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# Determining sexual maturity in male leopard sharks in San Francisco Bay, California

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The leopard shark (*Triakis semifasciata*) is endemic to the eastern North Pacific ranging from Mazatlan, Mexico and the Gulf of California to Oregon. It is an inshore species generally found in shallow water < 91 m deep (Smith 2001; Ebert 2003). This species is one of the most researched elasmobranchs along the Pacific Coast of North America with several aspects of its biology well defined (Ackerman 1971; Russo 1975, 2013, 2015, 2018, 2019; Talent 1976, 1985; Smith 1984, 2001, 2005; Smith and Abramson 1990; Cailliet 1992; Kusher et al. 1992; Au and Smith 1997; Smith et al. 2003; Hight and Lowe 2007; Lewallen et al. 2007; Carlisle and Starr 2009, 2010; Nosal et al. 2013a,b, 2014; Launer 2014; Barker et al. 2015). Various reproductive studies have occurred for leopard sharks (Ackerman 1971; Talent 1985; Ebert and Ebert 2005; Smith 2005; Nosal et al. 2013a, b, 2014; Launer 2014; Russo 2015, 2018, 2019). Although these studies have found sexual segregation the specifics of male sexual maturity remain unknown. Additionally, little detail is known concerning the process of sexual maturation in males from known breeding locations within its range.

Male leopard sharks may reach sexual maturity at a total length (TL) ranging from 70 to 120 cm (Ebert 2003), a range currently thought to be based on various known breeding locations and research methods. However, details regarding the length at which calcification of claspers and production of mature spermatozoa occur in male leopard sharks are poorly known.

Several reports on calcification of claspers and production of spermatozoa of various species have been published (Pratt 1979; Joung and Hsu 2005; Huveneers et al. 2007; Awruch et al. 2008). Clark and Von Schmidt (1965) and Chen et al. (1988) have suggested that calcified claspers and the ability of the rhipidion to splay is a measure of sexual maturity. Yet, other studies maintain that a more reliable indicator of sexual maturity involves clasper length and calcification in combination with swollen testes and the presence of spermatozoa (Peres and Vooren 1991; Jensen et al. 2002; Lucifora et al. 2005; Conde-Moreno and Galvan-Magaña 2006; Awruch et al. 2008; Dharmadi and Wiadnyana 2013; Natanson and Gervelis 2013; Gracan and Lackovic 2016). The purpose of the present study was to determine whether calcification of claspers is correlated with production of spermatozoa and thus an indicator of sexual maturity in leopard sharks. Data first reported herein, shows that in male leopard sharks in South San Francisco Bay, there is a time delay between the length at which claspers calcify and spermatozoa is produced.

Between 1970 and 2001, data collection was conducted monthly primarily between the San Francisco Bay Bridge (37.800 N, 122.3667 W) and the entrance of Alviso Slough (37.450 N, 122.017 W) at the south end of San Francisco Bay (Figure 1). There were 224 catch events (one technique, location, date, and time) using long-lines (n = 146), rod and reel (n = 36), and otter trawl (n = 42) (Russo 2019).


**Figure 1.** Map of the study area of San Francisco Bay with all catch events (red circles) in this study restricted to South San Francisco Bay and close to known parturition or mating sites. Map courtesy of the East Bay Regional Park District.

I collected data on sex, external parasites, and general condition of every shark. All measurements of individual sharks were made in centimeters total length (cm TL), whereas groups of sharks were calculated in centimeters mean total length (cm MTL). I physically examined male claspers for flexibility, extent of calcification and length from the tip to the posterior margin of the cloacal opening. I also examined clasper tips for spurs, hooks, or spines used in holding the clasper in the female cloaca, but none were found in the field and samples were not removed for microscopic examination later (Pratt and Carrier 2004). Clasper length was plotted against total length for analysis with color designations at the points at which calcification of claspers and production of spermatozoa occurred. Both sperm sacs and seminal vesicles were examined for presence of spermatozoa in the field along with the condition of the vas deferens, which are coiled in mature sharks. I took samples back to the laboratory for microscopic examination.

In this study, 4,121 elasmobranchs were captured mostly from South San Francisco Bay including 2,478 (60.1%) leopard sharks composed of 1,299 males and 1,179 females (Russo 2019). I selected a group of 99 male leopard sharks as a representative sample of all potential stages of maturity (Table 1, Figure 2A). These individuals ranged in size from 44.4 to 124.4 cm TL (86.3 cm MTL ± 19.6 SD) and were captured in six male-dominated long line events during the months of expected sexual activity from April to early July (1977 - 1990) (Ebert and Ebert 2005, Russo 2015, 2018, 2019) and close to known parturition sites defined in Russo (2019) (Figure 1). Clasper length ranged from 2.5 to 14.6 cm (5 cm MTL ± 1.3 SD). This analysis broadly defined the length "markers" at which calcification of claspers occurred along with the production of spermatozoa. Calcified claspers ranged in length from 6.3 cm to 14.6 cm (12.4 cm MTL ± 2.04 SD). Of these, 44.4% (44/99) possessed calcified claspers and 32.3% had possessed spermatozoa (Table 1, Figure 2a). Of the 44 males with calcified claspers, 72.7% (n = 32) were sexually mature, while the remaining 27.3% (n = 12) with calcified claspers were sexually immature.

Additionally, there was a familiar relationship between clasper elongation and TL. This relationship shows rapid clasper elongation from 6.4 cm to 14 cm (+7.6 cm) between 86 and 101 cm TL, which is where calcification begins between 86 and 92.7 cm TL (Table 1), along with weight gain from 2 kg (86 cm TL) to > 6.3 kg (> 100 cm TL; Russo, unpublished data; Kusher et al. 1992). Similar patterns in rapid clasper elongation coinciding with weight gain prior to or during calcification have been found in school sharks (tope) (*Galeorhinus galeus*; Peres and Vooren 1991), bonnethead shark (*Sphyrna tiburo*; Kajiura et al. 2005), shortfin mako shark (*Isurus oxyrinchus*; Joung and Ysu 2005) and the blackspotted smoothhound (*Mustelus punctulatus*; Gracan and Lackovic 2016). Whereas most of these studies focused

**Table 1.** Summary of 99 males captured in six male-dominated catch events between 1977 and 1990. Examination of body and clasper lengths were used to determine length "markers" when calcification of claspers occurs and onset of spermatozoa. n = no calcification or spermatozoa found at these size ranges.

| Size range | | Clasper length | | | Number | |
|---|---|---|---|---|---|---|
| cm/cm | MTL | cm/cm | MTL | Specimens | Calcified | Spermatozoa |
| 44.4 – 86.3 | 68.5 | 2.5 – 7 | 4.4 | 50 | *n* | *n* |
| 86.4 – 91.4 | 90.7 | 6.4 – 10.1 | 7.6 | 12 | 7 | *n* |
| 92.7 – 124.4 | 104.1 | 8.9 – 14.6 | 12.7 | 37 | 37 | 32 |

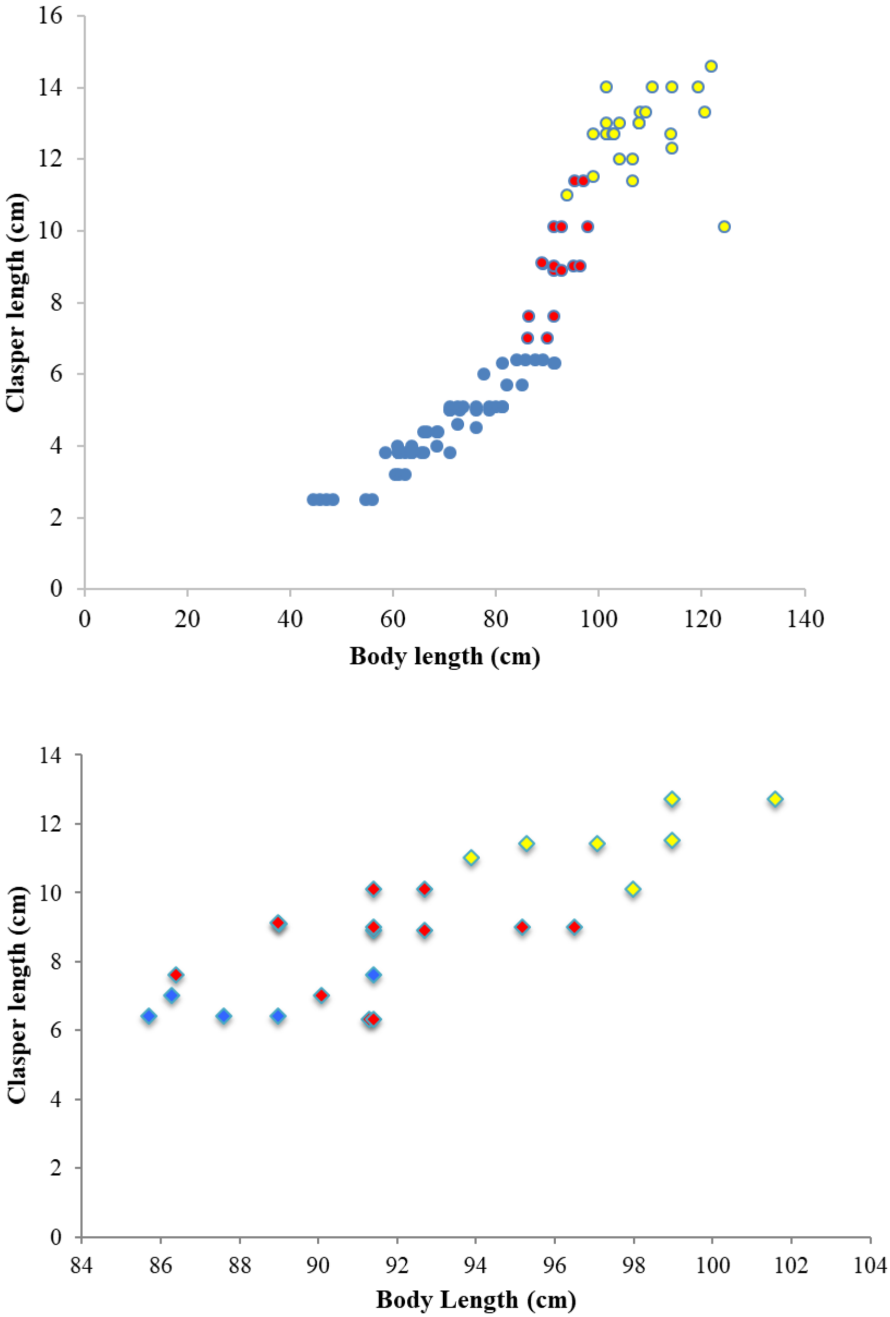


**Figure 2.** (a)Analysis of 99 male leopard sharks indicating the calcification of claspers (red) and the onset of spermatozoa with calcified claspers (yellow). (b) Analysis of 25 male leopard sharks from 85.7 cm to 101.6 cm TL indicating lengths at which calcification (red) and spermatozoa/calcification (yellow) occur. Blue indicates immature sharks without calcified claspers. Duplicate data points may be superimposed, one over another.

on clasper length, body length and testes weight, no study found or distinguished between the length at which calcification begins and the onset of spermatozoa production indicating any delay between the two episodes.

I conducted a separate analysis of 25 males, a subset of the larger sample of 99, ranging in TL from 85.7 cm (prior to calcification herein) to 101.6 cm (just beyond the 100% mature benchmark of 99 cm TL; Figure 2b). Here, claspers ranged from 6.3 to 12.7 cm long (9 cm MTL ± 1.9 SD), including 7 (28%) juveniles along with 18 (72%) males with calcified claspers and only 8 (32%) of those possessing spermatozoa. These results showed that claspers grew rapidly until individuals approached >103 cm TL when growth slowed as maximum size limits in this study approached (124.4 cm TL), along with a delay in the production of spermatozoa between calcification at 86.4 cm and 93.9 cm TL when the first spermatozoa were found.

The first indication of calcification occurred at 86.4 cm TL (claspers at 7.6 cm long), which indicated that clasper length compared to body length was highly variable. For example, the largest specimen measured at 124.4 cm TL had claspers at 10.1 cm, which was equivalent to another male at 91.4 cm TL, a difference of 33 cm in TL length. Between 86.4 cm TL and 91.4 cm TL ($n$ = 12), calcification was inconsistent appearing in only 7 (58.3%) males in this size range (Table 1). Claspers of males > 92.7 cm TL ($n$ = 35, 106.7 cm MTL ±7.8 SD) had a median clasper length of 12.7 cm (±1.4 SD) and were all calcified.

The production of spermatozoa was also inconsistent beginning at 93.9 cm TL and occurring among only 50% (n = 3) of the males between 93.9 cm TL and > 98.0 cm TL (n = 6) whereupon 100% of the males possessed spermatozoa. One male at 91.4 cm TL with 6.3 cm claspers represented the smallest claspers at which calcification occurred, yet it was still immature. The largest juvenile male with uncalcified claspers and no spermatozoa measured 91.4 cm TL. The smallest adult male with calcified claspers and spermatozoa was 93.9 cm TL.

Sexual maturity, as evidenced by presence of mature spermatozoa in seminal vesicles and sperm sacs as well as the coiled condition of the vas deferens, generally did not occur until males were > 93.9 cm TL with claspers at a minimum > 10.1 cm (Figure 2). Once calcification occurred there was a growth of 7.5 cm in body length combined with an increase in clasper length of 3.1 cm indicating a delay prior to sexual maturity as first reported here. Calcification and production of spermatozoa in this study occurred at a larger body length size (+16.4 cm) than the lower end “maturity” size (70 cm TL) given by Ebert (2003) but within his overall range. The range for sexual maturity in this study is relatively narrow (93.9 - 99 cm TL) compared to published estimates (100 - 105 cm TL [Kusher et al. 1992], 70 - 120 cm TL [Ebert 2003]) based on potential breeding locations, environmental conditions, changes in climate, and research methods. There is considerable variability in body length related to clasper length of individual leopard sharks possibly attributable to availability of food, parasites, general health, genetics and other environmental factors (Figure 2).

Based on data presented herein, sexual maturity cannot be inferred from length or the calcification of claspers alone in leopard sharks, given the potential variation in critical physical factors among individuals from one locality to another. Instead, clasper length and calcification in combination with presence of spermatozoa are the only reliable measure of sexual maturity for this species. Comparisons with male leopard sharks in other known breeding locations including an increased number of larger specimens should be made to refine our understanding of the reported 50 cm TL range for sexual maturity with leopard sharks, as well as factors that influence such differences.

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