 1.7: Density histogram (semi-transparent gray) of 2,596 positional fixes falling within the total 95% KUD (solid blue area) projected onto the best-fit line of these fixes.

Notes:

Land is indicated as solid black with white stippling along the coastline

Isobaths shown are 10, 20, 40, and 80 m; bathymetry data were acquired, processed, and distributed by the Seafloor Mapping Laboratory of California State University Monterey Bay

The intersection of the best-fit line and Rose Canyon Fault is extended upward by a dashed black line to show the relation of the fault line to the largest peak of the histogram



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ACKNOWLEDGEMENTS

I would like to thank the many people who assisted with this chapter, particularly J. Graham, E. Kisfaludy, N. Wegner, D. Cartamil, J. Long, M. Lührmann, M. Royer, A. Caillat, M. Taylor, M.C. Bernal, L. McCormick, A. Martin, A. Barker, J. Arce, J. Beckman, B. Frossard, J. Renfree, C. Jew, L. Bellquist, N. Ben-Aderet, D. Kacev, and E. Parnell. I would also like to thank my dissertation adviser and committee for constructive feedback: P. Hastings, R. Burton, N. Wegner, D. Woodruff, R. Norris, and M. Tresguerres. This work was conducted under University of California – San Diego Institutional Animal Care and Use Committee (IACUC) protocol No. S00080. Funding was provided by the Scripps Institution of Oceanography Graduate Department, Center for Marine Biodiversity and Conservation, and the Los Angeles Rod and Reel Club Foundation. A. Nosal was supported by Graduate Research Fellowship Program (GRFP) and Integrated Graduate Education and Research Traineeship (IGERT, No. 0333444) awards from the National Science Foundation. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

This chapter, in full, was submitted and accepted for publication as:

Nosal AP, Cartamil DC, Long JW, Lührmann M, Wegner NC, Graham, JB (*in press*). Demography and movement patterns of leopard sharks (*Triakis semifasciata*) aggregating near the head of a submarine canyon along the open coast of southern California, USA. *Environmental Biology of Fishes*, DOI: 10.1007/s10641-012-0083-5. The dissertation author is the primary investigator and author of this manuscript.

CHAPTER 2: LONG-TERM MOVEMENT PATTERNS OF AGGREGATING LEOPARD SHARKS (*TRIAKIS SEMIFASCIATA*) ALONG THE OPEN COAST OF SOUTHERN CALIFORNIA, USA

ABSTRACT

To understand the long-term spatiotemporal dynamics of aggregation behavior of the leopard shark (*Triakis semifasciata*), 20 females and 13 males were surgically fitted with coded acoustic transmitters and passively tracked for over three years by seven underwater acoustic receivers, deployed along 120 km of coastline from San Clemente, California to the Mexican border. Receiver locations included two known aggregation sites in La Jolla and Del Mar, California. Females exhibited strong site fidelity to La Jolla and males to Del Mar; shark abundance was higher at both aggregation sites during the day than at night. Female abundance in La Jolla was highest in late June – early December and was directly related to sea surface temperature and inversely related to swell height; seasonal arrival to and departure from the La Jolla aggregation site was highly synchronous and coincided with the summer and winter solstices, respectively. In contrast, male abundance in Del Mar was highest in late April – early October and was directly related to photoperiod and sea surface temperature; usage of the Del Mar aggregation site by males was generally less synchronous and predictable than that of the La Jolla aggregation site by females. Both sexes exhibited strong seasonal philopatry; 50.0% of females and 60.0% of males returned annually to La Jolla and Del Mar, respectively, during the 3-year study period. Sharks appear to overwinter along the mainland coast south of Los Angeles, offshore near Santa Catalina Island, or remain near to the aggregation sites year-round.

INTRODUCTION

The leopard shark (*Triakis semifasciata*) is a nearshore benthic species that forms aggregations throughout its range along the western coast of North America (Ebert and Ebert 2005, Hight and Lowe 2007, Chapter 1) and is vulnerable to overexploitation, particularly by bottom-set gill nets (Pondella and Allen 2008). Aggregation behavior in sharks, especially when combined with sexual segregation and seasonal philopatry, may locally exacerbate the effects of overfishing and habitat destruction (Heuter et al. 2004, Wearmouth and Sims 2008, Mucientes et al. 2009); elucidating the spatiotemporal details of these behaviors will ensure the design and implementation of effective management strategies. To that end, we used passive acoustic telemetry to quantify long-term abundance of adult leopard sharks at two aggregation sites along the mainland coast of southern California.

The first site was La Jolla, where mostly pregnant females (97.1%; Chapter 1) are known to aggregate in shallow water adjacent to shore; this site is protected by the no-take Matlahuayl State Marine Reserve (formerly the San Diego – La Jolla Ecological Reserve). Although the fine-scale, short-term (over several days) movement patterns of these females were resolved in Chapter 1, the long-term (over several years) patterns of diel and seasonal occurrence at the aggregation site remained unknown. Furthermore, the behavior of males was unknown because they were largely absent from this site. Thus, the second site was chosen to be 12 km north of La Jolla in Del Mar, where both females and males were suspected to aggregate due to frequent captures reported in online fishing forums; this site is not protected. The sampling periods of previous studies monitoring leopard sharks at other aggregation sites have not exceeded one full year (Hight and

Lowe 2007, Carlisle et al. 2009), thus precluding any long-term determination of seasonal philopatry and environmental factors related to shark movements. Furthermore, the movements of male leopard sharks have never been systematically measured; this study is the first to compare and contrast movement patterns between the sexes for this species.

The objectives of this study were 1) to quantify sex-specific aggregation behavior on a daily and seasonal time scale, 2) to quantify long-term (> 3 yr) seasonal philopatry at the aggregation sites, and 3) to identify environmental predictors of aggregation behavior. Leopard sharks were hypothesized to be most abundant at the aggregation sites during the daytime of the summer months. Numerous reports of male-mediated gene flow in elasmobranch species (Pardini et al. 2001, Keeney et al. 2005, Portnoy et al. 2010, Karl et al 2011) suggested that male leopard sharks would be more dispersive and less philopatric than females. Lastly, we hypothesized leopard shark aggregation behavior would be seasonally correlated with numerous environmental factors, particularly water temperature, wave height, and photoperiod.

METHODS

To determine daily and seasonal occurrence of leopard sharks at select locations along the coast, 20 females (16 in La Jolla and 4 in Del Mar) and 13 males (all in Del Mar) were captured and surgically fitted with coded acoustic transmitters (VEMCO V16-4H, 69 kHz, 60 – 180 s random transmission interval) and passively monitored by an array of seven underwater acoustic receivers (VEMCO VR2W). Sharks were monitored between 15 July 2009 and 4 September 2012 (1,148 d).

Capture and Surgical Implantation of Coded Acoustic Transmitters

Leopard sharks were captured by hook and line from a 5 m skiff (La Jolla: 32.853°, -117.262°; Del Mar: 32.958°, -117.277°), removed from the water, and placed ventral-side-up onto a custom-made surgical trough to induce tonic immobility. The incision site was dried and antiseptitized with povidone-iodine. A 3 cm longitudinal incision was made halfway between the pectoral and pelvic fins approximately 3 cm off the midline. A VEMCO V16-4H coded transmitter (coated with a 3:7 mixture of beeswax and paraffin wax and dipped in povidone-iodine to reduce the chance of immunological rejection; Holland et al. 1999) was inserted into the peritoneal cavity. The incision was closed with one continuous absorbable suture (2-0 Vicryl, Ethicon, Inc.) and treated with topical antibiotic (Neosporin, Johnson and Johnson Services, Inc.). The sex, fork length (FL), and total length (TL) were recorded and the shark was externally fitted with a “spaghetti” identification tag (Floy Tag FIM-96), inserted into the musculature and through the radials at the base of the first dorsal fin. To release the shark, the entire trough was placed into the water and gently overturned. Time out of water was 10.0 – 15.5 min; no complications were observed in the surgically operated sharks. In fact, shark M6 was recaptured and released 61 d after surgery; the incision had healed tightly into a thin scar with no suture remnants. A total of 140 sharks in La Jolla and 45 sharks in Del Mar, including those surgically implanted with acoustic transmitters, were equipped with FIM-96 tags between June – November of 2007 – 2011. [Note: Sharks were not anesthetized during surgical implantation because tricaine methanesulfonate (MS-222; the only anesthetic approved for fish by the United States Food and Drug Administration) requires a 21 d withdrawal period before human

consumption. This could not be guaranteed because leopard sharks are commonly fished and consumed and holding post-operative sharks in captivity for 21 d was deemed impractical and would have imposed further stress. All procedures were approved and performed in accordance with Protocol S00080 of the Institutional Animal Care and Use Committee of the University of California – San Diego.]

Passive Acoustic Monitoring of Shark-Borne Transmitters

VR2W receivers were mounted to dead-weight moorings, consisting of 20 mm diameter nylon rope, a ~100 kg steel clump weight, and a 30 cm diameter subsurface buoy positioned 2 m below mean lower low water (MLLW). The receiver was attached to the nylon rope 5 m below MLLW using heavy-duty UV-resistant cable ties. Detection data were downloaded quarterly from receivers *in situ* using an underwater Bluetooth cable (VEMCO) and receiver batteries were changed yearly at sea onboard the 5 m skiff. Moorings were located at 5 – 9 m below MLLW at the mouth of the Tijuana River (TJ; 32.554°, -117.131°), Pacific Beach (PB; 32.800°, -117.265°), La Jolla (LJ; 32.854°, -117.265°), Dike Rock (DR; 32.873°, -117.255°), Del Mar (DM; 32.958°, -117.272°), Carlsbad (CB; 33.078°, -117.315°), and San Clemente pier (SC; 33.418°, -117.624°). The TJ, PB, LJ, DR, and DM receivers were deployed in June 2009, the CB receiver in June 2010, and the SC receiver in August 2010; these seven receivers spanned approximately 120 km of coastline in San Diego and Orange Counties, California (Fig. 2.1). Acoustic detection range for each receiver was determined *in situ* and was approximately 300 m. The SC receiver was owned and operated by the Lowe Laboratory of California State University – Long Beach (CSULB).

Data Analyses

Temporal variation in tagged shark abundance was analyzed by converting raw detections to the number of individual sharks detected per day (abundance d^{-1} , to assess seasonal trends) and per 10-min time bin (abundance $damin^{-1}$; to assess diel trends). Time of day was expressed in Pacific Daylight Time (UTC-7) and all means were given with standard deviation (SD). Abundance d^{-1} was compared between 2010 and 2011 for males and females at the LJ and DM receivers using a Mann-Whitney *U* Test.

To assess seasonal patterns of sex-specific aggregation behavior, the following analyses were conducted for both females at the La Jolla receiver and males at the Del Mar receiver. Simple and multiple linear regression were used to determine the fine-scale seasonal relationship between shark abundance d^{-1} and five environmental variables: daily mean sea surface temperature (SST), swell height, and swell direction (all sampled twice h^{-1} at the Torrey Pines Outer Buoy, Coastal Data Information Program), daily mean barometric pressure (sampled once h^{-1} at the Scripps Institution of Oceanography pier, NOAA Tides and Currents), and photoperiod (United States Naval Observatory). These analyses were repeated on a courser scale, by pooling and averaging shark abundance d^{-1} and the above environmental measurements in 24 half-month bins, from the first to fifteenth day of the month (15 d; “early” month) and from the sixteenth to the last day of the month (13 – 16 d; “late” month). For convenience of discussion and conducting the following analyses, the “high season” was defined as the 12 consecutive half-months with the highest mean shark abundance d^{-1} and the “low season” was defined as the opposing 12 half-months. Shark abundance $damin^{-1}$ was averaged over all days in the high season to assess diel trends in aggregation behavior; the resulting 144 data points

were compared between day and night (delineated by times of mean sunrise and sunset during the high season; United States Naval Observatory) using a Mann-Whitney *U* Test. The effect of tide was assessed by pooling and averaging shark abundance damin^{-1} within two hours of every high and low tide and comparing these using a Mann-Whitney *U* Test.

RESULTS

As reported in Chapter 1, the 140 sharks tagged in La Jolla were 97.1% female, 2.9% male, and 110 – 164 cm TL (mean = 138.7 ± 10.8 cm). The 45 sharks tagged in Del Mar were 48.9% female, 51.1% male, and 105 – 154 cm (mean = 130.5 ± 11.2 cm). Females in La Jolla were significantly larger (mean TL = 138.9 ± 10.6 cm, range: 110 – 164 cm) than those in Del Mar (mean TL = 129.3 ± 11.2 cm, range: 105 – 154; $U = 2179$, $n = 158$, $p < 0.001$). No significant difference was detected in male TL between La Jolla (mean TL = 130.7 ± 15.6 cm, range: 116 – 147 cm) and Del Mar (mean TL = 131.6 ± 11.3 cm, range: 105 – 152; $U = 46$, $n = 27$, $p = 1.000$).

Sexual Segregation and Daily Site Fidelity

The VR2W receivers recorded 777,831 detections from the 33 shark-borne transmitters (range: 7 – 109,057 detections per shark; Table 2.1). Females tagged in La Jolla were detected most often at the LJ receiver (mean = 288.4 ± 257.1 d shark⁻¹), 24.9 times more than at the DM receiver (mean = 11.6 ± 15.7 d shark⁻¹; $U = 244.0$, $n = 32$, $p < 0.001$). In contrast, males were detected most often at the DM receiver (mean = 280.8 ± 183.6 d shark⁻¹), 8.8 times more than at the LJ receiver (mean = 32.0 ± 59.1 d shark⁻¹; $U = 155.5$, $n = 26$, $p < 0.001$; Fig. 2.2). Females tagged in Del Mar were detected equally at

the LJ (mean = 168.8 ± 162.7 d shark⁻¹) and DM receivers (mean = 177.3 ± 189.5 d shark⁻¹; $U = 9.0$, $n = 8$, $p = 0.886$).

Male abundance d⁻¹ was lower in 2011 than in 2010 at the DM receiver ($U = 86597$, $n = 730$, $p < 0.001$), but higher at the LJ receiver ($U = 99280$, $n = 730$, $p < 0.001$). Female abundance d⁻¹ was not significantly different between 2010 and 2011 at the LJ receiver ($U = 71560$, $n = 730$, $p = 0.082$), but marginally lower at the DM receiver ($U = 72377$, $n = 730$, $p = 0.042$). There was no significant difference between 2010 and 2011 in SST ($U = 70329$, $n = 730$, $p = 0.190$), barometric pressure ($U = 70635$, $n = 730$, $p = 0.157$), or swell direction ($U = 68295$, $n = 730$, $p = 0.552$); however, swell height was marginally lower in 2011 (mean = 1.06 ± 0.31 m, range: 0.48 – 2.46 m) than in 2010 (mean = 1.16 ± 0.44 m, range: 0.55 – 3.49 m; $U = 72910$, $n = 730$, $p = 0.026$).

Daily Patterns of Shark Abundance

Shark abundance damin⁻¹ was significantly higher during the day than at night for both females at the LJ receiver ($U = 3707$, $n = 144$, $p < 0.001$) and males at the DM receiver ($U = 4889$, $n = 144$, $p < 0.001$); abundance damin⁻¹ dropped precipitously for both sexes at both receivers at sunset. Female abundance damin⁻¹ remained low at the LJ receiver during the morning hours, even after sunrise, when water temperature was coldest (approximately 0700 – 1100 hrs), whereas male abundance damin⁻¹ began to rise immediately after sunrise (Fig. 2.3). Lastly, shark abundance damin⁻¹ was significantly higher within 2 h of high tide than within 2 h of low tide for females at the LJ receiver ($U = 707984$, $n = 2154$, $p < 0.001$), but not for males at the DM receiver ($U = 561323$, $n = 2113$, $p = 0.812$).

Seasonal Patterns and Environmental Predictors of Shark Abundance

High aggregation season was late June – early December for females at the LJ receiver (peak in late August – late September) and late April – early October for males at the DM receiver (peak in early July). Seasonal arrival of females to and departure from La Jolla were highly synchronous, coinciding with the summer and winter solstices, respectively (Fig. 2.4). Some females were transient and scarcely detected at the LJ receiver during the low season (e.g., sharks F1, F6, and F10), while others were more resident and detected sporadically even during the low season (e.g., sharks F2, F4, and F8). Transient females made frequent roundtrip excursions from the LJ receiver during the high season, being detected as far north and south as the CB and TJ receivers, respectively; however, resident females made fewer detectable excursions (Fig. 2.5). Males exhibited greater interindividual variation in detection patterns and thus could not be readily classified as transient or resident (Fig. 2.6); however, males also made roundtrip excursions from the DM receiver, with more frequent and prolonged excursions to the LJ receiver in 2011 – 2012 than in 2009 – 2010 (Fig. 2.7).

Pooled and averaged on a half-month basis, female abundance d^{-1} at the LJ receiver was directly related to SST, and inversely related to barometric pressure, swell height, and swell direction ($p < 0.001$ for each correlation), but not related to photoperiod alone ($p = 0.156$; r^2 values given in Fig. 2.8). Female abundance d^{-1} lagged SST by approximately one half-month ($r^2 = 0.951$ when SST shifted +1 half-month). Multiple linear regression increased the coefficient of determination to 0.948 (adjusted $r^2 = 0.933$). Male abundance d^{-1} at the DM receiver was directly related to SST and photoperiod, inversely related to barometric pressure and swell direction ($p < 0.001$ for each

correlation), but not related to swell height alone ($p = 0.111$; r^2 values given in Fig. 2.8). Male abundance d^{-1} lagged photoperiod by approximately one half-month ($r^2 = 0.745$ when photoperiod shifted +1 half-month). Multiple linear regression increased the coefficient of determination to 0.797 (adjusted $r^2 = 0.741$).

On a day-to-day basis, female abundance d^{-1} at the LJ receiver and male abundance d^{-1} at the DM receiver were directly related to SST (females: $r^2 = 0.448$, males: $r^2 = 0.173$) and photoperiod (females: $r^2 = 0.076$, males: $r^2 = 0.213$), and inversely related to swell height (females: $r^2 = 0.105$, males: $r^2 = 0.010$), swell direction (females: $r^2 = 0.200$, males: $r^2 = 0.025$), and barometric pressure (females: $r^2 = 0.147$, males: $r^2 = 0.1306$, $p < 0.001$ for each correlation). Multiple linear regression increased the coefficient of determination to 0.498 for females (adjusted $r^2 = 0.496$) and 0.299 for males (adjusted $r^2 = 0.296$).

Seasonal Philopatry and Recaptures of Tagged Sharks

Females returned annually to the LJ receiver; 50.0% of females tagged in 2009 ($n = 12$) were detected 3 y after tagging, 52.6% of sharks tagged in 2009 – 2010 ($n = 19$) were detected 2 y after tagging, and 70.0% of sharks tagged in 2009 – 2011 ($n = 20$) were detected 1 y after tagging. Males also returned annually to the DM receiver; 60.0% of males tagged in 2009 ($n = 10$) were detected 3 y after tagging, and 69.2% and 84.6% of sharks tagged in 2009 – 2010 ($n = 13$) were detected 2 and 1 y after tagging, respectively.

Finally, 12 of the 185 sharks equipped with spaghetti identification tags (6.5%), including three sharks implanted with acoustic transmitters, were recaptured ($n = 9$) or found dead ($n = 3$). Three were captured in commercial gillnets: one male (shark M1;

August 2010) and one female (April 2011) 5 km off San Onofre State Beach and one female (August 2011) off Puerto Nuevo, Baja California, Mexico (40 km south of the Mexican border). Recreational fishers captured one female (shark F9) off San Clemente pier in November 2009, two females from the beach immediately north of SIO pier in July (shark F7) and August 2010, and one male (shark M6; released) in Del Mar in September 2009. The authors captured and released one female in Del Mar in July 2010 and one female in La Jolla in July 2011. Lastly, three females were found dead in the water or on the beach near the La Jolla aggregation site in September 2010 and December 2011, with injuries attributable to fatal attacks by California sea lions (*Zalophus californicus*).

DISCUSSION

This study presents the longest uninterrupted acoustic detection record for leopard sharks (3.15 y), providing novel insight into their long-term movement patterns. In addition, this was the first attempt to systematically track male leopard sharks, demonstrating that both sexes exhibit site-specific aggregation behavior and seasonal philopatry, which has important conservation implications.

Sexual Segregation and Daily Site Fidelity

The detection record showed 16 females tagged in La Jolla exhibited strong site fidelity to the LJ receiver and were rarely detected at the DM receiver, only 12 km away (Fig. 2.2). This finding was consistent with the hypothesis that pregnant females are particularly attracted to this shallow site for its calm warm water to accelerate gestation,

access to nearby foraging grounds, and scarcity of males to coerce physically costly superfluous matings (Chapter 1). In contrast, four females tagged in Del Mar frequented the LJ and DM receivers equally; the reason for this remains unclear. Although males exhibited strong site fidelity to the DM receiver (Fig. 2.2), male abundance declined at the DM receiver and increased at the LJ receiver in 2011 - 2012 compared to 2009 - 2010 (Figs. 2.4, 2.6). There was no concomitant change in measured environmental factors that could readily explain this shift; however, there was a massive die-off of giant kelp (*Macrocystis pyrifera*) at the Del Mar site due to turbidity stress and winter storms in 2010 - 2011 (E. Parnell, pers. comm.). Permanent band transect surveys indicated a 23-fold decline in annual mean density of *M. pyrifera* in the Del Mar kelp forest, from 0.095 and 0.105 plants m⁻² in 2009 and 2010, respectively, to 0.035 and 0.005 plants m⁻² in 2011 and 2012, respectively (E. Parnell, unpubl. data). This drastic decline in canopy-forming kelp may have reduced prey abundance for leopard sharks and suggests that habitat change may alter the movement patterns of these predatory fishes, which may have wider-reaching biological consequences. For example, the percentage of leopard shark litters having multiple fathers was significantly higher in mothers sampled from the La Jolla site in 2011 (83.3%) than in 2010 (20.0%; Chapter 3); this shift may have resulted from increased mating activity in 2011 because of the higher abundance of males in La Jolla.

Daily Patterns of Shark Abundance at Aggregation Sites

Female abundance at the LJ receiver was highest during the day, particularly in late afternoon when water temperature was highest (Fig. 2.3). The conspicuous morning

dip was consistent with actively tracked females (Chapter 1) being biased toward the warmer rocky reef during the coldest morning hours of 0700 – 1100 hrs (outside detection range; Fig. 2.1), and shifting toward the sand flat area later in the day (within detection range; see Fig. 1.5). Hight and Lowe (2007) reported a similar late-afternoon peak in leopard shark abundance in Big Fisherman's Cove (BFC) on Santa Catalina Island. Like these females, which selectively occupied the warmest areas of the cove throughout the day, females in LJ appeared to move shoreward with the flooding tide (outside of detection range, yielding lower abundance at high tide) to remain in warm, shallow water.

The sharp decline in female abundance at sunset (Fig. 2.3) likely reflects dispersal of the sharks to deeper and colder water, presumably to forage, which was observed in several actively tracked females at the La Jolla site (see Figs. 1.3, 1.4). Males in Del Mar also showed daytime aggregation behavior and likely foraged at night in the kelp forest, which was just outside detection range. Carlisle and Starr (2009) did not observe diel patterns of leopard shark aggregation behavior in Elkhorn Slough, California; however, food was apparently abundant throughout the intertidal mudflats in the slough, giving the sharks little reason to leave at night.

Seasonal Patterns and Environmental Predictors of Shark Abundance

Seasonally fluctuating SST explained 86.7% of the half-month variation and 44.8% of the day-to-day variation in female abundance at the LJ receiver. This strong relationship was not unexpected, given that water temperature strongly influences the behavior and physiology of elasmobranch fishes (Economakis and Lobel 1998, Matern et

al. 2000, Wallman and Bennett 2006, Hight and Lowe 2007). Previous work similarly found leopard shark abundance at aggregation sites was related to water temperature, and, in central California, a temperature of 10 – 12°C was thought to cue the seasonal arrival and departure of the sharks (Hopkins and Cech 2003, Hight and Lowe 2007, Carlisle and Starr 2009). In contrast, no absolute value of SST readily explained the synchronous year-to-year formation and dissolution of the aggregation at the LJ receiver; however, females may cue into increasing or decreasing SST in combination with photoperiod, as seasonal arrival and departure typically coincided with the longest and shortest days of the year (Fig. 2.4). Lastly, female abundance lagged SST by approximately one half-month (Fig. 2.8), with SST explaining 95.1% of the variation in female abundance if the latter were shifted backward one half-month. If SST indeed drives aggregation behavior, this half-month delay may prevent premature arrival or departure in response to unseasonal spikes or drops in water temperature, respectively, or it may reflect the time to physiologically translate and process the environmental cue to a behavioral response.

SST was also an important predictor of male abundance at the DM receiver, explaining 58.1% and 17.3% of the half-month and day-to-day variation, respectively. However, male abundance preceded SST by approximately one month (Fig. 2.8), suggesting water temperature may not directly elicit aggregation behavior in males. In contrast, photoperiod explained more of the variation in male abundance (63.6% and 21.3% of the half-month and day-to-day variation, respectively) and male abundance lagged photoperiod by approximately one-half month. Photoperiod is seldom considered to affect the movements of elasmobranch fishes; however, it is the most predictable environmental cue for animals residing in middle and high latitudes, and, along with

temperature, the most powerful environmental regulator of seasonal life-history events, such as gametogenesis, mating, and migration (Bradshaw and Holzapfel 2007). Even in elasmobranch fishes both temperature and photoperiod have been shown to affect hormone production, which could result in behavioral changes (Heupel et al. 1999, Mull et al. 2008, Mull et al. 2010). Thus, photoperiod cannot be ignored and should be considered alongside temperature as a potentially important environmental cue affecting seasonal changes in shark movement patterns.

Seasonal fluctuations in wave height and swell direction may also affect female abundance at the LJ receiver. In particular, the female high season (late June – early December) was delineated by a sharp seasonal drop and spike in wave height, respectively (Fig. 2.8). Additionally, the predominant swell direction shifted from the west during the low season to the southwest during the high season due to austral winter storms; the La Jolla aggregation site is particularly sheltered from SW swells by the Point La Jolla promontory (Fig. 2.1). In short, the shift in swell direction and decrease in wave height results in lower wave energy, likely facilitating the invasion of warm shallow water during the high season.

Although barometric pressure accounted for a significant amount of the variation in female and male abundance at the LJ and DM receivers, respectively, the absolute change in mean daily barometric pressure averaged only 1.77 ± 1.68 mb (range: 0.00 – 14.90 mb) on a day-to-day basis and 1.04 ± 0.68 mb (range: 0.05 – 2.98 mb) on a half-month basis. Blacktip sharks (*Charcharhinus limbatus*) were believed to have fled a shallow bay in response to a drop in barometric pressure of 13 mb that accompanied an approaching hurricane (Heupel et al. 2003); however, it is unlikely that the much lower

variation in barometric pressure measured in this study could have resulted in the observed behavioral responses in leopard sharks. Instead, barometric pressure was strongly negatively correlated with SST ($r^2 = 0.295$, $p < 0.001$) and photoperiod ($r^2 = 0.242$, $p < 0.001$); thus, its relationship with shark abundance was likely spurious.

Seasonal Philopatry and Recaptures of Tagged Sharks

Both males and females exhibited long-term seasonal philopatry to the LJ and DM receivers, respectively; 6 out of 12 of females and 6 out of 10 males were detected in each of the four high seasons in 2009 – 2012 (Figs. 2.4, 2.6). Moreover, these percentages likely underestimate the true extent of philopatry because at least 3 of the 22 sharks tagged in 2009 were caught and killed and thus could not have returned. Short-term seasonal philopatry was also high; 70% of females ($n = 20$) and 84.6% of males ($n = 13$) returned to La Jolla and Del Mar, respectively, during the following high season. In contrast, females in BFC on Santa Catalina Island and in Elkhorn Slough exhibited a lower degree of philopatry, with only 50.0% ($n = 10$) and 7.7% ($n = 13$) detected at the aggregation site one year after tagging (Hight and Lowe 2007, Carlisle and Starr 2009). Hight and Lowe (2007) suggested the BFC aggregation was not composed of the same individuals year after year because there were multiple suitable aggregation sites around Santa Catalina Island. This may explain the stronger philopatry observed by females in this study because there are few sheltered areas along the mainland coast of southern California. However, the only readily apparent feature that defines the Del Mar site is the adjacent kelp forest, which is hardly unique to this area. Nevertheless, males exhibited a

high level of seasonal philopatry to this site and, notwithstanding the availability of suitable alternative “homes,” returned to this particular location.

Varying degrees of philopatry have been reported in other shark species (see Hueter et al. 2005 for review), often attributed to natal homing of juveniles and adult females to nursery areas (Feldheim et al. 2002, 2004). However, immature leopard sharks were rarely observed at the La Jolla aggregation site (Chapter 1), suggesting this is not a major nursery or pupping ground. Therefore, the yearly return of adult females to La Jolla is likely not related to natal homing. Alternatively, this site was likely encountered by chance during the lifetime of the shark and adopted as a favorable “home base” with predictable foraging grounds nearby; the same is likely true for males in Del Mar. Numerous studies reporting discordance in population structure based on analyzing nuclear DNA (biparentally inherited; less structure) and mitochondrial DNA (maternally inherited; more structure) suggest gene flow is largely male-mediated; thus, females are generally believed to exhibit a higher degree of philopatry than males, which are in turn more dispersive (Pardini et al. 2001, Keeney et al. 2005, Portnoy et al. 2010, Karl et al 2011). However, we found no evidence of sex-specific philopatry in the leopard shark, indicating both sexes may have limited dispersal.

When sharks were absent from the aggregation site, the more resident females (e.g., sharks F2, F4, and F8) apparently never strayed far from the LJ receiver, even during the low season (Fig. 2.4), and were only occasionally detected at the PB, DR, and DM receivers (Fig. 2.5). However, the more transient females (e.g., sharks F1, F6, and F10) were almost entirely absent from the LJ receiver during the low season (Fig. 2.4) and detected extensively north at the CB and SC receivers (Fig. 2.5). Males were

generally less predictable and were detected sporadically both north and south of the DM receiver during the low season (Figs. 2.6, 2.7). However, no sharks of either sex (except shark F20) were ever detected at the CSULB receiver arrays off Palos Verdes and Los Angeles Harbor (Fig. 2.1), which were in continuous operation from July 2010 through the end of this study, and would likely have detected any tagged sharks near the coast in that area. This suggests the northward movement of leopard sharks away from the aggregation site during the low season may terminate somewhere along the mainland coast between San Clemente and Los Angeles, or that sharks may instead transit offshore to Santa Catalina Island. As the La Jolla site is not suspected to be a major pupping or mating ground, these important life history events may instead occur at these locations.

CSULB receivers in BFC detected two sharks during the low season: shark M6 in November 2009 and shark F11 in December 2009. Although shark F11 was not detected again on the mainland, shark M6 was detected back at the DM receiver 49 h and 4 min after being last detected at BFC (125 km away; Fig. 2.1), suggesting an average swimming speed of 2.55 km h^{-1} or $0.54 \text{ body lengths s}^{-1}$. This is already higher than the theoretical optimal cruising speed of $0.40 \text{ body lengths s}^{-1}$ determined for other sharks species (Weihs 1975), suggesting this shark swam a rather direct route from Santa Catalina Island to Del Mar, and thus spent approximately two days in the pelagic environment. Transiting first to the mainland and then south along the coast would have further increased the journey distance and average speed. BFC receivers were in operation only from October 2009 to March 2010, precluding further detection of tagged sharks in subsequent years of the study. Hight and Lowe (2007) similarly reported that mainland receivers detected two females tagged at BFC. One of these sharks was

detected in Encinitas, CA, between the DM and CB receivers. This shark was detected back in BFC and was ultimately captured back in Encinitas by a fisher in July 2011 (C. Lowe, pers. comm.). These findings suggest a strong link between leopard shark populations on Santa Catalina Island and the mainland coast of southern California and are consistent with genetic evidence suggesting these populations are not distinct (Lewallen et al. 2007); thus, it is possible that more of the sharks tagged in this study visited the island, particularly during the low season, perhaps to mate or give birth.

Conservation Implications

The strong philopatry demonstrated in this study indicates *T. semifasciata*, like other philopatric species, are susceptible to localized stock depletions resulting from fishing mortality or habitat destruction (Heuter et al. 2005). The aggregation behavior and sexual segregation reported in this study likely exacerbate this vulnerability due to the risk of sharks being captured *en masse* and the threat of sex-biased mortality (Wearmouth and Sims 2008, Jacoby et al. 2011). The predominantly female aggregation in La Jolla clearly benefits from the small no-take Matlahuayl State Marine Reserve. Individual females spent up to 68.3% of the study period (1148 d) and up to 98.8% of the high season within range of the LJ receiver, and therefore well within the reserve (Fig. 2.1). This is clear evidence that a modestly sized reserve is very effective at protecting leopard sharks on both a short- and long-term basis; other leopard shark aggregations would also benefit from small, strategically placed no-take reserves. Given that some sharks were detected at the TJ receiver, and likely crossed the international border, and that at least one shark was confirmed killed in a commercial gillnet in Baja California,

Mexico (this was likely underreported), there is clearly a need for binational conservation efforts to protect this and other trans-border species and a need to identify aggregation sites in Mexico and establish appropriate management plans to protect them.

Table 2.1: Summary of detection data for 33 leopard sharks (*Triakis semifasciata*) surgically implanted with coded acoustic transmitters and monitored by passive acoustic telemetry between 15 July 2009 and 4 September 2012.

Notes:

Abbreviations: ID, shark identification; TL, total length; FL, fork length; TJ, Tijuana River receiver; PB, Pacific Beach receiver; LJ, La Jolla receiver; DR, Dike Rock receiver; DM, Del Mar receiver; CB, Carlsbad receiver; SC, San Clemente pier receiver

^L Last detected by array at this receiver

[^] Captured and killed by recreational fisher on north side of Scripps Institution of Oceanography pier on 10 July 2010

^{*} Captured and killed by recreational fisher from San Clemente pier on 7 November 2009

^{^^} Detected in Big Fisherman's Cove, Santa Catalina Island, on 14 December 2009

^{**} Detected near Los Angeles Harbor on 6 July 2012

^{^*} Captured and killed by commercial gillnet fisher off San Onofre State Beach on 6 August 2010

ID	TL FL (cm)	Tagging Date & Location	Last Day Detected & Last Known Day At Liberty	Known Days At Liberty	Total Detections at all Receivers	Days Detected at Each Receiver						
						Percentage of Known Days at Liberty Detected at Each Receiver						
						TJ	PB	LJ	DR	DM	CB	SC
F1	128	15-Jul-09	04-Sep-12	1148	41110	0	0	423 ^L	107	53	60	0
	146	La Jolla	04-Sep-12			0.00%	0.00%	36.85%	9.32%	4.62%	5.23%	0.00%
F2	122	15-Jul-09	25-Aug-12	1138	68756	0	21	640	23	15 ^L	10	0
	141	La Jolla	25-Aug-12			0.00%	1.85%	56.24%	2.02%	1.32%	0.88%	0.00%
F3	123	15-Jul-09	21-Jul-09	7	280	0	0	7 ^L	1	0	0	0
	141	La Jolla	21-Jul-09			0.00%	0.00%	100.00%	14.29%	0.00%	0.00%	0.00%
F4	122	15-Jul-09	04-Sep-12	1148	72237	0	9	678 ^L	36	7	5	0
	141	La Jolla	04-Sep-12			0.00%	0.78%	59.06%	3.14%	0.61%	0.44%	0.00%
F5	123	15-Jul-09	27-Dec-10	531	27878	0	0	308 ^L	11	20	2	0
	141	La Jolla	27-Dec-10			0.00%	0.00%	58.00%	2.07%	3.77%	0.38%	0.00%
F6	122	15-Jul-09	30-Jul-12	1112	33170	1	46	402 ^L	70	33	65	7
	140	La Jolla	30-Jul-12			0.09%	4.14%	36.15%	6.29%	2.97%	5.85%	0.63%
F7	126	15-Jul-09	10-Jul-10	361	3773	0	0	54	8 ^L	4	1	0
	144	La Jolla	10-Jul-10 ^A			0.00%	0.00%	14.96%	2.22%	1.11%	0.28%	0.00%
F8	123	16-Jul-09	04-Sep-12	1147	109057	0	0	784 ^L	5	0	0	0
	140	La Jolla	04-Sep-12			0.00%	0.00%	68.35%	0.44%	0.00%	0.00%	0.00%
F9	126	16-Jul-09	27-Sep-09	115	4168	0	0	59	11	7 ^L	0	0
	145	La Jolla	07-Nov-09*			0.00%	0.00%	51.30%	9.57%	6.09%	0.00%	0.00%
F10	127	16-Jul-09	04-Sep-12	1147	31206	1	5	369 ^L	79	34	18	4
	145	La Jolla	04-Sep-12			0.09%	0.44%	32.17%	6.89%	2.96%	1.57%	0.35%
F11	126	16-Jul-09	30-Aug-09	152	3116	0	0	40 ^L	0	0	0	0
	142	La Jolla	14-Dec-09 ^{AA}			0.00%	0.00%	26.32%	0.00%	0.00%	0.00%	0.00%
F12	131	16-Jul-09	01-Aug-09	17	359	0	0	13 ^L	1	0	0	0
	150	La Jolla	01-Aug-09			0.00%	0.00%	76.47%	5.88%	0.00%	0.00%	0.00%
F13	102	23-Jun-10	02-Sep-12	803	7160	0	16	161	4	6 ^L	69	0
	118	La Jolla	02-Sep-12			0.00%	1.99%	20.05%	0.50%	0.75%	8.59%	0.00%
F14	122	23-Jun-10	03-Aug-11	407	20317	0	44 ^L	160	22	4	24	0
	142	La Jolla	03-Aug-11			0.00%	10.81%	39.31%	5.41%	0.98%	5.90%	0.00%
F15	122	23-Jun-10	15-Dec-11	541	43508	0	2	451 ^L	15	3	4	0
	142	La Jolla	15-Dec-11			0.00%	0.37%	83.36%	2.77%	0.55%	0.74%	0.00%
F16	112	16-Jul-10	15-Jul-12	731	818	0	1	4	0	26	19 ^L	3
	126	Del Mar	15-Jul-12			0.00%	0.14%	0.55%	0.00%	3.56%	2.60%	0.41%
F17	126	20-Jul-10	04-Sep-12	778	40193	0	1	330 ^L	131	301	0	0
	142	Del Mar	04-Sep-12			0.00%	0.13%	42.42%	16.84%	38.69%	0.00%	0.00%
F18	122	20-Jul-10	04-Sep-12	778	40206	1	0	285	85	377 ^L	0	0
	140	Del Mar	04-Sep-12			0.13%	0.00%	36.63%	10.93%	48.46%	0.00%	0.00%
F19	108	22-Jul-10	25-Oct-10	96	7752	1 ^L	0	56	5	5	0	0
	122	Del Mar	25-Oct-10			1.04%	0.00%	58.33%	5.21%	5.21%	0.00%	0.00%
F20	104	07-Sep-11	01-Jan-12	304	2683	0	4	66	1	0	0	0
	118	La Jolla	06-Jul-12 ^{**}			0.00%	1.32%	21.71%	0.33%	0.00%	0.00%	0.00%
M1	117	24-Jul-09	31-Jul-10	379	3261	0	0	0	0	114	1 ^L	0
	132	Del Mar	06-Aug-10 ^{A*}			0.00%	0.00%	0.00%	0.00%	30.08%	0.26%	0.00%
M2	105	27-Jul-09	04-Sep-12	1136	26571	0	10	57 ^L	11	527	2	0
	124	Del Mar	04-Sep-12			0.00%	0.88%	5.02%	0.97%	46.39%	0.18%	0.00%
M3	112	27-Jul-09	06-May-12	1015	34674	0	0	18	6	625 ^L	1	0
	128	Del Mar	06-May-12			0.00%	0.00%	1.77%	0.59%	61.58%	0.10%	0.00%
M4	110	27-Jul-09	03-Sep-12	1135	27701	0	0	31	8	380 ^L	8	2
	128	Del Mar	03-Sep-12			0.00%	0.00%	2.73%	0.70%	33.48%	0.70%	0.18%
M5	113	28-Jul-09	07-Nov-11	833	18519	0	0	3	4	386 ^L	0	0
	131	Del Mar	07-Nov-11			0.00%	0.00%	0.36%	0.48%	46.34%	0.00%	0.00%
M6	119	28-Jul-09	21-Sep-10	421	29804	0	0	2	2	324 ^L	0	0
	136	Del Mar	21-Sep-10			0.00%	0.00%	0.48%	0.48%	76.96%	0.00%	0.00%
M7	123	29-Jul-09	29-Jul-09	1	7	0	0	0	0	1 ^L	0	0
	137	Del Mar	29-Jul-09			0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%
M8	107	29-Jul-09	02-Sep-12	1132	33085	0	0	70	17	341 ^L	0	0
	123	Del Mar	02-Sep-12			0.00%	0.00%	6.18%	1.50%	30.12%	0.00%	0.00%
M9	122	29-Jul-09	22-Jun-12	1060	6068	0	3	21	56	321 ^L	2	0
	141	Del Mar	22-Jun-12			0.00%	0.28%	1.98%	5.28%	30.28%	0.19%	0.00%
M10	132	29-Jul-09	02-Sep-12	1132	2609	0	0	0	2	112 ^L	24	0
	152	Del Mar	02-Sep-12			0.00%	0.00%	0.00%	0.18%	9.89%	2.12%	0.00%
M11	124	20-Jul-10	03-Sep-12	777	14626	0	0	0	0	251 ^L	19	0
	142	Del Mar	03-Sep-12			0.00%	0.00%	0.00%	0.00%	32.30%	2.45%	0.00%
M12	122	20-Jul-10	02-Jan-11	167	2167	0	0	1	1 ^L	37	2	0
	140	Del Mar	02-Jan-11			0.00%	0.00%	0.60%	0.60%	22.16%	1.20%	0.00%
M13	128	22-Jul-10	04-Sep-12	776	20992	0	1	213 ^L	31	232	16	0
	147	Del Mar	04-Sep-12			0.00%	0.13%	27.45%	3.99%	29.90%	2.06%	0.00%

Figure 2.1: Study area showing locations of underwater acoustic receivers (red dots) and their approximate 300 m detection ranges (concentric red circles). **A:** Enlarged view of small green box in world map inset showing southern California. **B:** Central San Diego County, California (enlarged view of green box in A). The boundary of the Matlahuayl State Marine Reserve (formerly the San Diego – La Jolla Ecological Reserve) is indicated by the solid black line extending north from Point La Jolla and then east to Scripps Institution of Oceanography pier. **C:** Del Mar, California (enlarged view of upper green box in B). The approximate boundary of the Del Mar kelp forest is indicated by a dashed white line. **D:** La Jolla, California (enlarged view of lower green box in B). The approximate boundary of the rocky reef is indicated by a dashed white line

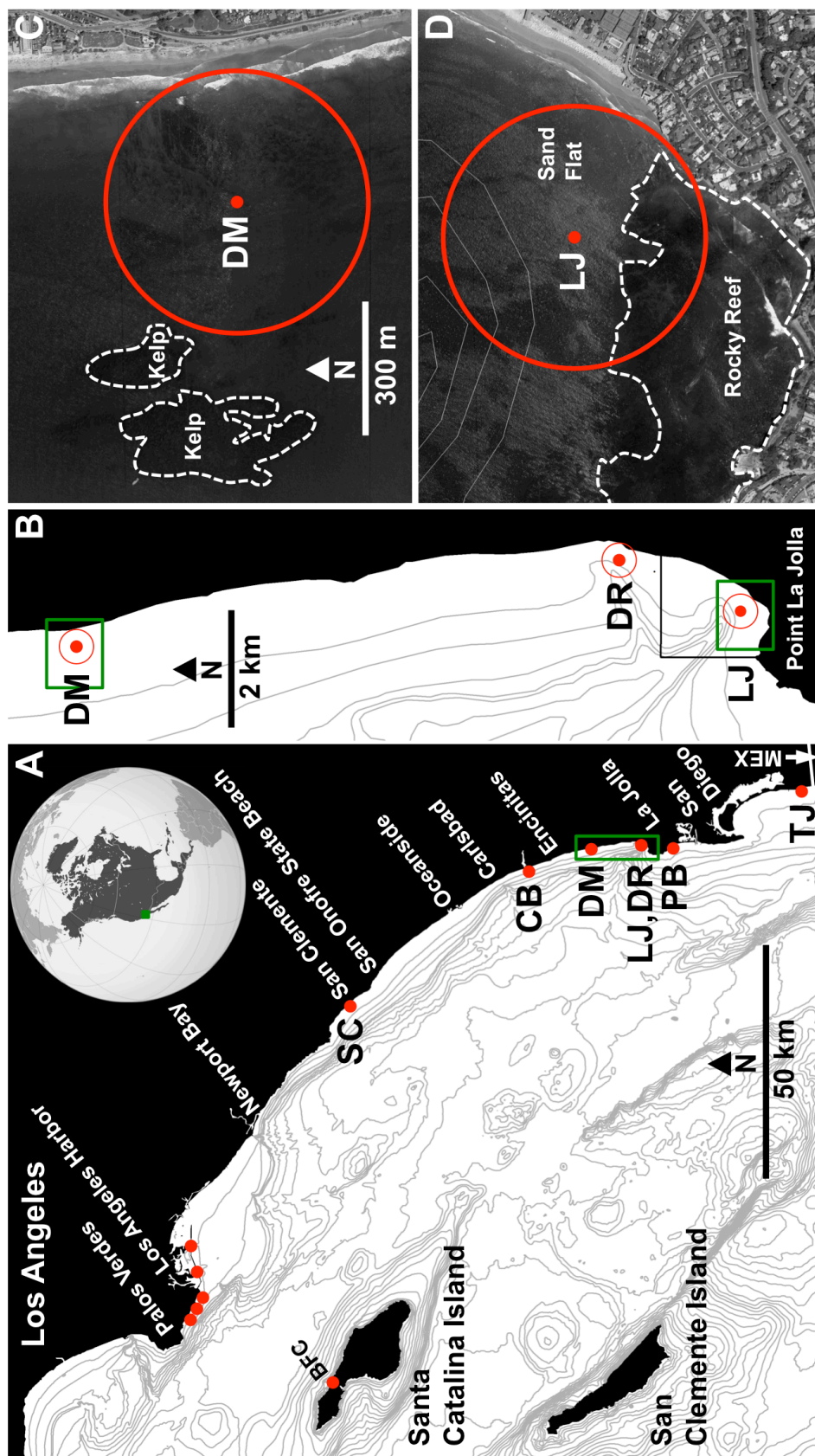
Notes:

Abbreviations: TJ, Tijuana River receiver; PB, Pacific Beach receiver; LJ, La Jolla receiver; DR, Dike Rock receiver; DM, Del Mar receiver; CB, Carlsbad receiver; SC, San Clemente pier receiver; BFC, Big Fisherman's Cove

Aerial views in C and D are from Google Earth Pro (Imagery Date: 1 February 2008)

All isobaths are given at 20 m intervals to 100 m, then at 100 m intervals

The approximate locations of the receivers near Los Angeles Harbor and Palos Verdes are shown in A, which were owned and operated by the Lowe Laboratory of CSULB



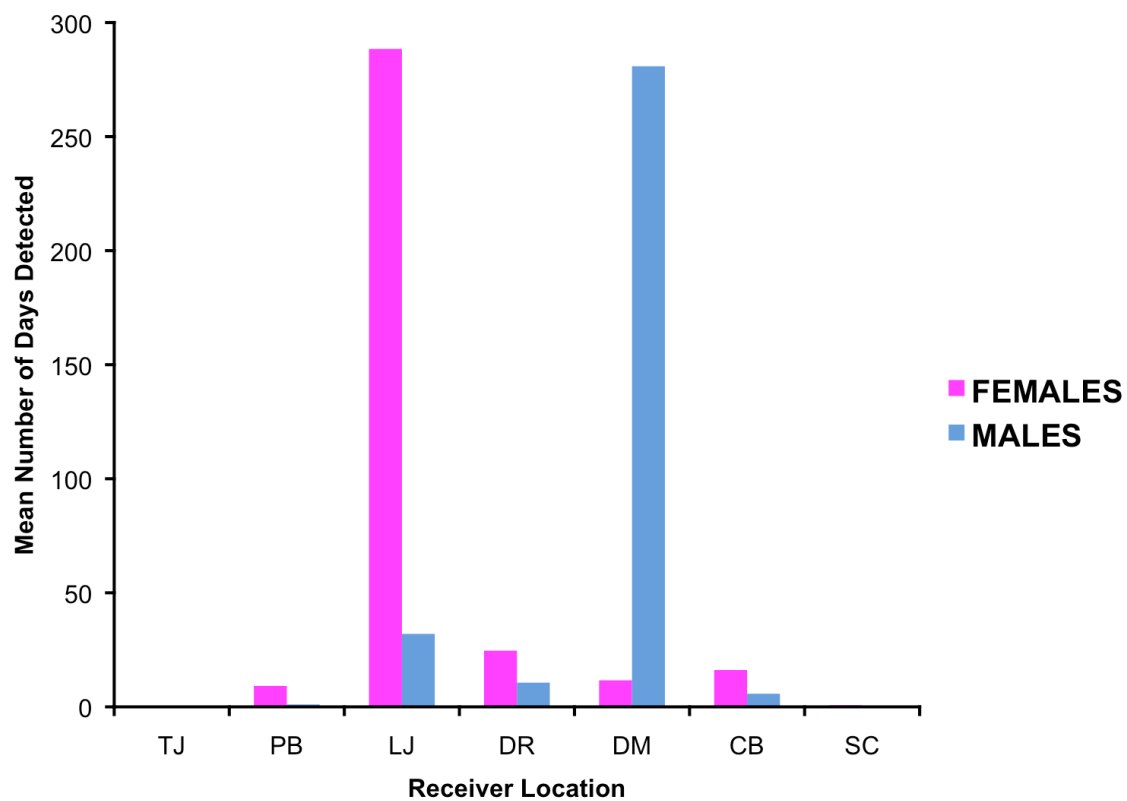


Figure 2.2: Mean number of days that male and female leopard sharks (*Triakis semifasciata*) were detected at underwater acoustic receivers.

Note:

Receiver location abbreviations: TJ, Tijuana River; PB, Pacific Beach; LJ, La Jolla; DR, Dike Rock; DM, Del Mar; CB, Carlsbad; SC, San Clemente pier

Figure 2.3: Mean number of individual female leopard sharks (*Triakis semifasciata*) detected damin⁻¹ during the high season (late June – early December) at the La Jolla receiver (pink line, top graph). Mean number of individual male *T. semifasciata* detected damin⁻¹ during the high season (late April – early October) at the Del Mar receiver (blue line, bottom graph).

Notes:

Mean hourly sea surface temperature (recorded at Scripps Institution of Oceanography pier) is shown as a solid black line on both graphs

Mean sunrise and sunset during the high season are shown as vertical dashed black lines with the range of sunrise and sunset during this period indicated by the gray bars

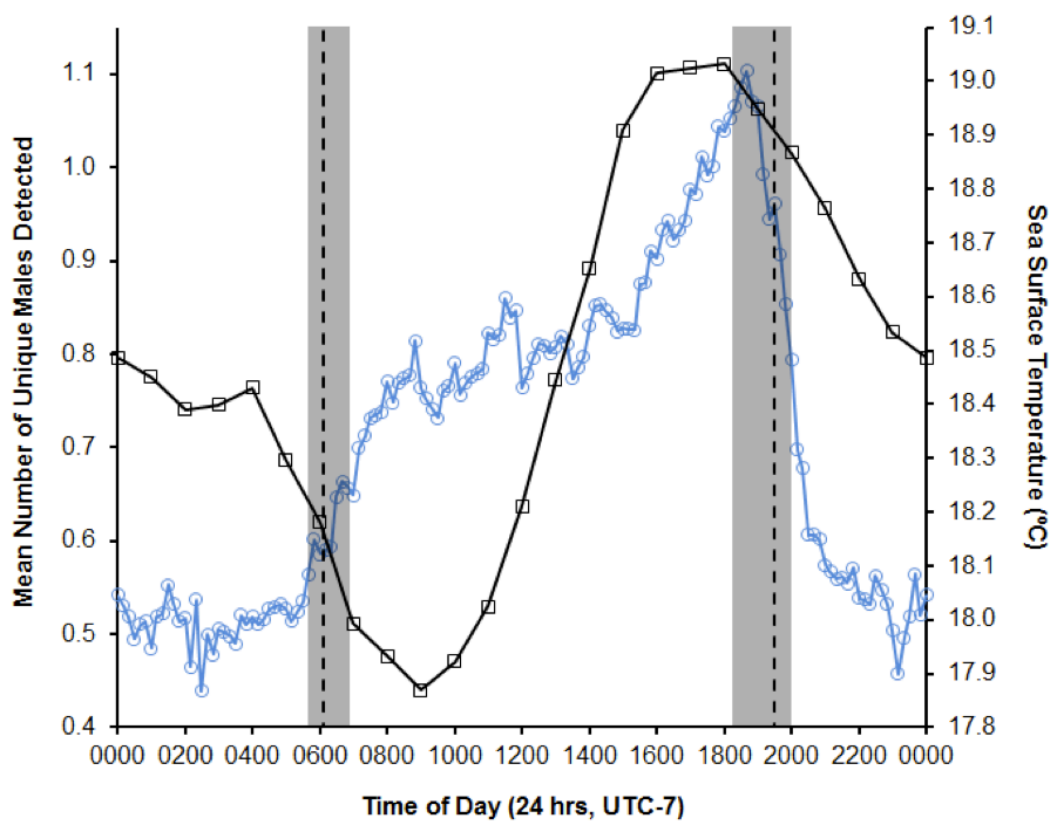
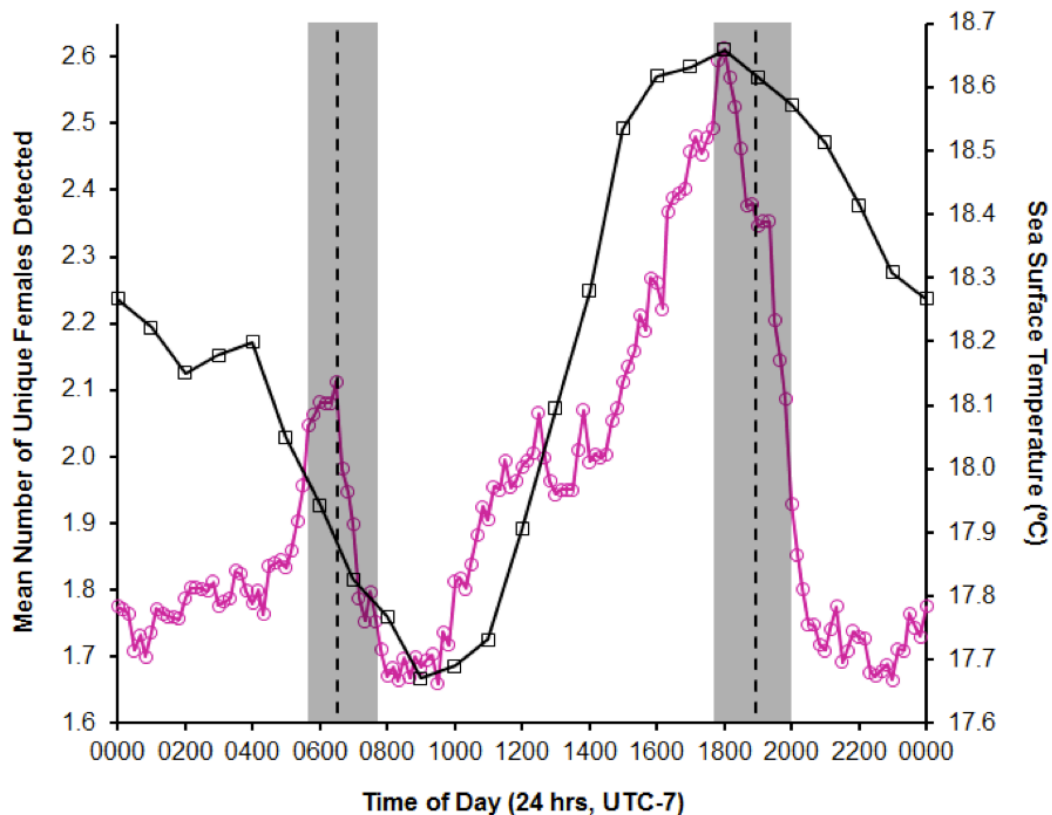


Figure 2.4: Female leopard sharks (*Triakis semifasciata*) at the La Jolla receiver. Six actograms are shown for the six females with the longest detection records. A compilation actogram is shown, including all tagged females ($n = 20$).

Notes:

Each actogram spans 24 h from left to right and 1,148 days from top to bottom

Each black pixel is a 10-min time bin in which that shark was detected at the La Jolla receiver

The compilation actogram is double plotted to show the nighttime portion uninterrupted

Plotted on the right is the number of individual females (pink) and males (blue) detected d^{-1} at the La Jolla receiver along with mean daily sea surface temperature (solid black line) and photoperiod (dotted black line)

The times of summer and winter solstices (± 1 week) are shown as semi-transparent horizontal peach bars

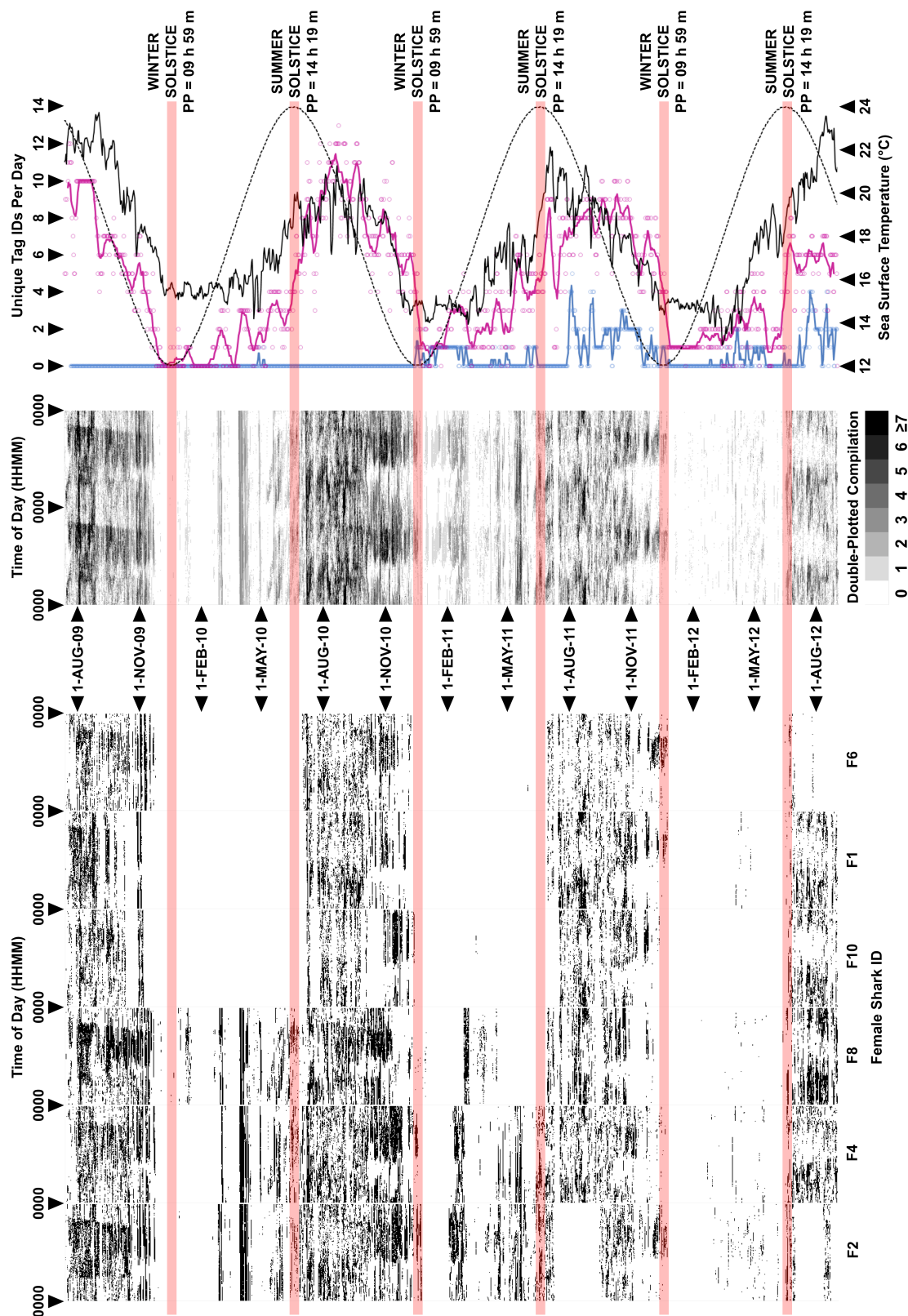


Figure 2.5: Detection records at selected receivers for each of the six female leopard sharks (*Triakis semifasciata*) with the longest detection records.

Notes:

Individual detections are shown as black dots and are connected by dashed lines

The times of the summer and winter solstices (± 1 week) are shown for reference as vertical semi-transparent peach bars

Abbreviations: TJ, Tijuana River receiver; PB, Pacific Beach receiver; LJ, La Jolla receiver; DR, Dike Rock receiver; DM, Del Mar receiver; CB, Carlsbad receiver; SC, San Clemente pier receiver

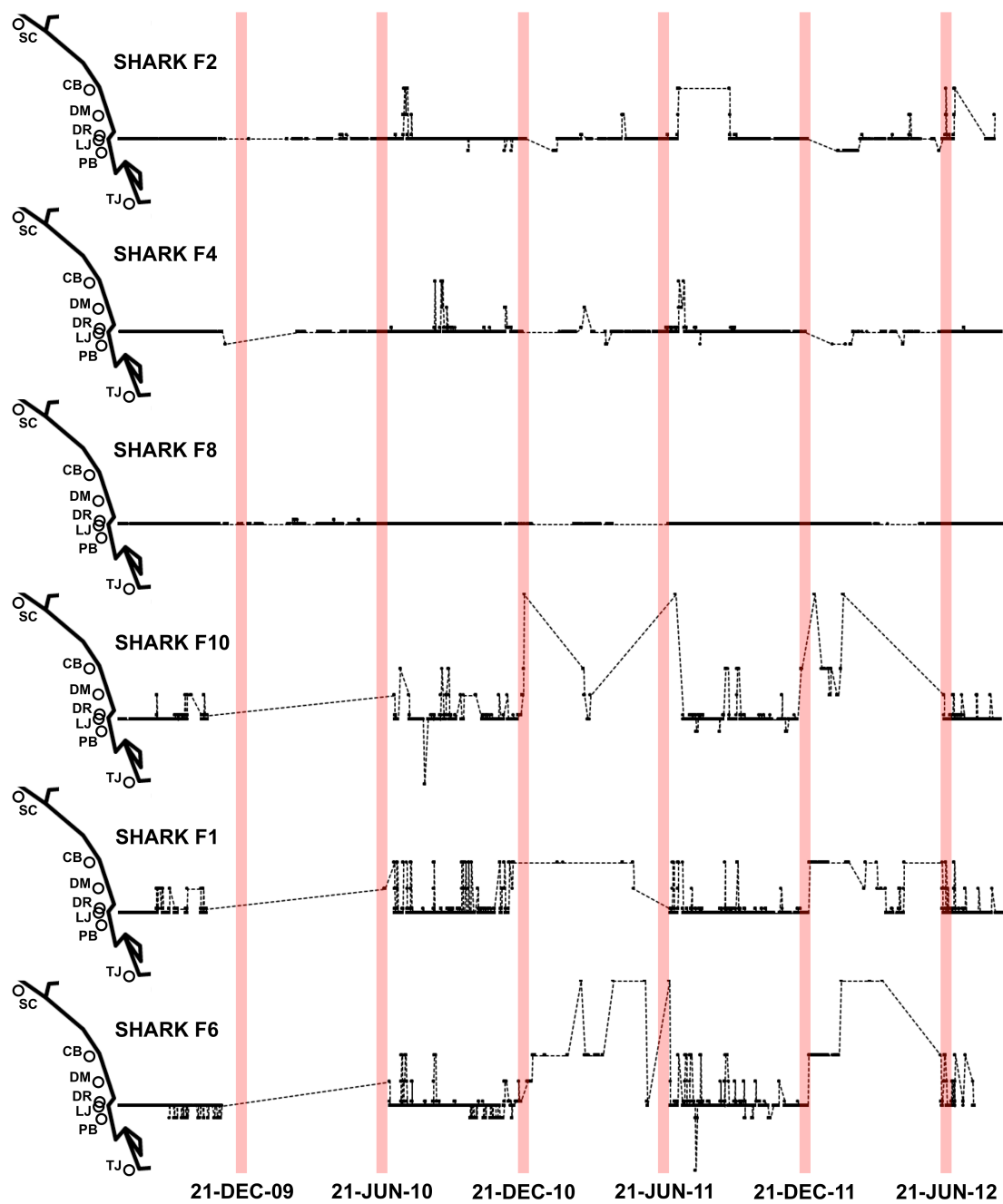


Figure 2.6: Male leopard sharks (*Triakis semifasciata*) at the La Jolla receiver. Six actograms are shown for the six males with the longest detection records. A compilation actogram is shown, including all tagged males ($n = 13$).

Notes:

Each actogram spans 24 h from left to right and 1,148 days from top to bottom

Each black pixel is a 10-min time bin in which that shark was detected at the La Jolla receiver

The compilation actogram is double plotted to show the nighttime portion uninterrupted

Plotted on the right is the number of individual males (blue) and females (pink) detected d^{-1} at the La Jolla receiver along with mean daily sea surface temperature (solid black line) and photoperiod (dotted black line)

The times of summer and winter solstices (± 1 week) are shown as semi-transparent horizontal peach bars

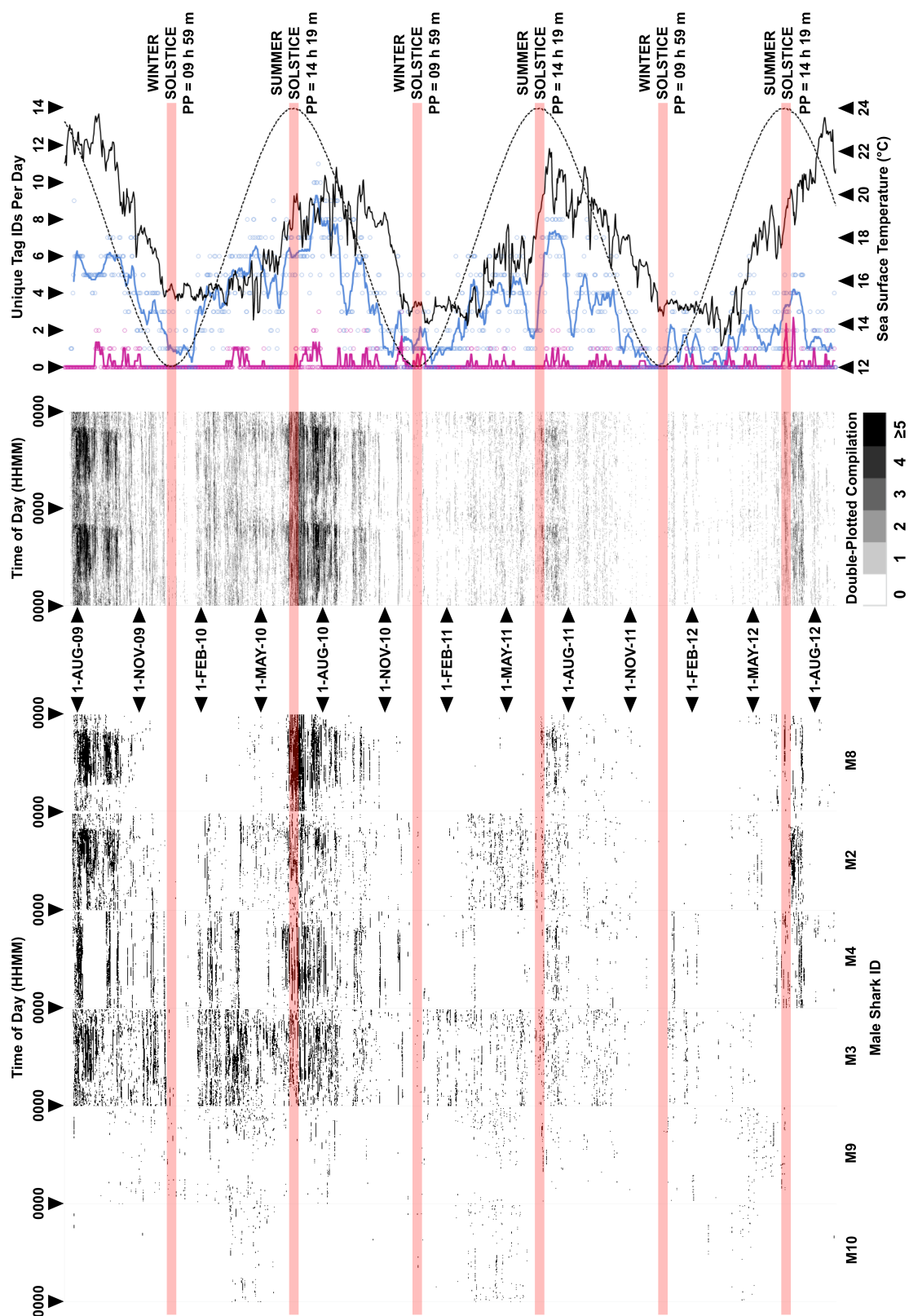


Figure 2.7: Detection records at selected receivers for each of the six male leopard sharks (*Triakis semifasciata*) with the longest detection records.

Notes:

Individual detections are shown as black dots and are connected by dashed lines

The times of the summer and winter solstices (± 1 week) are shown for reference as vertical semi-transparent peach bars

Abbreviations: TJ, Tijuana River receiver; PB, Pacific Beach receiver; LJ, La Jolla receiver; DR, Dike Rock receiver; DM, Del Mar receiver; CB, Carlsbad receiver; SC, San Clemente pier receiver

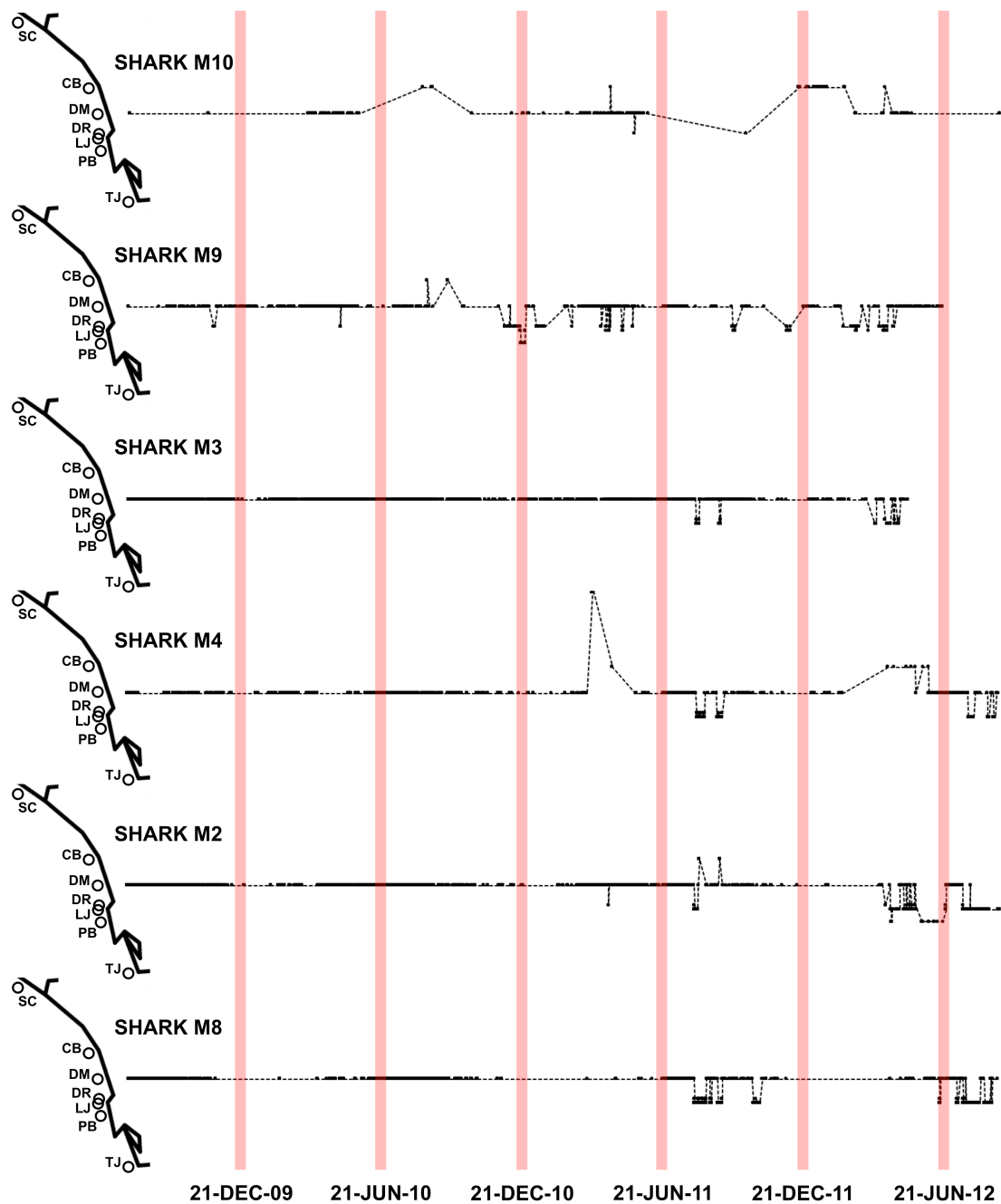
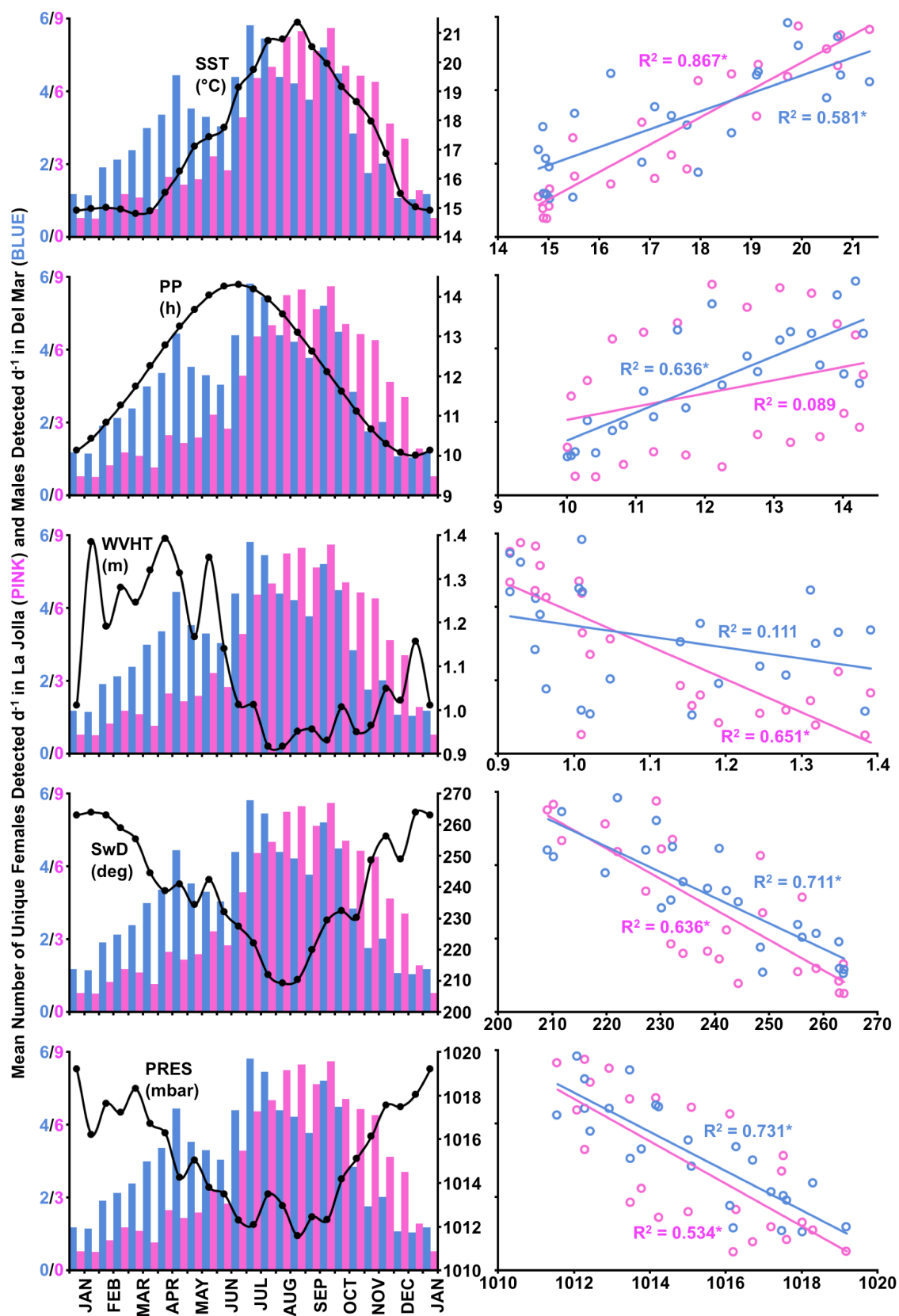


Figure 2.8: Number of individual female (pink) and male (blue) leopard sharks (*Triakis semifasciata*) detected d^{-1} , pooled and averaged in 24 half-month bins, plotted with measured environmental variables (left column). Simple linear regression of the number of individual females (pink) and males (blue), detected d^{-1} pooled and averaged in 24 half-month bins, with measured environmental variables (right column).

Note:

Abbreviations: SST, sea surface temperature; PP, photoperiod; WVHT, significant wave height; SwD, swell direction; PRES, barometric pressure



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ACKNOWLEDGEMENTS

I would like to thank the many people that assisted with this project: J. Graham, M Taylor, M. Royer, A. Caillat, M.C. Bernal, A. Martin, L. McCormick, N. Wegner, D. Cartamil, E. Kisfaludy, N. Ben-Aderat, L. Bellquist, P. Zerofski, C. McDonald, R. Walsh, D. Huang, C. Jew, A. Hardy, E. Lewallen, A. Randolph, D. Kacev, C. Lowe, K. Lyons, A. Barker, J. Beckman, J. Arce, J. Renfree, M. Cape, E. Parnell, C. Sepulveda, B. Wolfe, M. Espinoza, M. Farris, B. Ahr, R. Freedman, N.C. Lai, J. Garfield, P. Dockry, SeaLife Aquarium Carlsbad, and the Birch Aquarium at Scripps. I would also like to

thank my dissertation adviser and committee for constructive feedback: P. Hastings, R. Burton, N. Wegner, D. Woodruff, R. Norris, and M. Tresguerres.

Funding was provided by the Scripps Institution of Oceanography Graduate Department, Center for Marine Biodiversity and Conservation, Scripps Environmental Advocates, PADI Foundation, Project AWARE, SeaWorld and Busch Gardens Conservation Fund, Vemco, and generous donation from the Bohn Family. I would also like to thank the Scripps Development Office, particularly E. Riblet, M. Darling, and John Steinitz. Lastly, I would like to thank B. Beltaire and the individuals, families, and local businesses that sponsored leopard sharks: N. Hillgarth, Hughes Family, Posnock Family, Sundt Family, Whitney Family, Royer Family, Coastal Environments La Jolla, Speer Family, Surf Diva, La Jolla Kayak, Morris Family, Ocean Enterprises, Riblet Family, Bohn Family. A. Nosal was supported by Graduate Research Fellowship Program (GRFP) and Integrated Graduate Education and Research Traineeship (IGERT, No. 0333444) awards from the National Science Foundation.

This work was conducted under University of California – San Diego Institutional Animal Care and Use Committee (IACUC) protocol No. S00080.

CHAPTER 3: MULTIPLE PATERNITY IN LEOPARD SHARK (*TRIAKIS SEMIFASCIATA*) LITTERS SAMPLED FROM A FEMALE-DOMINATED AGGREGATION IN LA JOLLA, CA, USA

ABSTRACT

The number of sires per litter was determined for the leopard shark (Triakidae: *Triakis semifasciata*) to investigate the potential effect of sex-biased aggregation behavior on the frequency of multiple paternity (F_{MP}). Four highly polymorphic microsatellite markers were developed and used to genotype 449 pups from 22 litters (mean $\pm$ SD = 20.4 ± 7.0 pups per litter) sampled from pregnant individuals (dams) collected from a female-dominated leopard shark aggregation in La Jolla, California, USA. Multiple paternity was detected in 8 of 22 litters ($F_{MP} = 36.4\%$), all with two sires per litter. Significant interannual variability in F_{MP} was observed between two years of the study (2010: $F_{MP} = 20.0\%$, $n = 10$, and 2011: $F_{MP} = 83.3\%$, $n = 6$). Paternal skew deviated significantly from the expected ratio of 50:50 in four of the eight multiply sired litters, with the dominant male (having higher reproductive success) siring up to 95.5% of the pups in a given litter (mean $\pm$ SD = $91.7 \pm 0.3\%$). These high skews and the presence of unfertilized ova in eight of the 22 litters (36.4%) suggest that females may mate multiple times to promote sperm competition or combat sperm limitation, respectively. However, the most probable explanation for genetic polyandry in the leopard shark appears to be “convenience polyandry,” whereby females concede to multiple mating attempts because the associated costs (e.g., incurring injury) are less than those of resistance. The relatively low F_{MP} for leopard sharks supports the hypothesis that female-dominated aggregations may function as a refuge to avoid harassment by males.

INTRODUCTION

Multiply sired litters (multiple paternity) are common in elasmobranch fishes; however, the percentage of litters sired by multiple males (frequency of multiple paternity, F_{MP}) exhibits strong inter- and intraspecific variability (reviewed in Byrne and Avise 2012). Recent work suggests F_{MP} is set by the encounter rate between potential mates (Daly-Engel et al. 2010), which varies among and within species depending on the extent of sexual segregation (reviewed in Wearmouth and Sims 2008). For example, protracted sexual segregation might account for the low F_{MP} in the shortspine spurdog (*Squalus mitsukurii*, $F_{MP} = 11.1\%$; Daly-Engel et al. 2010) and the spiny dogfish (*Squalus acanthias*, $F_{MP} = 17.2\%$; Veríssimo et al. 2011), whereas the high F_{MP} in the brown smoothhound (*Mustelus henlei*, $F_{MP} = 93.0\%$) might be due to the formation of dense mixed-sex mating aggregations (Byrne and Avise 2012). Predominantly female aggregations are particularly common in sharks (reviewed in Jacoby et al. 2011) and are often hypothesized to function as a means of reducing male harassment in the form of excessive and potentially injurious mating attempts (Klimley 1985, Economakis and Lobel 1998, Sims et al. 2001). If this hypothesis is true, then female avoidance of superfluous copulations should limit genetic polyandry and result in a low F_{MP} . The present study tests this hypothesis using the leopard shark (Triakidae: *Triakis semifasciata*) as a model.

The leopard shark is a nearshore benthic species that forms dense aggregations of mature females throughout its range along the western coast of North America (Ebert and Ebert 2005, Hight and Lowe 2007, Chapter 1). *Triakis semifasciata* has an annual reproductive cycle; females give birth to 6 – 36 pups in April – June following a gestation

period of 10 – 11 months (Ebert 2003, Castro 2011). By logical extension, mating, ovulation, and fertilization should occur within a narrow window (1 – 2 months) following parturition. Sperm storage and the potential for multiply sired litters are likely in *T. semifasciata*, given the pronounced oviducal gland in mature females (Ebert and Ebert 2005), where sperm storage has been documented in other triakid sharks, such as *Mustelus canis* (Conrath and Musick 2002), *M. antarcticus* (Storrie et al. 2008), and *M. asterias* (Farrell et al. 2010). The present study examines F_{MP} for leopard shark litters acquired from pregnant females aggregating off La Jolla, California, USA. This site-specific aggregation, which forms annually in June – December and consists of 97.1% mature females, has been hypothesized to function, in part, as a refuge from males after mating elsewhere (Chapter 1); thus, F_{MP} was hypothesized to be low.

METHODS

The number of fathers was determined for each of 22 leopard shark litters, acquired from pregnant individuals (dams) collected from the La Jolla aggregation site (32.853°N, 117.263°W) between September 2007 and October 2011.

Sample Collection and DNA Extraction

Seventeen dams were captured by hook and line between the months of September and November (well after ovulation and fertilization, so as to not artificially reduce the period in which additional copulations might have been procured) and transported to an open flow-through aquarium facility at Scripps Institution of Oceanography. In captivity, eight females gave birth naturally to litters of term pups,

three were sacrificed, and six died of undetermined causes and prematurely delivered some or all of their pups shortly before death. Dead dams were dissected, whereas surviving dams and pups were donated to other laboratories, educational facilities, or released with permission from the California Department of Fish and Game. In addition to live-collected sharks, five pregnant females were found dead at the aggregation site during the course of the study (also between the months of September and November), salvaged, and included in the multiple paternity analysis.

The number of pups per litter (litter size), sex ratio of the litter (if embryos had developed claspers), and total length (TL) of each pup and dam were recorded. In sacrificed or salvaged dams, the uterine location of each pup (left or right uterine horns) was also recorded. Fin clips were collected from all dams and pups and preserved in 95% ethanol and stored at -80°C. To obtain baseline population genetic information, fin clips were collected from an additional 126 adult *Triakis semifasciata* at the aggregation site (tagged and released) during the same period. Total genomic DNA was extracted from each fin clip using a DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions.

Microsatellite Marker Development and Genotyping

Four microsatellite markers (*Trse-A1*, *Trse-A103*, *Trse-D2*, and *Trse-D12*) were developed from enriched DNA libraries constructed specifically for *Triakis semifasciata* and screened according to the methods described by Jones et al. (2002b). Briefly, total genomic DNA was digested with a cocktail of seven blunt-end restriction enzymes. Resulting fragments in the size range of 300 to 750 base pairs were hybridized to 5-

prime-biotinylated oligonucleotides (microsatellite probes) and subjected to streptavidin magnetic bead capture (Millipore, Billerica, MA). Next, captured fragments were amplified, cloned, and sequenced. Microsatellite-containing fragment sequences were identified by inspection and PCR primers were designed to anneal to flanking regions using DesignerPCR v1.03 (Research Genetics, Inc., Huntsville, AL). Forward primers were 5-prime-labeled with 6-FAM, HEX, or TET dyes for fluorescent visualization (Table 2).

Optimized PCR reactions for loci *Trse-A1*, *Trse-D2*, and *Trse-D12* consisted of 25 μ l of 1x GoTaq Green Master Mix (Promega Corporation, Fitchburg, WI), 10 μ M each forward and reverse primer, and 50 – 100 ng of DNA template. For locus *Trse-A103*, the optimized PCR reaction consisted of 25 μ l of 1x iTaq Buffer (Bio-Rad Laboratories, Inc., Hercules, CA), 1.5 mM $MgCl_2$, 200 μ M each dNTP, 10 μ M each forward and reverse primer, and contained 1.25 units iTaq DNA Polymerase (Bio-Rad Laboratories, Inc., Hercules, CA) and 50 – 100 ng DNA template. PCR amplification on a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA) consisted of an initial denaturation at 95°C for 3 min, followed by 33, 28, 32, or 35 cycles (for loci *Trse-A1*, *Trse-A103*, *Trse-D2*, and *Trse-D12*, respectively) of 30 s at 95°C, 30 s at 56°C, and 1 min at 72°C, followed by a final extension at 72°C for 45 min. PCR products (including negative controls), ROX-labeled DNA ladder (MegaBACE ET550-R; GE Healthcare Life Sciences, Piscataway, NJ), and locus-specific allelic standards (positive controls) were resolved concurrently on 0.4 mm thick, large-format (33 x 39 cm) vertical polyacrylamide denaturing gels according to the methods described by Gruenthal and Burton (2008). Gels were electrophoresed at 60 W for 2 – 4 h and fluorescently scanned

on a Typhoon 9410 Variable Mode Imager (Molecular Dynamics, Inc., Sunnyvale, CA). Allele sizes were visualized using ImageQuant software (Molecular Dynamics, Inc., Sunnyvale, CA) and manually scored, independently by two laboratory personnel. Discrepancies between scorers were rare, and settled by re-screening those particular individuals to confirm the genotype.

Statistical Analyses

For each microsatellite locus, the number of alleles, allelic frequency distribution, observed and expected heterozygosity, and conformance to the expectations of Hardy-Weinberg equilibrium (HWE) were determined for the population sample ($n = 148$, including the 22 dams) using Genepop v4.1 (Rousset, 2008). MicroChecker v1 (van Oosterhout et al., 2004) was used to infer potential genotyping errors due to null alleles and stutter peaks. The probability of detecting multiple paternity (PrDM) was calculated in PrDM v1 (Neff and Pitcher, 2002). This program uses a Monte Carlo simulation to generate 10,000 reconstructed genotypes of multiply sired litters based on the number of microsatellite loci, the number of alleles, and allelic frequency distribution at each locus, as well as the maternal genotype (optional), and various combinations of user-defined litter sizes, numbers of sires (at least two), and skews of sire reproductive success in the litter (paternal skew). PrDM is the proportion of 10,000 multiply sired litters that contain at least three unique alleles, not maternally inherited (i.e., paternal alleles), at one or more loci and would therefore be correctly identified as multiply sired. Due to the nature of Monte Carlo simulations resulting in minor fluctuations in replicated PrDM calculations, the program was always run 10 times and the returned PrDM values were averaged. As

an *a priori* power assessment of the marker suite, PrDM was calculated for six hypothetical two-sire paternal skews (50:50, 60:40, 70:30, 80:20, 90:10, and 95:5) across hypothetical litter sizes of 1 – 40 pups, without specifying a maternal genotype.

Genotypic arrays of each litter were evaluated manually to ensure all progeny shared a maternal allele at each locus and to count the number of paternal alleles. A litter having three or more paternal alleles at one or more loci was considered to be multiply sired. Gerud v2.0 (Jones, 2005) was used to confirm these findings, estimate the minimum number of sires for each litter, and determine the genotype of each sire. If no single solution of paternal genotypes could explain the progeny genotypic array, alternative solutions were ranked by relative probability based on patterns of Mendelian segregation and expected genotypic frequencies in the population (Jones, 2005). The most probable solution of paternal genotypes was used to assign a sire to each of the progeny and thus determine the paternal skew for each litter. As a *post hoc* power assessment, PrDM was calculated for each litter using the same two-sire paternal skews as above; however, the actual litter size and maternal genotype were specified. This was particularly relevant for apparently singly sired litters. The Bayesian program FMM v1 (Neff et al., 2002) was used to determine the expected frequency of multiple paternity (F_{MP} = number of multiply sired litters / total number of litters analyzed * 100) and 95% confidence interval (CI), given the allelic frequency distribution of the population.

The following tests were conducted to relate patterns of multiple paternity to the biology of *T. semifasciata*. A Mann-Whitney *U* test was used to determine whether dam TL and litter sizes were each significantly different between multiply and singly sired litters. A χ^2 test was used to determine if paternal skew in multiply sired litters was

significantly different from 50:50, and whether paternal skew inside each uterine horn was significantly different from the overall paternal skew. Lastly, a Fisher's Exact Test was used to determine whether the number of singly and multiply sired litters differed significantly between dams collected in 2010 and 2011, and whether the number of litters containing unfertilized ova differed between those sired by single and multiple males.

RESULTS

Mean TL $\pm$ SD of the dams ($n = 22$) was 142.6 ± 7.0 cm (range: 130 – 156 cm). Litters of term pups ($n = 8$) were born in captivity between 4 April and 1 July (mean date of birth $\pm$ SD = 16 May $\pm$ 34 d). Mean litter size $\pm$ SD ($n = 20$) was 21.8 ± 5.6 pups (range: 11 – 33 pups); litters H and K were excluded from this calculation because each was partially consumed by several large swell sharks (*Cephaloscyllium ventriosum*) being held temporarily in the same tank at the time of birth. The surviving pups from litters H ($n = 9$) and K ($n = 4$) were nevertheless genotyped; thus, the mean number of progeny genotyped $\pm$ SD was 20.4 ± 7.0 pups (range: 4 – 33 pups). The overall sex ratio of female to male pups did not deviate from 50:50 (181:182, $\chi^2 = 0.003$, $n = 363$, d.f. = 1, $p = 0.958$), nor did the overall ratio of pups found in the left to right horns of the uterus (99:101, $\chi^2 = 0.020$, $n = 200$, d.f. = 1, $p = 0.888$). These findings are summarized in Table 3.1. A positive linear relationship was observed between litter size and dam TL (excluding litters H and K; Fig. 3.1) with a slope (b) that was significantly different from zero ($n = 20$, $r^2 = 0.429$, $b = 0.526$, $p = 0.002$). Lastly, 1 – 3 unfertilized eggs were found in each of eight litters (36.4%), either at the anterior end of one or both uterine horns, or

delivered along with the pups. Mating scars were not evident in any of the 148 females captured.

The suite of four microsatellite markers exhibited moderate to high polymorphism (9 – 23 alleles per locus; Fig. 3.2) in the screened population sample ($n = 148$, including the 22 dams) and conformed to the expectations of Hardy-Weinberg equilibrium (Table 3.2). There was no evidence of linkage disequilibrium between the four loci (tested in Genepop v4.1; Rousset, 2008). An *a priori* power analysis revealed that even without knowing the maternal genotype, a probability of detecting multiple paternity (PrDM) of 0.950 could be achieved for paternal skews (2 sires) of 50:50, 60:40, 70:30, and 80:20 with minimum litter sizes of 6, 7, 9, and 14, respectively (Fig. 3.3). Given that the sizes of 17 of the 22 litters genotyped (77.3%) exceeded 14 pups, our marker suite was quite powerful. However, a multiply sired litter with a paternal skew of 90:10 was more difficult to detect, requiring 30 and 16 pups to achieve a PrDM of 0.950 and 0.800, respectively, and a skew of 95:5 further reduced PrDM, requiring at least 32 pups to achieve an estimate close to 0.800.

In total, 22 dams and 449 pups were genotyped for paternity analysis. Multiple paternity was detected in 8 of 22 litters ($F_{MP} = 36.4\%$). No more than four paternal alleles were observed at each locus, consistent with having two or fewer sires per litter (Table 3.1). The Bayesian maximum likelihood estimate of F_{MP} was 38% (95% CI: 19 – 57%), which closely approximated our finding of 36.4%. Paternal skew was high in the multiply sired litters; the dominant of the two males (having higher reproductive success) sired up to 95.5% of the pups in a given litter (mean $\pm$ SD = $76.0 \pm 18.1\%$). Paternal skew deviated significantly from the expected ratio of 50:50 in multiply sired litters R, T,

U, and V ($\chi^2 = 13.500 - 20.571$, d.f. = 1, $n = 22 - 28$, $p < 0.0002$), but not in litters E, H, K, and S ($\chi^2 < 3.841$, d.f. = 1, $n = 4 - 30$, $p > 0.05$). Of the eight multiply sired litters, GERUD 2.0 produced a unique paternal skew solution for five litters and two possible paternal skew solutions for three litters (Table 3.1). However, the most likely (reported) solutions for two of these litters (T and V) were $> 4 \times 10^3$ times more probable than their alternatives. Two nearly equally likely paternal skew solutions (3:1 and 2:2) were returned for litter K. Given that high paternal skew decreases PrDM, the empirically derived F_{MP} of 36.4% might underestimate the true F_{MP} . For example, if one half of the apparently singly sired litters ($n = 7$) had paternal skews of 90:10 (two thirds, $n = 4.67$) or 90:5 (one third, $n = 2.33$), and given a mean *post-hoc* PrDM of 0.865 (90:10) and 0.647 (95:5) for the apparently singly sired litters, then 1 – 2 of these might actually have been sired by multiple males, and not detected. Thus, F_{MP} for *T. semifasciata* might be as high as 40.9 – 45.5%, which still falls within the 95% CI given above. Lastly, the number of multiply and singly sired litters from dams collected in 2010 ($n = 10$) and 2011 ($n = 6$), and thus F_{MP} (20.0% in 2010; 83.3% in 2011), differed significantly between those years (Fisher's Exact Test, $n = 16$, $p < 0.035$).

In comparing singly and multiply sired litters, no significant differences were found in dam TL (Mann-Whitney Test, $U = 73$, $n = 22$, $p = 0.259$) or litter size (excluding litters H and K; Mann-Whitney Test, $U = 52$, $n = 20$, $p = 0.435$). In litter S, the paternal skew was significantly different from the overall skew of 17:13 in both the left (13:1, $\chi^2 = 7.476$, $n = 14$, d.f. = 1, $p = 0.006$) and right uterine horns (4:12, $\chi^2 = 6.543$, $n = 16$, d.f. = 1, $p = 0.011$); however, no other litter exhibited this pattern.

DISCUSSION

The present study is the first to demonstrate multiple paternity and estimate F_{MP} for *Triakis semifasciata*. The observed F_{MP} of 36.4% is not among the lowest reported for sharks (e.g., *Squalus mitsukurii*, $F_{MP} = 11.1\%$; Daly-Engel et al. 2010); however, it certainly falls to the lower end of the spectrum of F_{MP} reported for other species (reviewed in Byrne and Avise 2012) and thus generally supports the hypothesis that female aggregation behavior limits genetic polyandry. Nevertheless, multiply sired litters were detected; the simplest explanation for these is that they arose as a consequence of sexual conflict without any benefit to the females. However, it is important to address alternative explanations for these multiply sired litters and for the low observed F_{MP} .

First, Pratt and Carrier (2001) suggested repeated copulations might be required in some shark species to ensure complete fertilization of ova; thus, multiple mating could increase fecundity. Consistent with this hypothesis were unfertilized ova present in eight of the 22 leopard shark litters examined (36.4%), which, in dissected dams ($n = 6$), were located at the anterior end of either or both uterine horns, nearest the oviducal gland. Assuming conservation of uterine position throughout gestation, thus indicative of ovulation sequence (Smale and Compagno 1997, Smale and Goosen 1999), these unfertilized ova were last to pass through the sites of fertilization, when sperm had perhaps already been depleted. However, because unfertilized ova were found in both singly and multiply sired litters with no significant difference between the two, multiple mating clearly did not ensure complete fertilization of ovulated ova. Moreover, there was no significant difference in litter size between multiply and singly sired litters, suggesting mating multiply does not increase fecundity in *T. semifasciata*.

Second, females might benefit indirectly from multiple mating because the simultaneous presence of sperm from multiple males could promote sperm competition and cryptic female choice among ejaculates (Fitzpatrick et al. 2012). If sperm competitiveness is heritable, then male progeny of males with competitive sperm should have greater reproductive success (Keller and Reeve 1995). Alternatively, if sperm competitiveness is related to genetic quality of the corresponding male more generally, then promoting sperm competition could increase the chances that offspring will inherit “good genes” (Sheldon 1994, Yasui 1997, Jennions and Petrie 2000). Finally, cryptic female choice among ejaculates may similarly increase offspring genetic quality or complementarity (Olsson and Madsen 2001, Simmons 2005, Fitzpatrick et al. 2012). Consistent with these hypotheses are the high paternal skews observed in four multiply sired *T. semifasciata* litters (mean = 91.7:8.3), which suggest some form of post-copulatory sperm selection, or else are an artifact of mating order. However, direct evidence supporting “good genes” hypotheses (e.g., trading up, genetic bet-hedging) is lacking in elasmobranch fishes (Daly-Engel et al. 2007, DiBattista et al. 2008a).

Finally, interspecific differences in oviducal gland ultrastructure could generate variation in sperm storage capability, and perhaps affect F_{MP} (Pratt 1993). For example, Daly-Engel et al. (2010) suggested the underdeveloped oviducal gland in squalid sharks could limit sperm storage and account for the low F_{MP} in *S. mitsukurii*. Comparatively low F_{MP} in *S. acanthias* supports this hypothesis (Lage et al. 2008, Veríssimo et al. 2011), as does the high F_{MP} for *M. henlei* (Byrne and Avise 2012), a member of the family Triakidae, in which complex partitioning and sperm storage in the oviducal gland have been documented (Conrath and Musick 2002, Storrie et al. 2008, Farrell et al. 2010).

However, limited sperm storage does not readily explain the low F_{MP} observed for *T. semifasciata*, which should have an oviducal gland similar in structure to *M. henlei*.

Therefore, the most likely explanation for the low F_{MP} observed for *T. semifasciata* is a reduced mate encounter rate caused by female-specific aggregation behavior. However, there may be exceptions to this rule, given the difference observed in F_{MP} between 2010 (20.0%) and 2011 (83.3%). During a concurrent study of leopard shark movement patterns using passive acoustic telemetry (Chapter 2), the mean number of individual males detected d^{-1} at the La Jolla aggregation (males were tagged in July 2009 at a site 12 km north of the La Jolla aggregation) was 0.009 (range: 0 – 1 male detected d^{-1}) in 2010 and 0.784 (range: 0 – 5 males detected d^{-1}) in 2011. The increased male presence at the La Jolla aggregation site in 2011 suggests the mate encounter rate was also likely higher during this year, which in turn led to increased mating activity and a higher F_{MP} . Given this interannual variability in F_{MP} , multiple paternity likely arises in *Triakis semifasciata* as a consequence of sexual conflict; despite the need for cooperation for successful intromission, female sharks may concede to most, if not all, mating attempts if the costs of resistance and attempted escape (e.g., incurring injury) are expected to exceed the costs of capitulation (Portnoy et al. 2007, DiBattista et al. 2008a, Daly-Engel et al. 2010). Such “convenience polyandry” does not necessarily render moot more elaborate evolutionary explanations of multiple paternity (e.g., bet-hedging, trading up, combating sperm depletion); however, it would seem unlikely, for example, that female leopard sharks would hedge their bets or trade up to more attractive males more in one year than another.

In conclusion, the results of this study support the hypothesis that genetic polyandry is limited by female aggregation behavior and that F_{MP} should largely reflect the probability of encountering mates during each reproductive cycle (Daly-Engel et al. 2010). The lack of mating scars in sampled females suggests mating may not be as violent as in other species, suggesting at least for *T. semifasciata* that avoiding mating may not be the primary incentive for aggregation; rather, avoiding male harassment may be an additional benefit to food availability or behavioral thermoregulation (Chapter 1).

Table 3.1: Summary information for litters of *Triakis semifasciata*.

Notes:

Multiply sired litters are indicated in bold

Abbreviations: ID, dam identification letter; TL, dam total length in cm; SIZE, litter size in number of pups; F:M, ratio of female to male pups in a given litter; # DL, number of pups and unfertilized eggs (in parentheses) delivered; # LT, number of pups found in left horn of uterus upon dissection of dam; # RT, number of pups found in right horn of uterus upon dissection of dam; MEAN TL $\pm$ SD, mean total length of pups in a given litter $\pm$ standard deviation in cm; DEVELOP. STAGE, stage of development of pups at time of litter acquisition expressed as premature (PREM) or term (TERM) based on the presence or absence of a yolk sac, respectively; # SIREs, minimum number of sires suggested by GERUD; SKEW, skew of male reproductive success; # PAT. GEN., number of paternal genotype solutions returned by GERUD; ALT. SKEW, number of alternative skew solutions to that reported in previous column; PrDM, probability of detecting multiple paternity for six given paternal skew scenarios

[^] Litter size artificially low because some pups were consumed by swell sharks (see text)

* Significant departure from expected 50:50 ratio ($\chi^2 = 4.545$, $n = 22$, d.f. = 1, $p = 0.033$)

^M Mortality: dam died of unknown causes (see text)

^S Salvaged: dam was found dead in the wild

^X Sacrificed: dam was sacrificed in captivity

^D Dissected: all dead dams were dissected to obtain pups

^R Released: dams were released after they gave birth

DAM			LITTER												1 - PrDM									
ID	TL (cm)	DATE ACQUIRED	DATE ACQUIRED	ACQUIRED	SIZE	F:M	# DL	# LT	# RT	MEAN TL ± SD (cm)	DEVELOP. STAGE	# SIREs (SKEW)	# PAT. GEN. (ALT. SKEW)	0.50: 0.50	0.60: 0.40	0.70: 0.30	0.80: 0.20	0.90: 0.10	0.95: 0.05					
A	150	25-09-2007		11-02-2008	25	15:10	25 (0)	0 (0)	0 (0)	20.1 ± 0.6	PREM ^{M,D}	1 (25:0)	1 (0)	0.000	0.000	0.001	0.005	0.078	0.286					
B	142	25-09-2007		04-04-2008	22	10:12	22 (0)	n.d.	n.d.	21.5 ± 0.7	TERM ^R	1 (22:0)	1 (0)	0.000	0.001	0.001	0.009	0.105	0.333					
C	132	26-09-2007		28-06-2008	11	7:4	11 (0)	n.d.	n.d.	21.2 ± 0.6	TERM ^R	1 (11:0)	1 (0)	0.003	0.006	0.025	0.093	0.325	0.577					
D	140	14-08-2009		14-08-2009	15	n.d.	0 (0)	7 (1)	8 (0)	4.1 ± 0.7	PREM ^{S,D}	1 (15:0)	1 (0)	0.001	0.002	0.007	0.041	0.216	0.474					
E	130	25-11-2009		25-11-2009	13	5:8	0 (0)	8 (0)	5 (2)	13.5 ± 0.8	PREM ^{S,D}	2 (7:6)	1 (0)	0.001	0.003	0.013	0.062	0.266	0.523					
F	145	21-08-2010		21-08-2010	22	n.d.	0 (0)	11 (0)	11 (0)	4.3 ± 0.8	PREM ^{S,D}	1 (22:0)	1 (0)	0.001	0.001	0.001	0.011	0.109	0.336					
G	154	03-09-2010		03-09-2010	25	n.d.	0 (0)	14 (1)	11 (1)	4.9 ± 0.9	PREM ^{S,D}	1 (25:0)	1 (0)	0.000	0.000	0.001	0.005	0.079	0.287					
H	147	23-09-2010		27-12-2010	9 ^A	4:5	9 (0)	0 (0)	0 (0)	12.8 ± 0.6	PREM ^{M,D}	2 (5:4)	2 (0)	0.007	0.014	0.047	0.145	0.397	0.638					
I	147	18-09-2009		18-06-2010	33	22:11	33 (0)	n.d.	n.d.	21.0 ± 0.5	TERM ^R	1 (33:0)	1 (0)	0.000	0.000	0.001	0.001	0.035	0.191					
J	142	20-07-2010		20-07-2010	24	n.d.	0 (0)	11 (0)	13 (0)	3.8 ± 0.7	PREM ^{S,D}	1 (24:0)	1 (0)	0.000	0.001	0.001	0.007	0.087	0.301					
K	150	23-09-2010		09-01-2011	4 ^A	2:2	4 (0)	0 (0)	0 (0)	15.8 ± 0.6	PREM ^{M,D}	2 (3:1)	72 (1)	0.199	0.228	0.315	0.464	0.687	0.833					
L	140	23-09-2010		09-01-2011	13	7:6	0 (0)	5 (2)	8 (1)	13.0 ± 0.9	PREM ^{X,D}	1 (13:0)	1 (0)	0.001	0.013	0.012	0.061	0.265	0.523					
M	137	23-09-2010		19-04-2011	21	10:11	21 (2)	n.d.	n.d.	21.8 ± 0.7	TERM ^R	1 (21:0)	1 (0)	0.001	0.001	0.002	0.012	0.118	0.355					
N	137	23-09-2010		02-05-2011	22	7:15	22 (2)	n.d.	n.d.	19.5 ± 2.0	TERM ^R	1 (22:0)	1 (0)	0.000	0.001	0.001	0.008	0.097	0.319					
O	132	23-09-2010		03-05-2011	18	7:11	18 (0)	n.d.	n.d.	20.2 ± 0.7	TERM ^R	1 (18:0)	1 (0)	0.001	0.001	0.003	0.027	0.175	0.426					
P	143	23-09-2010		01-07-2011	21	13:8	21 (0)	n.d.	n.d.	20.7 ± 0.5	TERM ^R	1 (21:0)	1 (0)	0.001	0.001	0.002	0.012	0.117	0.348					
Q	139	03-10-2011		06-10-2011	25	9:16	0 (0)	12 (1)	13 (0)	15.5 ± 0.6	PREM ^{X,D}	1 (25:0)	1 (0)	0.000	0.001	0.001	0.006	0.078	0.287					
R	156	03-10-2011		26-01-2012	24	12:12	21 (0)	1 (1)	2 (1)	20.6 ± 0.6	PREM ^{M,D}	2 (21:3)	1 (0)	0.000	0.001	0.001	0.006	0.086	0.300					
S	148	04-10-2011		15-02-2012	30	17:13	0 (0)	14 (0)	16 (0)	20.2 ± 0.4	PREM ^{X,D}	2 (17:13)	1 (0)	0.000	0.001	0.001	0.002	0.047	0.223					
T	145	04-10-2011		21-03-2012	22	6:16*	3 (0)	9 (0)	10 (0)	20.6 ± 0.5	PREM ^{M,D}	2 (20:2)	4 (1)	0.000	0.001	0.001	0.010	0.107	0.333					
U	136	03-10-2011		24-03-2012	22	12:10	11 (0)	7 (0)	4 (0)	18.3 ± 1.1	PREM ^{M,D}	2 (21:1)	30 (0)	0.001	0.001	0.001	0.010	0.107	0.332					
V	145	04-10-2011		27-04-2012	28	16:12	28 (0)	n.d.	n.d.	21.6 ± 0.4	TERM ^R	2 (26:2)	21 (1)	0.000	0.001	0.001	0.003	0.059	0.248					

Table 3.2: Summary of microsatellite characteristics for *Triakis semifasciata*.

Note:

Abbreviations: T_a, annealing temperature; Cycl. #, number of PCR cycles; Size, size in base-pairs of the cloned allele from which the primers were designed; N_i, number of individuals screened; N_a, allelic diversity; H_E, expected heterozygosity; H_O, observed heterozygosity; P_{HW}, *p*-values from Hardy-Weinberg exact tests for homozygote excess; P_E, exclusion probability

Locus	Primer Sequences	5' Label	T _a (°C)	Cycl. #	Repeat Motif	Size (bp)	N _i	N _a	H _E	H _O	P _{HW}	P _E
<i>TrSe-A1</i>	F: 5'-TGTCCTTTTGTAATTCCTAATCC-3' R: 5'-CGGGAGTATGGTGATTC-3'	HEX	56	33	(CA) ₁₄	239	148	9	0.785	0.791	0.502	0.580
<i>TrSe-A103</i>	F: 5'-CACCAGCAATCTGTCACTTG-3' R: 5'-CTGTCTTAGCAATGGGTCTGT-3'	6-FAM	56	28	(CA) ₁₀ C(CA) ₁₈	123	148	23	0.868	0.885	0.674	0.735
<i>TrSe-D2</i>	F: 5'-CAGTATCTGGGATGGACTCTA-3' R: 5'-AAGCAGTGTGAGTGGTAGTAGG-3'	TET	56	32	(GATA) ₁₅	287	148	17	0.878	0.872	0.541	0.763
<i>TrSe-D12</i>	F: 5'-CCTGCCTGGTTATTGACC-3' R: 5'-CCTGACTGAGGTGTGTAAGATT-3'	HEX	56	35	(CTAT) ₁₆	140	148	18	0.873	0.885	0.78	0.757

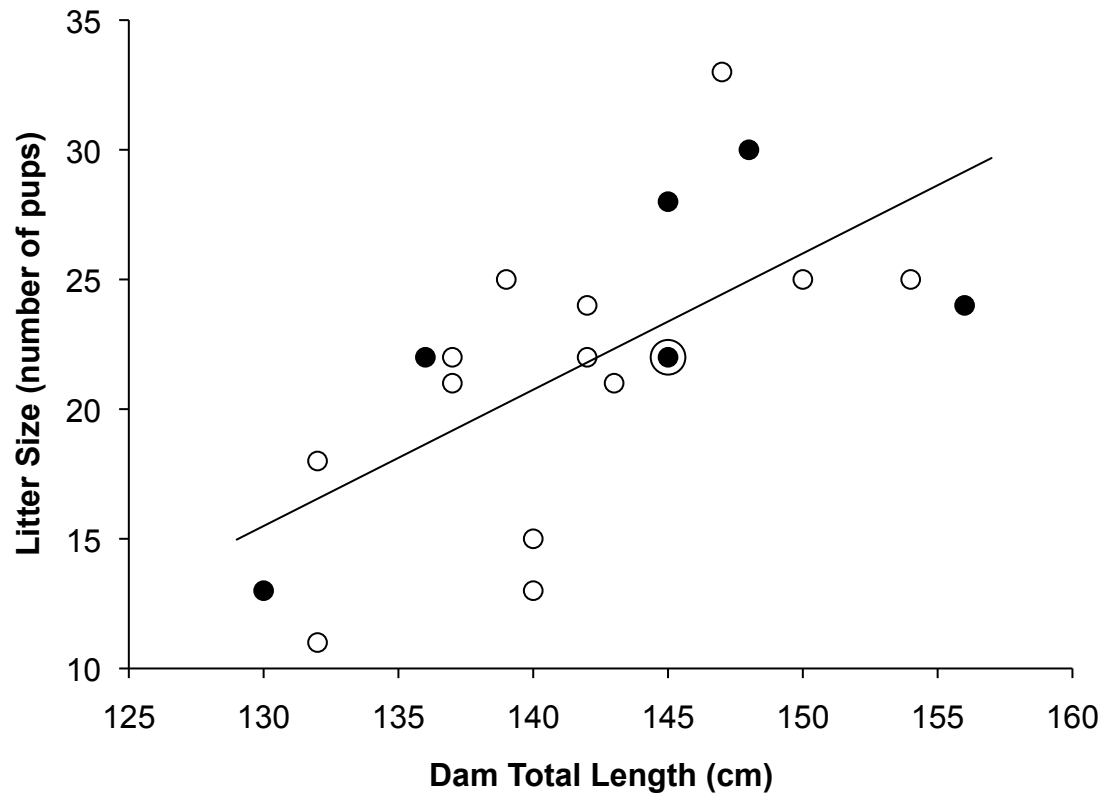


Figure 3.1: Linear regression of *Triakis semifasciata* litter size versus dam total length.

Notes:

Litters H and K are excluded from linear regression because these litter sizes were artificially low (see text)

Singly sired litters appear as open circles and multiply sired litters appear as closed circles

Both a singly sired and a multiply sired litter occur at (145, 22)

Regression statistics: $r^2 = 0.429$, $p = 0.002$; litter size = $0.526 \times (\text{dam total length}) - 52.826$

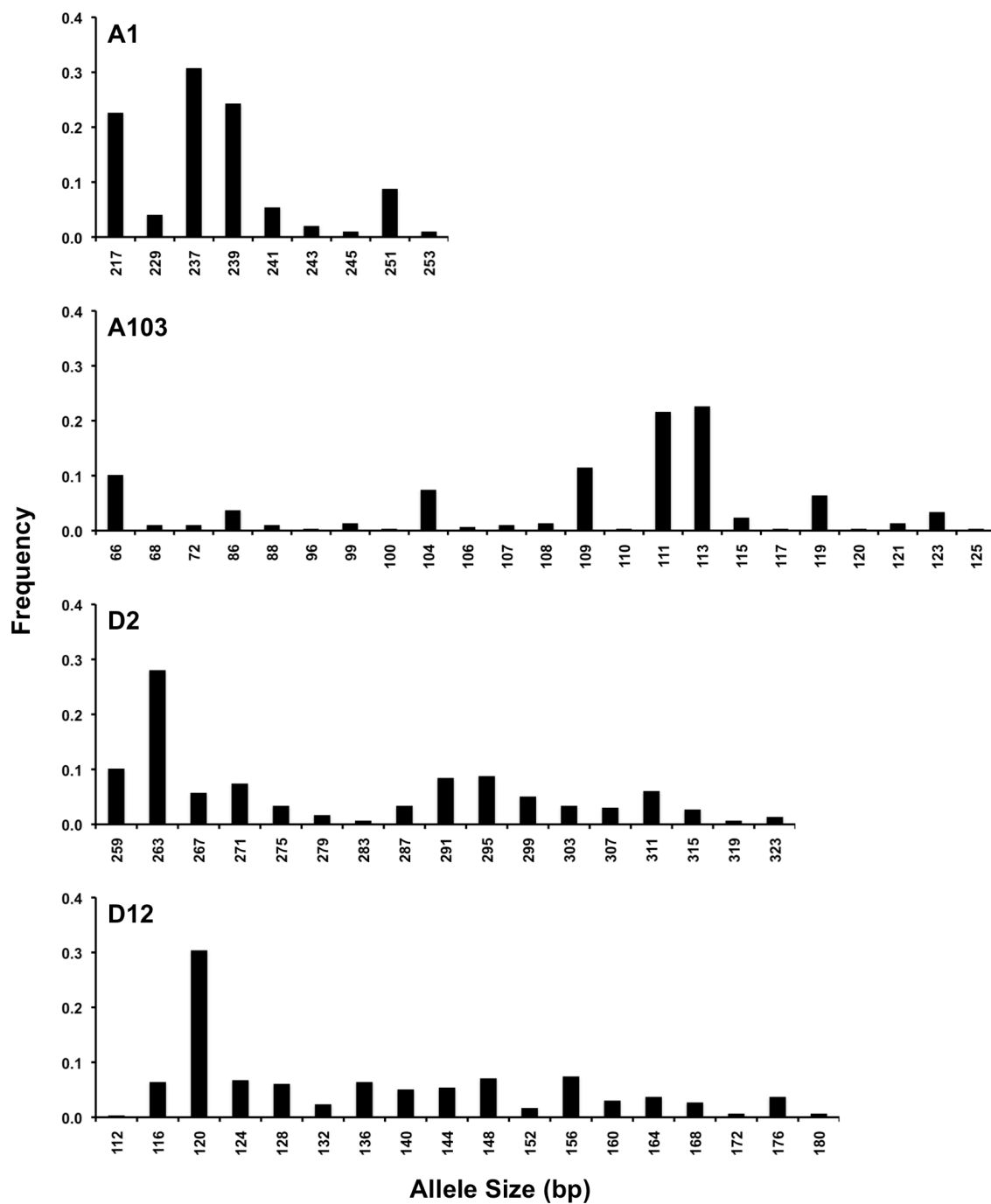


Figure 3.2: Allelic frequency distribution of four microsatellite loci (A1, A103, D2, and D12) for 148 presumably unrelated *Triakis semifasciata* individuals.

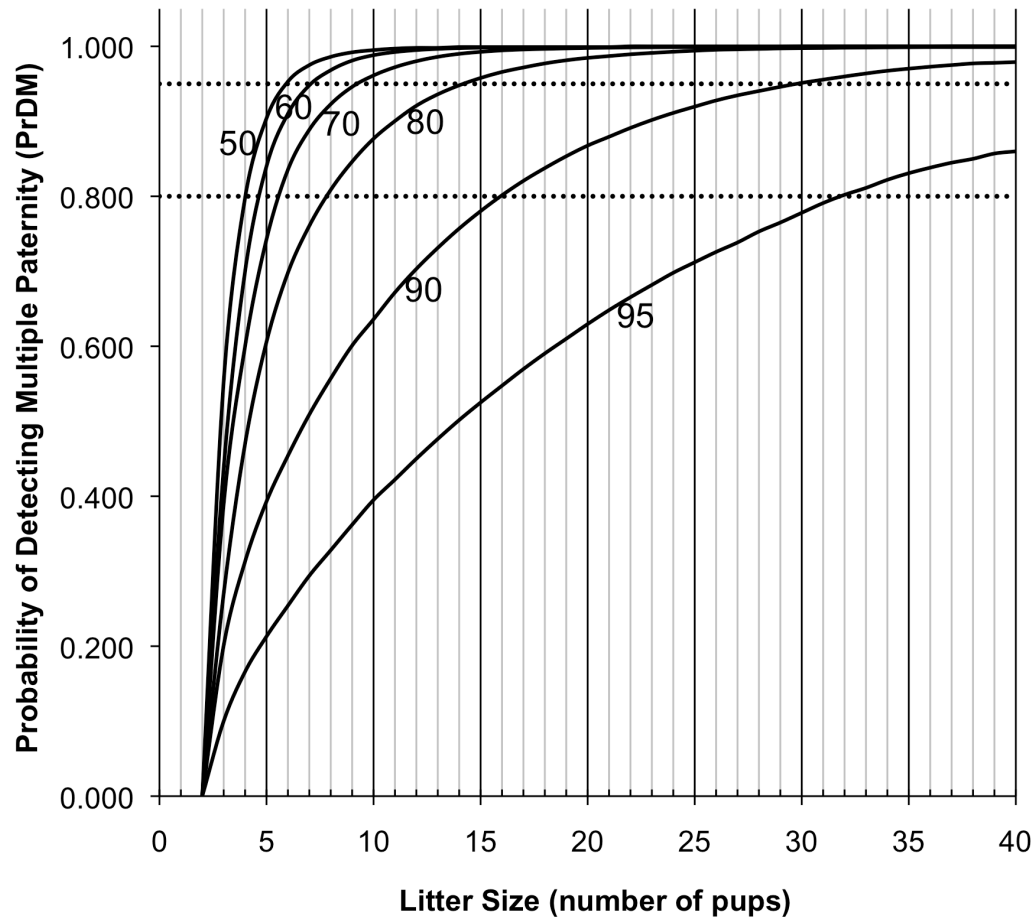


Figure 3.3: Probability of detecting multiple paternity (PrDM) versus leopard shark (*Triakis semifasciata*) litter size (number of pups) indicated by solid black lines for paternal skew scenarios of 50:50 (50), 60:40 (60), 70:30 (70), 80:20 (80), 90:10 (90), and 95:5 (95).

Notes:

PrDMs of 0.950 and 0.800 are indicated by horizontal dotted lines for reference

These results are specific to the four microsatellite loci (A1, A103, D2, and D12) and 148 presumably unrelated individuals screened in this study

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ACKNOWLEDGEMENTS

I thank the many people who volunteered their time in various aspects of this project, particularly S. Pennebaker, A. Barker, E. Kisfaludy, T. Smith, M. Ouyang, P. Zerofski, and C. Jew. Funding was provided by the Center for Marine Biodiversity and Conservation at Scripps Institution of Oceanography and a generous donation from the Nosal Family. A. Nosal was supported by Graduate Research Fellowship Program (GRFP) and Integrated Graduate Education and Research Traineeship (IGERT, No. 0333444) awards from the National Science Foundation. This work was conducted under University of California – San Diego Institutional Animal Care and Use Committee (IACUC) protocol No. S00080.

This chapter, in full, was submitted for publication as: Nosal AP, Lewallen EA, Burton RS (*in review*). Multiple paternity in leopard shark (*Triakis semifasciata*) litters sampled from a female-dominated aggregation in La Jolla, CA, USA. *Journal of Experimental Marine Biology and Ecology*. The dissertation author is the primary investigator and author of this manuscript.

CONCLUSIONS OF THE DISSERTATION

This is the first study of leopard shark aggregation behavior along the open coast; previous work was limited to bays, estuaries, and sheltered coves. However, the La Jolla aggregation site (the primary focus of this dissertation) actually resembles a sheltered cove in some ways, which likely explains the abundance of sharks there. This dissertation showed that leopard sharks aggregating in La Jolla spent most of their time in very shallow water close to shore (often near the surf); this is where the warmest water is found due to solar insolation of the surface water (Chapter 1). Thus, these ectothermic sharks, which are mostly pregnant females, likely benefit thermally by increasing the rate of embryonic development. Anomalous small waves at the aggregation site likely facilitate the invasion of the warmest and shallowest water available; the alongshore gradient in wave height is due to bathymetric wave refraction by the nearby La Jolla submarine canyon and may contribute to the proximate cause dictating the specific location of the aggregation. The “incubation hypothesis” is further supported by the fact that seasonal abundance of females at this location was strongly positively correlated with sea surface temperature, and strongly negatively correlated with swell height; diel abundance was also positively correlated with sea surface temperature (Chapter 2). Changing sea surface temperature, swell height, and other environmental variables such as photoperiod, may cue the seasonal arrival to and departure from the aggregation site.

The La Jolla aggregation may also function as a “home base” to facilitate central-place foraging. Sharks departed the aggregation site at night, likely dispersing to deeper

offshore locations to feed, as evidenced by some actively tracked sharks and the non-destructive diet analysis finding canyon-dwelling market squid (*Loligo opalescens*) to be the primary diet component (Chapter 1). Thus, maintaining a central position relative to known foraging grounds, as opposed to being nomadic, may increase foraging efficiency by reducing search time.

Lastly, the scarcity of males at the La Jolla aggregation site may benefit females by reducing harassment in the form of excessive and potentially injurious matings attempts. Consistent with this hypothesis is the generally low frequency of multiple paternity (F_{MP}) observed in litters sampled from mothers collected in La Jolla (Chapter 3). However, F_{MP} was significantly higher in 2011 than in 2010, coinciding with an increase in male abundance at the La Jolla aggregation site in 2011 compared to 2010; thus, there may have been more mating activity in 2011 resulting in more multiply fathered litters.

Future studies should attempt to further resolve the mechanism governing the formation of the aggregation, particularly the interaction between social and asocial forces that maintain group cohesion. In addition, it remains unclear why males generally avoid the La Jolla aggregation site; future work should investigate intersexual differences in habitat and temperature preference, which could explain the observed sexual segregation.